Chk1 and Chk2 kinases in checkpoint control and cancer

Jiri Bartek* and Jiri Lukas

Department of Cell Cycle and Cancer, Institute of Cancer Biology, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen, Denmark
*Correspondence: bartek@biobase.dk

Accumulation of mutations and chromosomal aberrations is one of the hallmarks of cancer cells. This enhanced genetic instability is fueled by defects in the genome maintenance mechanisms including DNA repair and cell cycle checkpoint pathways. Here, we discuss the emerging roles of the mammalian Chk1 and Chk2 kinases as key signal transducers within the complex network of genome integrity checkpoints, as candidate tumor suppressors disrupted in sporadic as well as some hereditary malignancies and as potential targets of new anticancer therapies.

Genomic instability, checkpoint defects, and cancer

The multistep evolution of cancer reflects accumulation of genetic changes that lead to transformation of normal cells to cancer cells and development from normal tissues into benign, and eventually invasive, malignant tumors. The accumulating alterations of tumor suppressor genes and protooncogenes facilitate tumorigenesis and, through selection of genetic variants, such alterations can also affect responses to radiotherapy and chemotherapy. Recent evidence implicates DNA repair and the so-called genome integrity checkpoints as the culprits whose defects are largely responsible for the enhanced genetic instability of cancer cells (Hoeijmakers, 2001; Bartek and Lukas, 2001; Khanna and Jackson, 2001). The checkpoint pathways are phyllogenetically conserved signaling cascades activated in response to DNA damage or errors in cell cycle events such as DNA replication or chromosome segregation (Zhou and Elledge, 2000). The activated checkpoints delay cell cycle progression to facilitate DNA repair, and they can also eliminate the hazardous damaged cells through induction of cell death, thereby protecting the organism against cancer. The checkpoint network must not only sense the damage, but also promptly spread such signals to reach the downstream cellular effector proteins. In this review, we highlight the roles of mammalian kinases Chk1 and Chk2 (the latter also known as Cds1 or CHEK2), two critical messengers of the genome integrity checkpoints, and particularly their involvement in the evolution of human cancer.

Chk1 and Chk2 in checkpoint signaling

Chk1 and Chk2 are structurally unrelated yet functionally overlapping serine/threonine kinases activated in response to diverse genotoxic insults (reviewed in Bartek et al., 2001; McGowan 2002). The key mission of Chk1 and Chk2 is to relay the checkpoint signals from the proximal checkpoint kinases of the phosphatidylinositol 3-kinase family, particularly ATM and ATR, and likely also the newly identified ATX (Abraham, 2001; Shiloh, 2003; Kastan and Lim, 2000; and R. Abraham, personal communication), which phosphorylate and activate Chk1 and/or Chk2 (Figure 1). Chk2 is a stable protein expressed throughout the cell cycle (Lukas et al., 2001), it appears to be largely inactive in the absence of DNA damage, it is activated mainly by ATM in response to double-strand DNA breaks (DSBs), and its activation involves dimerization and autophosphorylation (Figure 1A). In contrast, the labile Chk1 protein is largely restricted to S and G2 phases (Lukas et al., 2001), it is active even in unperturbed cell cycles (Kaneko et al., 1999; Zhao et al., 2002; Sørensen et al., 2003), and although it is further activated in response to DNA damage or stalled replication, this may not require Chk1 dimerization or autophosphorylation (Figure 1B). The original concept of rather strict dependency of Chk1 on ATR, and Chk2 on ATM, has recently been softened by reports of various "crosstalks" among these kinases (Figure 1), exemplified by phosphorylation/activation of Chk1 by ATM in response to ionizing radiation (Gatei et al., 2003; Sørensen et al., 2003), the identification of a novel checkpoint cascade signaling via ATM-Chk1 to Tlk kinases and thereby likely to chromatin remodeling in response to various stresses (Groth et al., 2003), reports of ATM-independent activation of Chk2 (Hirao et al., 2002), as well as by the ATX kinase whose links to Chk1 and Chk2 remain to be elucidated.

An exciting recent development in this field has been the identification of a group of large, BRCT domain-containing proteins including 53BP1, BRCA1, and MDC1 as "mediators" of checkpoint responses (Wang et al., 2000, 2002; DiTullio et al., 2002; Fernandez-Capetillo et al., 2002; Yarden et al., 2002; Goldberg et al., 2003; Stewart et al., 2003; Lou et al., 2003; Lee et al., 2000). The emerging role of these proteins is to modulate diverse checkpoint events, including activation of Chk1 and Chk2, and promotion of other ATM-mediated phosphorylation events through protein-protein interactions involved in "matchmaker" and/or recruitment functions (Figure 1). While BRCA1 is an established tumor suppressor (Scully and Livingston, 2000) and 53BP1-deficient mice are tumor prone (Ward et al., 2003), it remains to be seen whether 53BP1 and MDC1 are also targeted in human cancer.

