ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 10, 2008

DOI: 10.1089/ars.2008.2071

Comprehensive Invited Review

Cell Cycle Regulation by Oncogenic Tyrosine Kinases in Myeloid Neoplasias: From Molecular Redox Mechanisms to Health Implications

Margret S. Rodrigues, Mamatha M. Reddy, and Martin Sattler

Reviewing Editors: Douglas Conklin, David Gius, Nicholas Heintz, Elke Heiss, David Jourd'heuil, Alicia Jozkowicz, and Hugo Monteiro

I. Introduction 1814
II. Source of ROS in BCR–ABL Transformation 1815
A. ROS are linked to a hyperactive glucose metabolism 1815
B. Warburg effect and signaling requirements for ROS 1815
C. Rac and NADPH oxidases 1817
III. Regulation of Protein Function by ROS 1818
IV. Regulation of BCR–ABL Function by Oxidative Stress 1819
V. Role of Hypoxia in the Regulation of ROS 1820
A. HIF-1α activation and ROS 1820
B. Targeting HIF-1 pathways 1821
C. NF-κB activation by hyperactive glucose metabolism and ROS 1822
D. Redox regulation of NF-κB 1823
VI. Cell Cycle Regulation Through Redox Mechanisms 1823
A. Protein tyrosine phosphatases 1824
1. PTEN 1824
2. CDC25 phosphatases 1825
B. The FoxO subfamily of forkhead box proteins 1826
C. The Jak2/STAT5 pathway 1827
D. A redox switch for Ras family proteins 1828
VII. Heat-Shock Proteins 1829
VIII. Nrf2—-A Crucial Oxidative Stress Response Regulator 1831
IX. Transformation and Genomic Instability 1832
X. Targeting ROS in Cancer Therapy 1833
XI. Conclusions 1835

Abstract

Neoplastic expansion of myeloid cells is associated with specific genetic changes that lead to chronic activation of signaling pathways, as well as altered metabolism. It has become increasingly evident that transformation relies on the interdependency of both events. Among the various genetic changes, the oncogenic BCR–ABL ty-

Department of Medical Oncology, Dana–Farber Cancer Institute, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts.

rosine kinase in patients with Philadelphia chromosome positive chronic myeloid leukemia (CML) has been a focus of extensive research. Transformation by this oncogene is associated with elevated levels of intracellular reactive oxygen species (ROS). ROS have been implicated in processes that promote viability, cell growth, and regulation of other biological functions such as migration of cells or gene expression. Currently, the BCR–ABL inhibitor imatinib mesylate (Gleevec) is being used as a first-line therapy for the treatment of CML. However, BCR–ABL transformation is associated with genomic instability, and disease progression or resistance to imatinib can occur. Imatinib resistance is not known to cause or significantly alter signaling requirements in transformed cells. Elevated ROS are crucial for transformation, making them an ideal additional target for therapeutic intervention. The underlying mechanisms leading to elevated oxidative stress are reviewed, and signaling mechanisms that may serve as novel targeted approaches to overcome ROS-dependent cell growth are discussed. *Antioxid. Redox Signal*. 10, 1813–1848.

I. Introduction

The role of oxidative stress has been a focus of cancer research for years, and despite the considerable progress in the field, little is known about the molecular mechanisms contributing to cancer growth. It has been widely accepted that oxidative stress through ROS (reactive oxygen species) leads to DNA lesions and genomic mutations. ROS refers to sequential intermediates generated by univalent reductions of molecular oxygen through stepwise electron transfer from the superoxide radical ($O_2\bullet^-$), to hydrogen peroxide (H_2O_2), and then to the hydroxyl radical (•OH), which can be reduced to water. Probably the most relevant ROS for the regulation of signaling mechanisms is H_2O_2, due to its long half-life relative to other ROS. The importance of ROS in signaling of cancer cells is evident by the growth inhibitory and pro-apoptotic effects of antioxidants. The molecular mechanisms that regulate these biological effects are just beginning to emerge. It is clear, however, that there is no master switch that regulates all biological functions.

ROS are thought to be involved in the pathogenesis of many different types of cancer. They can play a role in processes that promote cell growth and regulate other biological functions such as gene expression or migration of cells (320). Interestingly, Suh *et al*. demonstrated that increased production of ROS can lead to a transforming phenotype (351). Overexpression of the $O_2\bullet^-$-generating NADPH oxidase catalytic subunit Nox1 in Ras-transformed NIH3T3 fibroblasts increases cell growth and induces tumors in athymic mice. ROS are also formed in normal cells in response to a variety of stimuli including UV irradiation or hematopoietic growth factors, such as thrombopoietin (TPO), steel factor (SF), interleukin-3 (IL-3), granulocyte–macrophage colony stimulating factor (GM–CSF), and others (166, 320). Platelet-derived growth factor (PDGF)-dependent increase of H_2O_2 was shown to induce tyrosine phosphorylation, mitogen-activated protein (MAP) kinase stimulation, DNA synthesis, and chemotaxis (354). In all these cases, it was suggested that ROS act as second messengers to regulate activities of redox-sensitive enzymes (355, 365).

In addition to the direct induction of ROS through mitochondrial pathways, levels of ROS can be regulated indirectly though a variety of pathways. For example, thiols such as thioredoxin or glutathione reduce ROS, and it is possible that growth stimuli in hematopoietic cells affect the activity of one or more of these pathways. Superoxide dismutase generates H_2O_2 from $O_2\bullet^-$, while catalase reduces H_2O_2 to water. The glutathione peroxidases, which also include the phospholipid hydroperoxide glutathione peroxidase, reduce peroxides by using reduced glutathione (GSH) as an electron donor and generating the oxidized dimeric form of glutathione (GSSG). Another group of peroxidases are the peroxiredoxins (Prxs), previously characterized to be more widely expressed and with higher binding affinities to hydroperoxides, making them a more efficient scavenger of H_2O_2 (399). In eukaryotic cells, peroxiredoxins are proposed to function both as antioxidants as well as regulators of H_2O_2-mediated signaling. This proposed dual mechanism has been conceptualized in the "floodgate hypothesis" (399). The normal peroxiredoxin catalytic cycle in resting cells involves the cyclic oxidation of the peroxidactic (catalytic) cysteine to sulfenic acid by H_2O_2, followed by the formation of an intracellular disulfide bond. This in turn is subsequently reduced by thioredoxin to its initial state. This way, peroxiredoxins act as a peroxide floodgate, preventing H_2O_2 signaling until the floodgate is opened. When a cell produces a transient intracellular burst of H_2O_2, peroxiredoxins are subject to overoxidation. Thioredoxins are inactivated under these conditions, preventing the reduction of H_2O_2, thus rendering the floodgate open and allowing for the signaling of H_2O_2. There are also mechanisms of ROS regulation that do not directly involve enzyme activities. ROS levels can further be regulated by exogenous antioxidants such as α-tocopherols, β-carotene, or ascorbic acid. Spontaneous dismutation of $O_2\bullet^-$ results in the generation of H_2O_2. Reduction of H_2O_2 in the presence of Fe^{2+} or certain other metal ions can yield •OH through the Fenton reaction. In addition to enzymes, antioxidants, and sulfhydryl groups, ROS can be quenched by reacting with cellular molecules such as lipids and DNA bases (137).

Cancer cells typically display chronic activation of signaling mechanisms that lead to deregulation of growth. ROS may contribute to these mechanisms, but elevated levels may also be dependent on deregulated pathways. Consistent with an important role of ROS in signaling, there is an imbalance between ROS production and scavenging. For example, transformation of hematopoietic cells by the oncogenic tyrosine kinase BCR–ABL is associated with a chronic increase of intracellular ROS (319). The BCR–ABL fusion protein in CML (chronic myelogenous leukemia) is generated by the Philadelphia (Ph) chromosome translocation t(9;22)(q34;q11), and has elevated ABL tyrosine kinase ac-

tivity that is critical for transformation. In addition to BCR–ABL, myeloproliferative diseases and myeloid leukemias are frequently associated with mutations that activate additional tyrosine kinases, including the Jak2V617F mutation associated with myeloproliferative disorders (MPD), TEL-ABL, FLT3.ITD, and TEL-PDGFR associated with acute myeloid leukemias (AML) and others (for review, see ref. 22).

CML is considered a model disease in cancer biology, since the BCR–ABL oncogene is well defined and is not only required but also likely sufficient for transformation by itself. BCR–ABL-transformed cells show reduced growth factor requirements, enhanced viability, and altered adhesion as a result of constitutive activation of signaling pathways such as $p21^{RAS}$, STAT5, and phosphatidylinositol 3–kinase (PI3K) (Fig. 1). Interestingly, the peroxiredoxin family member Prx-1 (PAG) was shown to interact with the Src homology-3 (SH3) domain of c-ABL (393). This domain is of significance since mutation of the c-ABL SH3 generates a transforming phenotype in fibroblasts but not in hematopoietic cells (170, 280). The interaction of Prx-1 with ABL results in the inhibition of tyrosine phosphorylation and inhibition of cytostatic effects induced by overexpression of c-Abl, effectively linking redox mechanisms to the functional activation of ABL. This functional interaction may be partially disrupted in the BCR–ABL oncoprotein (393). Indirect evidence for elevated levels of ROS in primary CML cells has previously been provided (165). Not only ROS by themselves, but also mechanisms that lead to ROS production, as well as redox-sensitive enzymes, may provide ideal targets for future therapeutic intervention. In this review, the major molecular mechanisms and targets of ROS in cancer cells that are known to contribute to cell growth are summarized with an emphasis on transformation by the BCR–ABL oncogenic tyrosine kinase.

II. Source of ROS in BCR–ABL Transformation

A. ROS are linked to a hyperactive glucose metabolism

A key factor in discerning the role and function of ROS is to understand the mechanism of their generation. This will be instrumental in understanding their contribution in signal transduction, viability, growth, and possibly genomic instability. The exact mechanisms whereby different oncogenic tyrosine kinases regulate intracellular ROS have not yet been fully determined, however, there may be considerable overlap. The origin of ROS associated with BCR–ABL transformation is linked to the mitochondrial electron transport chain and is under metabolic control in the cell (185, 319). In BCR–ABL transformation, the electron transport inhibitor rotenone and the Glut1 glucose transport inhibitor phloretin lead to a reduction of ROS similar to that in cells treated with the ABL kinase inhibitor imatinib (185, 319). Therefore, generation of ROS depends mainly on the supply of energy in the form of glucose or pyruvate.

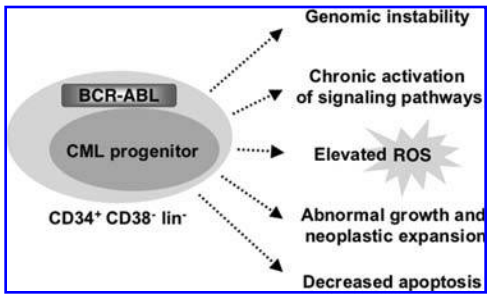

FIG. 1. Phenotypes of CML progenitors. In contrast to $CD34^+CD38^-lin^-$ CML progenitors, signaling and biology of normal $CD34^+$ progenitors is transient and ligand dependent.

Increases in intracellular ROS associated with BCR–ABL can be reduced by the glucose analog 2-DOG (2-deoxy-D-glucose). Phosphorylation of 2-DOG to 2-DOG-6-phosphate reduces the amount of intracellular glucose-6-phosphate formation, which has been suggested to be involved in the regulation of ROS levels (73). In cells transformed by the BCR–ABL or v-Abl tyrosine kinases, the uptake of glucose through the glucose transporter is tightly regulated by the oncoprotein itself (8, 28, 33). Activated ABL kinases are likely to regulate glucose uptake by increasing the affinity of Glut1 for glucose and regulating transporter activity, but there may be additional mechanisms (7, 9, 103). Targeting the glucose pathway with 2-DOG demonstrated potent anti-cancer activity of this drug *in vitro* by causing apoptosis, reduced cell growth, and cooperative effects with imatinib. 2-DOG can also reduce cell growth in hematopoietic cells transformed by a mechanism different from that of BCR–ABL, including leukemia, lymphoma, and multiple myeloma cell lines (185).

Identifying targets for other signal transduction inhibitors that are complementary to imatinib without adding toxicity to normal cells is of great interest. This may be achieved through pharmacological inhibitors with increased specificity such as the 2-DOG derivative 2-fluorodeoxy-D-glucose, which efficiently inhibits glycolysis but may have a smaller impact on cellular glycosylation (200). However, targeting glycolysis may also inhibit cell growth and ROS levels not only in BCR–ABL transformed cell lines, but also to a certain extent in normal, growth factor-dependent cells. This is in part due to the fact that transformation by BCR–ABL as well as stimulation with hematopoietic growth factors upregulates intracellular ROS levels in hematopoietic cells (319, 320). However, the ROS pathway is chronically active only in BCR–ABL transformed cells, suggesting that indeed a combination of traditional therapy with improved inhibitors of glucose pathways may be beneficial for the treatment of CML.

B. Warburg effect and signaling requirements for ROS

Cells transformed by BCR–ABL and other cancer cells tend to have an active glycolysis, even under normoxic conditions, and produce lactate to generate energy ('Warburg effect') (389). The enzymes involved in the glycolytic pathway have been well characterized, but the significance of this dysregulation remains at the center of discussion since its discovery ~80 years ago by Otto Warburg. This apparent energetically inefficient way of glucose metabolism was viewed by Warburg as a major requirement for transformation of cancer cells, whereas others have considered this effect as an epiphenomenon of transformation (115). It is suggested here that the Warburg effect contributes to elevated ROS by pro-

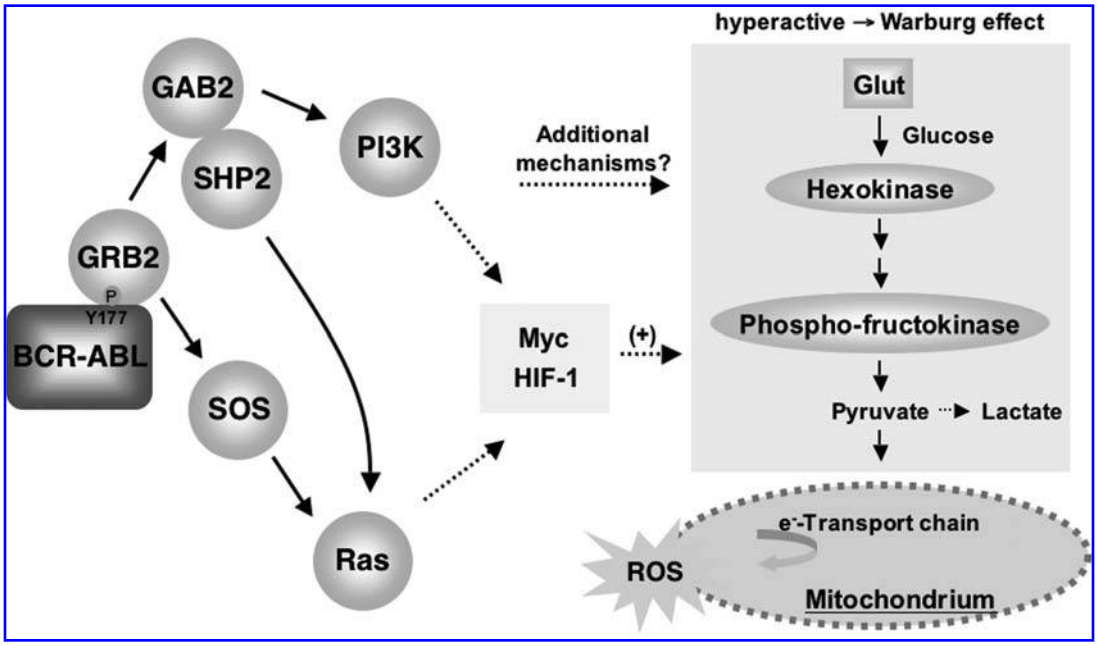

FIG. 2. Hypothetical model for the regulation of glycolysis and the induction of ROS by BCR–ABL. BCR–ABL regulates the activation of the PI3K and Ras pathway in part through autophosphorylation of Tyr177, a binding site for the GRB2 adapter protein. GRB2/SOS or GRB2/GAB2/SHP2 complexes bound to BCR–ABL activate Ras signaling, GRB2/GAB2 complexes activate the phosphatidylinositol-3′ kinase (PI3K) pathway. Myc and HIF-1 are key regulators of glycolytic genes, downstream of PI3K and Ras. There are likely additional mechanisms that contribute to increased glycolysis by the oncoprotein under normoxic conditions (Warburg effect). This hyperactive glucose metabolism is required for mitochondrial ROS production.

viding substrate through a hyperactive glucose metabolism and fueling the mitochondrial electron transport chain (Fig. 2). Therefore, in BCR–ABL transformed cells, elevated ROS are expected to depend on mechanisms that regulate glucose metabolism. There are likely other advantages for cancer cells to utilize a hyperactive glucose metabolism, such as the ability to generate ATP under hypoxic conditions. The signaling requirements for the Warburg effect in cancer cells are not well understood. In a recent study using microarrays to evaluate the gene expression profile of BCR–ABL transformed cells, it was demonstrated that the rate of glycolysis is significantly increased in BCR–ABL expressing cells in a PI3K-dependent manner, linking altered glucose metabolism to a specific signaling event (154).

The PI3K/mTOR pathway is also of special interest for BCR–ABL transformation due to its requirement for the induction of elevated levels of intracellular ROS through increased glucose metabolism (185). This is not a unique requirement, and PI3K activation is, for example, also essential in the PDGF receptor-dependent production of hydrogen peroxide (20). Activation of the PI3K pathway has already been shown to be important for transformation by BCR–ABL (341). Inhibition of either PI3K or mTOR with specific inhibitors cooperates with imatinib in BCR–ABL transformed cells, while having reduced or little effect on normal bone marrow cells (188, 251). Evidence that the PI3K pathway is critical for the generation of ROS comes from the finding that BCR–ABL generated reduced levels of ROS in the Tyr177Phe mutant, which is partially defective in its ability to activate PI3K (185, 316). This BCR–ABL autophosphorylation site is also a major binding site for the GRB2 adapter protein through its SH2 domain (3, 281, 288). Tyr177 is also required for full activation of the Ras pathway in Rat-1 cells (281), but may be activated through different mechanisms in hematopoietic cell lines (71). In addition, mutation of Tyr177 to Phe reduces the myeloproliferative disease induced by BCR–ABL in favor of B- and T-lymphoid leukemias with prolonged latency in mice (249, 412). ROS levels were found to be 77% higher in the BCR–ABL expressing cells compared to the parental growth factor deprived cells and introduction of the Tyr177Phe mutation in BCR–ABL failed to increase intracellular levels of ROS.

Confirmation that PI3K is likely to be important comes from the observation that expression of an activated allele of PI3K in nontransformed cells results in production of ROS by itself (185). In these experiments, clonal populations of BaF3 cells with doxycycline-inducible myristoylated p110PI3K (myr-p110PI3K) were used and characterized for altered levels of intracellular ROS in response to doxycycline. Whereas doxycycline treatment by itself does not alter intracellular ROS levels, induction of Myr-p110PI3K expression led to a significant increase in ROS from 43% to 54% compared to untreated cells (185). The functional activation of the PI3K pathway in these cell lines was confirmed by immunoblotting using phospho-specific antibodies against activation sites in AKT and p70S6K, and a significant induction of phosphorylation at Ser473 in AKT and Thr421/Ser424 in p70S6K was observed, demonstrating the activation of PI3K-dependent signaling pathways in cells expressing myr-p110PI3K. Overall, these data suggest that activation of the PI3K pathway is sufficient for the induction of elevated levels of ROS.

BCR–ABL activates PI3K likely through recruitment of the scaffolding adapter Grb2/Gab2 complex to autophosphorylated Tyr177 in BCR (316). Gab2 and its associated proteins may therefore be key mediators of ROS signaling in BCR–ABL-transformed cells. It is of note that Gab2 is an essential mediator of BCR–ABL transformation. Gab2 belongs to a family of PH-domain containing adapter proteins that includes mammalian Gab1 (159) and Gab3 (397), as well as *Drosophila* DOS (daughter of sevenless) (151, 290). Gab2 is the target of several tyrosine kinases in response to multiple cell stimuli (130, 131, 227, 250, 261, 396, 414). Signaling studies and additional genetic approaches have implicated Gab2 as a positive component of growth factor, cytokine, and antigen receptor signal transduction that enhances signaling (130, 131, 223, 227, 250, 261, 396, 414). However, Gab2 may also have negative regulatory roles in T-cell receptor signaling (287, 406). In BCR–ABL transformed cells, Gab2 is constitutively tyrosine phosphorylated (130). As in the requirement for BCR–ABL driven ROS production, this phosphorylation requires the Grb2 SH3 binding site in Gab2 as well as Tyr177 of BCR–ABL. This suggests that Gab2 phosphorylation by BCR–ABL is mediated by a Grb2/Gab2 complex. This signaling complex is required for full activation of the PI3K/Akt or Ras/Erk pathways and may also have an important role for the induction of ROS since BCR–ABL is unable to transform bone marrow cells from *Gab2* (−/−) mice (316). Although mice with Gab2 gene disruption have an apparently normal hematopoietic phenotype with normal numbers of stem cells and peripheral blood cells, the responsiveness of early hematopoietic cells to growth factors is diminished (413). Gab2-deficient c-Kit$^+$Lin$^-$Sca-1$^+$ cells responded poorly to stem cell factor, FLT3 ligand, or thrombopoietin, and deficiency of Gab2 in c-Kit$^+$Lin$^-$ cells interferes with signaling in the PI3K and MAPK pathway in response to IL-3 (413). Additionally, Gab2 may not be the only route from Tyr177 to the activation of the PI3K pathway (183) and thus subsequent ROS production.

Interestingly, Gab2 phosphorylation dependent activation of PI3K has also been associated with $O_2\bullet^-$ formation in Fcγ and fMLP (formyl-methionyl-leucyl-phenylalanine)-stimulated cells (253). It is therefore likely that Gab2 may also have a predominant role in the regulation of ROS in BCR–ABL transformed cells. It still remains to be established whether the likely requirement for Gab2/PI3K in the induction of ROS also involves the regulation of the mTOR pathway, a pathway that was identified as a key regulator of ROS (185). This was done by treating BCR–ABL transformed cells with rapamycin, an inhibitor of mTOR activity, and with wortmannin, an inhibitor of PI3K activity, as a positive control. Both inhibitors significantly lowered intracellular ROS, reducing the ROS levels by 29% with wortmannin and by 56% with rapamycin treatment, compared to control cells (185). Neither inhibitor reduced intracellular ROS to levels found in untransformed cells, suggesting that there are additional pathways involved in the regulation of ROS. Also, rapamycin was more effective than wortmannin in reducing ROS, which may indicate that mechanisms other than PI3K regulate ROS through mTOR.

The activity of mTOR is regulated at least in part through the PI3K effecter AKT and can be specifically inhibited by rapamycin. mTOR has previously been implicated in nutrient sensing by activating S6kinase and inducing gene expression (90, 282). Involvement of mTOR in the glucose-dependent regulation of ROS in BCR–ABL-transformed cells remains to be determined, although there is already indirect evidence in favor of this. Most, if not all, glycolytic enzymes are under the transcriptional control of Myc and/or HIF-1 (124). In response to H_2O_2, Myc is also known to increase promotor activity of γ-glutamyl-cysteine synthetase, a rate-limiting enzyme for GSH biosynthesis which is required for the maintenance of intracellular redox balance (27).

Myc expression is known to depend on Tyr177 in BCR–ABL, and activated ABL induces Myc through the Ras pathway (71, 416) and active Akt (340). Finally, HIF-1 expression is dependent on an active PI3K/mTor pathway (239). There are additional mechanisms that may contribute to the dysregulation of these molecules and pathways, leading to elevated ROS. Targeting ROS by antioxidants as part of a regular diet, such as vitamin C or vitamin E, is thought to be beneficial in reducing the risk for certain cancers and this may depend in part on HIF-1 (16, 114). It is striking that inhibition of signaling mechanisms that lead to induction of ROS are equally important for transformation. Targeting these mechanisms is therefore likely to have beneficial effects for cancer treatment.

C. Rac and NADPH oxidases

BCR–ABL mediated transformation of hematopoietic cells is dependent on various downstream signaling molecules such as RAS and PI3K, and some of the properties of BCR–ABL expressing cells are dependent on these effectors. Specific inhibition of signaling molecules downstream of BCR–ABL leads to a decreased transforming phenotype in hematopoietic cells (for review, see ref. 385). The small GTPase Rac is an important downstream effector of BCR–ABL and was previously shown to be required for leukemogenesis (342). The family of Rac proteins includes three highly homologous members that are ubiquitously expressed, with the exception of Rac2, which seems more restricted to hematopoietic cells (134, 252, 336). Complex formation of BCR–ABL with the hematopoietic specific protein Vav, a GDP/GTP exchange factor, was shown to be necessary for the activation of Rac (25). In murine models with Rac1 and Rac2 gene disruption, the combination of both proteins has been shown to be required for BCR–ABL-mediated transformation (366). The small molecule Rac inhibitor NSC23766 showed efficacy in this model as well.

Rac functions as an important regulatory subunit of the NADPH oxidase (Nox) complex. NADPH oxidase complexes, in addition to mitochondria, generate significant amounts of ROS by catalyzing the reduction of molecular oxygen to superoxide anion (289). NADPH oxidase is a multi-subunit enzyme consisting of a catalytic subunit gp91phox (also termed as Nox2) and regulatory subunits p47phox, p67phox, p22phox, and Rac. It was originally identified as an important mediator of host defense by phagocytes (17). However, later studies reported the presence of Nox enzymes in various nonphagocytic cells and tissues (for review, see refs. 117, 203). Localized production of ROS is important to achieve a precise redox signaling effect in response to the activation of specific receptors. NADPH oxidases are now shown to localize to specific subcellular components such as membrane ruffles (275), lamellipodial focal

complexes (401), caveolae, lipid rafts (376, 381, 408), endoplasmic reticulum (13, 377), the perinuclear compartment (217), and endosomes (218) (for review, see also ref. 375). The family of Nox is comprised of Nox1-5 and Duox1-2 (related dual oxidases). Function of Nox is regulated by a multitude of factors (205). ROS generated by Nox are necessary for various normal physiological functions such as angiogenesis, cell proliferation, and integrin signaling (204).

Due to the apparent connection between Nox and ROS, it is likely that Nox-derived ROS can influence cancer development and progression. Association between Nox activity and human cancer has already been reported (for review, see ref. 204). Nox are suggested to be the major producers of ROS in many cancers including melanoma (41), glioblastoma (66), prostate cancer (40, 219), and likely others. Recently, it was shown in FLT3/ITD-positive AML cell lines and primary AML cells that there is increased ROS production that correlates with activation of STAT5 and Rac1. Treatment of cells with FLT3 inhibitor reduced the levels of ROS (309). However, Nox as the major or only source of ROS in transformation is a somewhat contentious point since the transformation process correlates with increased cell growth and thus mitochondrial activity. For example, in ovarian cancer cells, it was observed that elevated levels of ROS were reduced by the Nox inhibitor diphenylene iodonium as well as the mitochondrial electron chain inhibitor rotenone or specific knockdown of p47phox (402). Both ROS producing processes may be connected or dependent on each other and further studies are needed to have a better understanding about the role of Nox in ROS dependent transformation by BCR–ABL.

III. Regulation of Protein Function by ROS

Probably the most relevant form of redox regulated protein function for transformation involves the oxidation of cysteine residues in the active site of protein tyrosine phosphatases (PTPases), leading to a loss of enzymatic activity (312, 368). Modification of the thiol group in these cysteine residues can either be transient or reversible and may not be limited to these classes of proteins. It is conceivable that cysteine oxidation leads to conformational changes or makes functional groups inaccessible, resulting in overall altered protein function. Cysteine oxidation in PTPases involves changes in either S redox state, such as in PTP1B (23, 24), or S–S state, such as in the dual-specific phosphatidylinositol-3′ phosphatase PTEN (213) (see also Section VI. A). Nevertheless, it should be cautiously pointed out that these modifications do not necessarily alter protein function in a way that one would anticipate supports transformation. It is expected that protein function is regulated at multiple levels and that redox regulation may be superceded by other mechanisms. The accessibility of oxidized proteins could be restricted, expression altered, or intracellular oxidative stress could be more compartmentalized than previously thought. Additionally, oxidative stress could affect some proteins to a greater extent than others. Likely, multiple regulatory mechanisms coexist at the same time.

Transformed cells are constantly exposed to intracellular oxidative stress that has the potential to oxidize redox sensitive groups. The source of ROS does not have to be necessarily endogenous but can also result from the interaction of the cancer cell with the microenvironment. In CML, ROS levels in the cancer stem cell may be in part regulated by the bone marrow microenvironment, which may also provide stimuli to maintain viability and induce self-renewal during targeted therapy. It is critical to maintain ROS at levels that induce neither apoptosis nor necrosis or cause detrimental levels of protein, DNA, or lipid oxidation. Oxidized thiol groups are constantly 'recycled' through antioxidant mechanisms that involve either thiol-containing compounds or compounds with antioxidant activity (*e.g.*, ascorbate). Probably the most important regulator of this process is glutathione. The ratio between reduced glutathione (GSH) and oxidized glutathione (GSSG) is a good indicator of the intracellular redox status. Glutathione works in concert with thioredoxin and nicotinamide adenine dinucleotide phosphate (NADPH) to reduce thiol groups and to maintain the cellular redox status at physiological levels (for review, see ref. 102). Thioredoxin has a dual function and has also been implicated in sensing changes in redox status. It is found in a complex with Ask1 (apoptosis signal-regulated kinase1). When bound to Ask1, reduced thioredoxin inhibits its kinase activity, which is restored when oxidized thioredoxin is released (127, 307). A similar process seems to regulate the functional activation of JNK (c-Jun N-terminal kinase). The activity of JNK is inhibited by GST-π (glutathione S-transferase-π), which is in a complex with JNK. Oxidized GST-π dissociates from JNK and enables the kinase to function (1, 2). Even though these are unique examples, there are likely additional proteins and interactions involved in response to changes of the intracellular redox status.

In contrast to other oxidative protein modifications, the reversible sulfhydryl group-dependent oxidative regulation of cellular proteins is thought to be a rather specific process that can direct protein function in a defined way. Nevertheless, recent evidence suggests that protein carbonylation bears an unexpected degree of specificity (4, 153). Carbonylation is a result of increased levels of intracellular ROS and it is expected that mechanisms involved in regulating ROS would also indirectly regulate protein carbonylation. Oxidative carbonylation leads to an irreversible modification that can be caused either through reaction with other reactive carbonyl groups in cellular molecules, or through metal-catalyzed oxidation. Even though there is abundant evidence that this process occurs in human diseases, its role in cancer has not been well defined (77, 215). A recent finding demonstrating that transiently increased carbonylation correlates with activation of the endothelin-1 receptor in smooth muscle cells hints at a potential role of this process in mitogenic signaling (398). As a result of this modification, proteins are more likely to undergo proteolytic degradation or aggregation (266). In this context, the elimination of carbonylated proteins during differentiation of embryonic stem cells has been suggested to serve as a 'rejuvenation process'. The interesting finding here was that the load of damaged proteins was limited to a small number of defined proteins, including tubulin, actin, and the chaperones Hsp90, Gr75, and Hsc70, thus hinting at the specificity of this process (153). Chaperones have an important role in ROS-dependent protein maturation and oncogenic transformation (see also Section VII). Therefore, this mechanism involving metal-catalyzed oxidation may open a venue to target cancer cell growth through

metal chelators as a possible class of nonthiol dependent antioxidants. It would now be interesting to see whether carbonylation of chaperones or other proteins is dependent on oncogenic signaling, and if this plays a role in transformation of myeloid neoplasias or human cancers in general.

IV. Regulation of BCR–ABL Function by Oxidative Stress

The ABL tyrosine kinase in the oncogenic BCR–ABL fusion protein is constitutively active and its activity may increase over time as the disease progresses from CML stable phase to blast crisis (318). Also, the kinase activity of BCR–ABL is regulated *in vivo* by cellular proteins that are thought to bind reversibly and inhibit autophosphorylation of the kinase (74). Transformation of hematopoietic cells by BCR–ABL results in an increase in ROS compared to quiescent untransformed cells. This increase in ROS is directly due to BCR–ABL kinase activity since it can be blocked by the ABL tyrosine kinase inhibitor imatinib (319). BCR–ABL mimics some signaling events induced by activated growth factor receptors (318), and similar to BCR–ABL, activation of growth factor receptors coincides with an increase of intracellular ROS levels. These include the receptor for PDGF (354), TGF-β (268), and GM-CSF (320). However, unlike ligand-stimulated growth factor receptors that cause only a transient increase in intracellular ROS levels, BCR–ABL is capable of inducing chronically high levels of ROS. In addition, antioxidants or antioxidant enzymes have been shown to reduce tyrosine phosphorylation events in cells stimulated with these growth factors. This is of interest, since transformation by BCR–ABL requires constitutive activation of its ABL tyrosine kinase activity (88, 225).

Biological effects of BCR–ABL are therefore likely to be stimulated through tyrosine phosphorylation of cellular proteins, which in turn are regulated by PTPases. ROS such as H_2O_2 have been implicated in the regulation of PTPase activities (84). In hematopoietic cell line models, cellular protein tyrosine phosphatase activity is decreased by BCR–ABL as well as H_2O_2 and the phosphatase inhibitor pervanadate (319). Additional PTPases that have not been directly implicated in BCR–ABL function are discussed later (Section VI, A). Likely candidates that might regulate BCR–ABL function are PTPases that are known to interact with the BCR–ABL kinase such as SHP-1 (362), SHP-2 (361), or PTP1B (206). The high level of tyrosine phosphorylation of SHP-1 (362) and SHP-2 (361) in BCR–ABL expressing cells demonstrates an imbalance between kinase and PTPase activities. Since BCR–ABL transformed cells have increased levels of ROS and decreased PTPase activity, it is likely that elevated ROS contribute to these changes in PTPase activity. It will therefore be of interest to determine if oncogenic tyrosine kinases like BCR–ABL are not only involved in generating ROS and regulate the function of redox sensitive proteins but are also regulated by redox-sensitive PTPases themselves (Fig. 3). Reduced PTPase activity in BCR–ABL transformed cells is consistent with the known reduced alkaline phosphatase activity in CML cells (278). Both *in vitro* activities overlap since PTPase activity as well as alkaline phosphatase activity can be measured with the same substrates, including tyrosine phosphorylated peptides and p-nitrophenyl-phosphate (262, 292, 346). It will be of interest to identify specific PTPases

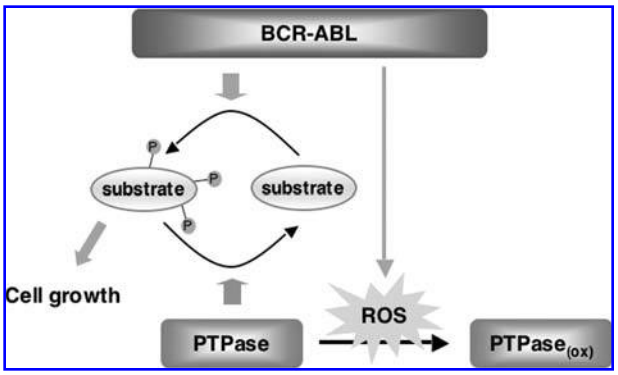

FIG. 3. The yin and yang of BCR–ABL and protein tyrosine phosphatases (PTPases). The BCR–ABL tyrosine kinase activity is required for cell growth through phosphorylation of specific ABL substrates, including BCR–ABL itself. Dephosphorylation of BCR–ABL substrates is regulated through PTPases. PTPase activity can be inhibited through BCR–ABL-dependent ROS production. Oxidized (ox) PTPases fail to dephosphorylate BCR–ABL and its phospho substrates.

that contribute to the observed reduction in PTPase activity and to evaluate their response to changes in the cellular redox status.

Not surprisingly, there is a significant overlap in tyrosine phosphorylation patterns of signaling proteins induced by BCR–ABL transformation and in H_2O_2 stimulated cells, including c-CBL, SHC, or SHP-2. This also includes c-Abl, the cellular form of the BCR–ABL oncogene. On the other hand, ROS-dependent tyrosine phosphorylation of cellular proteins can be decreased by reducing agents such as pyrrolidine dithiocarbamate (PDTC) and *N*-acetylcysteine (NAC) (166, 320, 384). In BCR–ABL transformed cell line models, antioxidants such as PDTC and NAC decrease tyrosine phosphorylation of cellular proteins (319). Reduced tyrosine phosphorylation of BCR–ABL substrates is consistent with activation of cellular PTPases, but could also be due to reduced BCR–ABL kinase activity. It is also possible that in addition to BCR–ABL, other tyrosine kinases previously activated by BCR–ABL are reduced in their activity. This could be either a result of decreased BCR–ABL activity or a result of direct inhibition by antioxidants. Indeed, BCR–ABL *in vitro* tyrosine kinase activity towards its substrate CRKL is reduced in PDTC-treated cells. Also, the *in vitro* kinase activity towards CRKL in ABL immune complexes was found to be elevated by H_2O_2 as well as pervanadate treatment of untransformed cells (319). Thus, these data would favor a mechanism whereby cellular ROS levels alter activity of one or more PTPases, which then regulate the kinase activity of both the BCR–ABL oncoprotein and c-ABL.

ROS contribute to several cellular functions and are known to be involved in the regulation of signal transduction, gene expression, and proliferation. The growth-promoting effect of ROS and the growth-inhibiting effect of antioxidants are of interest. The available data suggest that the mechanism involves enhanced tyrosine phosphorylation, possibly by inhibiting one or more PTPases. ROS can regulate protein function in part through oxidation of redox-sensitive cysteine residues in some proteins. It is likely that fur-

ther characterization of redox-sensitive PTPases and other proteins will be helpful in understanding the signaling of BCR–ABL or other tyrosine kinase oncogenes and in particular their mechanism of transformation. Elevated ROS in BCR–ABL transformed cells would also be expected to contribute to the activation of additional effector kinases, including Src family kinases (45), Pyk2 (RAFTK) (356), or others. In particular the Src family kinase Hck, Fgr and Lyn have been implicated in BCR–ABL mediated transformation, disease progression, and imatinib resistance of CML and Ph+ ALL (78, 163, 221, 349, 390).

Overall, the data at hand are consistent with a model in which increased ROS amplify BCR–ABL signaling, possibly amplified by simultaneous reduction in the activity of one or more PTPases (Fig. 3). Since BCR–ABL kinase activity is required for all aspects of transformation, altered glucose metabolism and elevated ROS are a direct consequence of BCR–ABL dependent signaling events. Increased glycolysis and ROS production are not known to be sufficient for transformation in hematopoietic cells but are likely to be required for this process. Drugs that alter ROS metabolism or reactivate PTPases are expected to antagonize BCR–ABL transformation and point at new targets for treatment of CML. Ideally, drugs that would be efficient in CML treatment would lower ROS by directly targeting the ROS producing enzymes or would supplement the antioxidative potential of cells. Such drugs could be used in combination with BCR–ABL kinase inhibitors such as imatinib to block BCR–ABL signaling and thus progression of the disease.

V. Role of Hypoxia in the Regulation of ROS

Human cancers are often exposed to low oxygen levels, a condition also termed hypoxia. Cells increase glucose uptake and glycolysis under hypoxic conditions in order to compensate for the decrease in respiration and ATP production (the Pasteur effect). The Pasteur effect is dependent upon hypoxia-inducible factor (HIF)-mediated transactivation of genes encoding glycolytic enzymes (329). Physiological responses triggered by hypoxia impact all critical aspects of cancer progression including transformation, differentiation, glucose metabolism, genetic instability, increased ROS levels, and other cellular functions, allowing cancer cells not only to survive but to continue to proliferate and metastasize.

A. HIF-1α activation and ROS

Hypoxia is known to activate an α/β heterodimeric transcription factor, HIF, and its activity is affected not only by cellular oxygen levels but also by ROS that include $O_2^{\bullet -}$, hydrogen peroxide, and hydroxyl radical produced during hypoxia (58). Hypoxic conditions lead to stabilization of the HIF-1α protein, which can then trigger downstream HIF-target genes involved in various biological processes. Some of these genes include those involved in the development and functioning of the vascular system (such as VEGF and TGF-β3) (52, 107), erythropoiesis (such as erythropoietin and transferrin) (176, 301), cell proliferation and viability (such as p21, CDC25A, and BNIP3) (44, 56, 140), and energy metabolism (such as lactate dehydrogenase A and glucose transporter1 and 3) (105, 119) (Fig. 4). Of particular interest are the genes responsible for energy metabolism that eventually lead to increased glucose uptake and mediate the switch to glycolysis in the case of metabolic crisis such as hypoxia.

HIF-1α is stabilized under hypoxia but is rapidly degraded under normoxic conditions by the ubiquitin–proteasome pathway, a process mediated by prolyl hydroxylase. Prolyl hydroxylases are enzymes that function as critical oxygen sensors, requiring oxygen and 2-oxoglutarate as substrates, and a nonheme iron as a cofactor. They target HIF-1α through the hydroxylation of two conserved proline residues (Pro402 and Pro564) in the oxygen-dependent degradation domain of HIF-1α and trigger the VHL gene product binding for proteasomal degradation via the von Hippel–Lindau ubiquitylation complex (164, 180). Changes in the cellular levels of either the substrate, cofactors, or both would have a possible outcome on the prolyl hydroxylase activity. This is evident under conditions of low cellular oxygen levels. Previous studies have demonstrated that chemical inhibitors blocking the activity of prolyl hydroxylases, such as iron chelators, or dimethyloxallyl glycine, which competes with 2-oxoglutarate for binding at the hydroxylase, prevent HIF proline hydroxylation, causing accumulation, and hence stabilization of HIF-1α (169).

A number of groups have previously reported increased levels of ROS under hypoxic conditions, however others have reported the reverse (63, 286). A possible explanation for this controversy is the basal metabolism of the cell type, and the degree and duration of oxidative stress experienced. Although the role of hypoxia has been widely studied in solid tumors, few studies demonstrate the effects of hypoxia on hematological malignancies. In one study, it was shown that hypoxia could modulate the BCR–ABL-dependent leukemic phenotype. By using CD34+ cells from CML patients, it was shown that hypoxia inhibited the proliferation, in addition to maintaining the primitiveness of these cells, better than normoxic conditions. Inhibition of BCR–ABL activity, accompanied with activation of MAPK, also contributed to the survival of these cells, thus implying a modulatory role of hypoxia in the CML cell population (85). On the other hand, there is evidence of an upregulation of VEGF, downstream of HIF-1, as well as HIF-1 in primary leukemic cells (239), suggesting that these cells may require elevated levels of HIF-1 accompanied by elevated levels of ROS in order to stimulate growth signals.

To evaluate redox changes on the stabilization of the HIF-1α subunit, the mitochondria-targeted antioxidant mitoubiquinone (MitoQ) was used (314). It was found that under hypoxic conditions, MitoQ cancelled out the induction of ROS along with the destabilization of HIF-1α, thus suggesting that mitochondrial ROS are intrinsically linked to HIF-1α during hypoxia. While the mechanistic investigations by which the ROS signals are transduced to HIF-1α is unclear, multiple oncogenes may lead to elevated ROS, including BCR–ABL (319). Murine pre-B cells expressing constitutive tyrosine kinase activity such as BCR–ABL have been shown to promote the expression of functionally active HIF-1 (239), thus linking BCR–ABL to ROS and HIF-1α.

Another growth factor pathway of particular importance for tumor growth and HIF activation includes the epidermal growth factor (EGF) pathway. Signaling through the EGF receptor (EGFR) is common in tumor cells and ultimately contributes to the production of factors responsible for tumor cell growth and survival (222). More recently, a role for an

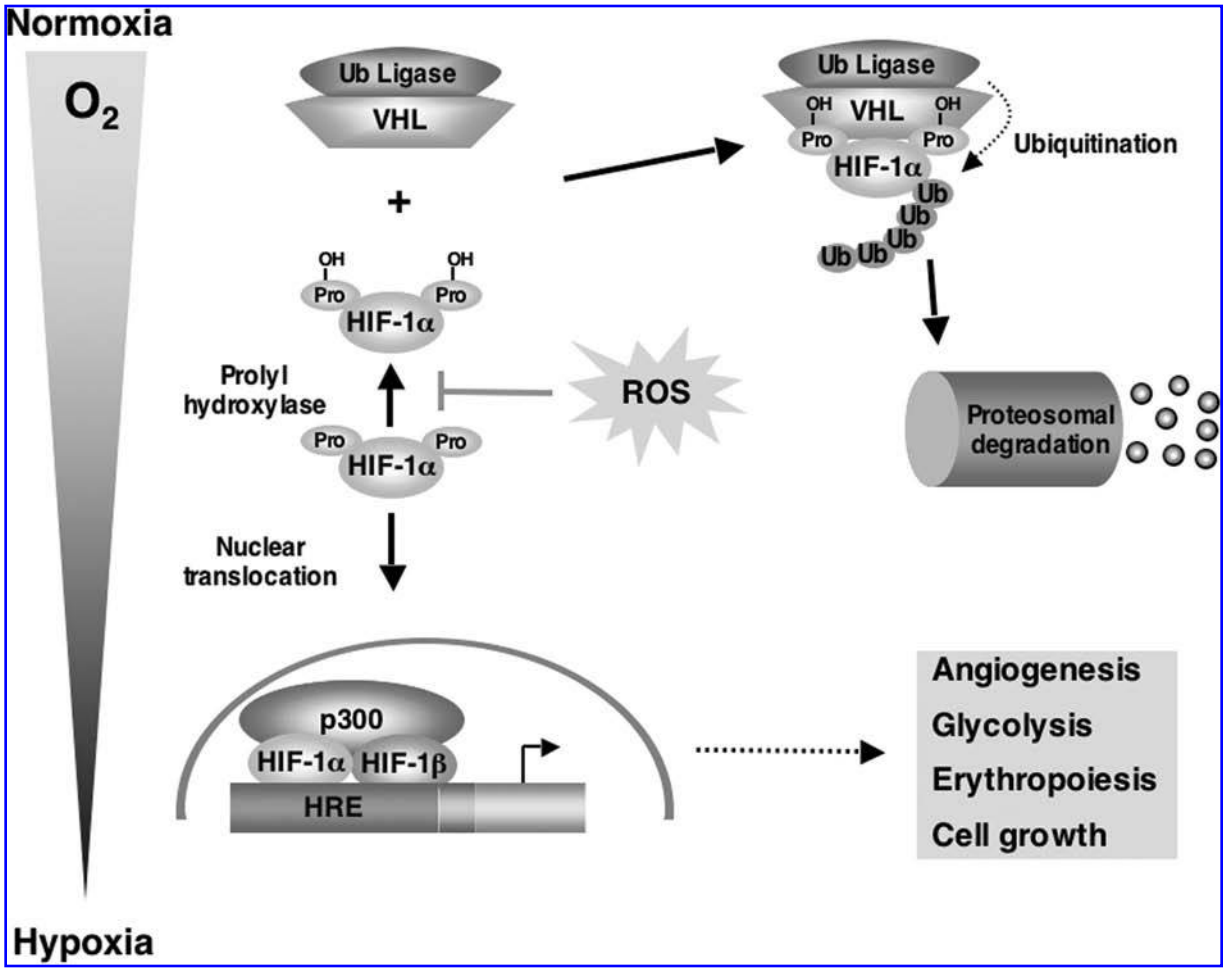

FIG. 4. Regulation of HIF-1α by ROS. Under normoxic conditions, HIF-1α is present in the cytoplasm. Prolyl hydroxylase enzyme is activated under normoxic condition. This allows for the complex formation with the von Hippel Lindau protein (pVHL), followed by a rapid degradation of HIF-1α by the ubiquitin–proteasome system. Under hypoxic conditions, HIF-1α is stabilized in its dehydroxylated state due to an inactive hydroxylase enzyme. The stable HIF-1α translocates to the nucleus, where it accumulates and forms a dimer with the constitutively expressed HIF-1β in the presence of a transcriptional coactivator p300, resulting in the intact HIF-1 complex. This complex binds to the hypoxia response element (HRE), facilitating the transcriptional activity of hypoxia-induced genes. Elevated levels of ROS inhibit the prolyl hydroxylase activity.

alternate and novel mechanism, the *'HIF-1α-Myc pathway'* has been identified in the regulation of hypoxia. In this pathway, HIF-1α counteracts the repressive activity of Myc by displacing Myc from the CDKN1A promoter via protein–protein interactions. This pathway functions independent of the hypoxia response element (HRE) and also contributes to the hypoxic downregulation of DNA repair genes, MSH2 and NBS1, both of which have been shown to be activated by Myc. In the same study, the cell cycle gene CDC25A was identified by microarray analysis to be downregulated under hypoxic conditions (140). This is in agreement with others that indicate cell cycle arrest by inhibiting CDC25A under hypoxia.

B. Targeting HIF-1 pathways

ROS have more recently emerged as promising targets for anticancer drug discovery. The use of potential targets, both at the level of HIF-1 and HIF-1 downstream genes, in addition to those that regulate angiogenesis, are of major interest. Due to the chemoresistant status of cancer cells induced by hypoxia, it is important to develop and test novel therapeutic strategies targeting hypoxia. Among the glycolytic enzymes, hexokinase, GAPDH, lactate dehydrogenase, and phosphofructokinase have been indicated as potential therapeutic targets with known chemical inhibitors. Several compounds have been evaluated for their role in inducing cell death in cancer cells with mitochondrial defects or cells under hypoxia, including 2-DOG. A more potent inhibitor is 3-bromopyruvate (3-BrPA), inhibiting hexokinase II. Using human leukemia and lymphoma cells, the glycolytic pathway could be inhibited with 3-BrPA inducing severe depletion of cellular ATP in addition to significant cell death in these cells. Moreover, this study also showed that by depleting ATP, 3-BrPA could effectively kill cells with a multidrug-resistant (MDR) phenotype (404). This approach of targeting the gly-

colytic pathway to minimize ROS may have broad applications in cancer therapy.

A number of anticancer agents have been shown to inhibit HIF-1 activity, but with indirect evidence to prove their specificity to HIF-1 (for review see ref. 277). They are categorized into two classes: small molecule inhibitors (targeting Hsp90, topoisomerase, microtubule modifier, Trx-1 inhibitor, histone deacytylase, proteasome, and others) and inhibitors of signal transduction pathways (targeting PI3K, mTOR, MEK, tyrosine kinase, ErbB2, EGFR, and COX2). Further, a high throughput biological screen identified small molecule inhibitors of the HIF-1 pathway. Using the National Cancer Institute "Diversity Set", comprising a library of 200 compounds representative of the greater chemical diversity, four compounds with HIF-1 inhibiting as well as anticancer properties were identified (294). Later, in an independent study screening for additional HIF-1 inhibiting molecules, two novel inhibitors, NSC-134754 and NSC-643735, were identified. In addition to inhibiting HIF-1 activity, both at the transcriptional as well as the translational level, NSC-134754 also inhibited Glut-1 expression (65).

C. NF-κB activation by hyperactive glucose metabolism and ROS

The transcription factor, NF-κB (nuclear factor-κB) is one of the major intracellular targets of the glucose metabolism pathway and oxidative stress (96). Increased levels of ROS and increased glucose metabolism can both lead to NF-κB activation (254, 284). Aberrant regulation of NF-κB leads to a number of chronic diseases including autoimmunity, diabetes, and hematopoietic disorders. In resting cells, NF-κB is present in the cytoplasm as an inactive heterodimer consisting of the p50 and p65 subunits complexed with an inhibitor protein subunit, IκBα. The activation of this molecule involves the translocation of the heterodimer to the nucleus, thus regulating the expression of a large number of genes (283). It can be activated by a wide array of exogenous and endogenous entities, including the BCR–ABL oncogene, hyperglycemia, ROS and DNA damage. In response to oxidative stress, the IκBα subunit is phosphorylated at Ser32 and 36, leading to ubiquitination of lysine 21 and 22 and dissociation from the complex. This results in degradation of IκBα by the 26S proteasome and translocation of the functional NF-κB to the nucleus, where it acts as transcriptional activator (144) (Fig. 5). Previous studies show that the activation of NF-κB and the subsequent upregulation of NF-κB dependent gene expression are significantly induced by the generation of ROS and that this response is a cell type and stimulus specific phenomenon (37).

In addition to the induction of ROS in BCR–ABL transformed hematopoietic cells (265, 319), the requirement for NF-κB activation in BCR–ABL-mediated tumorigenesis and transformation has been well documented (139, 187, 297). The constitutive activation of NF-κB was also shown in Ph+ primary CML blasts. In an attempt to examine the mechanisms involved in BCR–ABL-induced NF-κB/Rel activation, IκB kinase (IKK) activity was not found to be deregulated. Although the exact mechanism of NF-κB activation is not known, phosphorylation at Tyr42 of the IκBα subunit may be involved (325). Alternatively, increased activity of an additional IκBα kinase or decreased activity of a related phosphatase may explain this process. On the other hand, Ras was shown to be involved in the activation of NF-κB/Rel, but the mechanism was independent of Grb2 and Grb10 binding to the BCR–ABL oncoprotein. There was also variable involvement of the small GTPase Rac in NF-κB activation (187).

Many published reports to date stress the importance of understanding the role of NF-κB activation mechanisms upon stimulation of oxidative stress. It has been shown in leukemic cells that NF-κB activation involves the tyrosine phosphorylation of IκBα (357). In a recent study with leukemic cells, the role of another molecule, SHIP-1 (SH2-containing inositol 5′-phosphatase-1) in NF-κB activation in response to H_2O_2 ($\leq$200 μM) was described. It was shown that in SHIP-1 expressing leukemic cells exposed to oxidative stress treatment, NF-κB was activated in an IKK-dependent manner without tyrosine phosphorylation of IκBα (120).