The fact that Chk1 and Chk2 perform partly redundant roles becomes evident from the spectra of their known substrates (Bartek et al., 2001; McGowan, 2002), most of which are shared by both kinases (Figure 2). Through targeting these downstream effector proteins that also include recent additions such as the Tlk kinases (Groth et al., 2003), the PML protein (Yang et al., 2002), the PLK3 kinase (Xie et al., 2002), or the E2F1 transcription factor (Stevens et al., 2003), Chk1 and Chk2 regulate fundamental cellular functions such as DNA replication and cell cycle progression, chromatin restructuring, and apoptosis (Figure 2). Consistent with the fact that some substrates of Chk2 such as Cdc25A or p53 are soluble, mobile proteins, and with the need to rapidly spread the checkpoint signal from localized sites of DNA damage to such targets, live-cell imaging of

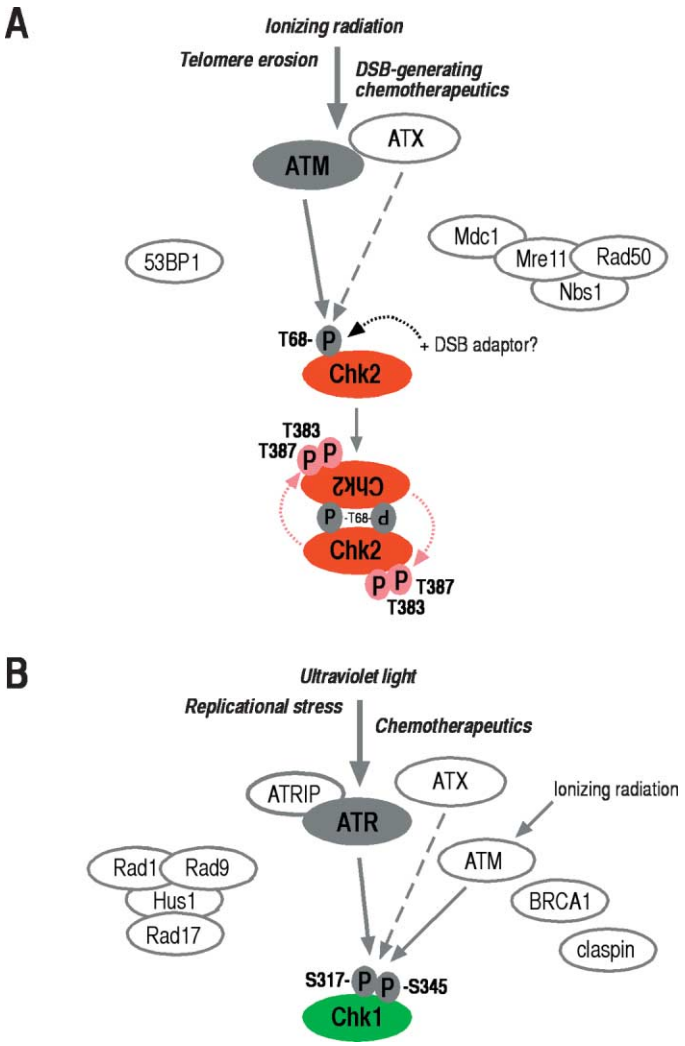

Figure 1. Upstream regulation of Chk1 and Chk2 under genotoxic stress

A: Ionizing radiation, telomere erosion, and radiomimetic drugs generate DNA double-strand breaks (DSB) and activate the ATM kinase (Shiloh, 2003). ATM phosphorylates the N-terminal regulatory domain of Chk2, exemplified here by the most prominent, threonine 68 phosphorylation (Bartek et al., 2001). This in turn promotes homodimerization and intermolecular transphosphorylation of Chk2 on its C-terminal kinase domain (Ahn et al., 2002; Xu et al., 2002; Lee and Chung, 2001), a modification required for a full activation of Chk2 toward heterologous substrates. A recent report indicates that the ATM-dependent phosphorylation of Chk2 cannot occur freely in the nucleoplasm, but requires a specific DSB-associated adaptor protein(s) (Lukas et al., 2003). In addition, other checkpoint proteins may coregulate the physiological velocity and/or timing of Chk2 activation. These factors include a DSB-interacting protein (53BP1), DNA ends-processing "MRN" nuclease complex (Mre11/Rand50/Nbs1), and its newly identified binding partner Mdc1 (see text for details). The exact functional interplay among these factors is yet to be determined; here, we schematically indicate the reported protein-protein interaction patterns.
B: Ultraviolet light, stalled replication, and some drugs activate ATR, the major upstream kinase phosphorylating and activating Chk1 (Feijoo et al., 2001; Heffernan et al., 2002; Liu et al., 2000; Shiloh, 2003; Zhao and Piwnica-Worms, 2001). The exact DNA intermediates that lead to activation of human ATR are not known but its recruitment to DNA requires ATRIP, a DNA-interacting adaptor protein (Cortez et al., 2001). Several recent studies show that ATM can also phosphorylate Chk1 in cells exposed to IR, although to a lesser extent compared to the ATR-mediated effect after other types of DNA damage (Gatei et al., 2003; Sørensen et al., 2003). Both ATR and ATM target the SQ-rich C terminus of Chk1, including serines 317 and 345, respectively. These phosphorylations may directly lead to Chk1 activation. Optimal activation of Chk1 also requires a cooperative action of other factors including the multifunctional BRCA1 tumor suppressor (Yarden et al., 2002), the claspin adaptor molecule (Kumagai and Dunphy, 2000), and the PCNA-like DNA sliding clamp (Rad9/Rad1/Hus1) together with its loading factor (Rad17) (Weiss et al., 2002; Zou et al., 2002).