Previous studies have confirmed that hypoxia-induced ROS enhances the DNA binding of NF-κB through a redox-dependent mechanism. Moreover, it was shown that an enhanced generation of ROS could also lead to the sensitization of cancer cells to hypoxia and the activation of NF-κB through c-Src stimulation (224). It has also been determined that the expression of BCR–ABL leads to activation of NF-

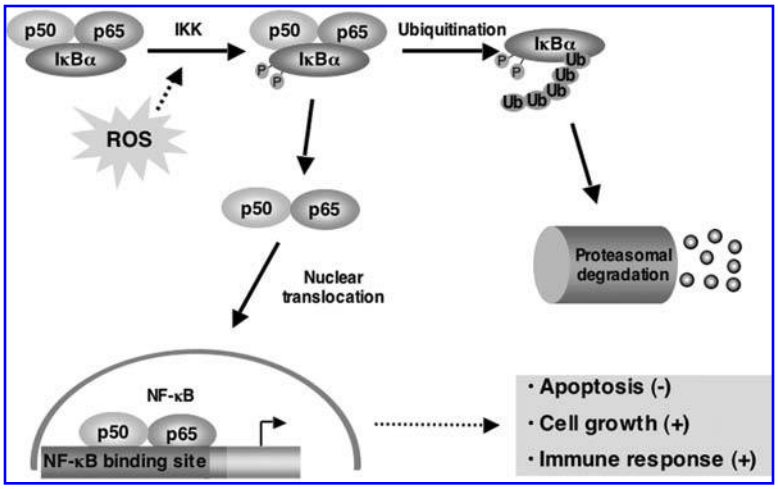

FIG. 5. ROS-induced activation of NF-κB. NF-κB (p50/p65 dimer) is present in the cytoplasm as an inactive complex with a member of the IκB inhibitor protein family (IκBα). Elevated ROS lead to activation of the IκBα kinase (IKK). IKK phosphorylates the NF-κB complex-associated IκBα. This is followed by a rapid degradation of IκBα by the ubiquitin-proteasome system. Active NF-κB rapidly translocates from the cytoplasm to the nucleus where it binds specifically to DNA elements within the promoter regions of target genes and activates their transcription.

κB-dependent transcription through the nuclear translocation of NF-κB as well as by increasing the transactivation function of the RelA/p65 subunit of NF-κB (141). Although NF-κB has been shown to be anti-apoptotic, BCR–ABL has been suggested to utilize alternative and multiple pathways to protect from cell death (297). This is corroborated by others whereby BCR–ABL signals to NF-κB activation through a Ras-dependent pathway (71, 122). Further studies on key intermediate regulators of NF-κB activation would be required to better understand and identify critical events associated with this process. Since NF-κB may not be the only anti-apoptotic molecule in CML and related myeloid neoplasia, combination therapies are more likely to be successful.

D. Redox regulation of NF-κB

Accumulating evidence for the role of NF-κB in redox regulation provides a rationale for targeting the functional inhibition of NF-κB in BCR–ABL-positive leukemias. The fact that some antioxidants have already demonstrated to be strong inhibitors of NF-κB activation suggests that this may be one of the pathways by which they protect against cancers. NAC and pyrrolidine dithiocarbamate (PDTC) are two antioxidants that have been extensively used. Others include vitamin E derivatives, α-lipoic acid, glutathione (GSH), and thioredoxin (Txn). Inhibition of NF-κB activation can also be achieved by the overexpression of antioxidant enzymes, including superoxide dismutase, glutathione peroxidase, or thioredoxin peroxidase. NAC acts by increasing intracellular levels of glutathione, directly scavenging oxidants such as H_2O_2, hydroxyl radical, and hypochlorous acid, and blocking NF-κB activation by preventing a decrease in intracellular thiol levels (37). Furthermore, it was demonstrated that NAC blocked NF-κB activation by diverse stimuli such as H_2O_2 in Jurkat T cells, and attributed this to the suppression of ROS (327). A general inhibitor of NF-κB is PDTC. In an independent study, it was first shown that PDTC inhibited NF-κB activation by IL-1, TNF, PMA, LPS, and H_2O_2 in a number of human lymphocytic cells as well as a mouse fibroblast cell line (326). This provided strong evidence for a central role of ROS in NF-κB activation.

Although accumulating evidence supports the notion that ROS lead to the activation of NF-κB, it should be pointed out that this effect may be cell type and pathway specific. H_2O_2 can negatively regulate cytokine-induced NF-κB activation indirectly through the inhibition of IKK activity (121). Furthermore, high levels of oxidized GSH can block the ubiquitination of IκBα, exerting an inhibitory effect of ROS on NF-κB (173). Despite the role of antioxidants in inhibiting NF-κB activation, there is evidence that common antioxidants such as NAC and PDTC may inhibit NF-κB activation independent of their antioxidant function (42, 143, 337). This is also supported by additional studies indicating the role of ROS as antagonists, rather than agonists of NF-κB (for review, see refs. 121, 135). Finally, redox regulation of NF-κB may occur upstream from its activation. A recent study demonstrated a positive-feedback mechanism for NF-κB, where TNF-α-induced activation of NF-κB led to the upregulation of NADPH oxidase activity. This increase in NADPH oxidase activity resulted in increased ROS levels and a further activation of NF-κB (116).

Additionally, the use of either antisense oligonucleotides or transcription factor decoys to inhibit NF-κB activation results in loss of adhesion in tumor cells in culture (345), and reduction of tumor formation in nude mice (155). Recently, the sensitization of CML cells to Etoposide-induced apoptosis with the use of the NF-κB inhibitor MG132 (prevents degradation of IκB), Bay11-7082 (inhibits cytokine-induced IκBα phosphorylation), and Resveratrol (inhibits IκB kinase activity) was reported (255). These results indicate the role of NF-κB antagonists as chemosensitizers in the targeted therapy of CML.

VI. Cell Cycle Regulation Through Redox Mechanisms

Cellular redox state is a delicate balance between the levels of reactive ROS produced during metabolism and the antioxidant systems that scavenge them. Though ROS are notorious for their deleterious effects on disruption of membrane integrity and DNA damage, literature supporting a cell proliferative role of ROS at low thresholds is available (299). Evidence for a threshold-dependent dual role for ROS in regulating multiple biological processes was shown in NIH3T3 cells, whereby 0.02–0.13 μM H_2O_2 enhanced cellular proliferation and treatment with 0.25–2 μM H_2O_2 resulted in increased cell death (209). Thus, these significant differences in dose-dependent biological effects by H_2O_2 may open a venue for targeted cancer therapies that can shift the balance towards increased H_2O_2 levels and thus cell death (see also Section X).

It is interesting to note that several cell cycle regulatory proteins have redox-sensitive motifs such as cysteine residues and metal co-factors in their active sites. This indicates that changes in the intracellular redox state could play a major role in regulating G0/G1 to S to G2 and M cell cycle progression. This hypothesis of a redox cycle within the cell cycle is supported by the observation that intracellular protein bound and nonprotein sulfhydryl (–SH) and disulfide (–SS-) groups show cyclic changes in concentration during different stages of cell cycle (69, 158, 236, 241, 245, 246).

The concept of a redox cycle regulating the cell cycle is supported by a recent report (374). Budding yeast exhibit a metabolic redox cycle consisting of a reductive nonrespiratory phase and an oxidative respiratory phase. This metabolic redox cycle coordinates with periods of gene expression regulating essential cellular and metabolic events (374). Several genes regulating DNA replication and cell cycle progression are expressed during its reductive phase with cell cycle initiation occurring very late during the oxidative phase. A transient increase in pro-oxidant levels early in G1 is required for these cells to transit from G1 into the S phase. Inhibiting this pro-oxidant event using an antioxidant such as NAC has been shown to arrest cells in the G1 phase (246). In support of this, modulation of antioxidant enzymes such as catalase (43) and GPx4 (386) has been shown to affect cellular proliferation rate, as well as the G1 to S phase transition respectively. Overall, intracellular redox state appears to be a critical cell cycle progression regulator.

The genomic mutations that lead to transformation in myeloid leukemias can be quite different, but it is apparent that cell growth is associated with deregulated tyrosine kinase activity at some level (22). The kinase activity of

BCR–ABL and related tyrosine kinase oncogenes is required for factor independent growth and self renewal of stem cells. Inhibition of the oncogenic kinase leads to G1 cell cycle arrest, supporting the important role of these oncogenes for progression from G1 to S phase of the cell cycle. Oxidative stress may be sufficient to activate cellular signaling to promote cell cycle progression, but is insufficient to support cycling of hematopoietic cells (320). It is likely that ROS support various signaling events and modulate or amplify activities of multiple signaling pathways. General molecular mechanisms that lead cells into S-phase are controlled by cyclin D (D1, D2 or D3), cyclin E (E1 or E2) and cyclin-dependent kinases (cdk), together forming cyclin-dependent kinase complexes (for review, see ref. 334). The major targets of these complexes are the retinoblastoma protein (Rb) tumor suppressor family proteins (Rb, p107, and p130). As a consequence of Rb protein phosphorylation, members of the E2F family of transcription factors become activated. Genes that are regulated by E2F transcription factors are required for S-phase entry. The detailed mechanisms are not of interest here, and we focus instead on processes that are sensitive to changes in the cellular redox status, initiated by tyrosine kinase oncogenes.

A. Protein tyrosine phosphatases

The extent of tyrosine phosphorylation in a cell depends on the activity of protein tyrosine kinases and protein tyrosine phosphatases. Under normal signal transduction processes, the activity of protein tyrosine kinases and phosphatases is tightly regulated. There are ~100 genes that belong to the PTPase superfamily in the human genome. The PTPase superfamily includes tyrosine-specific protein phosphatases, the CDC25 family of phosphatases, the VH1-like dual specificity phosphatases, the low molecular weight phosphatases, and lipid phosphatases. (14, 99, 369). During normal signaling, PTPases remove the phosphate group from proteins phosphorylated on tyrosine residues, and this mechanism of protein phosphatases is crucial for the transient nature of signal transduction events.

In cells with oncogenic tyrosine kinases, this attenuating mechanism is often deregulated. Of interest here is that PTPases are highly sensitive to oxidation because of a critical thiol group in the active site. All PTPases contain a highly conserved 11 amino acid sequence that contains a critical cysteine residue: (Ile/Val)–His–Cys–Xxx–Ala–Gly–Xxx–Xxx–Arg–(Ser/Thr)–Lys–Gly. Under normal cellular conditions this cysteine is in thiolate form (84) and is involved in the formation of a cysteinyl–phosphate intermediate within the enzyme catalytic mechanism. Upon exposure to ROS, the cysteine residue present in the active site gets oxidized, and therefore acts as sensor of redox status in the cell. Oxidation or mutation of this cysteine residue inactivates phosphatase activity, thus implying a ubiquitous role for the active site cysteine in response to ROS (23, 132).

It has now been shown that PTPases are transiently inactivated by ROS, resulting in enhanced protein tyrosine phosphorylation. In a different cellular context, PTPase oxidation can show various degree of susceptibility towards oxidants (391). This could be partly due to different cellular levels of antioxidant activity (244, 300). Previous results have directly demonstrated that oxidative stress can efficiently reduce the cellular PTPase activity and increase tyrosine phosphorylation of cellular proteins (106, 198, 355). In A431 cells, treatment with epidermal growth factor led to an increase in intracellular H_2O_2 production. This increase in H_2O_2 resulted in selective oxidation of catalytic cysteine residue present in the active site of protein tyrosine phosphatase 1B (212). *In vitro* studies showed the reversible oxidation and inactivation of protein tyrosine phosphatases in Rat-1 cells (244). H_2O_2 can specifically inhibit the protein tyrosine phosphatase activity of LAR (Leukocyte Antigen Related) and PTP1 (84). PTP1 is an intracellular tyrosine specific phosphatase and LAR is a receptor-like protein tyrosine phosphatase. PTP1B and LAR are shown to be important in the down-regulation of insulin receptor mediated signaling events (5, 6, 199, 330). These phosphatases, in the presence of low concentrations of H_2O_2, oxidize the cysteine residue present in the phosphatase signature motif to a specific cysteine sulfenic acid intermediate, and this is rapidly converted to a cysteine sulfenyl–amide intermediate, resulting in a large conformational change in the active site accompanied by inhibition of substrate binding (311). The cysteine sulfenic acid intermediate cannot act against the phosphorylated substrates. If high levels of oxidative stress continue to persist in the cell, the sulfenic acid form of cysteine will be irreversibly oxidized to sulfinic and sulfonic acid. One known notable exception to the irreversible mechanism is the ATP-dependent reduction of sulfinic acid residues in some peroxiredoxins by sulfiredoxins (32, 175). The modified cysteine sulfenic acid can be fully reactivated with thiols such as reduced glutathione (Fig. 6) (84). The activity of tyrosine phosphatases is also altered in BCR–ABL transformed cells. Overall, there is a fine balance between phosphatases and kinases in the homeostatic mechanism of the cell, and it is likely that changes in the oxidative state in the cell lead to an imbalance that may amplify the signal sent from an activated tyrosine kinase.

1. PTEN. PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a dual-specific tyrosine/lipid phos-

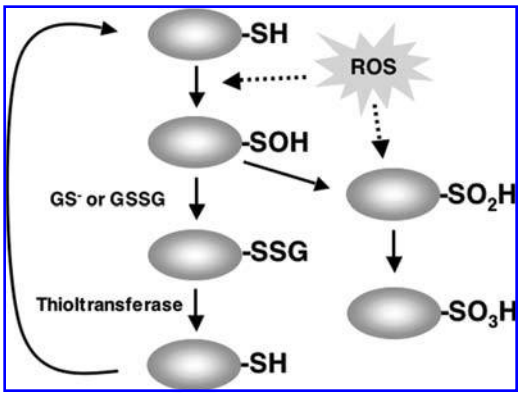

FIG. 6. Regulation of redox sensitive cysteines in PTPases. Upon exposure to oxidative stress, cysteine present in the active site of PTPases undergoes oxidation to form cysteine sulfenic acid. In the continued presence of ROS, active site cysteine will be irreversibly oxidized to sufinic and sulfonic acid forms. The sulfenic acid form can be reduced to cysteine by glutathionylation (see also ref. 24).

phatase that dephosphorylates phosphotidylinositol 3,4,5-trisphosphate (PIP_3) by removing a phosphate group from the 3′-hydroxyl position of the phosphoinositide inositol ring. It is an important signaling molecule that regulates cell growth, migration, and apoptosis, and is mutated in various types of human cancers (67, 228). PTEN has a predominant role in regulating PI3K function, and has been shown to have particular importance in the attenuation of downstream signaling molecules and thus PI3K-dependent ROS signaling. The PI3K/AKT pathway is stimulated by a variety of growth factors, cytokines, cell–matrix interactions, and the lipid products generated by PI3K activity. PIP_3 has been shown to control many biological functions including cell proliferation, cell survival, growth, motility, and insulin responses (210). Deregulated activation of the PI3K pathway aids tumor formation by supporting S-phase entry as well as causing resistance to apoptotic signals that would normally help to restrict uncontrolled cell growth. By counteracting PI3K/AKT signaling cascade, PTEN acts as an important tumor suppressor (18, 55, 80, 110, 347, 352, 358). Interestingly, there is also genetic evidence in *Caenorhabditis elegans* linking the PTEN pathway to metabolism. Under nutrient deprivation, larva development leads to formation of the dauer form, an alternative diapause third stage larva, and G2 arrest of germline precursors. Mutation of the PTEN homolog daf18 can suppress this phenotype, and this may involve the insulin receptor-like molecule daf-2 and the PI3K homolog age-1 (109, 248). Whether a similar connection between PTEN and metabolism (and thus oxidative stress) exists in mammalian cells or even in human cancers, still needs to be determined.

Similar to the redox dependent regulation of protein dephosphorylation by PTPases, an increase in cellular ROS has the potential to shift the balance towards increased phosphotidylinositol-3′-phosphates through reduced PTEN activity (Fig. 7). *In vitro* treatment of cells with hydrogen peroxide leads to a time and concentration dependent increase in oxidation of PTEN (213). Inactivation of PTEN by H_2O_2 treatment also resulted in elevated concentration of PIP_3 in the PTEN-null glioblastoma cell line U87MG (214). H_2O_2-treated PTEN likely forms disulfide bonds between sulfhydryl groups of cysteine residues. Mutational analyses of PTEN suggested that Cys124 present in the signature motif of PTEN forms a disulfide bond with Cys71 (213). The oxidized PTEN can be converted to its active form by reducing agents, suggesting a reversible redox regulation of PTEN under physiological conditions. Interestingly, it has been suggested that inactivation of PTEN by itself can lead to oxidative stress and activation of PI3K, hinting at a positive feedback mechanism (214).

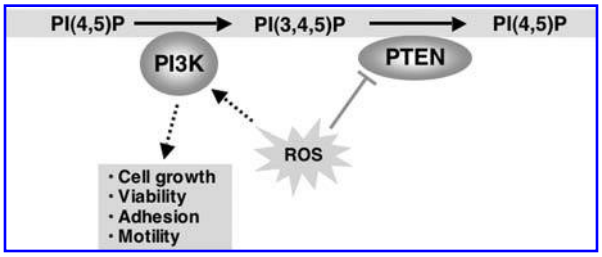

FIG. 7. Regulation of PTEN by ROS. The dual-specific lipid and protein tyrosine phosphatase PTEN dephosphorylates bioactive phosphatidylinositols in the 3′ position. For example, phosphatidylinositol(3,4,5)triphosphate [PI(3,4,5)P] is dephosphorylated by PTEN to PI(4,5)P. In the presence of ROS, PTEN is inhibited resulting in an increase of 3′ phosphorylated phosphatidylinositols generated by PI3K.

Even though PTEN was originally described as a tumor suppressor and a frequent target of mutations in solid tumors, mutations in hematologic malignancies are rare and have thus far been described in some forms of drug resistant T-cell lymphoblastic leukemia (92, 272). Nevertheless, the ROS-dependent inactivation of PTEN implies that H_2O_2 produced under disease conditions contributes to inhibition of apoptosis, resulting in tumor formation. Large amounts of ROS produced by tumor cells may therefore result in increased tumor cell proliferation (49, 79, 264). In hematopoietic cells transformed by BCR–ABL, activation of PI3K is sufficient to lead to an increase in ROS (185). However, the exact role of PTEN in the direct regulation of ROS and PI3K in hematopoietic cells still needs to be determined. It is possible that there are additional PI3K independent pathways that contribute to the regulation of ROS by BCR–ABL.

2. CDC25 phosphatases. Cell division cycle 25 (CDC25) proteins are a highly conserved family of dual specificity phosphatases, and in mammalian cells, three isoforms (A, B, C) have been identified (257, 304). The only known targets of CDC25 phosphatases are CDK–cyclin complexes (35) that serve as the master regulators of cell cycle progression. CDC25 proteins function by removing the inhibitory phosphate on CDKs to promote G1–S and G2–M transitions through regulation of CDK1 and CDK2 (35). CDC25 phosphatases are also key cell cycle checkpoint control components that are critical to maintain genomic stability. When these pathways are defective, cells continue to divide, and DNA lesions are potentially passed on to daughter cells, thus resulting in the loss of genome integrity (for review, see ref. 36).

CDC25 phosphatase functions are tightly regulated during normal cell division, as well as in response to checkpoint activation by multiple mechanisms such as inhibitory and activating phosphorylation, changes in intracellular localization and interactions with the 14-3-3 family of proteins. For example, sequestration of CDC25C into the cytoplasm during interphase leads to the inhibition of mitotic entry due to maintained CDK1 phosphorylation (35, 152). In various human cancers, the CDC25A and CDC25B isoforms have been reported to be overexpressed (36, 51, 111). However, the mechanisms by which CDC25 isoforms become deregulated during tumorigenesis are not completely understood. A recent study with the sesquiterpene Zerumbone in NB4 promyelocytic leukemia cells suggests that activation of the checkpoint kinase Chk1 correlated with inhibition of CDC25C and association with 14-3-3 proteins through the Ser216 phosphorylation site (403).

CDC25 proteins contain a cellular redox sensitive cysteine residue in their active site (344). An additional regulatory feature of CDC25s are cysteine residues within the conserved DCR (Asp–Cys–Arg) motif. In the presence of altered redox environment, these extra invariant cysteines (Cys384 in CDC25A, Cys426 in CDC25B, and Cys330 in CDC25C) are

capable of forming intramolecular disulfide bonds in alternate structures that render the CDC25s inactive. In CDC25A, this intramolecular disulfide bond can be formed as a result of tungstate treatment, presumably via oxidation to sulfenic acid and subsequent attack by Cys384 (98). In the case of CDC25B, disulfide bridging was observed in a crystal form lacking a bound anion in the active state (298). On the other hand, because sulfenic acid is unstable, further oxidation to sulfinic acid (Cys-SO_2^-) or sulfonic acid (Cys-SO_3^{2-}) is also observed, and leads to irreversible inactivation of the enzyme. Thus, reversible intramolecular disulfide bond formation by the active site cysteine with the closely positioned invariant cysteine might be a safety mechanism to prevent irreversible inactivation of these proteins by ROS. Stability of CDC25 proteins is influenced by this disulfide bond formation, and therefore ROS play a key role in tumorigenesis where stability of CDC25 can be a driving factor (Fig. 8).

In vitro, CDC25C is rapidly degraded upon H_2O_2 oxidative challenge through a nonproteasomal pathway. H_2O_2 treatment leads to an intramolecular disulfide bond between the active site cysteine and the invariant cysteine near the active site (321). Interestingly, CDC25A levels remained unaltered in these experiments, hinting at a certain degree of specificity in the redox response of CDC25 proteins. Also, a double mutant incapable of forming a disulfide bond was found to be insensitive to the oxidation and has lower binding to the 14-3-3 protein compared to disulfide bond forming wild-type protein. It was suggested that disulfide bond formation upon oxidation works as a switch for CDC25C to modulate its interaction with 14-3-3, and that formation of intramolecular disulfide bonds could be a protective mechanism against an irreversible oxidation of the active site sulfhydryl group to sulfinic species (321).

The deregulation of CDC25 proteins is thought to occur largely at the post-translational level. In acute myeloid leukemia, increased proliferation in response to integrin activation by fibronectin correlated with accelerated S-phase entry and accumulation of CDC25A (101). The accumulation of CDC25A was a result of decreased proteasomal degradation due to post-translational modifications, thus resulting in increased protein stability. Evidence for a direct role of ROS levels in the regulation of CDC25 phosphatases in CML is not available; however, in a BCR–ABL transformed cell line, the oncogenic kinase activity is required for elevated CDC25A expression and cell growth (240). Inhibition of BCR–ABL also correlates with persistent phosphorylation of CDK2 (232). It is possible that increased ROS seen during BCR–ABL mediated transformation would alter CDC25 proteins so as to result in the deregulation of their function, thus contributing to cellular transformation. This potential disease mechanism requires further investigation to gain a better understanding of the role of CDC25 in the pathogenesis of CML.

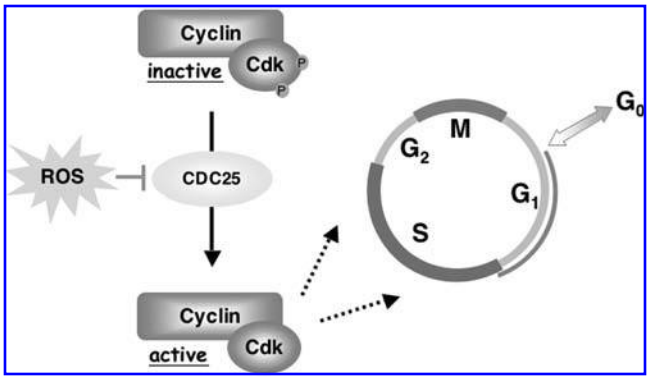

FIG. 8. Role of ROS in CDC25 function. CDC25 phosphatases remove the inhibitory phosphates present on Cdk proteins, facilitating the formation of an active Cyclin/Cdk complex. Upon exposure to ROS, the redox-sensitive cysteine present in the active site of CDC25 forms a disulfide bond with a neighboring cysteine to inhibit further irreversible oxidation, thus preventing cell cycle progression. This pathway may be altered in transformed cells.

B. The FoxO subfamily of forkhead box proteins

The family of forkhead box (Fox) containing transcription factors include a common conserved domain required for DNA binding. Among the Fox family members (FoxA–FoxS), the FoxO proteins and their orthologues have been implicated in the oxidative stress response and may also serve to regulate longevity (94, 146, 267, 388). The FoxO proteins were originally identified in translocations associated with human cancers (15, 34, 81, 112, 156, 276). In mice, FoxO proteins are required for maintenance of the hematopoietic stem cell pool, and mice lacking FoxO were found to have elevated ROS and decreased expression of proteins regulating oxidative stress (371). FoxO3 is likely to be required to protect erythrocytes from oxidative stress and maintain a normal lifespan (234). All three human FoxO proteins, FoxO1a (FKHR), FoxO3a (FKHR-L1), and FoxO4 (AFX), can be phosphorylated by Akt (30, 46, 195, 296, 359). The activation status of FoxO proteins is regulated by their phosphorylation through activated Akt, a process which leads to inactivation and translocation of FoxO from the nucleus to the cytoplasm (Fig. 9). Dephosphorylation of cytoplasmic FoxO converts it to the active form with nuclear localization.

The activity of FoxO proteins can also be positively regulated by JNK (267, 387), a member of the MAPK family, activated in response to cellular stress. It is this activity of JNK that is also thought to contribute to altered lifespan. JNK and Akt have opposing effects on FoxO function. Regulation of FoxO in response to stress involves deacetylation through the sirtuins (Sirt) a family of mammalian NAD-dependent deacetylases. Sirt1 and FoxO proteins form a complex in response to oxidative stress, whereby Sirt1 deacetylates the transcription factor. As a result, FoxO proteins are less efficient in inducing apoptosis but show increased efficiency in regulating genes that cause cell cycle arrest and protect cells from oxidative stress. Nevertheless, deacetylation by Sirt1 may have differential effects on the transcriptional activity of FoxO proteins, dependent on the cellular context (47, 75, 256, 378). FoxO proteins also activate a feedback loop in response to oxidative stress by inducing the expression of detoxifying enzymes such as Mn superoxide dismutase (194). Tyrosine kinase oncogenes with a constitutively active PI3K/Akt pathway would be expected to suppress transcriptional activation of FoxO target genes. The PI3K/Akt pathway has already been shown to be crucial for transformation by BCR–ABL (340, 341) as well as other oncoproteins,

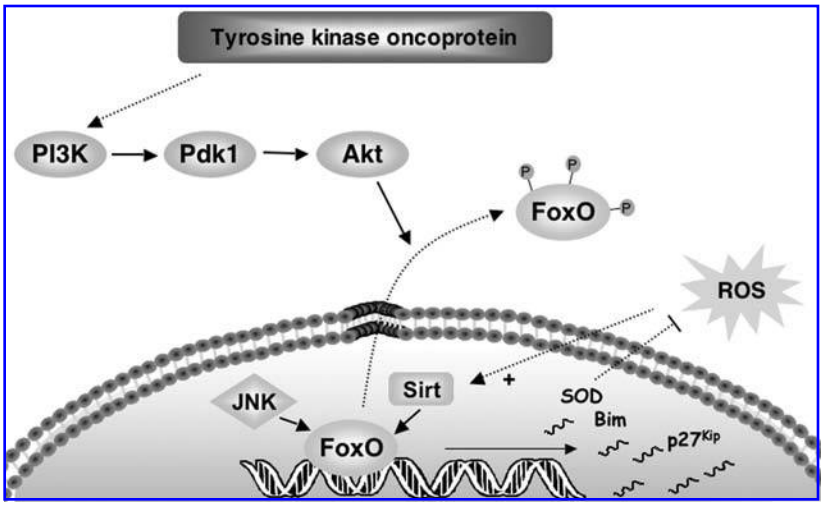

FIG. 9. FoxO transcription factors are involved in oxidative stress response. Pro-apoptotic FoxO proteins are targets of the Akt kinase, downstream of PI3K. Phosphorylated FoxO shuttles from the nucleus to the cytoplasm. Phosphorylation of FoxO by JNK partially opposes Akt effects. Sirt1 deacetylates FoxO in response to oxidative stress and increases the efficiency of the transcription factor to induce genes that cause cell cycle arrest and protect from oxidative stress. Deacetylation of FoxO is likely to have an altered transcriptional response and may be less efficient in inducing apoptotic signals, depending on the cellular context.

indicating an important role for their down-stream targets in this process.