A and **B:** ATX is a new member of the ATM/ATR kinase family, which could be activated both by UV light and in response to DSB (R. Abraham, personal communication). As such, it likely contributes to Chk1 and Chk2 activation by diverse types of genotoxic stress.

Chk2 in human cells exposed to subnuclear DNA damage revealed immediate redistribution of the activated Chk2 throughout the nucleus. Thus, the repeatedly observed "foci" of activated Chk2 represent an unfortunate artifact attributable to crossreactive antibodies against the Thr68-phosphorylated Chk2, and the new data (Lukas et al., 2003) document the rapid mobility and the lack of accumulation at sites of DNA damage, supporting the role of Chk2 as the checkpoint signal spreader. The lack of nuclear foci formation after DNA damage distinguishes Chk2 from many other DNA damage checkpoint proteins, yet it is shared with Chk1 (C. Lukas, personal communication), again pointing to analogous functions of these two checkpoint transducers.

Lessons learned from Chk1 and Chk2 knockout and knockdown models

Despite their overlapping roles in checkpoint signaling, the biological requirements for Chk1 and Chk2 function are strikingly different, as Chk1 (Liu et al., 2000; Takai et al., 2000) but not Chk2 (Hirao et al., 2002; Takai et al., 2002) is essential for mammalian development and viability. The early embryonic lethality of the Chk1-deficient mice and the acute lethality of Chk1-deficient embryonic cells (Liu et al., 2000; Takai et al., 2000) allowed only limited analysis of the consequences of Chk1 absence for checkpoint functions, yet these studies implicated Chk1 in the G2/M DNA damage response and the S-M checkpoint in response to incomplete DNA replication. In contrast, complete deficiency of Chk1 in avian somatic DT-40 lymphoma cells (Zachos et al., 2003) can be tolerated and does not affect cell division, while it abolished DNA damage-induced G2 arrest, undermined replication checkpoint responses, and sensitized cells to killing upon perturbations of DNA structure or metabolism. RNAi-mediated knockdown of Chk1 in human cells revealed an essential role of this kinase in the control of Cdc25A protein turnover and thereby in both normal S phase and the intra-S phase DNA damage checkpoint (Zhao et al., 2002; Sørensen et al., 2003) and confirmed the requirement for Chk1 in the G2/M checkpoint in response to ionizing radiation (Gatei et al., 2003) and some DNA-damaging drugs (Xiao et al., 2003).

The Chk2-deficient mice are viable, fertile, and do not show a tumor-prone phenotype except when exposed to carcinogens, or possibly later in their lives if left unperturbed (Takai et al., 2002; Hirao et al., 2002). Although there are some discrepancies in the results of the different studies with Chk2-deficient mice and cells derived from them, the observed phenotype was dominated by increased resistance of the *Chk2*$^{-/-}$ mice to ionizing radiation, and cellular defects in p53 function, some checkpoint responses, and especially in apoptosis (Takai et al., 2002; Hirao et al., 2002; Hirao et al., 2000; Jack et al., 2002). Chk2-deficient HCT-15 human colon carcinoma cells have also been used to study Chk2 function, particularly in the intra-S phase checkpoint in response to DSBs (Falck et al., 2001a, 2001b, 2002), a role recently confirmed by S phase checkpoint mal-

function manifested as partial radiation-resistant DNA synthesis after RNAi-mediated downregulation of Chk2 (or Chk1) in other human cell types (Sørensen et al., 2003; Zhao et al., 2002). Compared with human cells, the S phase checkpoint defect was not as obvious in the slowly proliferating primary *Chk2*$^{-/-}$ mouse fibroblasts, but it became apparent when these cells were immortalized (Hirao et al., 2002).

Collectively, these studies supported partly overlapping roles of Chk1 and Chk2 in multiple checkpoints, revealed the distinct requirements for Chk1 versus Chk2 for embryonic development and viability, and underscored the conditional nature and low penetrance of Chk2 as a tumor suppressor in mice.

Chk2 variants predisposing to breast and colon cancer

The first evidence that genetic alteration in Chk2 may predispose to cancer was the finding by Bell et al. (1999) of rare germline mutations in the *Chk2* gene in families with Li-Fraumeni syndrome (LFS). LFS is a familial cancer syndrome characterized by multiple tumors at young age, with a predominance of breast cancer and sarcomas, and it is often linked to germline mutations in the *p53* gene. The same Chk2 protein-truncating mutation (Figure 3A), 1100delC (Bell et al., 1999; Lee et al., 2001), was also identified in a small subset of LFS families in Finland (Vahteristo et al., 2001). The fact that *p53* was wild-type in all these cases suggested that germline mutations of *Chk2* may represent an alternative genetic defect predisposing to LFS.

The recurrent finding of the 1100delC variant (Figure 3) in LFS prompted two large parallel studies of *Chk2* status in hereditary breast cancer, both concluding that *Chk2* 1100delC is a low-penetrance breast cancer susceptibility allele with a high degree of statistical significance (Vahteristo et al., 2002; Meijers-Heijboer et al., 2002). Interestingly, *Chk2* 1100delC confers no increased cancer risk in breast cancer families with mutations in the two previously identified breast cancer susceptibility genes, *BRCA1* and *BRCA2*, consistent with the concept that *Chk2* and *BRCA1/2* all participate in the DNA damage response network whose function can be undermined by any of these mutations. At the molecular level, the 1100delC truncation eliminates the kinase domain and activity of Chk2 (Wu et al., 2001). Furthermore, the 1100delC protein is unstable (J. Falck, personal communication) and the remaining wild-type allele is often lost in the tumors (Lee et al., 2001), which together results in a complete loss of Chk2 function and a gross reduction or lack of the overall Chk2 protein, a phenomenon that facilitates detection of tumors with Chk2 1100delC by immunohistochemistry (Figure 4; Vahteristo et al., 2002). Interestingly, most recent data indicate that the 1100delC variant is particularly common in families predisposed to combined breast and colon cancer (Schutte et al., 2003).

Unlike the 1100delC variant whose carrier frequency of 1.1%–1.4% is similar in normal populations of Western Europe, North America, and Finland (Vahteristo et al., 2002; Meijers-Heijboer et al., 2002), the missense variant Chk2 I157T, originally detected in rare LFS families (Bell et al., 1999; Vahteristo et al., 2001), is much more common in normal Finnish population (at 5%–6%; Allinen et al., 2001; Kilpivaara et al., our unpublished results) than elsewhere (Lee et al., 2001; Schutte et al., 2003). Recent data from a large Finnish study show that Chk2 I157T is significantly more frequent among unselected cohort of breast cancer patients, suggesting that this variant of Chk2 may contribute to breast cancer in the Finnish population (Kilpivaara et al., our unpublished results). Mechanistically, the I157T may contribute to tumorigenesis by a dominant-negative effect on the remaining wild-type Chk2, rather than through loss of function as the 1100delC variant. Thus, the Chk2 I157T protein is stable and itself deficient in recognizing its physiological