Progress has been made in understanding and identifying the role of FoxO targets in cell growth and viability, but it is clear that there are additional activities regulated through FoxO that are not well explained with known targets on hand. A major transcriptional target for FoxO proteins is the cyclin-dependent kinase (cdk) inhibitor $p27^{Kip}$, effectively leading to G1 cell cycle arrest and inhibiting S-phase entry (243). In BCR–ABL-transformed cells, the FoxO3a transcription factor has a negative role in cell growth (31, 192). Expression of cyclin D2 is required for transformation by BCR–ABL (174); it is under transcriptional control and depends on BCR–ABL kinase activity (83, 273). Activation of FoxO3a correlates with repression of cyclin D, but it has not been shown that this effect is regulated directly through altered transcription by FoxO through direct DNA binding. Instead, it has been suggested that BCR–ABL inhibition leads to activation of FoxO3a, and this will induce expression of Bcl-6. In a second step, the transcriptional repressor Bcl-6 binds to the Stat5/Bcl-6 consensus site in the cyclin D2 promoter, thus regulating transcriptional expression of the gene, indirectly through FoxO3a (100).

Similarly, constitutively active forms of the FLT3 receptor tyrosine kinase lead to activation of the PI3K/Akt pathway, likely through a PI3K binding site in the carboxy terminal region of the FLT3 receptor (302). Pharmacologic inhibition of PI3K results in growth arrest and apoptosis of human AML cell lines expressing the FLT3-ITD tyrosine kinase oncoprotein. FLT3 stimulation by its natural ligand and constitutively activated FLT3-ITD (internal tandem duplication) has been shown to activate Akt and phosphorylate the FoxO3a transcription factor (179, 322). Further, FLT3-ITD-induced phosphorylation of FoxO3a correlated with suppression of the FoxO-target genes $p27^{Kip}$ and Bim, a pro-apoptotic Bcl-2 family member (322). FLT3-ITD is therefore likely to promote cell survival and proliferation through inhibition of FoxO3a-mediated upregulation of the $p27^{Kip}$ and Bim genes.

C. The Jak2/STAT5 pathway

The Jak/STAT pathway has been implicated in a variety of solid tumors as well as hematologic disorders. Similar to BCR–ABL (319), activated Jak2 has been associated with increased intracellular ROS (384, 395). A Jak kinase inhibitor was found to reduce ROS levels by ~50% after a 2-day treatment, compared to untreated cells. These changes were observed in cell lines transfected to express the oncogenes compared to parental cells or cells treated with specific tyrosine kinase inhibitors when compared to control cells. The importance of the Jak2 pathway in the growth of hematopietic cells has become apparent with the identification of activating mutations in myeloproliferative diseases and myeloid leukemias. A single activating point mutation leading to a V617F (Val617Phe) substitution in Jak2 has been reported in patients with polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis, as well as in several myeloproliferative disorders, and infrequently in myelodysplastic syndromes (26, 172, 177, 197, 216, 350, 415). In these diseases, hematopoietic stem cells are mostly characterized by hyper-responsiveness to growth factors, as well as partial factor-independent growth.

The family of Janus kinases includes four members (Jak1-3 and Tyk2) containing seven regions with significant sequence homology between the kinases, termed Jak homology (JH) domains (405). The V617F substitution lies within the pseudokinase domain of Jak2, and is likely to lead to deregulated kinase activity. The tyrosine kinase domain (JH1 domain) is located within the carboxy terminus of the protein, and the adjacent pseudokinase domain (JH2 domain) shows close homology to the JH1 domain. JH2 domains are thought to negatively regulate the kinase activity associated with the JH1 domain (226, 306). This domain is also required for transformation by Jak2V617F and induction of ROS (395). The detailed molecular requirements for Jak2 activation are still being worked out and may require autophosphorylation on multiple sites.

Another notable aspect of signaling by Jak2V617F is the apparent requirement for expression of specific cytokine receptors. Even though Jak2V617F can directly transform murine cells (172), the efficient induction of cell cycle progression may require growth factor receptor co-expression (384). In this model, Jak2V617F readily induced partial growth factor independence in BaF3 cells only in the presence of the erythropoietin receptor (EpoR), but wild-type Jak2 did not increase cell growth in BaF3EpoR cells. The role

of active Jak2 on cell cycle distribution was determined in the Jak2V617F positive erythroleukemia cell line HEL in response to a Jak2 inhibitor (384). On average, Jak2 inhibition led to an increase in the percentage of cells in G1-phase by 28%, whereas the percentage of cells in S-phase decreased by 21%, and those in G2/M-phase decreased by 7%. An intriguing possibility could be that different *in vivo* disease phenotypes associated with Jak2V617F are due to lineage specific genes such as certain growth factor receptors, or they may simply correlate with the expression levels of the Jak2 kinase. The direct involvement of any of these mechanisms in a proliferative phenotype would require further evaluation.

Also, oncogenic fusion proteins of Jak2 with ETV6, PCM1, or BCR have been described in different leukemias and myeloproliferative disorders (129, 202, 279, 295). It has been suggested that elevated Jak2 tyrosine kinase activity contributes to transformation, likely in part through the STAT5 (signal transducer and activator of transcription 5) transcription factor. Genetic modeling suggests that STAT5 genes support immature hematopoiesis, as mice with a targeted disruption of STAT5A and STAT5B genes have reduced myeloid progenitor counts (363). Furthermore, STAT5 may also be required for normal fetal erythropoiesis (343). A close link of Jak2 kinase activity to STAT5 activation is also consistent with the fact that Jak2 knockout mice have severely disrupted hematopoiesis that resembles the phenotype in mice with erythropoietin or erythropoietin receptor gene disruption (184, 220, 258, 274, 400). Although activated Jak2 is likely to be required for transformation in these diseases, it may not be sufficient.

Interestingly, Jak2V617F signaling has also been linked to increased ROS production. Both induction of Jak2 activity in Jak2V617F transformed cells and constitutive active STAT5 expressed in murine cells increase ROS, likely in part through the JakV617F/STAT5 pathway (384). Nevertheless, constitutively active STAT5 may not necessarily act only as transcriptional activator to induce ROS, but has also the potential to activate the PI3K pathway through recruitment of the scaffold Gab2 (142). Similar to BCR–ABL transformation, the activation of PI3K may provide a crucial signal for the Jak2/STAT5-dependent induction of ROS. On the other hand, several antioxidants were found to dramatically reduce Jak2V617F-induced cell growth in a dose-dependent manner, consistent with an important role of a high oxidative state for cell growth in these cells. The effect of antioxidants on cell growth was tested using HEL cells, and included NAC, Tiron (a vitamin E analog), and PDTC. Treatment with 30 m*M* NAC, 3 μM Tiron, or 3 μM PDTC led to a reduction in cell growth and reduced the cell number by >90% (384). These results suggest that a high oxidative state is important to maintain cell growth in HEL cells. This is in agreement with previous findings that treatment of a growth factor deprived hematopoietic cell line with hydrogen peroxide is sufficient to promote G1 to S-phase cell cycle transition (320) and reducing agents induce G1 cell cycle arrest in a variety of models (186, 246, 331). The role of ROS in cell cycle progression is not universal and even though antioxidants inhibit proliferation, ROS are unlikely to be sufficient for cell growth. In the human megakaryocytic cell line M07e, hydrogen peroxide is insufficient to cause S to M-phase cell cycle transition (320). Thus, though elevated ROS are required for cell cycle progression, they are more likely to support cellular growth functions than to initiate or cause them.

The kinase activity of Jak2 is required for G1 to S-phase progression, since inhibition of Jak2V617F with a pyridone-containing tetracycle Jak kinase inhibitor (367) specifically leads to G1 cell cycle arrest and reduced cell growth in HEL cells (384). The observed reduction in cell growth correlated with reduced tyrosine phosphorylation of STAT5 on its activation site. STAT5 is a known substrate of Jak2 (128) and activation of STAT5 has also been implicated in transformation by the BCR–ABL oncogene (338). This is of interest because activation of STAT5 by itself is sufficient to transform hematopoietic cells (270). It is possible that activation of this transcription factor is a common feature in myeloproliferative disorders and myeloid leukemias. Therefore, downstream targets of STAT5 deserve additional attention. Reduced cell growth may be a direct consequence of reduced STAT5 phosphorylation, or may involve other Jak2 substrates, such as VAV (335). The main mechanism involved in inhibition of growth by Jak inhibition is G1 cell cycle arrest with decreased cyclin D2 expression and increased $p27^{Kip}$ expression (384) (Fig. 10). These experiments were performed in cell lines expressing Jak2V617F compared to parental cells, or in HEL cells in response to a Jak2 tyrosine kinase inhibition.

Cyclin D proteins and $p27^{Kip}$ are critical cell cycle regulators responsible for S phase entry (237, 285, 308, 372). It is possible that regulation of the cell cycle inhibitor $p27^{Kip}$ downstream of STAT5, in combination with redox-dependent processes, may significantly contribute to the regulation of G1/S-phase transition. The molecular mechanisms involved in the suppression of $p27^{Kip}$ by the constitutively active Jak2/STAT5 pathway are of future interest and require further evaluation. Overall, activation of the Jak2/STAT5 pathway seems to be sufficient for the induction of elevated levels of ROS and cyclin D2, as well as the suppression of the cell cycle inhibitor $p27^{Kip}$. Due to the fact that a major effect of inhibiting active Jak2V617F is G1 cell cycle arrest, it is likely that the efficacy of targeting this pathway will be increased *in vivo* by combination with antioxidant therapy or drugs that regulate the expression of cyclin D2 or $p27^{Kip}$.Future studies should look to further define the molecular mechanisms involved in transformation by activated forms of Jak2, and to identify targets that would aid in the development and implementation of targeted therapies.

D. A redox switch for Ras family proteins

Ras belongs to a family of small GTPases that includes Rac, Rho, and others. There are a number of downstream effectors of Ras, including serine and threonine kinases such as RAF, extracellular signal-regulated kinase, and PI3K, all of which serve to regulate cell growth, apoptosis, and differentiation (328). In BCR–ABL transformation, Ras activity is regulated indirectly in part through Tyr177, an autophosphorylation site in BCR (412). This site has been identified as a major Grb2 SH2 binding site in BCR–ABL (3, 281, 288). Grb2 is constitutively linked through SH3 domain interactions to SOS, resulting in activation of Ras. A BCR–ABL mutant with a phenylalanine substitution (Tyr177Phe) reduced Ras activation in some assays (3, 281, 288). In hematopoietic cells, this BCR–ABL mutant can still activate Ras

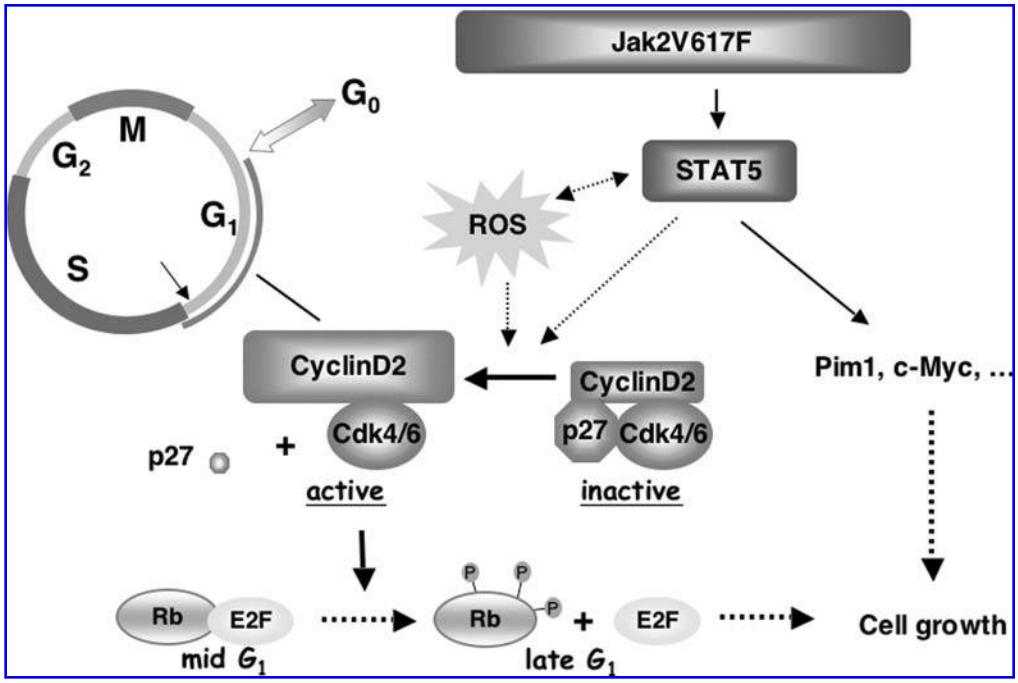

FIG. 10. Hypothetical model for the regulation of the Jak2/STAT5 pathway through ROS. STAT5 is a substrate of Jak2V617F and deregulated activation by itself is sufficient to promote growth in hematopoietic cells. Jak2 inhibition correlates with reduced ROS, G1 cell cycle arrest, decreased cyclin D2 expression, and increased $p27^{Kip}$ expression. Cyclin D proteins and $p27^{Kip}$ are critical cell cycle regulators responsible for S-phase entry. Regulation of cyclin D2 and the cell cycle inhibitor $p27^{Kip}$ in combination with redox-dependent processes promotes G1/S-phase transition downstream of Jak2V617F/STAT5, likely through increased Rb phosphorylation and E2F activation as well as the expression of STAT5 specific genes.

and can transform, suggesting that there are multiple pathways in BCR–ABL-transformed hematopoietic cells that lead to activation of Ras.

Ras activity itself is regulated in part through a redox switch within Ras. Normally, Ras is active when bound to GTP, and intrinsic GTP hydrolysis converts Ras to the inactive GDP-bound form. In addition to guanine nucleotide exchange factors, oxidation of Cys^{118} by ROS in Ras has been suggested to activate its guanine nucleotide exchange activity to convert Ras to the GTP-bound state (207). Also, Ras is not only regulated by ROS but reactive nitrogen species can increase its activity as well (208). A major target of Ras is Raf kinase and its downstream effectors, which are crucial for cell growth. Cys^{118} is contained within the nucleotide binding pocket, and it is thought that oxidation of Ras enhances the release of GDP from Ras, thus enabling it to return to its active GTP-bound form (Fig. 11). A mechanism by which oxidized Ras regulates this process through $O_2^{-}\bullet$ has recently been proposed, and it involves the formation of a 5-Oxo-GDP intermediate with low affinity to Ras (149). In the case of nitrogen dioxide radicals ($\bullet NO_2$), a reactive nitrogen species, it has been shown in more detail that its interaction with Cys^{118} in Ras leads to radical formation, which is transferred to GDP. GDP then forms intermediates with $\bullet NO_2$ until it is decarboxylated and forms the 5-nitro-GDP derivative, which dissociates from Ras and allows GTP binding (147, 150). In addition to Ras, there are likely other Ras family proteins or Ras related proteins that are regulated through an analogous mechanism (148).

VII. Heat-Shock Proteins

Cells react to oxidative stress in a carefully orchestrated response, mainly regulated through inducible protective mechanisms within the cells. As part of this response, heat-shock proteins (Hsps) are induced or activated, including Hsp70 and Hsp90 (123, 339). Hsps can act as molecular chaperones and help various proteins in folding and subsequent maturation. Altering the protein stability of tyrosine kinase, oncoproteins may represent a useful strategy for treatment of the associated disease. Hsp90 is an abundant cytosolic protein that gains full function only with the help of partner proteins such as Hsp70, Hsp40, p23, Hip, and Hop (57). Inactivation of Hsp90 using benzoquinone ansamycins such as geldanamycin (GA), or its less toxic analogues such 17-AAG (17-allylamino-17-demethoxygeldanamycin), results in dissociation of Hsp90 from its client proteins and is followed by rapid degradation of proteins that require this chaperone for maturation or stability (324, 348). GA and 17-AAG downregulate intracellular BCR–ABL protein levels by shifting the binding of BCR–ABL from Hsp90 to Hsp70. The proteasome inhibitor PS-341 reduced both GA and 17-AAG-mediated downregulation of BCR–ABL levels and inhibited apoptosis of HL-60/BCR–ABL and K562 cells (260). Therefore, it has been suggested that degradation of BCR–ABL is of proteasomal nature (Fig. 12).

Although GA targets only Hsp90, the relative levels of both Hsp90 and Hsp70 may be important for growth and transformation of cells (263). GA and 17-AAG have also

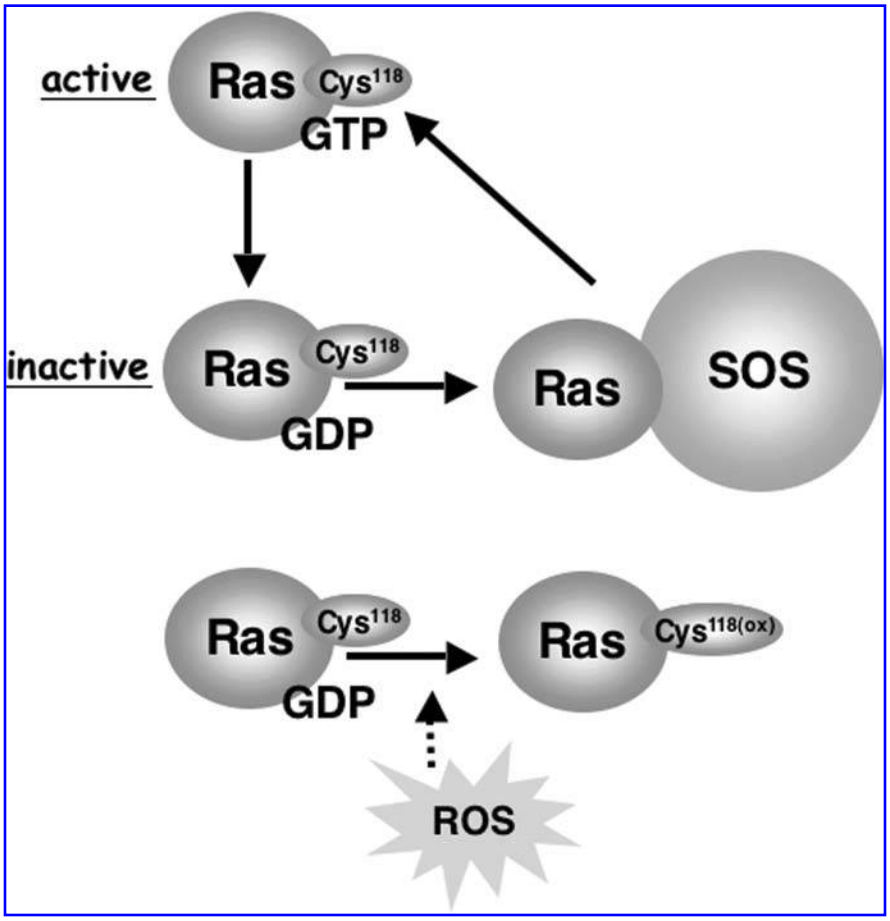

FIG. 11. Redox-mediated regulation of Ras. The Ras oncogene cycles between an inactive GDP-bound and active GTP bound form to modulate its association with downstream targets. The Ras nucleotide exchange factor SOS catalyzes the replacement of GDP with GTP from Ras. In the presence of ROS, oxidation of cysteine 118 in Ras, enhances the release of GDP, enabling it to return to the active GTP-bound form.

been shown to be effective against the imatinib-resistant mutations T315I and E255K of the BCR–ABL kinase *in vitro* (125). The combination of imatinib and 17-AAG led to synergistic effects in primary chronic phase CML cells (291), but 17-AAG alone may not be sufficient in cells that overexpress BCR–ABL as the leading mechanism of imatinib resistance (370). Even though Hsp70 and Hsp90 are not directly involved in cell cycle progression, they are crucial for the functional expression of the BCR–ABL tyrosine kinase and have an important role in the initial step of transformation. Targeting ROS may therefore in part reduce the functional expression of BCR–ABL through reduced

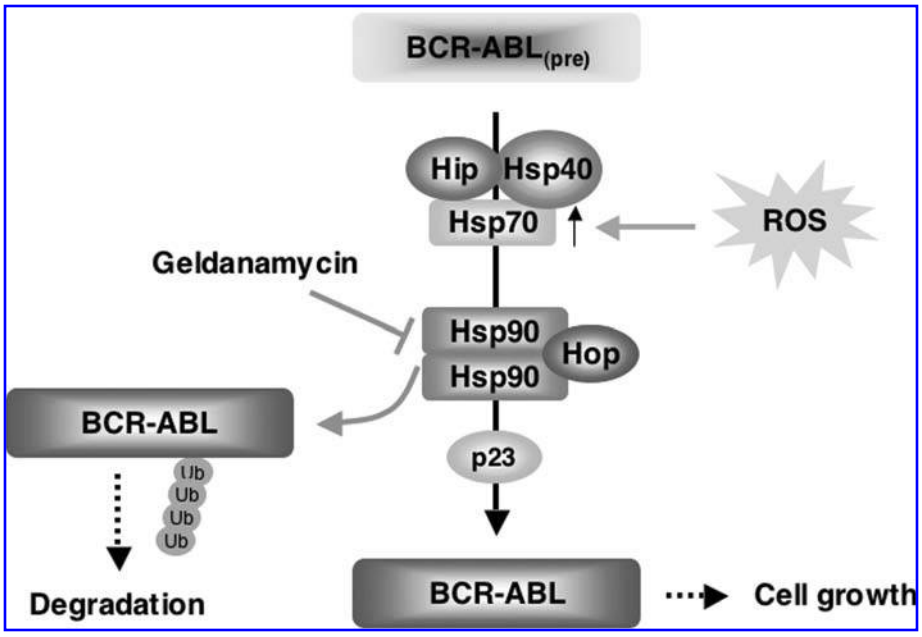

FIG. 12. Regulation of BCR–ABL maturation by heat shock proteins (Hsps). Maturation of the BCR–ABL precursor (pre) is regulated through the interaction of Hsps and co-chaperones, including Hsp40, Hsp70, Hsp90, Hip, Hop, p23, and likely others. Expression of Hsp70 is required for activation of Hsp90 and the maturation of BCR–ABL. Hsp70 expression is induced by ROS and requires BCR–ABL kinase activity. Inhibition of Hsp90 by geldanamycin leads to ubiquitination of BCR–ABL and proteasomal degradation, resulting in reduced expression.

Hsp70 levels, and thus disruption of its interaction with Hsp90.

VIII. Nrf2—A Crucial Oxidative Stress Response Regulator

Nrf2 (NF-E2-related factor-2) is a key element in the regulation of the oxidative stress response and is instrumental for the induction of several antioxidant enzymes through antioxidant responsive elements (ARE) in their respective promoters. In recent years Nrf2 has been studied predominantly as a factor involved in oxidative and chemical induced environmental stress (171, 191). There is little to no direct evidence that Nrf2 is required for normal homeostatic mechanisms; however, there is abundant evidence that Nrf2 is required to maintain cellular oxidative stress homeostasis. Mice with Nrf2 gene disruption exhibit no apparent defects during embryogenesis, undergo normal development and are fertile, thus suggesting that Nrf2 function can be substituted by other factors, such as the related transcription factors NFE2 or Nrf1 (61). However, this possibility is not supported by findings that combined deficiency of NFE2 and Nrf2 had no significant effect on erythroid maturation and globin gene expression in mice (235). Also, targeted disruption of Nrf1 resulted in anemia and was embryonic lethal in mice, demonstrating that Nrf2 cannot substitute for Nrf1 function (59). On the other hand, mice with Nrf2 gene disruption are more sensitive to some chemical compounds, and Nrf2 is required for detoxification that involves expression of γ-glutamylcysteine synthase, glutathione S-transferase, and NADPH quinone oxidoreductase (60, 62, 93, 167, 201, 293). This clearly signifies a unique role for Nrf2 in the cellular stress response, and implicates it as a master regulator for this process. Due to the function of Nrf2 in the oxidative stress response, its role in diseases associated with oxidative stress has been well recognized, including lung injury, inflammation, lung cancer, or other cancers and diseases. Nevertheless, little is known about the role of Nrf2 in hematologic malignancies. It is conceivable that the major regulatory mechanism for Nrf2 and its transcriptional targets are quite similar in different cellular contexts. Even though Nrf2 may not directly be involved in driving cell cycle progression, its function as critical regulator of cellular redox homeostasis makes it a pivotal player in cellular growth pathways.

The activity and expression of Nrf2 is under tight post-transcriptional control by its negative regulator, Keap1 (Kelch-like ECH-associated protein 1). In the absence of oxidative stress, unmodified Keap1 binds tightly to Nrf2. Keap1 is localized to the cytoplasm and thus inhibits Nrf2 nuclear translocation (168). It should be noted that this function of Keap1 is somewhat controversial due to the fact that there is no good evidence that the stoichiometry of the Nrf2-Keap1 complex changes upon stress stimuli. As a result of nuclear translocation, the binding of Nrf2 to ARE sites and transcriptional activation is initiated. In addition to potentially retaining Nrf2 in the cytoplasm, Keap1 also targets Nrf2 for ubiquitination and subsequently proteasomal degradation (410). Redox-sensitive cysteine residues in Keap1 are likely to function as a sensor for oxidative stress and trigger dissociation of Keap1 from Nrf2. The Nrf2-Keap1 complex recruits cullin family ubiquitin E3 ligases, preferably Cul3 (72, 190, 411), that functions to transfer activated ubiquitin to Nrf2, thus promoting poly-ubiquitination and proteasomal degradation. The role of Keap1 in this process may not only be to prevent Nrf2 from nuclear translocation, but also to help Cul3 and other proteins in this complex recognize the target protein for ubiquitination. Although this model appears straight-forward, several reports support alternative models of action, and additional research is required to explain the nuclear shuttling of Nrf2 as well as its transcriptional regulation (409). In the nucleus, Nrf2 forms heteromeric complexes with Jun or small Maf family transcription factors, resulting in transcriptional activation of cytoprotective phase 2 genes in response to ARE site binding (233, 380) (Fig. 13).

The role of Nrf2 in CD34+ hematopoietic cells has not been well characterized. Nrf2 can be induced in response to hematopoietic growth factors, including thrombopoietin as well as erythropoietin (364). The breast cancer susceptibility gene 1 (BRCA1) was found to increase Nrf2 activity by stimulating ARE-dependent transcription (19). BRCA1 was shown, at least in one report, to be reduced in expression by BCR–ABL (86). This introduces the possibility that ROS in BCR–ABL transformation are elevated in part through a compromised Nrf2 due to lack of BRCA1 expression. How-

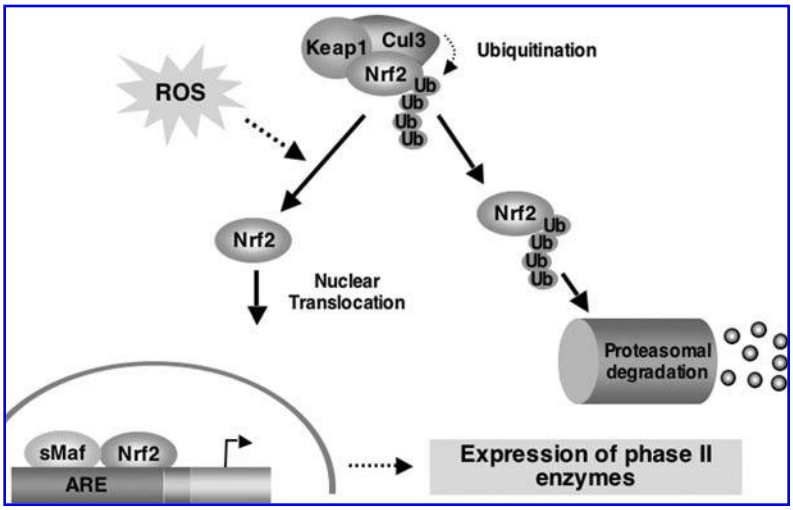

FIG. 13. Schematic model of Nrf2 regulation by ROS. Nrf2 exists as a complex with Keap1, which recruits Cul3-containing E3 ubiquitin ligase. In response to Nrf2-inducers such as ROS, Keap1 undergoes modification, resulting in decreased activity of the Keap1-containing E3 ubiquitin ligase, and subsequent stabilization of Nrf2. Increased amounts of Nrf2 are likely to saturate the binding sites of Keap1, resulting in nuclear translocation of free Nrf2. Nrf2 then forms a dimer with small Maf family transcription factors (sMaf), which bind to ARE to activate the transcription of its downstream genes.

ever, these findings, as well as a casual relationship between BRCA1 expression and Nrf2 function, still need to be confirmed in the context of CML.

Another potential regulatory mechanism of Nrf2 function in CML involves the Bach2 transcription factor. It has recently been suggested that this protein contributes to reduction of the apoptotic response to oxidative stress in BCR–ABL transformed cells (407). The gene was originally identified as a transcriptional target of BCR–ABL kinase activity in lymphoid cells (82). Bach2 is of interest since it can inhibit the expression of at least one major Nrf2 target protein, heme oxygenase-1 (HO-1) (407). In BCR–ABL expressing cells, Bach2 is phosphorylated through a PI3K/S6kinase-dependent mechanism that inhibits nuclear translocation. Furthermore, overexpression of Bach2 leads to reduced cell growth when compared to control transfected cells (407).

In CML, BCR–ABL kinase activity is required for induction of HO-1, and hemin-induced overexpression leads to imatinib resistance *in vitro* (238). HO-1 is also one of the major transcriptionally repressed genes in response to Bach2 in BCR–ABL positive cells (407). Under physiological conditions, HO-1 expression is induced by Nrf2 and inhibited by Bach1, both interacting with the same promoter element (11, 353). In conjunction with a small Maf protein, Bach1 acts as a transcriptional repressor and binds to ARE, thus quenching the target gene expression and opposing the function and gene expression of the Nrf2 transactivator (271, 353). Considering the central role of Nrf2 in the antioxidant stress response, a better understanding of the functional regulation of Nrf2 expression and nuclear translocation is likely to yield potential targets for drug development. Enhancing Nrf2 function is expected to be beneficial for the treatment of cancers with elevated ROS, and is likely to directly affect viability and growth of cancer cells.

IX. Transformation and Genomic Instability

Of particular relevance to human CML is the fact that an increase in ROS could also have long-term consequences for genetic stability. BCR–ABL is likely sufficient to cause CML, but over time other genetic events occur and the disease progresses to an acute leukemia. CML is typically characterized by phases of variable duration, starting with an initial chronic phase (CP), followed by progression to accelerated phase (AP) and finally resulting in blast crisis (BC) (315). There are three different sized BCR–ABL fusion proteins that can differ in the associated clinical phenotype. Occasionally, BCR–ABL fusions are associated with Ph positive acute lymphoblastic leukemia (ALL) or the rare chronic neutrophilic leukemia (97, 305, 383). Cells expressing the chimeric BCR–ABL oncoprotein show signs typical of malignant transformation, including excessive cell growth of immature myeloid cells with lack of differentiation and inhibition of apoptosis (193). In addition, the BCR–ABL oncoprotein decreases an apoptotic reaction to mutagenic stimuli, which in turn results in a survival advantage to neoplastic clones (95). In many model systems, BCR–ABL completely abrogates growth factor dependence, and has been associated with reduced requirement for growth factors in primary hematopoietic cells. It is clear, however, that there are other activities of BCR–ABL that remain poorly understood, in particular the propensity for CML to evolve into blast crisis. It is this phenotype that gives direct clues to the genomic instability associated with BCR–ABL transformation, and is of interest in the context of ROS.

The intracellular ROS levels are not only quenched by enzymes, antioxidants, and sulphydryl groups, but also by reacting with DNA bases. Both $O_2\bullet^-$ and •OH are radicals with unpaired electrons that have the potential to cause cellular damage. For example, •OH can modify the DNA bases adenosine, guanosine, thymidine, or cytosine, and lead to derivatives such as 5-formamido-4,6-diamino-pyrimidine, 8-hydroxy-guanine, thymine glycol, and 5-hydroxy-cytosine, respectively (138). Although these modifications can be efficiently removed by DNA repair mechanisms, we had previously formulated the original hypothesis that in BCR–ABL-transformed cells a persistent increase in ROS could lead to accumulation of genomic mutations (319). Recently, it was shown that BCR–ABL induced chronic oxidative DNA damage results in double-strand breaks and that these lesions are repaired imperfectly, resulting in a high rate of point mutations (265). In stable phase CML, ROS have the potential to lead to additional mutations that could contribute to progression of CML (Fig. 14).

Mutations that cause resistance to the small molecule kinase inhibitor imatinib are of major clinical relevance in the treatment of CML. Resistance to imatinib is defined as the lack of complete hematological response in patients with chronic phase disease or as a lack of return to chronic phase for patients in acute phase, in blast crisis CML, or with Ph+ ALL. Imatinib was developed by Druker and colleagues (imatinib mesylate, Gleevec/Glivec, CGP57148B), as an ABL tyrosine kinase inhibitor (88). It is an inhibitor of the 2-phenylamino pyrimidine class that binds to and stabilizes the inactive form of BCR–ABL (48, 323). Targeted therapy with imatinib has led to a revolution in the treatment of CML. With this treatment, ~95% of patients in chronic phase can

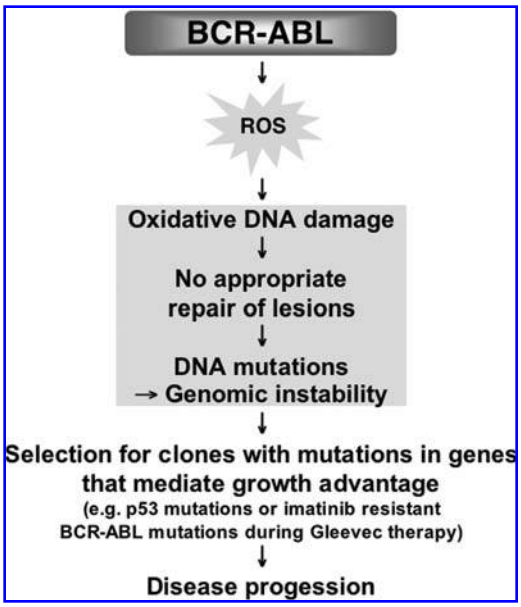

FIG. 14. Hypothetical model (flow chart) for the role of oxidative stress in disease progression of CML.

achieve a complete hematologic remission and ~60% can achieve a major cytogenetic response. However, most patients in blast crisis either do not respond or relapse shortly after an initial response (303). Imatinib also possesses inhibitory properties against the ARG, PDGFR, and c-Kit tyrosine kinase family members (48, 269). The long-term effects of imatinib and its ability to cure CML as a single agent are unknown. The primary effect of imatinib on BCR–ABL expressing progenitor cells seems to be inhibition of proliferation rather than induction of apoptosis (29, 160). Thus, it is possible that as a single drug, imatinib may not be sufficient to completely eradicate BCR–ABL-positive stem cells from the body as it holds the activity of the tyrosine kinase oncoprotein dormant. The resistant cells include CD34+ CD38- leukemic "stem cells" that are thought to be the source of cells that can lead to hematologic relapse due to new mutations (70). *In vivo*, it is thought that these cells are not dependent on BCR–ABL because of viability and growth signals from the microenvironment, and that the notion of "oncogene addiction" does not apply to stem cells. The stromal cell microenvironment is likely to play a crucial role in providing a sanctuary for this cell population. It is an unanswered question, whether these cells show increased DNA lesions and if they have a susceptibility towards stromal cell derived stimuli, leading to DNA lesions during therapeutic treatment.

There are several molecular mechanisms that lead to imatinib resistance, however, the major cause seems to be genomic instability associated with BCR–ABL transformation. The most frequent clinically relevant mechanisms that alter imatinib sensitivity in BCR–ABL transformed cells are point mutations within the ABL kinase domain. Imatinib-resistant mutations may precede exposure to imatinib in some cases, and may ultimately expand as the patient is treated with imatinib. To date, >50 different point mutations encoding distinct single amino-acid substitutions in the BCR–ABL kinase domain have been identified in 50–90% of relapsed CML patients (38, 39, 126, 157, 332). These mutations are more frequently observed in relapsed patients when compared to primary resistant patients (38, 157). Mutations of residues located in BCR–ABL domains (P-loop, T315 proximal region, catalytic domain, activation loop) prevent the kinase from achieving the conformation required to bind imatinib (39, 360, 382). Resistance to imatinib can also be caused by rare overexpression of the BCR–ABL protein due to gene amplification (126, 211). In pharmacological mechanisms of imatinib drug resistance, transmembrane proteins involved in ion transport (50, 145, 162, 229, 230) and plasma protein binding (113) have been implicated.

There are additional mutations in Ph+ cells that appear to bear more relevance during disease progression from stable phase to blast crisis. However, there does not appear to be a particular mutation that drives transformation in any specific way. It is possible that accumulation of additional mutations may be sufficient for transformation by themselves, independent of BCR–ABL. In one study involving 36 patients with paired cytogenetic analyses (pretherapy/refractory or resistant disease), 19 patients showed a variety of chromosomal aberrations in addition to the Ph chromosomal translocation (157). Also, it has recently been suggested that the tumor suppressor p53 is selectively activated by imatinib in BCR–ABL expressing cells and 6% (3 out of 50 samples) of CML patients showed p53 mutations that were not present in the pre-imatinib samples (394). Thus, it is possible that p53 is one potentially important regulator of imatinib resistance in CML. These different mechanisms causing imatinib resistance are consistent with genomic instability associated with BCR–ABL transformation.

The underlying cause for genomic instability by BCR–ABL or other tyrosine kinase oncogenes is poorly understood. In general, genomic instability is thought to be a two-phase process, in which at first mostly random lesions or modifications are introduced into genomic DNA. In a second step, DNA checkpoints fail to efficiently arrest cells and activate appropriate DNA repair mechanisms. There are six major DNA repair pathways required for the maintenance of genomic stability including homologous double-strand break repair, nonhomologous endjoining, base excision repair, nucleotide excision repair, mismatch repair, and translesional DNA synthesis (for review, see ref. 12). An inactivation or reduced activity of any one of these pathways results in various genomic alterations that augment genomic instability, and ultimately have the potential to alter cellular signaling pathways and result in a transforming phenotype or drug resistance. It has long been known that there are frequent cytogenetic changes in addition to the Philadelphia chromosome that may contribute to progression from stable phase to blast crisis CML (10, 108). Similarly, in murine models, BCR–ABL is sufficient to cause a lethal transforming phenotype (76, 118, 182, 242), and the oncoprotein has been suggested to cause secondary mutations as well (53, 54, 86, 189, 310). In murine cell line models, oxidative stress contributes to oxidative DNA damage and the induction of imatinib-resistant BCR–ABL mutants (196, 265). Therefore, mutations depend on signaling mechanisms downstream of Tyr177 in BCR–ABL through increased ROS (196). This mechanism is sufficient to be the major cause for genomic instability, although it should be noted that there are additional factors that have the potential to contribute to this as well (21, 87, 89, 161, 231).

X. Targeting ROS in Cancer Therapy

One current goal in targeted therapies for malignancies with activated tyrosine kinases is to identify new compounds that can bind to and inhibit the kinase and are less affected by mutations through their static conformation. Due to the role of the BCR–ABL tyrosine kinase oncogene, specific inhibitors are not only expected to reduce transforming activity of the kinase, but also to reduce intracellular ROS. Second generation ABL inhibitors are designed to have increased potency of inhibition, bind to the active and inactive ABL kinase conformation, or target the substrate binding pocket in ABL. AMN107 (nilotinib, Novartis, Basel, Switzerland) is an anilio-pyrimidine derivative that is structurally related to imatinib (392) and was found to be 10- to 25-fold more potent compared to imatinib in reduction of both autophosphorylation and proliferation. However, clones carrying Y253H, E255V, G250E, and T315I mutations are all markedly resistant at *in vitro* doses (392). The pyridol pyrimidine BMS-354825 (dasatinib, Bristol-Myers Squibb, New York, NY) is a novel ABL inhibitor that also shows activity towards Src kinases (333). The T315I substitution is at least one known BMS-354825 resistant mutant (333). Unlike imatinib, the ABL

inhibitor ON012380 was specifically designed by Onconova Therapeutics (Princeton, NJ) to block the substrate binding site rather than the ATP binding site and shows activity towards the T315I mutant (133). Similarly, the ABL inhibitor adaphostin (NSC 680410) shows activity against imatinib resistant mutants (64). There are additional ABL inhibitors at various stages of testing, but inhibitors with sufficient efficacy for treatment of imatinib resistant CML have yet to be identified. Additional approaches would reduce the ROS production through targeted approaches towards ROS producing pathways as outlined above. Nevertheless, dietary supplements that are expected to reduce oxidative stress have not found to be beneficial for the treatment of most cancers (136). It is conceivable that a more direct approach with novel small molecule drugs that mimic antioxidant enzyme activities, including superoxide dismutase and catalase, will be more successful. The SOD mimetic M40403 (Metaphore Pharm, Fort Lee, NJ) has shown efficacy in targeting cancer cells and reduces a hyperactive glucose metabolism as well (104, 313). Similar approaches would have to be carefully tested since some apoptotic mechanisms require elevated ROS (Fig. 15).

On the other hand, further elevation of ROS in cancer cells may be sufficient to trigger apoptosis or necrosis. It should be emphasized that cellular oxidative stress is not simply the result of increased ROS production, but has been generally viewed as an imbalance between oxidative stress inducing and scavenging mechanisms. There is regulation on several levels, including compartmentalization, different sensitivity of signaling pathways to changes in ROS, and differential expression of key regulatory proteins, which overall may lead to a specific outcome. Thus, a general change in ROS is the result of individual signaling events and the cellular response to it. The cause for increased ROS and the biological result, from a signaling point of view, may depend on the cellular context and open the possibility for individually targeted approaches (for review, see ref. 178).

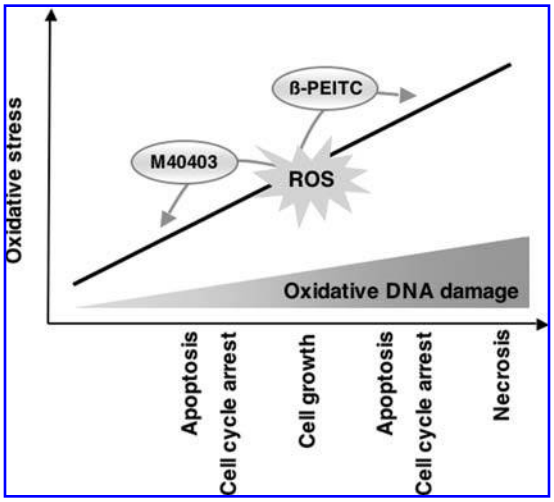

FIG. 15. Effect of altered ROS levels on cancer cell biology. Increased intracellular levels of ROS cannot only contribute to oxidative DNA damage, but also have the potential to alter viability or cell growth. Either high or low amounts of ROS lead to apoptosis or cell cycle arrest. Excessive amounts of ROS will result in necrosis. Antioxidants, such as the superoxide dismutase mimetic M40403, can lower ROS and inhibition of glutathione peroxidase through β-phenylethyl isothiocyanate (β-PEITC) can increase ROS, both resulting in the killing of cancer cells.

Inhibition of superoxide dismutase by 2-methoxyestradiol had been identified as a targeted approach to kill leukemia cells but not normal lymphocytes through elevation of intracellular ROS to levels that induce cell death (165). However, even though the efficacy of this and related drugs has been demonstrated, the actual growth inhibiting mechanism may include other primary targets. It cannot be excluded that increased ROS are simply a required signaling effect during drug-induced apoptosis, instead of being the cause for cell death. In BCR–ABL transformed cells, 2-methoxyestradiol is more likely to act through destabilization of microtubules (317). Cells undergo a mitotic arrest in G2/M phase in response to this drug, which is also evident by an overall increase in cell size. In these experiments, no significant increase in ROS was observed (317). Nevertheless, it is of note that this approach may be suitable to target therapy-related drug resistance with clinically relevant mutations in the BCR–ABL kinase domain.

More recently, inhibition of glutathione peroxidase through β-phenylethyl isothiocyanate has been shown to be effective against BCR–ABL transformed cells, likely through a high oxidative state as a result of the depleted glutathione pool (373). β-Phenylethyl isothiocyanate is already known to be an inhibitor of glutathione peroxidase activity (379), but it is likely that there are additional targets that may be in part inhibited through covalent binding (247). If glutathione peroxidase is indeed the major relevant target for this drug, accentuation of intracellular oxidative stress may prove to be a useful strategy to specifically target cancer cells. Even though this strategy may be effective, it could have some obvious drawbacks on genomic stability.

Elevated ROS are known to be associated with genomic instability. For example, mice with disruption of the CuZn-SOD gene have an increased rate of liver cancer (91). Also, mice with targeted disruption of Prdx1, a member of the peroxiredoxin family of antioxidant enzymes, have a shortened lifespan due to the development of severe hemolytic anemia and malignant cancers, including lymphomas, sarcomas, and carcinomas. Prdx1-deficient fibroblasts show decreased proliferation and increased sensitivity to oxidative DNA damage, ultimately implicating Prdx1 as an important defense against oxidants in aging mice (259). Thus, the long-term consequences of this 'high redox' approach on genomic stability should be carefully considered. To date, the only known curative treatment for CML is bone marrow transplantation from matched donors. A magic bullet would have the ability to eradicate all neoplastic clones that harbor the BCR–ABL transcript, however, it may still be sufficient to develop drugs that at least inhibit the expansion of BCR–ABL-positive stem cells. This goal will not be easily achieved with currently available drugs, and alternative strategies are urgently needed. It seems that ROS themselves, as well as pathways that lead to or synergize with elevated ROS, are additional potential targets for drug development and may suppress or delay the induction of resistant mutants and disease progression.

XI. Conclusions

ROS play an important role in processes that promote cell growth, viability, and regulate other biological functions such as gene expression or migration of cells. However, they have also been implicated in processes that are detrimental to complex eukaryotic life, including aging, a variety of chronic diseases, and cancer development. The exact mechanism of ROS action has not been entirely elucidated, and further characterization of redox sensitive proteins will be helpful in understanding the signaling pathways involving ROS and, in particular, its contribution to transformation. It is possible that *in vivo* ROS simply synergize with cellular functions instead of regulating them. The source of ROS is not necessarily endogenous, as it is true for BCR–ABL transformation, but can also be exogenous, for example through the inhalation of tobacco smoke (68) or through chronic inflammation (181). In CML, previous data suggest a model in which ROS do not only support cell growth but also significantly contribute to genomic instability by causing a persistent increase in oxidative DNA adducts and a lack of appropriate DNA repair (196, 319). Since a hyperactive glucose metabolism induced by BCR–ABL is crucial for the generation of ROS (185), the identification of molecular mechanisms that lead to this dysregulation is beneficial for the development of novel therapies. Increased glucose metabolism is a common feature among metabolically active hematopoietic cells and the efficacy of targeting this pathway will likely be increased *in vivo* by combination with standard therapy, such as imatinib treatment. New therapeutic approaches that reduce ROS have the potential to be of tremendous value, not only in CML, but likely also in other cancers with elevated ROS.

Acknowledgments

This work was supported by grants from the American Cancer Society, the United States Department of Defense, and the Adams Barr Program in Innovative Cancer Research.

Abbreviations

17-AAG, 17-allylamino-17-demethoxygeldanamycin; AP, accelerated phase; ARE, antioxidant responsive element; BC, blast crisis; Cdk, cyclin-dependent kinase; CML, chronic myelogenous leukemia; 2-DOG, 2-deoxy-D-glucose; CP, chronic phase; EGFR, epidermal growth factor receptor; GA, geldanamycin; GM–CSF, granulocyte–macrophage colony stimulating factor; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF, hypoxia-inducible factor; HO, heme oxygenase; HRE, hypoxia response element; Hsp, heat-shock protein; IKK, IκB kinase; IL-3, interleukin-3; ITD, internal tandem duplication; JH, Jak homology; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MDR, multidrug resistant; NAC, *N*-acetylcysteine; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; $O_2\bullet^-$, superoxide; PDGF, platelet-derived growth factor; Ph, Philadelphia chromosome; PI3K, phosphatidylinositol 3-kinase; PDTC, pyrrolidine dithiocarbamate; PTPase, protein tyrosine phosphatase; Rb, retinoblastoma protein; ROS, reactive oxygen species; SF, steel factor; SH2/SH3, Src homology-2/3; Sirt, sirtuins; TPO, thrombopoietin; Txn, thioredoxin.

References

1. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai Z. Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321–1334, 1999.
2. Adler V, Yin Z, Tew KD, and Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. *Oncogene* 18: 6104–6111, 1999.
3. Afar DE, Goga A, McLaughlin J, Witte ON, and Sawyers CL. Differential complementation of BCR–ABL point mutants with c-Myc. *Science* 264: 424–426, 1994.
4. Aguilaniu H, Gustafsson L, Rigoulet M, and Nystrom T. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299: 1751–1753, 2003.
5. Ahmad F, Considine RV, and Goldstein BJ. Increased abundance of the receptor-type protein-tyrosine phosphatase LAR accounts for the elevated insulin receptor dephosphorylating activity in adipose tissue of obese human subjects. *J Clin Invest* 95: 2806–2812, 1995.
6. Ahmad F, Li PM, Meyerovitch J, and Goldstein BJ. Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. *J Biol Chem* 270: 20503–20508, 1995.
7. Ahmed N and Berridge MV. Distinct regulation of glucose transport by interleukin-3 and oncogenes in a murine bone marrow-derived cell line. *Biochem Pharmacol* 57: 387–396, 1999.
8. Ahmed N and Berridge MV. Regulation of glucose transport by interleukin-3 in growth factor-dependent and oncogene-transformed bone marrow-derived cell lines. *Leuk Res* 21: 609–618, 1997.
9. Ahmed N and Berridge MV. Transforming oncogenes regulate glucose transport by increasing transporter affinity for glucose: contrasting effects of oncogenes and heat stress in a murine marrow-derived cell line. *Life Sci* 63: 1887–1903, 1998.
10. Ahuja H, Bar–Eli M, Arlin Z, Advani S, Allen SL, Goldman J, Snyder D, Foti A, and Cline M. The spectrum of molecular alterations in the evolution of chronic myelocytic leukemia. *J Clin Invest* 87: 2042–2047, 1991.
11. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, and Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 274: 26071–26078, 1999.
12. Altieri F, Grillo C, Maceroni M, and Chichiarelli S. DNA damage and repair: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10: 891–938, 2008.
13. Ambasta RK, Kumar P, Griendling KK, Schmidt HH, Busse R, and Brandes RP. Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. *J Biol Chem* 279: 45935–45941, 2004.
14. Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, Jansen PG, Andersen HS, Tonks NK, and Moller NP. Structural and evolutionary relationships among protein tyrosine phosphatase domains. *Mol Cell Biol* 21: 7117–7136, 2001.
15. Anderson MJ, Viars CS, Czekay S, Cavenee WK, and Arden KC. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. *Genomics* 47: 187-199, 1998.
16. Aruoma OI. Free radicals: Dietary advantages and disadvantages. In: *Oxidative Stress and the Molecular Biology of*