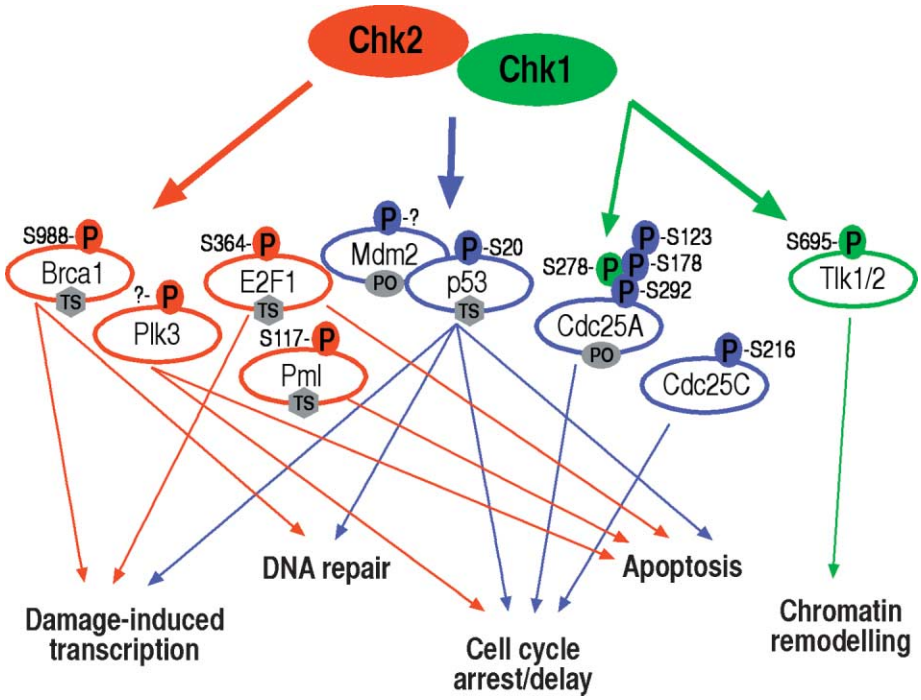

Figure 2. Chk1 and Chk2 as mediators of the checkpoint signaling network

Following their activation, Chk1 and Chk2 phosphorylate unique (green and red, respectively) and overlapping (blue) downstream effectors that further propagate the checkpoint signaling. Depending on the type of stress, velocity of DNA damage, and cellular context, this leads to (1) switch to the stress-induced transcription program (E2F1, Brca1, p53), (2) direct or indirect initiation of DNA repair (BRCA1, p53), (3) acute delay (degradation of Cdc25A) and/or sustained block (Cdc25C, p53, Plk3) of cell cycle progression, (4) apoptosis (Pml1, p53, E2F1), and (5) modulation of the chromatin remodeling pathways (Tlk1/2). The known target sites of Chk1 (green), Chk2 (red), and both Chk1 and Chk2 (blue) on the individual substrates are shown. Some of the Chk1/Chk2 downstream effectors are classified as protooncogenes (PO) or tumor suppressors (TS), as indicated.

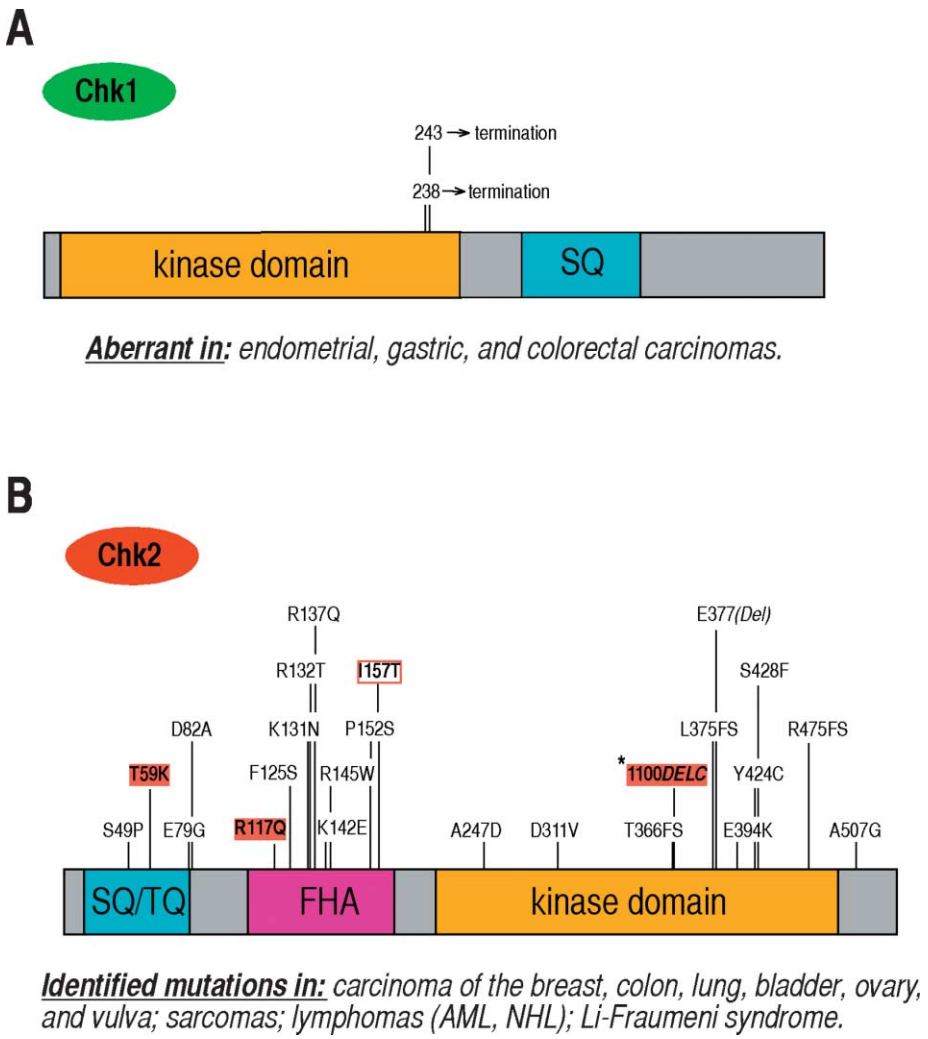

Figure 3. Structure of human Chk1 and Chk2 and their aberrations in cancer

A: Structure of human Chk1. Tumor types with the reported aberrations of Chk1 gene are indicated (bottom). Specific nature and locations of the majority of Chk1 genetic abnormalities have not yet been determined. SQ, a serine/glutamine-rich region with multiple ATM/ATR/(ATX) phosphorylation sites.

B: Structure of human Chk2. Positions of tumor-associated mutations are indicated (top) together with the list of tumor types, where these mutations were identified (bottom). Red boxes highlight three mutations associated with familial cancer; the asterisk indicates the most frequent 1100delC mutation found both in sporadic and familial cancer; this mutation is indicated by its nucleotide, rather than amino acid position, due to the established designation of this variant. The I157T mutant (red-framed) seems to be associated with higher incidence of sporadic breast cancer in Finland (see text). SQ/TQ, a regulatory domain containing multiple ATM/(ATX)-recognition sites; FHA, a forkhead-associated domain required for Chk2 homodimerization and other protein-protein interactions.

substrates p53 (Falck et al., 2001b), Cdc25A (Falck et al., 2001a), and BRCA1 (Li et al., 2002), and it undermines the function of wild-type Chk2 when expressed in the same cells, resulting in checkpoint defects in response to ionizing radiation (Falck et al., 2001a).

These and other (Ingvarsson et al., 2002; Sodha et al., 2002) studies document the involvement of Chk2 defects in breast cancer and colon cancer susceptibility, and indicate distinct modes of action for different genetic variants of this tumor suppressor. They furthermore provide pioneering examples of pinpointing low-penetrance breast cancer predisposing genes, of which Chk2 likely represents only the tip of the iceberg. Given their low penetrance, testing for these Chk2 variants alone would likely have little predictive value for individual patients. However, it may eventually allow risk assessment for breast cancer at the population, or even individual level as part of a broader panel of analogous variants suitable for profiling and screening, and thereby prove useful for chemoprevention or lifestyle counseling.

Chk1 and Chk2 abnormalities in sporadic tumors

In contrast to the functionally overlapping Chk2 kinase which qualifies as a tumor suppressor, cancer-associated defects of Chk1 are extremely rare, and so far seem limited to carcinomas of the colon, stomach, and endometrium (Bertoni et al., 1999; Menoyo et al., 2001, Vassileva et al., 2002). Frameshift mutations due to insertion or deletion of single adenine in the polyadenine tract of the *Chk1* gene have been reported in colon and endometrial carcinomas with microsatelite instability (Bertoni et al., 1999). The resulting truncated Chk1 proteins (Figure 3A) are predicted to be defective due to the lack of the C-terminal end of the catalytic domain and the complete loss of the SQ-rich regulatory domain, yet given their heterozygous state, the overall functional impact of these mutations in cancer cells remains uncertain. A shorter isoform of Chk1 mRNA, predicted to encode a protein which lacks a conserved subdomain in the catalytic domain of Chk1, has been detected in a subset of small cell lung carcinomas (Haruki et al., 2000). The deleted part of the catalytic domain is predicted to be involved in substrate selectivity, and the significance of the predominant expression of this alternative Chk1 isoform in fetal lung and in small cell carcinomas, but not in normal adult lung tissue or other types of lung tumors, remains to be established. Despite the fact that Chk1 function and its analogy with Chk2 fit a candidate tumor suppressor gene, the complete deficiency of Chk1 in mice results in early embryonic lethality (see above), and it is yet to be seen whether noncancerous somatic cells lacking Chk1 are viable. On the other hand, deletion of *Chk1* in a p53-deficient chicken tumor cell line is tolerable (Zachos et al., 2003), as are the heterozygous truncation mutations of *Chk1* in some human tumors (Bertoni et al., 1999). Thus, it is plausible that either hypomorphic mutations of *Chk1* or Chk1 defects that occur during progression of cancer at the stage when cancer cells are less prone to apoptosis (such as those with mutant *p53*) may contribute to enhanced genetic instability in some tumors.