Antioxidant Defenses, edited by Scandalios JG. Plainview, NY: CSHL Press, 1997.
17. Babior BM, Kipnes RS, and Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 52: 741–744, 1973.
18. Backman S, Stambolic V, and Mak T. PTEN function in mammalian cell size regulation. *Curr Opin Neurobiol* 12: 516–522, 2002.
19. Bae I, Fan S, Meng Q, Rih JK, Kim HJ, Kang HJ, Xu J, Goldberg ID, Jaiswal AK, and Rosen EM. BRCA1 induces antioxidant gene expression and resistance to oxidative stress. *Cancer Res* 64: 7893–7909, 2004.
20. Bae YS, Sung JY, Kim OS, Kim YJ, Hur KC, Kazlauskas A, and Rhee SG. Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. *J Biol Chem* 275: 10527–10531, 2000.
21. Bakalova R, Ohba H, Zhelev Z, Ishikawa M, Shinohara Y, and Baba Y. Cross-talk between BCR–ABL tyrosine kinase, protein kinase C and telomerase-a potential reason for resistance to Glivec in chronic myelogenous leukaemia. *Biochem Pharmacol* 66: 1879–1884, 2003.
22. Banerji L and Sattler M. Targeting mutated tyrosine kinases in the therapy of myeloid leukaemias. *Expert Opin Ther Targets* 8: 221–239, 2004.
23. Barrett WC, DeGnore JP, Keng YF, Zhang ZY, Yim MB, and Chock PB. Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B. *J Biol Chem* 274: 34543–34546, 1999.
24. Barrett WC, DeGnore JP, Konig S, Fales HM, Keng YF, Zhang ZY, Yim MB, and Chock PB. Regulation of PTP1B via glutathionylation of the active site cysteine 215. *Biochemistry* 38: 6699–6705, 1999.
25. Bassermann F, Jahn T, Miething C, Seipel P, Bai RY, Coutinho S, Tybulewicz VL, Peschel C, and Duyster J. Association of BCR–ABL with the proto-oncogene Vav is implicated in activation of the Rac-1 pathway. *J Biol Chem* 277: 12437–12445, 2002.
26. Baxter EJ, Scott LM, Campbell PJ, East C, Fouroucklas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, and Green AR. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 365: 1054–1061, 2005.
27. Benassi B, Fanciulli M, Fiorentino F, Porrello A, Chiorino G, Loda M, Zupi G, and Biroccio A. c-Myc phosphorylation is required for cellular response to oxidative stress. *Mol Cell* 21: 509–519, 2006.
28. Bentley J, Walker I, McIntosh E, Whetton AD, Owen–Lynch PJ, and Baldwin SA. Glucose transport regulation by p210 BCR–ABL in a chronic myeloid leukaemia model. *Br J Haematol* 112: 212–215, 2001.
29. Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL, Arber DA, Slovak ML, and Forman SJ. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 101: 4701–4707, 2003.
30. Biggs WH, 3rd, Meisenhelder J, Hunter T, Cavenee WK, and Arden KC. Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* 96: 7421–7426, 1999.
31. Birkenkamp KU, Essafi A, van der Vos KE, da Costa M, Hui RC, Holstege F, Koenderman L, Lam EW, and Coffer PJ. FOXO3a induces differentiation of BCR–ABL-transformed cells through transcriptional down-regulation of Id1. *J Biol Chem* 282: 2211–2220, 2007.
32. Biteau B, Labarre J, and Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. *Nature* 425: 980–984, 2003.
33. Boren J, Cascante M, Marin S, Comin–Anduix B, Centelles JJ, Lim S, Bassilian S, Ahmed S, Lee WN, and Boros LG. Gleevec (STI571) influences metabolic enzyme activities and glucose carbon flow toward nucleic acid and fatty acid synthesis in myeloid tumor cells. *J Biol Chem* 276: 37747–37753, 2001.
34. Borkhardt A, Repp R, Haas OA, Leis T, Harbott J, Kreuder J, Hammermann J, Henn T, and Lampert F. Cloning and characterization of AFX, the gene that fuses to MLL in acute leukemias with a t(X;11)(q13;q23). *Oncogene* 14: 195–202, 1997.
35. Boutros R, Dozier C, and Ducommun B. The when and wheres of CDC25 phosphatases. *Curr Opin Cell Biol* 18: 185–191, 2006.
36. Boutros R, Lobjois V, and Ducommun B. CDC25 phosphatases in cancer cells: key players? Good targets? *Nature Rev* 7: 495–507, 2007.
37. Bowie A and O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol* 59: 13–23, 2000.
38. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, Herrmann R, Lynch KP, and Hughes TP. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 99: 3472–3475, 2002.
39. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, Taylor K, Herrmann R, Seymour JF, Arthur C, Joske D, Lynch K, and Hughes T. Detection of BCR–ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood* 102: 276–283, 2003.
40. Brar SS, Corbin Z, Kennedy TP, Hemendinger R, Thornton L, Bommarius B, Arnold RS, Whorton AR, Sturrock AB, Huecksteadt TP, Quinn MT, Krenitsky K, Ardie KG, Lambeth JD, and Hoidal JR. NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. *Am J Physiol Cell Physiol* 285: C353–369, 2003.
41. Brar SS, Kennedy TP, Sturrock AB, Huecksteadt TP, Quinn MT, Whorton AR, and Hoidal JR. An NAD(P)H oxidase regulates growth and transcription in melanoma cells. *Am J Physiol Cell Physiol* 282: C1212–1224, 2002.
42. Brennan P and O'Neill LA. Effects of oxidants and antioxidants on nuclear factor kappa B activation in three different cell lines: evidence against a universal hypothesis involving oxygen radicals. *Biochim Biophys Acta* 1260: 167–175, 1995.
43. Brown MR, Miller FJ, Jr., Li WG, Ellingson AN, Mozena JD, Chatterjee P, Engelhardt JF, Zwacka RM, Oberley LW, Fang X, Spector AA, and Weintraub NL. Overexpression of human catalase inhibits proliferation and promotes apoptosis in vascular smooth muscle cells. *Circ Res* 85: 524–533, 1999.
44. Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. *Proc Natl Acad Sci USA* 97: 9082–9087, 2000.
45. Brumell JH, Burkhardt AL, Bolen JB, and Grinstein S. Endogenous reactive oxygen intermediates activate tyrosine kinases in human neutrophils. *J Biol Chem* 271: 1455–1461, 1996.

46. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, and Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96: 857–868, 1999.
47. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, and Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303: 2011–2015, 2004.
48. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno–Jones S, Druker BJ, and Lydon NB. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther* 295: 139–145, 2000.
49. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18: 775–794, 1995.
50. Burger H, van TH, Boersma AW, Brok M, Wiemer EA, Stoter G, and Nooter K. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. *Blood* 104: 2940–2942, 2004.
51. Cangi MG, Cukor B, Soung P, Signoretti S, Moreira G, Jr., Ranashinge M, Cady B, Pagano M, and Loda M. Role of the Cdc25A phosphatase in human breast cancer. *J Clin Invest* 106: 753–761, 2000.
52. Caniggia I, Mostachfi H, Winter J, Gassmann M, Lye SJ, Kuliszewski M, and Post M. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J Clin Invest* 105: 577–587, 2000.
53. Canitrot Y, Falinski R, Louat T, Laurent G, Cazaux C, Hoffmann JS, Lautier D, and Skorski T. p210 BCR/ABL kinase regulates nucleotide excision repair (NER) and resistance to UV radiation. *Blood* 102: 2632–2637, 2003.
54. Canitrot Y, Lautier D, Laurent G, Frechet M, Ahmed A, Turhan AG, Salles B, Cazaux C, and Hoffmann JS. Mutator phenotype of BCR–ABL transfected Ba/F3 cell lines and its association with enhanced expression of DNA polymerase beta. *Oncogene* 18: 2676–2680, 1999.
55. Cantley LC and Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA* 96: 4240–4245, 1999.
56. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, and Keshert E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394: 485–490, 1998.
57. Carrello A, Allan RK, Morgan SL, Owen BA, Mok D, Ward BK, Minchin RF, Toft DO, and Ratajczak T. Interaction of the Hsp90 cochaperone cyclophilin 40 with Hsc70. *Cell Stress Chaperones* 9: 167–181, 2004.
58. Cash TP, Pan Y, and Simon MC. Reactive oxygen species and cellular oxygen sensing. *Free Radic Biol Med* 43: 1219–1225, 2007.
59. Chan JY, Kwong M, Lu R, Chang J, Wang B, Yen TS, and Kan YW. Targeted disruption of the ubiquitous CNC-bZIP transcription factor, Nrf-1, results in anemia and embryonic lethality in mice. *EMBO J* 17: 1779–1787, 1998.
60. Chan K, Han XD, and Kan YW. An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. *Proc Natl Acad Sci USA* 98: 4611–4616, 2001.
61. Chan K, Lu R, Chang JC, and Kan YW. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. *Proc Natl Acad Sci USA* 93: 13943–13948, 1996.
62. Chanas SA, Jiang Q, McMahon M, McWalter GK, McLellan LI, Elcombe CR, Henderson CJ, Wolf CR, Moffat GJ, Itoh K, Yamamoto M, and Hayes JD. Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. *Biochem J* 365: 405–416, 2002.
63. Chandel NS and Budinger GR. The cellular basis for diverse responses to oxygen. *Free Radic Biol Med* 42: 165–174, 2007.
64. Chandra J, Tracy J, Loegering D, Flatten K, Verstovsek S, Beran M, Gorre M, Estrov Z, Donato N, Talpaz M, Sawyers C, Bhalla K, Karp J, Sausville E, and Kaufmann SH. Adaphostin-induced oxidative stress overcomes BCR/ABL mutation-dependent and -independent imatinib resistance. *Blood* 107: 2501–2506, 2006.
65. Chau NM, Rogers P, Aherne W, Carroll V, Collins I, McDonald E, Workman P, and Ashcroft M. Identification of novel small molecule inhibitors of hypoxia-inducible factor-1 that differentially block hypoxia-inducible factor-1 activity and hypoxia-inducible factor-1alpha induction in response to hypoxic stress and growth factors. *Cancer Res* 65: 4918–4928, 2005.
66. Cheng G, Cao Z, Xu X, van Meir EG, and Lambeth JD. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269: 131–140, 2001.
67. Cho SH, Lee CH, Ahn Y, Kim H, Ahn CY, Yang KS, and Lee SR. Redox regulation of PTEN and protein tyrosine phosphatases in H(2)O(2) mediated cell signaling. *FEBS Lett* 560: 7–13, 2004.
68. Church DF and Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* 64: 111–126, 1985.
69. Conour JE, Graham WV, and Gaskins HR. A combined in vitro/bioinformatic investigation of redox regulatory mechanisms governing cell cycle progression. *Physiol Genomics* 18: 196–205, 2004.
70. Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, Barow M, Mountford JC, and Holyoake TL. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML, but does not eliminate the quiescent fraction. *Blood* 107: 4532–4539, 2006.
71. Cortez D, Kadlec L, and Pendergast AM. Structural and signaling requirements for BCR–ABL-mediated transformation and inhibition of apoptosis. *Mol Cell Biol* 15: 5531–5541, 1995.
72. Cullinan SB, Gordan JD, Jin J, Harper JW, and Diehl JA. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. *Mol Cell Biol* 24: 8477–8486, 2004.
73. Da-Silva WS, Gomez–Puyou A, Gomez–Puyou MT, Moreno–Sanchez R, De Felice FG, De Meis L, Oliveira MF, and Galina A. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: Steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. *J Biol Chem* 279: 39846–39855, 2004.
74. Dai ZH and Pendergast AM. Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. *Genes Dev* 9: 2569–2582, 1995.

75. Daitoku H, Hatta M, Matsuzaki H, Aratani S, Ohshima T, Miyagishi M, Nakajima T, and Fukamizu A. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci USA* 101: 10042–10047, 2004.
76. Daley GQ, Van Etten RA, and Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 247: 824–830, 1990.
77. Dalle–Donne I, Giustarini D, Colombo R, Rossi R, and Milzani A. Protein carbonylation in human diseases. *Trends Molec Med* 9: 169–176, 2003.
78. Danhauser–Riedl S, Warmuth M, Druker BJ, Emmerich B, and Hallek M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res* 56: 3589–3596, 1996.
79. Datta R, Hallahan DE, Kharbanda SM, Rubin E, Sherman ML, Huberman E, Weichselbaum RR, and Kufe DW. Involvement of reactive oxygen intermediates in the induction of c-jun gene transcription by ionizing radiation. *Biochemistry* 31: 8300–8306, 1992.
80. Davies MA, Kim SJ, Parikh NU, Dong Z, Bucana CD, and Gallick GE. Adenoviral-mediated expression of MMAC/PTEN inhibits proliferation and metastasis of human prostate cancer cells. *Clin Cancer Res* 8: 1904–1914, 2002.
81. Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, and Barr FG. Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. *Cancer Res* 54: 2869–2872, 1994.
82. Deininger MW, Vieira S, Mendiola R, Schultheis B, Goldman JM, and Melo JV. BCR–ABL tyrosine kinase activity regulates the expression of multiple genes implicated in the pathogenesis of chronic myeloid leukemia. *Cancer Res* 60: 2049–2055, 2000.
83. Deininger MW, Vieira SA, Parada Y, Banerji L, Lam EW, Peters G, Mahon FX, Kohler T, Goldman JM, and Melo JV. Direct relation between BCR–ABL tyrosine kinase activity and cyclin D2 expression in lymphoblasts. *Cancer Res* 61: 8005–8013, 2001.
84. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
85. Desplat V, Faucher JL, Mahon FX, Dello Sbarba P, Praloran V, and Ivanovic Z. Hypoxia modifies proliferation and differentiation of CD34(+) CML cells. *Stem Cells* 20: 347–354, 2002.
86. Deutsch E, Jarrousse S, Buet D, Dugray A, Bonnet ML, Vozenin–Brotons MC, Guilhot F, Turhan AG, Feunteun J, and Bourhis J. Down-regulation of BRCA1 in BCR–ABL-expressing hematopoietic cells. *Blood* 101: 4583–4588, 2003.
87. Dierov J, Dierova R, and Carroll M. BCR/ABL translocates to the nucleus and disrupts an ATR-dependent intra-S phase checkpoint. *Cancer Cell* 5: 275–285, 2004.
88. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, and Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of BCR–ABL positive cells. *Nat Med* 2: 561–566, 1996.
89. Drummond MW, Hoare SF, Monaghan A, Graham SM, Alcorn MJ, Keith WN, and Holyoake TL. Dysregulated expression of the major telomerase components in leukaemic stem cells. *Leukemia* 19: 381–389, 2005.
90. Edinger AL and Thompson CB. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. *Mol Biol Cell* 13: 2276–2288, 2002.
91. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, and Huang TT. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 24: 367–380, 2005.
92. Eng C. PTEN: one gene, many syndromes. *Hum Mutat* 22: 183–198, 2003.
93. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'-Connor T, Harada T, and Yamamoto M. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. *Toxicol Sci* 59: 169–177, 2001.
94. Essers MA, Weijzen S, de Vries–Smits AM, Saarloos I, de Ruiter ND, Bos JL, and Burgering BM. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* 23: 4802–4812, 2004.
95. Evans CA, Owen–Lynch PJ, Whetton AD, and Dive C. Activation of the Abelson tyrosine kinase activity is associated with suppression of apoptosis in hemopoietic cells. *Cancer Res* 53: 1735–1738, 1993.
96. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23: 599–622, 2002.
97. Fainstein E, Marcelle C, Rosner A, Canaani E, Gale RP, Dreazen O, Smith SD, and Croce CM. A new fused transcript in Philadelphia chromosome positive acute lymphocytic leukaemia. *Nature* 330: 386–388, 1987.
98. Fauman EB, Cogswell JP, Lovejoy B, Rocque WJ, Holmes W, Montana VG, Piwnica–Worms H, Rink MJ, and Saper MA. Crystal structure of the catalytic domain of the human cell cycle control phosphatase, Cdc25A. *Cell* 93: 617–625, 1998.
99. Fauman EB and Saper MA. Structure and function of the protein tyrosine phosphatases. *Trends Biochem Sci* 21: 413–417, 1996.
100. Fernandez de Mattos S, Essafi A, Soeiro I, Pietersen AM, Birkenkamp KU, Edwards CS, Martino A, Nelson BH, Francis JM, Jones MC, Brosens JJ, Coffer PJ, and Lam EW. FoxO3a and BCR–ABL regulate cyclin D2 transcription through a STAT5/BCL6-dependent mechanism. *Mol Cell Biol* 24: 10058–10071, 2004.
101. Fernandez–Vidal A, Ysebaert L, Didier C, Betous R, De Toni F, Prade–Houdellier N, Demur C, Contour–Galcera MO, Prevost GP, Ducommun B, Payrastre B, Racaud–Sultan C, and Manenti S. Cell adhesion regulates CDC25A expression and proliferation in acute myeloid leukemia. *Cancer Res* 66: 7128–7135, 2006.
102. Filomeni G, Rotilio G, and Ciriolo MR. Disulfide relays and phosphorylative cascades: partners in redox-mediated signaling pathways. *Cell Death different* 12: 1555–1563, 2005.
103. Fiorentini D, Hakim G, Bonsi L, Bagnara GP, Maraldi T, and Landi L. Acute regulation of glucose transport in a human megakaryocytic cell line: difference between growth factors and H(2)O(2). *Free Radic Biol Med* 31: 923–931, 2001.
104. Fiorentini D, Prata C, Maraldi T, Zambonin L, Bonsi L, Hakim G, and Landi L. Contribution of reactive oxygen species to the regulation of Glut1 in two hemopoietic cell lines differing in cytokine sensitivity. *Free Radic Biol Med* 37: 1402–1411, 2004.
105. Firth JD, Ebert BL, Pugh CW, and Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the

erythropoietin 3′ enhancer. *Proc Natl Acad Sci USA* 91: 6496–6500, 1994.
106. Flohe L, Brigelius–Flohe R, Saliou C, Traber MG, and Packer L. Redox regulation of NF-kappa B activation. *Free Radic Biol Med* 22: 1115–1126, 1997.
107. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, and Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 16: 4604–4613, 1996.
108. Foti A, Ahuja HG, Allen SL, Koduru P, Schuster MW, Schulman P, Bar–Eli M, and Cline MJ. Correlation between molecular and clinical events in the evolution of chronic myelocytic leukemia to blast crisis. *Blood* 77: 2441–2444, 1991.
109. Fukuyama M, Rougvie AE, and Rothman JH. *C. elegans* DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline. *Curr Biol* 16: 773–779, 2006.
110. Furnari FB, Huang HJ, and Cavenee WK. The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. *Cancer Res* 58: 5002–5008, 1998.
111. Galaktionov K, Lee AK, Eckstein J, Draetta G, Meckler J, Loda M, and Beach D. CDC25 phosphatases as potential human oncogenes. *Science* 269: 1575–1577, 1995.
112. Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher FJ, 3rd, Emanuel BS, Rovera G, and Barr FG. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. *Nat Genet* 5: 230–235, 1993.
113. Gambacorti–Passerini C, Gunby RH, Piazza R, Galietta A, Rostagno R, and Scapozza L. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol* 4: 75–85, 2003.
114. Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, Bhujwalla ZM, Felsher DW, Cheng L, Pevsner J, Lee LA, Semenza GL, and Dang CV. HIF-dependent antitumorigenic effect of antioxidants *in vivo*. *Cancer Cell* 12: 230–238, 2007.
115. Garber K. Energy boost: the Warburg effect returns in a new theory of cancer. *J Natl Cancer Inst* 96: 1805–1806, 2004.
116. Gauss KA, Nelson–Overton LK, Siemsen DW, Gao Y, DeLeo FR, and Quinn MT. Role of NF-kappaB in transcriptional regulation of the phagocyte NADPH oxidase by tumor necrosis factor-alpha. *J Leuk Biol* 82: 729–741, 2007.
117. Geiszt M and Leto TL. The Nox family of NAD(P)H oxidases: host defense and beyond. *J Biol Chem* 279: 51715–51718, 2004.
118. Gishizky ML, Johnson–White J, and Witte ON. Efficient transplantation of BCR–ABL-induced chronic myelogenous leukemia-like syndrome in mice. *Proc Natl Acad Sci USA* 90: 3755–3759, 1993.
119. Gleadle JM and Ratcliffe PJ. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: evidence against a regulatory role for Src kinase. *Blood* 89: 503–509, 1997.
120. Gloire G, Charlier E, Rahmouni S, Volanti C, Chariot A, Erneux C, and Piette J. Restoration of SHIP-1 activity in human leukemic cells modifies NF-kappaB activation pathway and cellular survival upon oxidative stress. *Oncogene* 25: 5485–5494, 2006.
121. Gloire G, Legrand–Poels S, and Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol* 72: 1493–1505, 2006.
122. Goga A, McLaughlin J, Afar DE, Saffran DC, and Witte ON. Alternative signals to RAS for hematopoietic transformation by the BCR–ABL oncogene. *Cell* 82: 981–988, 1995.
123. Gomer CJ, Ryter SW, Ferrario A, Rucker N, Wong S, and Fisher AM. Photodynamic therapy-mediated oxidative stress can induce expression of heat shock proteins. *Cancer Res* 56: 2355–2360, 1996.
124. Gordan JD, Thompson CB, and Simon MC. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell* 12: 108–113, 2007.
125. Gorre ME, Ellwood–Yen K, Chiosis G, Rosen N, and Sawyers CL. BCR–ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR–ABL chaperone heat shock protein 90. *Blood* 100: 3041–3044, 2002.
126. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, and Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 293: 876–880., 2001.
127. Gotoh Y and Cooper JA. Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. *J Biol Chem* 273: 17477–17482, 1998.
128. Gouilleux F, Wakao H, Mundt M, and Groner B. Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *EMBO J* 13: 4361–4369, 1994.
129. Griesinger F, Hennig H, Hillmer F, Podleschny M, Steffens R, Pies A, Wormann B, Haase D, and Bohlander SK. A BCR-JAK2 fusion gene as the result of a t(9;22)(p24;q11.2) translocation in a patient with a clinically typical chronic myeloid leukemia. *Genes Chromosomes Cancer* 44: 329–333, 2005.
130. Gu H, Pratt JC, Burakoff SJ, and Neel BG. Cloning of p97/Gab2, the major SHP2-binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation. *Mol Cell* 2: 729–740., 1998.
131. Gu H, Saito K, Klaman LD, Shen J, Fleming T, Wang Y, Pratt JC, Lin G, Lim B, Kinet JP, and Neel BG. Essential role for Gab2 in the allergic response. *Nature* 412: 186–190., 2001.
132. Guan KL and Dixon JE. Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. *J Biol Chem* 266: 17026–17030, 1991.
133. Gumireddy K, Baker SJ, Cosenza SC, John P, Kang AD, Robell KA, Reddy MV, and Reddy EP. A non-ATP-competitive inhibitor of BCR–ABL overrides imatinib resistance. *Proc Natl Acad Sci USA* 102: 1992–1997, 2005.
134. Haataja L, Groffen J, and Heisterkamp N. Characterization of RAC3, a novel member of the Rho family. *J Biol Chem* 272: 20384–20388, 1997.
135. Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. *Biochem Biophys Res Commun* 316: 969–977, 2004.
136. Halliwell B. Oxidative stress and cancer: have we moved forward? *Biochem J* 401: 1–11, 2007.
137. Halliwell B. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition. *Mutat Res* 443: 37–52, 1999.
138. Halliwell B and Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett* 281: 9–19, 1991.
139. Hamdane M, David–Cordonnier MH, and D'Halluin JC. Activation of p65 NF-kappaB protein by p210BCR–ABL in a myeloid cell line (P210BCR–ABL activates p65 NF-kappaB). *Oncogene* 15: 2267–2275, 1997.
140. Hammer S, To KK, Yoo YG, Koshiji M, and Huang LE. Hypoxic suppression of the cell cycle gene CDC25A in tumor cells. *Cell Cycle* 6: 1919–1926, 2007.

141. Hanson JL, Anest V, Reuther–Madrid J, and Baldwin AS. Oncoprotein suppression of tumor necrosis factor-induced NF kappa B activation is independent of Raf-controlled pathways. *J Biol Chem* 278: 34910–34917, 2003.
142. Harir N, Pecquet C, Kerenyi M, Sonneck K, Kovacic B, Nyga R, Brevet M, Dhennin I, Gouilleux–Gruart V, Beug H, Valent P, Lassoued K, Moriggl R, and Gouilleux F. Constitutive activation of Stat5 promotes its cytoplasmic localization and association with PI3-kinase in myeloid leukemias. *Blood* 109: 1678–1686, 2007.
143. Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, Karin M, and Kikugawa K. Evidence that reactive oxygen species do not mediate NF-kappaB activation. *EMBO J* 22: 3356–3366, 2003.
144. Hayden MS and Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 132: 344–362, 2008.
145. Hegedus T, Orfi L, Seprodi A, Varadi A, Sarkadi B, and Keri G. Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1. *Biochim Biophys Acta* 1587: 318–325, 2002.
146. Henderson ST and Johnson TE. daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. *Curr Biol* 11: 1975–1980, 2001.
147. Heo J and Campbell SL. Mechanism of p21Ras S-nitrosylation and kinetics of nitric oxide-mediated guanine nucleotide exchange. *Biochemistry* 43: 2314–2322, 2004.
148. Heo J and Campbell SL. Mechanism of redox-mediated guanine nucleotide exchange on redox-active Rho GTPases. *J Biol Chem* 280: 31003–31010, 2005.
149. Heo J and Campbell SL. Superoxide anion radical modulates the activity of Ras and Ras-related GTPases by a radical-based mechanism similar to that of nitric oxide. *J Biol Chem* 280: 12438–12445, 2005.
150. Heo J, Prutzman KC, Mocanu V, and Campbell SL. Mechanism of free radical nitric oxide-mediated Ras guanine nucleotide dissociation. *J Mol Biol* 346: 1423–1440, 2005.
151. Herbst R, Carroll PM, Allard JD, Schilling J, Raabe T, and Simon MA. Daughter of sevenless is a substrate of the phosphotyrosine phosphatase Corkscrew and functions during sevenless signaling. *Cell* 85: 899–909, 1996.
152. Hermeking H and Benzinger A. 14-3-3 proteins in cell cycle regulation. *Semin Cancer Biol* 16: 183–192, 2006.
153. Hernebring M, Brolen G, Aguilaniu H, Semb H, and Nystrom T. Elimination of damaged proteins during differentiation of embryonic stem cells. *Proc Natl Acad Sci USA* 103: 7700–7705, 2006.
154. Hickey FB and Cotter TG. Identification of transcriptional targets associated with the expression of p210 BCR–ABL. *Eur J Haematol* 76: 369–383, 2006.
155. Higgins KA, Perez JR, Coleman TA, Dorshkind K, McComas WA, Sarmiento UM, Rosen CA, and Narayanan R. Antisense inhibition of the p65 subunit of NF-kappa B blocks tumorigenicity and causes tumor regression. *Proc Natl Acad Sci USA* 90: 9901–9905, 1993.
156. Hillion J, Le Coniat M, Jonveaux P, Berger R, and Bernard OA. AF6q21, a novel partner of the MLL gene in t(6;11)(q21;q23), defines a forkhead transcriptional factor subfamily. *Blood* 90: 3714–3719, 1997.
157. Hochhaus A, Kreil S, Corbin AS, La RP, Muller MC, Lahaye T, Hanfstein B, Schoch C, Cross NC, Berger U, Gschaidmeier H, Druker BJ, and Hehlmann R. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 16: 2190–2196, 2002.
158. Hoffman A, Spetner LM, and Burke M. Cessation of cell proliferation by adjustment of cell redox potential. *J Theoret Biol* 211: 403–407, 2001.
159. Holgado–Madruga M, Emlet DR, Moscatello DK, Godwin AK, and Wong AJ. A Grb2-associated docking protein in EGF- and insulin-receptor signalling. *Nature* 379: 560–564., 1996.
160. Holyoake TL, Jiang X, Jorgensen HG, Graham S, Alcorn MJ, Laird C, Eaves AC, and Eaves CJ. Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3. *Blood* 97: 720–728, 2001.
161. Hoser G, Majsterek I, Romana DL, Slupianek A, Blasiak J, and Skorski T. Fusion oncogenic tyrosine kinases alter DNA damage and repair after genotoxic treatment: role in drug resistance? *Leuk Res* 27: 267–273, 2003.
162. Houghton PJ, Germain GS, Harwood FC, Schuetz JD, Stewart CF, Buchdunger E, and Traxler P. Imatinib mesylate is a potent inhibitor of the ABCG2 (BCRP) transporter and reverses resistance to topotecan and SN-38 *in vitro*. *Cancer Res* 64: 2333–2337, 2004.
163. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, and Li S. Requirement of Src kinases Lyn, Hck and Fgr for BCR–ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet* 36: 453–461, 2004.
164. Huang LE, Gu J, Schau M, and Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci USA* 95: 7987–7992, 1998.
165. Huang P, Feng L, Oldham EA, Keating MJ, and Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* 407: 390–395, 2000.
166. Huang RP, Wu JX, Fan Y, and Adamson ED. UV activates growth factor receptors via reactive oxygen intermediates. *J Cell Biol* 133: 211–220, 1996.
167. Iida K, Itoh K, Kumagai Y, Oyasu R, Hattori K, Kawai K, Shimazui T, Akaza H, and Yamamoto M. Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. *Cancer Res* 64: 6424–6431, 2004.
168. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
169. Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K, Yang H, Sorokina I, Conaway RC, Conaway JW, and Kaelin WG, Jr. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA* 99: 13459–13464, 2002.
170. Jackson PK, Paskind M, and Baltimore D. Mutation of a phenylalanine conserved in SH3-containing tyrosine kinases activates the transforming ability of c-Abl. *Oncogene* 8: 1943–1956, 1993.
171. Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic Biol Med* 36: 1199–1207, 2004.
172. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur–Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, and Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 434: 1144–1148, 2005.