Somatic mutations of *Chk2* have been found in small sub-

sets of diverse types of sporadic human malignancies (Figure 3B), including carcinomas of the breast (Sullivan et al., 2002), lung (Haruki et al., 2000), vulva (Reddy et al., 2002), urinary bladder (J. Bartkova and P. Guldberg, personal communication), colon (Bell et al., 1999), and ovary (Miller et al., 2002), osteosarcomas (Miller et al., 2002), and lymphomas (Hofman et al., 2001; Hangaishi et al., 2002; Tavor et al., 2001; Tort et al., 2002). The majority of these mutations are missense or truncation mutations, clustered in three domains of Chk2: the N-terminal SQ/TQ-rich regulatory domain, the protein-protein interaction FHA domain, or the C-terminal catalytic domain (Figure 3B). Some of these *Chk2* mutants have been characterized functionally at the protein level and found aberrant in several different ways. Thus, some of the FHA domain (e.g., R145W) or kinase domain (D311Val, 1100delC) mutants are unstable proteins, targeted by the proteasome-mediated degradation machinery, and as a consequence, they are expressed at much lower levels than the wild-type Chk2 protein (Bartkova et al., 2001; Lee et al., 2001; Matsuoka et al., 2001). Mutants within the catalytic domain show decreased or lost kinase activity (Matsuoka et al., 2001; Wu et al., 2001), while those in the FHA domain are often defective in recognizing their substrates such as Cdc25A, p53, or BRCA1 (Falck et al., 2001a, 2001b; Li et al., 2002), and some of them show a dominant-negative effect when coexpressed with the wild-type Chk2 (Falck et al., 2001a), a scenario reminiscent of their heterozygous state in human tumors. Other mutants (e.g., the "unstable" ones mentioned above) are unlikely to grossly interfere with the remaining wild-type Chk2 and may represent "classical" loss-of-function mutants, an interpretation consistent with the lack of the second allele due to loss of heterozygosity seen in subsets of such tumors. The latter tumors show gross reduction or loss of Chk2 staining in immunohistochemical analyses, an approach which also identified a significant subset of human tumors with low or undetectable Chk2 protein in the apparent absence of any mutations in the Chk2 gene (Bartkova et al., 2001; Vahteristo et al., 2002; Sullivan et al., 2002; Tort et al., 2002). Epigenetic silencing of gene expression through promoter methylation has been excluded as a cause of this phenotype (Sullivan et al., 2002; Tort et al., 2002), and the finding of normal mRNA levels of Chk2 in such cases suggested potential posttranscriptional aberrations of Chk2 as a plausible way to downregulate Chk2 levels during oncogenesis.

Apart from Chk2 mutations or protein downregulation, a constitutive phosphorylation of the activatory threonine 68 of Chk2 has been found in human cancer cell lines, particularly those with mutant p53, and in subsets of human primary breast and colon carcinomas (DiTullio et al., 2002). Such checkpoint activation in cells without any external DNA damage, and in tumors untreated by chemotherapy or radiotherapy, raises the question about the origin of the stimulus that evoked such a constitutive response of the ATM-Chk2 pathway. Regardless of the activating insult, this persistent activation of Chk2 likely increases the selective pressure in human cancers to mutate p53 (DiTullio et al., 2002), an important aspect of cancer biology that is also addressed in the next section.