173. Jaspers I, Zhang W, Fraser A, Samet JM, and Reed W. Hydrogen peroxide has opposing effects on IKK activity and IkappaBalpha breakdown in airway epithelial cells. *Am J Resp Cell Mol Biol* 24: 769–777, 2001.
174. Jena N, Deng M, Sicinska E, Sicinski P, and Daley GQ. Critical role for cyclin D2 in BCR/ABL-induced proliferation of hematopoietic cells. *Cancer Res* 62: 535–541, 2002.
175. Jeong W, Park SJ, Chang TS, Lee DY, and Rhee SG. Molecular mechanism of the reduction of cysteine sulfinic acid of peroxiredoxin to cysteine by mammalian sulfiredoxin. *J Biol Chem* 281: 14400–14407, 2006.
176. Jiang BH, Semenza GL, Bauer C, and Marti HH. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. *Am J Physiol* 271: C1172–1180, 1996.
177. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, Score J, Seear R, Chase AJ, Grand FH, White H, Zoi C, Loukopoulos D, Terpos E, Vervessou EC, Schultheis B, Emig M, Ernst T, Lengfelder E, Hehlmann R, Hochhaus A, Oscier D, Silver RT, Reiter A, and Cross NC. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood* 106: 2162–2168, 2005.
178. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 8: 1865–1879, 2006.
179. Jonsson M, Engstrom M, and Jonsson JI. FLT3 ligand regulates apoptosis through AKT-dependent inactivation of transcription factor FoxO3. *Biochem Biophys Res Commun* 318: 899–903, 2004.
180. Kallio PJ, Wilson WJ, O'Brien S, Makino Y, and Poellinger L. Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. *J Biol Chem* 274: 6519–6525, 1999.
181. Kawanishi S, Hiraku Y, Pinlaor S, and Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biological chemistry* 387: 365–372, 2006.
182. Kelliher MA, McLaughlin J, Witte ON, and Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci USA* 87: 6649–6653, 1990.
183. Kharas MG and Fruman DA. ABL oncogenes and phosphoinositide 3-kinase: mechanism of activation and downstream effectors. *Cancer Res* 65: 2047–2053, 2005.
184. Kieran MW, Perkins AC, Orkin SH, and Zon LI. Thrombopoietin rescues in vitro erythroid colony formation from mouse embryos lacking the erythropoietin receptor. *Proc Natl Acad Sci USA* 93: 9126–9131, 1996.
185. Kim JH, Chu SC, Gramlich JL, Pride YB, Babendreier E, Chauhan D, Salgia R, Podar K, Griffin JD, and Sattler M. Activation of the PI3K/mTOR pathway by BCR–ABL contributes to increased production of reactive oxygen species. *Blood* 105: 1717–1723, 2005.
186. Kim KY, Rhim T, Choi I, and Kim SS. N-acetylcysteine induces cell cycle arrest in hepatic stellate cells through its reducing activity. *J Biol Chem* 276: 40591–40598, 2001.
187. Kirchner D, Duyster J, Ottmann O, Schmid RM, Bergmann L, and Munzert G. Mechanisms of BCR–ABL-mediated NF-kappaB/Rel activation. *Exp Hematol* 31: 504–511, 2003.
188. Klejman A, Rushen L, Morrione A, Slupianek A, and Skorski T. Phosphatidylinositol-3 kinase inhibitors enhance the anti-leukemia effect of STI571. *Oncogene* 21: 5868–5876, 2002.
189. Klucher KM, Lopez DV, and Daley GQ. Secondary mutation maintains the transformed state in BaF3 cells with inducible BCR/ABL expression. *Blood* 91: 3927–3934, 1998.
190. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, and Yamamoto M. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* 24: 7130–7139, 2004.
191. Kobayashi M and Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* 7: 385–394, 2005.
192. Komatsu N, Watanabe T, Uchida M, Mori M, Kirito K, Kikuchi S, Liu Q, Tauchi T, Miyazawa K, Endo H, Nagai T, and Ozawa K. A member of Forkhead transcription factor FKHRL1 is a downstream effector of STI571-induced cell cycle arrest in BCR–ABL-expressing cells. *J Biol Chem* 278: 6411–6419, 2003.
193. Konopka JB, Watanabe SM, and Witte ON. An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. *Cell* 37: 1035–1042, 1984.
194. Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, and Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419: 316–321, 2002.
195. Kops GJ, de Ruiter ND, De Vries–Smits AM, Powell DR, Bos JL, and Burgering BM. Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398: 630–634, 1999.
196. Koptyra M, Falinski R, Nowicki MO, Stoklosa T, Majsterek I, Nieborowska-Skorska M, Blasiak J, and Skorski T. BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood* 108: 319–327, 2006.
197. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, and Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 352: 1779–1790, 2005.
198. Krejsa CM, Nadler SG, Esselstyn JM, Kavanagh TJ, Ledbetter JA, and Schieven GL. Role of oxidative stress in the action of vanadium phosphotyrosine phosphatase inhibitors. Redox independent activation of NF-kappaB. *J Biol Chem* 272: 11541–11549, 1997.
199. Kulas DT, Zhang WR, Goldstein BJ, Furlanetto RW, and Mooney RA. Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR. *J Biol Chem* 270: 2435–2438, 1995.
200. Kurtoglu M, Maher JC, and Lampidis TJ. Differential toxic mechanisms of 2-deoxy-D-glucose versus 2-fluorodeoxy-D-glucose in hypoxic and normoxic tumor cells. *Antioxid Redox Signal* 9: 1383–1390, 2007.
201. Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, and Kensler TW. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J Biol Chem* 278: 8135–8145, 2003.
202. Lacronique V, Boureux A, Valle VD, Poirel H, Quang CT, Mauchauffe M, Berthou C, Lessard M, Berger R, Ghysdael J, and Bernard OA. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* 278: 1309–1312, 1997.
203. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
204. Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med* 43: 332–347, 2007.

205. Lambeth JD, Kawahara T, and Diebold B. Regulation of Nox and Duox enzymatic activity and expression. *Free Radic Biol Med* 43: 319–331, 2007.
206. LaMontagne KR, Flint AJ, Franza BR, Pendergast AM, and Tonks NK. Protein tyrosine phosphatase 1B antagonizes signalling by oncoprotein tyrosine kinase p210 BCR–ABL *in vivo*. *Mol Cell Biol* 18: 2965–2975, 1998.
207. Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, and Quilliam LA. A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. *J Biol Chem* 272: 4323–4326, 1997.
208. Lander HM, Ogiste JS, Pearce SF, Levi R, and Novogrodsky A. Nitric oxide-stimulated guanine nucleotide exchange on p21ras. *J Biol Chem* 270: 7017–7020, 1995.
209. Laurent A, Nicco C, Chereau C, Goulvestre C, Alexandre J, Alves A, Levy E, Goldwasser F, Panis Y, Soubrane O, Weill B, and Batteux F. Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res* 65: 948–956, 2005.
210. Lawlor MA and Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci* 114: 2903–2910, 2001.
211. le Coutre P, Tassi E, Varella–Garcia M, Barni R, Mologni L, Cabrita G, Marchesi E, Supino R, and Gambacorti–Passerini C. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood* 95: 1758–1766, 2000.
212. Lee SR, Kwon KS, Kim SR, and Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J Biol Chem* 273: 15366–15372, 1998.
213. Lee SR, Yang KS, Kwon J, Lee C, Jeong W, and Rhee SG. Reversible inactivation of the tumor suppressor PTEN by H2O2. *J Biol Chem* 277: 20336–20342, 2002.
214. Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, and Downes CP. Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *EMBO J* 22: 5501–5510, 2003.
215. Levine RL. Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 32: 790–796, 2002.
216. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggon TJ, Wlodarska I, Clark JJ, Moore S, Adelsperger J, Koo S, Lee JC, Gabriel S, Mercher T, D'Andrea A, Frohling S, Dohner K, Marynen P, Vandenberghe P, Mesa RA, Tefferi A, Griffin JD, Eck MJ, Sellers WR, Meyerson M, Golub TR, Lee SJ, and Gilliland DG. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 7: 387–397, 2005.
217. Li JM and Shah AM. Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. *J Biol Chem* 277: 19952–19960, 2002.
218. Li Q, Harraz MM, Zhou W, Zhang LN, Ding W, Zhang Y, Eggleston T, Yeaman C, Banfi B, and Engelhardt JF. Nox2 and Rac1 regulate H2O2-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes. *Mol Cell Biol* 26: 140–154, 2006.
219. Lim SD, Sun C, Lambeth JD, Marshall F, Amin M, Chung L, Petros JA, and Arnold RS. Increased Nox1 and hydrogen peroxide in prostate cancer. *Prostate* 62: 200–207, 2005.
220. Lin CS, Lim SK, D'Agati V, and Costantini F. Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. *Genes Dev* 10: 154–164, 1996.
221. Lionberger JM, Wilson MB, and Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by BCR–ABL is suppressed by kinase-defective Hck. *J Biol Chem* 275: 18581–18585, 2000.
222. Liu LZ, Hu XW, Xia C, He J, Zhou Q, Shi X, Fang J, and Jiang BH. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells. *Free Radic Biol Med* 41: 1521–1533, 2006.
223. Liu Y, Jenkins B, Shin JL, and Rohrschneider LR. Scaffolding protein Gab2 mediates differentiation signaling downstream of Fms receptor tyrosine kinase. *Mol Cell Biol* 21: 3047–3056., 2001.
224. Lluis JM, Buricchi F, Chiarugi P, Morales A, and Fernandez–Checa JC. Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-{kappa}B via c-SRC and oxidant-dependent cell death. *Cancer Res* 67: 7368–7377, 2007.
225. Lugo TG, Pendergast AM, Muller AJ, and Witte ON. Tyrosine kinase activity and transformation potency of BCR–ABL oncogene products. *Science* 247: 1079–1082, 1990.
226. Luo H, Rose P, Barber D, Hanratty WP, Lee S, Roberts TM, D'Andrea AD, and Dearolf CR. Mutation in the Jak kinase JH2 domain hyperactivates Drosophila and mammalian Jak-Stat pathways. *Mol Cell Biol* 17: 1562–1571, 1997.
227. Lynch DK and Daly RJ. PKB-mediated negative feedback tightly regulates mitogenic signalling via Gab2. *EMBO J* 21: 72–82., 2002.
228. Maehama T, Taylor GS, and Dixon JE. PTEN and myotubularin: novel phosphoinositide phosphatases. *Annu Rev Biochem* 70: 247–279, 2001.
229. Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau–Gaudry F, Reiffers J, Goldman JM, and Melo JV. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* 101: 2368–2373, 2003.
230. Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Goldman JM, and Melo JV. Selection and characterization of BCR–ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood* 96: 1070–1079, 2000.
231. Majsterek I, Slupianek A, Hoser G, Skorski T, and Blasiak J. ABL-fusion oncoproteins activate multi-pathway of DNA repair: role in drug resistance? *Biochimie* 86: 53–65, 2004.
232. Mancini M, Brusa G, Zuffa E, Corrado P, Martinelli G, Grafone T, Barbieri E, and Santucci MA. Persistent Cdk2 inactivation drives growth arrest of BCR–ABL-expressing cells in response to dual inhibitor of SRC and ABL kinases SKI606. *Leuk Res* 31: 979–987, 2007.
233. Marini MG, Chan K, Casula L, Kan YW, Cao A, and Moi P. hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. *J Biol Chem* 272: 16490–16497, 1997.
234. Marinkovic D, Zhang X, Yalcin S, Luciano JP, Brugnara C, Huber T, and Ghaffari S. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin Invest* 117: 2133–2144, 2007.
235. Martin F, van Deursen JM, Shivdasani RA, Jackson CW, Troutman AG, and Ney PA. Erythroid maturation and globin gene expression in mice with combined deficiency of NF-E2 and nrf-2. *Blood* 91: 3459–3466, 1998.
236. Martirosyan A, Leonard S, Shi X, Griffith B, Gannett P, and Strobl J. Actions of a histone deacetylase inhibitor NSC3852 (5-nitroso-8-quinolinol) link reactive oxygen species to cell

differentiation and apoptosis in MCF-7 human mammary tumor cells. *J Pharmacol Exper Therap* 317: 546–552, 2006.
237. Matsushime H, Roussel MF, Ashmun RA, and Sherr CJ. Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell* 65: 701–713, 1991.
238. Mayerhofer M, Florian S, Krauth MT, Aichberger KJ, Bilban M, Marculescu R, Printz D, Fritsch G, Wagner O, Selzer E, Sperr WR, Valent P, and Sillaber C. Identification of heme oxygenase-1 as a novel BCR/ABL-dependent survival factor in chronic myeloid leukemia. *Cancer Res* 64: 3148–3154, 2004.
239. Mayerhofer M, Valent P, Sperr WR, Griffin JD, and Sillaber C. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood* 100: 3767–3775, 2002.
240. Mazzacurati L, Pattacini L, Brusa G, Mancini M, Benvenuti M, Barbieri E, Martinelli G, Baccarani M, Greenberger JS, and Santucci MA. Chk2 drives late G1/early S phase arrest of clonal myeloid progenitors expressing the p210 BCR–ABL tyrosine kinase in response to STI571. *Hematol J* 5: 168–177, 2004.
241. McDonald RE, Hughes DJ, and Davis BG. Modular control of lectin function: redox-switchable agglutination. *Angewand Chem (Int ed* 43: 3025–3029, 2004.
242. McLaughlin J, Chianese E, and Witte ON. *In vitro* transformation of immature hematopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. *Proc Natl Acad Sci USA* 84: 6558–6562., 1987.
243. Medema RH, Kops GJ, Bos JL, and Burgering BM. AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. *Nature* 404: 782–787, 2000.
244. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9: 387–399, 2002.
245. Menon SG and Goswami PC. A redox cycle within the cell cycle: ring in the old with the new. *Oncogene* 26: 1101–1109, 2007.
246. Menon SG, Sarsour EH, Spitz DR, Higashikubo R, Sturm M, Zhang H, and Goswami PC. Redox regulation of the G1 to S phase transition in the mouse embryo fibroblast cell cycle. *Cancer Res* 63: 2109–2117, 2003.
247. Mi L, Wang X, Govind S, Hood BL, Veenstra TD, Conrads TP, Saha DT, Goldman R, and Chung FL. The role of protein binding in induction of apoptosis by phenethyl isothiocyanate and sulforaphane in human non-small lung cancer cells. *Cancer Res* 67: 6409–6416, 2007.
248. Mihaylova VT, Borland CZ, Manjarrez L, Stern MJ, and Sun H. The PTEN tumor suppressor homolog in *Caenorhabditis elegans* regulates longevity and dauer formation in an insulin receptor-like signaling pathway. *Proc Natl Acad Sci USA* 96: 7427–7432, 1999.
249. Million RP and Van Etten RA. The Grb2 binding site is required for the induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. *Blood* 96: 664–670., 2000.
250. Miyakawa Y, Rojnuckarin P, Habib T, and Kaushansky K. Thrombopoietin induces phosphoinositol 3-kinase activation through SHP2, Gab, and insulin receptor substrate proteins in BAF3 cells and primary murine megakaryocytes. *J Biol Chem* 276: 2494–2502., 2001.
251. Mohi MG, Boulton C, Gu TL, Sternberg DW, Neuberg D, Griffin JD, Gilliland DG, and Neel BG. Combination of rapamycin and protein tyrosine kinase (PTK) inhibitors for the treatment of leukemias caused by oncogenic PTKs. *Proc Natl Acad Sci USA* 101: 3130–3135, 2004.
252. Moll J, Sansig G, Fattori E, and van der Putten H. The murine rac1 gene: cDNA cloning, tissue distribution and regulated expression of rac1 mRNA by disassembly of actin microfilaments. *Oncogene* 6: 863–866, 1991.
253. Momose H, Kurosu H, Tsujimoto N, Kontani K, Tsujita K, Nishina H, and Katada T. Dual phosphorylation of phosphoinositide 3-kinase adaptor Grb2-associated binder 2 is responsible for superoxide formation synergistically stimulated by Fc gamma and formyl-methionyl-leucyl-phenylalanine receptors in differentiated THP-1 cells. *J Immunol* 171: 4227–4234, 2003.
254. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Remuzzi A, Zoja C, and Remuzzi G. Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 101: 1905–1915, 1998.
255. Morotti A, Cilloni D, Pautasso M, Messa F, Arruga F, Defilippi I, Carturan S, Catalano R, Rosso V, Chiarenza A, Taulli R, Bracco E, Rege–Cambrin G, Gottardi E, and Saglio G. NF-kB inhibition as a strategy to enhance etoposide-induced apoptosis in K562 cell line. *Am J Hematol* 81: 938–945, 2006.
256. Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, and Guarente L. Mammalian SIRT1 represses forkhead transcription factors. *Cell* 116: 551–563, 2004.
257. Nagata A, Igarashi M, Jinno S, Suto K, and Okayama H. An additional homolog of the fission yeast cdc25+ gene occurs in humans and is highly expressed in some cancer cells. *New Biol* 3: 959–968, 1991.
258. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, and Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* 93: 397–409, 1998.
259. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, and Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 424: 561–565, 2003.
260. Nimmanapalli R, O'Bryan E, and Bhalla K. Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers BCR–ABL levels and induces apoptosis and differentiation of BCR–ABL-positive human leukemic blasts. *Cancer Res* 61: 1799–1804, 2001.
261. Nishida K, Yoshida Y, Itoh M, Fukada T, Ohtani T, Shirogane T, Atsumi T, Takahashi–Tezuka M, Ishihara K, Hibi M, and Hirano T. Gab-family adapter proteins act downstream of cytokine and growth factor receptors and T- and B-cell antigen receptors. *Blood* 93: 1809–1816., 1999.
262. Niu TQ, Liang XS, Yang J, Zhao ZH, and Zhou GW. Kinetic comparison of the catalytic domains of SHP-1 and SHP-2. *J Cell Biochem* 72: 145–150, 1999.
263. Nollen EA and Morimoto RI. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* 115: 2809–2816, 2002.
264. Nose K, Shibanuma M, Kikuchi K, Kageyama H, Sakiyama S, and Kuroki T. Transcriptional activation of early-response genes by hydrogen peroxide in a mouse osteoblastic cell line. *Eur J Biochem* 201: 99–106, 1991.
265. Nowicki MO, Falinski R, Koptyra M, Slupianek A, Stoklosa T, Gloc E, Nieborowska–Skorska M, Blasiak J, and Skorski T. BCR/ABL oncogenic kinase promotes unfaithful repair

of the reactive oxygen species-dependent DNA double-strand breaks. *Blood* 104: 3746–3753, 2004.
266. Nystrom T. Role of oxidative carbonylation in protein quality control and senescence. *EMBO J* 24: 1311–1317, 2005.
267. Oh SW, Mukhopadhyay A, Svrzikapa N, Jiang F, Davis RJ, and Tissenbaum HA. JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc Natl Acad Sci USA* 102: 4494–4499, 2005.
268. Ohba M, Shibanuma M, Kuroki T, and Nose K. Production of hydrogen peroxide by transforming growth factor-beta 1 and its involvement in induction of egr-1 in mouse osteoblastic cells. *J Cell Biol* 126: 1079–1088, 1994.
269. Okuda K, Weisberg E, Gilliland DG, and Griffin JD. ARG tyrosine kinase activity is inhibited by STI571. *Blood* 97: 2440–2448, 2001.
270. Onishi M, Nosaka T, Misawa K, Mui AL, Gorman D, McMahon M, Miyajima A, and Kitamura T. Identification and characterization of a constitutively active STAT5 mutant that promotes cell proliferation. *Mol Cell Biol* 18: 3871–3879, 1998.
271. Oyake T, Itoh K, Motohashi H, Hayashi N, Hoshino H, Nishizawa M, Yamamoto M, and Igarashi K. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol* 16: 6083–6095, 1996.
272. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E, Buteau J, Brown K, Perkins SL, Bhagat G, Agarwal AM, Basso G, Castillo M, Nagase S, Cordon–Cardo C, Parsons R, Zuniga–Pflucker JC, Dominguez M, and Ferrando AA. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat Med* 13: 1203–1210, 2007.
273. Parada Y, Banerji L, Glassford J, Lea NC, Collado M, Rivas C, Lewis JL, Gordon MY, Thomas NS, and Lam EW. BCR–ABL and interleukin 3 promote haematopoietic cell proliferation and survival through modulation of cyclin D2 and p27Kip1 expression. *J Biol Chem* 276: 23572–23580, 2001.
274. Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G, and Ihle JN. Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 93: 385–395, 1998.
275. Park HS, Lee SH, Park D, Lee JS, Ryu SH, Lee WJ, Rhee SG, and Bae YS. Sequential activation of phosphatidylinositol 3-kinase, beta Pix, Rac1, and Nox1 in growth factor-induced production of H2O2. *Mol Cell Biol* 24: 4384–4394, 2004.
276. Parry P, Wei Y, and Evans G. Cloning and characterization of the t(X;11) breakpoint from a leukemic cell line identify a new member of the forkhead gene family. *Genes Chromosomes Cancer* 11: 79–84, 1994.
277. Patiar S and Harris AL. Role of hypoxia-inducible factor-1alpha as a cancer therapy target. *Endocr Relat Cancer* 13 Suppl 1: S61–75, 2006.
278. Pedersen B. Functional and biochemical phenotype in relation to cellular age of differentiated neutrophils in chronic myeloid leukaemia. [Review]. *Br J Haematol* 51: 339–344, 1982.
279. Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, Monpoux F, Van Rompaey L, Baens M, Van den Berghe H, and Marynen P. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. *Blood* 90: 2535–2540, 1997.
280. Pendergast AM, Muller AJ, Havlik MH, Clark R, McCormick F, and Witte ON. Evidence for regulation of the human ABL tyrosine kinase by a cellular inhibitor. *Proc Natl Acad Sci USA* 88: 5927–5931, 1991.
281. Pendergast AM, Quilliam LA, Cripe LD, Bassing CH, Dai Z, Li N, Batzer A, Rabun KM, Der CJ, and Schlessinger J. BCR–ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. *Cell* 75: 175–185, 1993.
282. Peng T, Golub TR, and Sabatini DM. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* 22: 5575–5584, 2002.
283. Pickering BM, de Mel S, Lee M, Howell M, Habens F, Dallman CL, Neville LA, Potter KN, Mann J, Mann DA, Johnson PW, Stevenson FK, and Packham G. Pharmacological inhibitors of NF-kappaB accelerate apoptosis in chronic lymphocytic leukaemia cells. *Oncogene* 26: 1166–1177, 2007.
284. Pieper GM and Riaz ul H. Activation of nuclear factor-kappaB in cultured endothelial cells by increased glucose concentration: prevention by calphostin C. *J Cardiovasc Pharmacol* 30: 528–532, 1997.
285. Polyak K, Kato JY, Solomon MJ, Sherr CJ, Massague J, Roberts JM, and Koff A. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 8: 9–22, 1994.
286. Pouyssegur J and Mechta–Grigoriou F. Redox regulation of the hypoxia-inducible factor. *Biol Chem* 387: 1337–1346, 2006.
287. Pratt JC, Igras VE, Maeda H, Baksh S, Gelfand EW, Burakoff SJ, Neel BG, and Gu H. Cutting edge: gab2 mediates an inhibitory phosphatidylinositol 3′- kinase pathway in T cell antigen receptor signaling. *J Immunol* 165: 4158–4163., 2000.
288. Puil L, Liu J, Gish G, Mbamalu G, Bowtell D, Pelicci PG, Arlinghaus R, and Pawson T. BCR–ABL oncoproteins bind directly to activators of the Ras signalling pathway. *EMBO J* 13: 764–773, 1994.
289. Quinn MT and Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J Leuk Biol* 76: 760–781, 2004.
290. Raabe T, Riesgo–Escovar J, Liu X, Bausenwein BS, Deak P, Maroy P, and Hafen E. DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in Drosophila. *Cell* 85: 911–920., 1996.
291. Radujkovic A, Schad M, Topaly J, Veldwijk MR, Laufs S, Schultheis BS, Jauch A, Melo JV, Fruehauf S, and Zeller WJ. Synergistic activity of imatinib and 17-AAG in imatinib-resistant CML cells overexpressing BCR–ABL–Inhibition of P-glycoprotein function by 17-AAG. *Leukemia* 19: 1198–1206, 2005.
292. Rambaldi A, Terao M, Bettoni S, Bassan R, Battista R, Barbui T, and Garattini E. Differences in the expression of alkaline phosphatase mRNA in chronic myelogenous leukemia and paroxysmal nocturnal hemoglobinuria polymorphonuclear leukocytes. *Blood* 73: 1113–1115, 1989.
293. Ramos–Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, and Kensler TW. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci USA* 98: 3410–3415, 2001.

294. Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, and Melillo G. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res* 62: 4316–4324, 2002.
295. Reiter A, Walz C, Watmore A, Schoch C, Blau I, Schlegelberger B, Berger U, Telford N, Aruliah S, Yin JA, Vanstraelen D, Barker HF, Taylor PC, O'Driscoll A, Benedetti F, Rudolph C, Kolb HJ, Hochhaus A, Hehlmann R, Chase A, and Cross NC. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. *Cancer Res* 65: 2662–2667, 2005.
296. Rena G, Guo S, Cichy SC, Unterman TG, and Cohen P. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J Biol Chem* 274: 17179–17183, 1999.
297. Reuther JY, Reuther GW, Cortez D, Pendergast AM, and Baldwin AS, Jr. A requirement for NF-kappaB activation in BCR–ABL-mediated transformation. *Genes Dev* 12: 968–981, 1998.
298. Reynolds RA, Yem AW, Wolfe CL, Deibel MR, Jr., Chidester CG, and Watenpaugh KD. Crystal structure of the catalytic subunit of Cdc25B required for G2/M phase transition of the cell cycle. *J Mol Biol* 293: 559-568, 1999.
299. Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med* 31: 53–59, 1999.
300. Rhee SG, Kang SW, Jeong W, Chang TS, Yang KS, and Woo HA. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* 17: 183–189, 2005.
301. Rolfs A, Kvietikova I, Gassmann M, and Wenger RH. Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. *J Biol Chem* 272: 20055–20062, 1997.
302. Rottapel R, Turck CW, Casteran N, Liu X, Birnbaum D, Pawson T, and Dubreuil P. Substrate specificities and identification of a putative binding site for PI3K in the carboxy tail of the murine Flt3 receptor tyrosine kinase. *Oncogene* 9: 1755–1765, 1994.
303. Roy L, Guilhot J, Krahnke T, Guerci–Bresler A, Druker BJ, Larson RA, O'Brien S G, So C, Massimini G, and Guilhot F. Survival advantage from Imatinib compared to the combination Interferon-{alpha} plus Cytarabine in chronic phase CML: historical comparison between two phase III trials. *Blood* 108: 1478–1484, 2006.
304. Sadhu K, Reed SI, Richardson H, and Russell P. Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc Natl Acad Sci USA* 87: 5139–5143, 1990.
305. Saglio G, Guerrasio A, Rosso C, Zaccaria A, Tassinari A, Serra A, Rege–Cambrin G, Mazza U, and Gavosto F. New type of Bcr/Abl junction in Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood* 76: 1819–1824, 1990.
306. Saharinen P, Vihinen M, and Silvennoinen O. Autoinhibition of Jak2 tyrosine kinase is dependent on specific regions in its pseudokinase domain. *Mol Biol Cell* 14: 1448–1459, 2003.
307. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
308. Sala A and Calabretta B. Regulation of BALB/c 3T3 fibroblast proliferation by B-myb is accompanied by selective activation of cdc2 and cyclin D1 expression. *Proc Natl Acad Sci USA* 89: 10415–10419, 1992.
309. Sallmyr A, Fan J, Datta K, Kim KT, Grosu D, Shapiro P, Small D, and Rassool F. Internal tandem duplication of FLT3 (FLT3/ITD) induces increased ROS production, DNA damage and misrepair: implications for poor prognosis in AML. *Blood* 111: 3173–3182, 2008.
310. Salloukh HF and Laneuville P. Increase in mutant frequencies in mice expressing the BCR–ABL activated tyrosine kinase. *Leukemia* 14: 1401–1404, 2000.
311. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, and Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* 423: 769–773, 2003.
312. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid Redox Signal* 7: 560–577, 2005.
313. Samlowski WE, Petersen R, Cuzzocrea S, Macarthur H, Burton D, McGregor JR, and Salvemini D. A nonpeptidyl mimic of superoxide dismutase, M40403, inhibits dose-limiting hypotension associated with interleukin-2 and increases its antitumor effects. *Nat Med* 9: 750–755, 2003.
314. Sanjuan–Pla A, Cervera AM, Apostolova N, Garcia–Bou R, Victor VM, Murphy MP, and McCreath KJ. A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1alpha. *FEBS Lett* 579: 2669–2674, 2005.
315. Sattler M and Griffin JD. Molecular mechanisms of transformation by the BCR–ABL oncogene. *Semin Hematol* 40: 4–10, 2003.
316. Sattler M, Mohi MG, Pride YB, Quinnan LR, Malouf NA, Podar K, Gesbert F, Iwasaki H, Li S, Van Etten RA, Gu H, Griffin JD, and Neel BG. Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell* 1: 479–492, 2002.
317. Sattler M, Quinnan LR, Pride YB, Gramlich JL, Chu SC, Even GC, Kraeft SK, Chen LB, and Salgia R. 2-methoxyestradiol alters cell motility, migration, and adhesion. *Blood* 102: 289–296, 2003.
318. Sattler M and Salgia R. Activation of hematopoietic growth factor signal transduction pathways by the human oncogene BCR/ABL. *Cytokine Growth Factor Rev* 8: 63–79, 1997.
319. Sattler M, Verma S, Shrikhande G, Byrne CH, Pride YB, Winkler T, Greenfield EA, Salgia R, and Griffin JD. The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells. *J Biol Chem* 275: 24273–24278, 2000.
320. Sattler M, Winkler T, Verma S, Byrne CH, Shrikhande G, Salgia R, and Griffin JD. Hematopoietic growth factors signal through the formation of reactive oxygen species. *Blood* 93: 2928–2935, 1999.
321. Savitsky PA and Finkel T. Redox regulation of Cdc25C. *J Biol Chem* 277: 20535–20540, 2002.
322. Scheijen B, Ngo HT, Kang H, and Griffin JD. FLT3 receptors with internal tandem duplications promote cell viability and proliferation by signaling through Foxo proteins. *Oncogene* 23: 3338–3349, 2004.
323. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, and Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 289: 1938–1942, 2000.
324. Schneider C, Sepp–Lorenzino L, Nimmesgern E, Ouerfelli O, Danishefsky S, Rosen N, and Hartl FU. Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90. *Proc Natl Acad Sci USA* 93: 14536–14541, 1996.

325. Schoonbroodt S and Piette J. Oxidative stress interference with the nuclear factor-kappa B activation pathways. *Biochem Pharmacol* 60: 1075–1083, 2000.
326. Schreck R, Meier B, Mannel DN, Droge W, and Baeuerle PA. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 175: 1181–1194, 1992.
327. Schreck R, Rieber P, and Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J* 10: 2247–2258, 1991.
328. Scita G, Tenca P, Frittoli E, Tocchetti A, Innocenti M, Giardina G, and Di Fiore PP. Signaling from Ras to Rac and beyond: not just a matter of GEFs. *EMBO J* 19: 2393–2398, 2000.
329. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, and Johnson RS. Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. *Mol Cell Biol* 21: 3436–3444, 2001.
330. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, Kusari J, and Olefsky JM. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. *Diabetes* 45: 1379–1385, 1996.
331. Sekharam M, Trotti A, Cunnick JM, and Wu J. Suppression of fibroblast cell cycle progression in G1 phase by N-acetylcysteine. *Toxicol Appl Pharmacol* 149: 210–216, 1998.
332. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, and Sawyers CL. Multiple BCR–ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2: 117–125, 2002.
333. Shah NP, Tran C, Lee FY, Chen P, Norris D, and Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 305: 399–401, 2004.
334. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol* 24: 1770–1783, 2006.
335. Shigematsu H, Iwasaki H, Otsuka T, Ohno Y, Arima F, and Niho Y. Role of the vav proto-oncogene product (Vav) in erythropoietin-mediated cell proliferation and phosphatidylinositol 3-kinase activity. *J Biol Chem* 272: 14334–14340, 1997.
336. Shirsat NV, Pignolo RJ, Kreider BL, and Rovera G. A member of the ras gene superfamily is expressed specifically in T, B and myeloid hemopoietic cells. *Oncogene* 5: 769–772, 1990.
337. Shrivastava A and Aggarwal BB. Antioxidants differentially regulate activation of nuclear factor-kappa B, activator protein-1, c-jun amino-terminal kinases, and apoptosis induced by tumor necrosis factor: evidence that JNK and NF-kappa B activation are not linked to apoptosis. *Antioxid Redox Signal* 1: 181–191, 1999.
338. Sillaber C, Gesbert F, Frank DA, Sattler M, and Griffin JD. STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells. *Blood* 95: 2118–2125, 2000.
339. Sitia R and Molteni SN. Stress, protein (mis)folding, and signaling: the redox connection. *Sci STKE* 2004: pe27, 2004.
340. Skorski T, Bellacosa A, Nieborowska–Skorska M, Majewski M, Martinez R, Choi JK, Trotta R, Wlodarski P, Perrotti D, Chan TO, Wasik MA, Tsichlis PN, and Calabretta B. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. *EMBO J* 16: 6151–6161, 1997.
341. Skorski T, Kanakaraj P, Nieborowska–Skorska M, Ratajczak MZ, Wen SC, Zon G, Gewirtz AM, Perussia B, and Calabretta B. Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome-positive cells. *Blood* 86: 726–736, 1995.
342. Skorski T, Wlodarski P, Daheron L, Salomoni P, Nieborowska–Skorska M, Majewski M, Wasik M, and Calabretta B. BCR/ABL-mediated leukemogenesis requires the activity of the small GTP-binding protein Rac. *Proc Natl Acad Sci USA* 95: 11858–11862, 1998.
343. Socolovsky M, Fallon AE, Wang S, Brugnara C, and Lodish HF. Fetal anemia and apoptosis of red cell progenitors in Stat5a-/-5b-/- mice: a direct role for Stat5 in Bcl-X(L) induction. *Cell* 98: 181–191, 1999.
344. Sohn J and Rudolph J. Catalytic and chemical competence of regulation of cdc25 phosphatase by oxidation/reduction. *Biochemistry* 42: 10060–10070, 2003.
345. Sokoloski JA, Sartorelli AC, Rosen CA, and Narayanan R. Antisense oligonucleotides to the p65 subunit of NF-kappa B block CD11b expression and alter adhesion properties of differentiated HL-60 granulocytes. *Blood* 82: 625–632, 1993.
346. Sparks JW and Brautigan DL. Specificity of protein phosphotyrosine phosphatases. Comparison with mammalian alkaline phosphatase using polypeptide substrates. *J Biol Chem* 260: 2042–2045, 1985.
347. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, and Mak TW. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* 95: 29–39, 1998.
348. Stancato LF, Silverstein AM, Owens–Grillo JK, Chow YH, Jove R, and Pratt WB. The hsp90-binding antibiotic geldanamycin decreases Raf levels and epidermal growth factor signaling without disrupting formation of signaling complexes or reducing the specific enzymatic activity of Raf kinase. *J Biol Chem* 272: 4013–4020, 1997.
349. Stanglmaier M, Warmuth M, Kleinlein I, Reis S, and Hallek M. The interaction of the BCR–ABL tyrosine kinase with the Src kinase Hck is mediated by multiple binding domains. *Leukemia* 17: 283–289, 2003.
350. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, Gilliland DG, and Tefferi A. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and the myelodysplastic syndrome. *Blood* 106: 1207–1209, 2005.
351. Suh Y–A, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, and Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1. *Nature* 401: 79–82, 1999.
352. Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, Gavrilova N, Mueller B, Liu X, and Wu H. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. *Proc Natl Acad Sci USA* 96: 6199–6204, 1999.
353. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, and Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J* 21: 5216–5224, 2002.
354. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
355. Suzuki YJ, Forman HJ, and Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 22: 269–285, 1997.
356. Tai LK, Okuda M, Abe J, Yan C, and Berk BC. Fluid shear stress activates proline-rich tyrosine kinase via reactive oxygen species-dependent pathway. *Arterioscler Thromb Vasc Biol* 22: 1790–1796, 2002.

357. Takada Y, Mukhopadhyay A, Kundu GC, Mahabeleshwar GH, Singh S, and Aggarwal BB. Hydrogen peroxide activates NF-kappa B through tyrosine phosphorylation of I kappa B alpha and serine phosphorylation of p65: evidence for the involvement of I kappa B alpha kinase and Syk protein-tyrosine kinase. *J Biol Chem* 278: 24233–24241, 2003.
358. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, and Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 280: 1614–1617, 1998.
359. Tang ED, Nunez G, Barr FG, and Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. *J Biol Chem* 274: 16741–16746, 1999.
360. Tanis KQ, Veach D, Duewel HS, Bornmann WG, and Koleske AJ. Two distinct phosphorylation pathways have additive effects on Abl family kinase activation. *Mol Cell Biol* 23: 3884–3896, 2003.
361. Tauchi T, Feng GS, Shen R, Song HY, Donner D, Pawson T, and Broxmeyer HE. SH2-containing phosphotyrosine phosphatase Syp is a target of p210BCR–ABL tyrosine kinase. *J Biol Chem* 269: 15381–15387, 1994.
362. Tauchi T, Ohyashiki K, Yamashita Y, Sugimoto S, and Toyama K. SH2-containing phosphotyrosine phosphatase SHP-1 is involved in BCR–ABL signal transduction pathways. *Int J Oncol* 11: 471–475, 1997.
363. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, and Ihle JN. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 93: 841–850, 1998.
364. Terui K, Takahashi Y, Kitazawa J, Toki T, Yokoyama M, and Ito E. Expression of transcription factors during megakaryocytic differentiation of CD34+ cells from human cord blood induced by thrombopoietin. *Tohoku J Exper Med* 192: 259–273, 2000.
365. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol* 279: L1005–1028, 2000.
366. Thomas EK, Cancelas JA, Chae HD, Cox AD, Keller PJ, Perrotti D, Neviani P, Druker BJ, Setchell KD, Zheng Y, Harris CE, and Williams DA. Rac guanosine triphosphatases represent integrating molecular therapeutic targets for BCR–ABL-induced myeloproliferative disease. *Cancer Cell* 12: 467–478, 2007.
367. Thompson JE, Cubbon RM, Cummings RT, Wicker LS, Frankshun R, Cunningham BR, Cameron PM, Meinke PT, Liverton N, Weng Y, and DeMartino JA. Photochemical preparation of a pyridone containing tetracycle: a Jak protein kinase inhibitor. *Bioorg Med Chem Lett* 12: 1219–1223, 2002.
368. Tonks NK, Diltz CD, and Fischer EH. Characterization of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem* 263: 6731–6737, 1988.
369. Tonks NK and Neel BG. Combinatorial control of the specificity of protein tyrosine phosphatases. *Curr Opin Cell Biol* 13: 182–195, 2001.
370. Topaly J, Schad M, Laufs S, Melo JV, Zeller WJ, and Fruehauf S. Cross-resistance of imatinib mesylate and 17-AAG in imatinib-resistant cells that overexpress BCR–ABL. *Br-JHaematol* 121: 672–673, 2003.
371. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, McDowell EP, Lazo–Kallanian S, Williams IR, Sears C, Armstrong SA, Passegue E, DePinho RA, and Gilliland DG. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128: 325–339, 2007.
372. Toyoshima H and Hunter T. p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell* 78: 67–74, 1994.
373. Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, and Huang P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell* 10: 241–252, 2006.
374. Tu BP, Kudlicki A, Rowicka M, and McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. *Science* 310: 1152–1158, 2005.
375. Ushio–Fukai M. Localizing NADPH oxidase-derived ROS. *Sci STKE* 2006: re8, 2006.
376. Ushio–Fukai M, Griendling KK, Becker PL, Hilenski L, Halleran S, and Alexander RW. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 21: 489–495, 2001.
377. Van Buul JD, Fernandez–Borja M, Anthony EC, and Hordijk PL. Expression and localization of NOX2 and NOX4 in primary human endothelial cells. *Antioxid Redox Signal* 7: 308–317, 2005.
378. van der Horst A, Tertoolen LG, de Vries–Smits LM, Frye RA, Medema RH, and Burgering BM. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J Biol Chem* 279: 28873–28879, 2004.
379. van Lieshout EM, Peters WH, and Jansen JB. Effect of oltipraz, alpha-tocopherol, beta-carotene and phenethylisothiocyanate on rat oesophageal, gastric, colonic and hepatic glutathione, glutathione S-transferase and peroxidase. *Carcinogenesis* 17: 1439–1445, 1996.
380. Venugopal R and Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17: 3145–3156, 1998.
381. Vilhardt F and van Deurs B. The phagocyte NADPH oxidase depends on cholesterol-enriched membrane microdomains for assembly. *EMBO J* 23: 739–748, 2004.
382. von Bubnoff N, Schneller F, Peschel C, and Duyster J. BCR–ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* 359: 487–491, 2002.
383. Wada H, Mizutani S, Nishimura J, Usuki Y, Kohsaki M, Komai M, Kaneko H, Sakamoto S, Delia D, Kanamaru A, and et al. Establishment and molecular characterization of a novel leukemic cell line with Philadelphia chromosome expressing p230 BCR/ABL fusion protein. *Cancer Res* 55: 3192–3196, 1995.
384. Walz C, Crowley BJ, Hudon HE, Gramlich JL, Neuberg DS, Podar K, Griffin JD, and Sattler M. Activated Jak2 with the V617F point mutation promotes G1/S phase transition. *J Biol Chem* 281: 18177–18183, 2006.
385. Walz C and Sattler M. Novel targeted therapies to overcome imatinib mesylate resistance in chronic myeloid leukemia (CML). *Crit Rev Oncol/Hematol* 57: 145–164, 2006.
386. Wang HP, Schafer FQ, Goswami PC, Oberley LW, and Buettner GR. Phospholipid hydroperoxide glutathione peroxidase induces a delay in G1 of the cell cycle. *Free Radic Res* 37: 621–630, 2003.
387. Wang MC, Bohmann D, and Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121: 115–125, 2005.
388. Wang MC, Bohmann D, and Jasper H. JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Developmental cell* 5: 811–816, 2003.
389. Warburg O. Über die fakultative Anaerobiose der Krebszellen und ihre Anwendung auf die Chemotherapie.

Munchener medizinische Wochenschrift (1950) 103: 2504–2506, 1961.
390. Warmuth M, Bergmann M, Priess A, Hauslmann K, Emmerich B, and Hallek M. The Src family kinase Hck interacts with BCR–ABL by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. *J Biol Chem* 272: 33260–33270, 1997.
391. Weibrecht I, Bohmer SA, Dagnell M, Kappert K, Ostman A, and Bohmer FD. Oxidation sensitivity of the catalytic cysteine of the protein-tyrosine phosphatases SHP-1 and SHP-2. *Free Radic Biol Med* 43: 100–110, 2007.
392. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan–Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall–Meyers E, Kung AL, Mestan J, Daley GQ, Callahan L, Catley L, Cavazza C, Mohammed A, Neuberg D, Wright RD, Gilliland DG, and Griffin JD. Characterization of AMN107, a selective inhibitor of native and mutant BCR–ABL. *Cancer Cell* 7: 129–141, 2005.
393. Wen ST and Vanetten RA. The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. *Genes Develop* 11: 2456–2467, 1997.
394. Wendel HG, de Stanchina E, Cepero E, Ray S, Emig M, Fridman JS, Veach DR, Bornmann WG, Clarkson B, McCombie WR, Kogan SC, Hochhaus A, and Lowe SW. Loss of p53 impedes the antileukemic response to BCR–ABL inhibition. *Proc Natl Acad Sci USA* 103: 7444–7449, 2006.
395. Wernig G, Gonneville JR, Crowley BJ, Rodrigues MS, Reddy MM, Hudon HE, Walz C, Reiter A, Podar K, Royer Y, Constantinescu SN, Tomasson MH, Griffin JD, Gilliland DG, and Sattler M. The Jak2V617F oncogene associated with myeloproliferative diseases requires a functional FERM domain for transformation and for expression of the Myc and Pim proto-oncogenes. *Blood* 111: 3751–3759, 2008.
396. Wickrema A, Uddin S, Sharma A, Chen F, Alsayed Y, Ahmad S, Sawyer ST, Krystal G, Yi T, Nishada K, Hibi M, Hirano T, and Platanias LC. Engagement of Gab1 and Gab2 in erythropoietin signaling. *J Biol Chem* 274: 24469–24474., 1999.
397. Wolf I, Jenkins BJ, Liu Y, Seiffert M, Custodio JM, Young P, and Rohrschneider LR. Gab3, a new DOS/Gab family member, facilitates macrophage differentiation. *Mol Cell Biol* 22: 231–244., 2002.
398. Wong CM, Cheema AK, Zhang L, and Suzuki YJ. Protein carbonylation as a novel mechanism in redox signaling. *Circ Res* 102: 310–318, 2008.
399. Wood ZA, Poole LB, and Karplus PA. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300: 650–653, 2003.
400. Wu H, Liu X, Jaenisch R, and Lodish HF. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. *Cell* 83: 59–67, 1995.
401. Wu RF, Xu YC, Ma Z, Nwariaku FE, Sarosi GA, Jr., and Terada LS. Subcellular targeting of oxidants during endothelial cell migration. *J Cell Biol* 171: 893–904, 2005.
402. Xia C, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, and Jiang BH. Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Res* 67: 10823–10830, 2007.
403. Xian M, Ito K, Nakazato T, Shimizu T, Chen CK, Yamato K, Murakami A, Ohigashi H, Ikeda Y, and Kizaki M. Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas- and mitochondria-mediated pathway. *Cancer Sci* 98: 118–126, 2007.
404. Xu RH, Pelicano H, Zhou Y, Carew JS, Feng L, Bhalla KN, Keating MJ, and Huang P. Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. *Cancer Res* 65: 613–621, 2005.
405. Yamaoka K, Saharinen P, Pesu M, Holt VE, 3rd, Silvennoinen O, and O'Shea JJ. The Janus kinases (Jaks). *Genome Biol* 5: 253, 2004.
406. Yamasaki S, Nishida K, Hibi M, Sakuma M, Shiina R, Takeuchi A, Ohnishi H, Hirano T, and Saito T. Docking protein Gab2 is phosphorylated by ZAP-70 and negatively regulates T cell receptor signaling by recruitment of inhibitory molecules. *J Biol Chem* 276: 45175–45183., 2001.
407. Yoshida C, Yoshida F, Sears DE, Hart SM, Ikebe D, Muto A, Basu S, Igarashi K, and Melo JV. BCR–ABL signaling through the PI-3/S6 kinase pathway inhibits nuclear translocation of the transcription factor Bach2, which represses the antiapoptotic factor heme oxygenase-1. *Blood* 109: 1211–1219, 2007.
408. Zhang AY, Yi F, Zhang G, Gulbins E, and Li PL. Lipid raft clustering and redox signaling platform formation in coronary arterial endothelial cells. *Hypertension* 47: 74–80, 2006.
409. Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metabol Rev* 38: 769–789, 2006.
410. Zhang DD and Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol* 23: 8137–8151, 2003.
411. Zhang DD, Lo SC, Cross JV, Templeton DJ, and Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* 24: 10941–10953, 2004.
412. Zhang X, Subrahmanyam R, Wong R, Gross AW, and Ren R. The NH(2)-terminal coiled-coil domain and tyrosine 177 play important roles in induction of a myeloproliferative disease in mice by BCR–ABL. *Mol Cell Biol* 21: 840–853., 2001.
413. Zhang Y, Diaz–Flores E, Li G, Wang Z, Kang Z, Haviernikova E, Rowe S, Qu CK, Tse W, Shannon KM, and Bunting KD. Abnormal hematopoiesis in Gab2 mutant mice. *Blood* 110: 116–124, 2007.
414. Zhao C, Yu DH, Shen R, and Feng GS. Gab2, a new pleckstrin homology domain-containing adapter protein, acts to uncouple signaling from ERK kinase to Elk-1. *J Biol Chem* 274: 19649–19654., 1999.
415. Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz SB, and Zhao ZJ. Identification of an acquired JAK2 mutation in polycythemia vera. *J Biol Chem* 280: 22788–22792, 2005.
416. Zou X, Rudchenko S, Wong K, and Calame K. Induction of c-myc transcription by the v-Abl tyrosine kinase requires Ras, Raf1, and cyclin-dependent kinases. *Genes Dev* 11: 654–662, 1997.

Address reprint requests to:
Martin Sattler, Ph.D.
Department of Medical Oncology
Dana–Farber Cancer Institute
44 Binney Street
Boston, MA 02115

E-mail: martin_sattler@dfci.harvard.edu

Date of first submission to ARS Central, March 6, 2008; date of final revised submission, April 12, 2008; date of acceptance, April 12, 2008.

This article has been cited by:

1. A Y H Leung, C-H Man, Y-L Kwong. 2012. FLT3 inhibition: a moving and evolving target in acute myeloid leukaemia. *Leukemia* . [CrossRef]
2. Mamatha M Reddy, Anagha Deshpande, Martin Sattler. 2012. Targeting JAK2 in the therapy of myeloproliferative neoplasms. *Expert Opinion on Therapeutic Targets* 1-12. [CrossRef]
3. Tomasz Skorski. 2012. Genetic Mechanisms of Chronic Myeloid Leukemia Blastic Transformation. *Current Hematologic Malignancy Reports* . [CrossRef]
4. A. J. Montero, C. M. Diaz-Montero, Y. E. Deutsch, J. Hurley, L. G. Koniaris, T. Rumboldt, S. Yasir, M. Jorda, E. Garret-Mayer, E. Avisar, J. Slingerland, O. Silva, C. Welsh, K. Schuhwerk, P. Seo, M. D. Pegram, S. Glück. 2011. Phase 2 study of neoadjuvant treatment with NOV-002 in combination with doxorubicin and cyclophosphamide followed by docetaxel in patients with HER-2 negative clinical stage II–IIIc breast cancer. *Breast Cancer Research and Treatment* . [CrossRef]
5. M M Reddy, M S Fernandes, A Deshpande, E Weisberg, H V Inguilizian, O Abdel-Wahab, A L Kung, R L Levine, J D Griffin, M Sattler. 2011. The JAK2V617F oncogene requires expression of inducible phosphofructokinase/fructose-bisphosphatase 3 for cell growth and increased metabolic activity. *Leukemia* . [CrossRef]
6. P. S. Hole, R. L. Darley, A. Tonks. 2011. Do reactive oxygen species play a role in myeloid leukemias?. *Blood* **117**:22, 5816-5826. [CrossRef]
7. M M Reddy, M S Fernandes, R Salgia, R L Levine, J D Griffin, M Sattler. 2011. NADPH oxidases regulate cell growth and migration in myeloid cells transformed by oncogenic tyrosine kinases. *Leukemia* **25**:2, 281-289. [CrossRef]
8. Gabriella Leonarduzzi, Barbara Sottero, Giuseppe Poli. 2010. Targeting tissue oxidative damage by means of cell signaling modulators: The antioxidant concept revisited. *Pharmacology & Therapeutics* **128**:2, 336-374. [CrossRef]
9. Irene Colavita, Nicola Esposito, Rosanna Martinelli, Francesca Catanzano, Junia V. Melo, Fabrizio Pane, Margherita Ruoppolo, Francesco Salvatore. 2010. Gaining insights into the Bcr-Abl activity-independent mechanisms of resistance to imatinib mesylate in KCL22 cells: A comparative proteomic approach. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1804**:10, 1974-1987. [CrossRef]
10. E Weisberg, M Sattler, A Ray, J D Griffin. 2010. Drug resistance in mutant FLT3-positive AML. *Oncogene* **29**:37, 5120-5134. [CrossRef]
11. Danilo Perrotti, Catriona Jamieson, John Goldman, Tomasz Skorski. 2010. Chronic myeloid leukemia: mechanisms of blastic transformation. *Journal of Clinical Investigation* **120**:7, 2254-2264. [CrossRef]
12. Georg T. Wondrak . 2009. Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities. *Antioxidants & Redox Signaling* **11**:12, 3013-3069. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
13. Pablo E. Vivas-Mejía, Bulent Ozpolat, Xian Chen, Gabriel Lopez-Berestein. 2009. Downregulation of the c-MYC target gene, peroxiredoxin III, contributes to arsenic trioxide-induced apoptosis in acute promyelocytic leukemia. *International Journal of Cancer* **125**:2, 264-275. [CrossRef]
14. Dunyaporn Trachootham, Jerome Alexandre, Peng Huang. 2009. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?. *Nature Reviews Drug Discovery* **8**:7, 579-591. [CrossRef]