The Chk2-p53 connection

The widely accepted view that Chk2 phosphorylates the N-terminal activation domain of p53 and thereby regulates p53 in response to DSBs, supported by a large body of evidence (Chehab et al., 1999, 2000; Shieh et al., 2000; Falck et al., 2001b; Hirao et al., 2000, 2002; Takai et al., 2002; Lukas et al., 2003), has recently been challenged based on the following arguments. First, the p53-derived peptide containing the Chk2-targeted phosphorylation sites threonine 18 and serine 20 is a poor substrate for Chk2, and these sequences do not fit the consensus Chk2 phosphorylation site found in other Chk2 substrates (O'Neill et al., 2002; Ahn et al., 2003). Second, the elimination of Chk2 by gene knockout or its downregulation by RNAi resulted in only partial or no defects in the DNA damage-induced phosphorylation and stabilization of p53 (Hirao et al., 2000, 2002; Takai et al., 2002; Jallepalli et al., 2003; Ahn et al., 2003), which seemed surprising given the postulated key role of Chk2-mediated serine 20 phosphorylation in regulation of p53 protein turnover. Third, the occurrence of concomitant mutations of p53 and Chk2 in the same tumor contrasts with mutually exclusive cancer-associated aberrations of some other functionally linked genes such as p53 and mdm2, and the former phenomenon has been interpreted as evidence against the p53 and Chk2 tumor suppressors operating in the same linear pathway.

However, most recent biochemical, genetic, and functional analyses of the Chk2-p53 link reconcile most of these apparent discrepancies and further support the physiological role of Chk2 as a p53 kinase. Thus, unlike Cdc25A or Cdc25C which contain the Chk2 consensus sites and can serve as good substrates even in the form of short peptides, activation of Chk2 as kinase toward threonine 18 and serine 20 of p53 requires allosteric changes in Chk2 induced by its interaction with sequences in the core domain of native full-length p53. This novel and unexpected mode of Chk2 regulation as a p53 kinase (T. Hupp, personal communication) reveals existence of two classes of Chk2 substrates and highlights the emerging concept of protein kinase docking sites as modulators of substrate specificity. The phosphorylations at Thr18 or Ser20 attenuate MDM2 interaction with p53 and create a p300 phospho-consensus binding site, thereby operating as a molecular switch to convert p53 from a protein that binds its negative regulator MDM2 to a p300 binding protein subject to enhanced acetylation by p300 (Shieh et al., 2000; Schon et al., 2002; Dornan et al., 2003). Thus, Chk2 contributes to regulation of p53 stabilization, yet regulates also the activation of p53, consistent with the pronounced defect of p53-dependent transcription in Chk2-deficient mouse cells (Hirao et al., 2000; Takai et al., 2002) and with the rate-limiting effects of Chk2 on p53 function in human cells (Chehab et al., 2000; Falck et al., 2001b; Lukas et al., 2003).

Finally, when considering the selective advantages of cancer cells with single versus dual defects in the Chk2-p53 pathway, it is essential to realize that most types of cellular stresses (including some types of DNA damage) that activate p53 do not activate Chk2 and therefore can signal to p53 normally even in the absence of Chk2. And vice versa, Chk2 has several other important functions apart from targeting p53 (Figure 2); so in the absence of either Chk2 or p53, the other protein remains engaged in multiple checkpoint pathways. In addition, even in response to DSBs, the role of Chk2 as a p53 kinase is likely partly redundant, and at least the functionally overlapping kinase Chk1 has been found to undergo rapid activation in Chk2-deficient cells (Takai et al., 2002), consistent with a potential compensatory role in the absence of Chk2. Given these biological features of the Chk2-p53 interplay, it is understandable why *Chk2* deletion or mutation does not recapitulate the effects of p53 inactivation in human cancer cells. Thus, despite the fact that Chk2

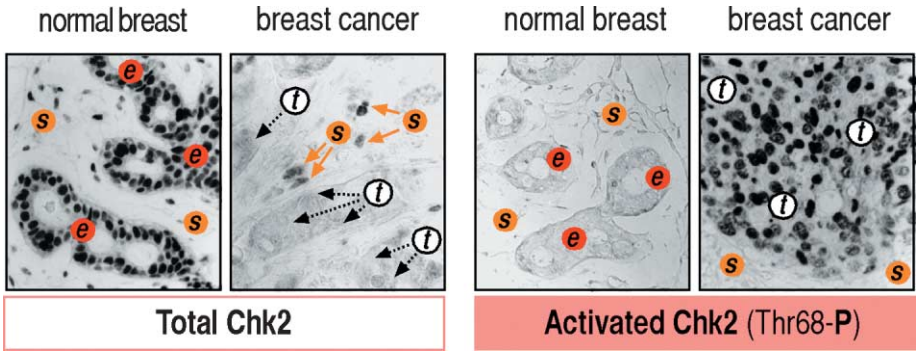

Figure 4. Examples of Chk2 aberrations in human breast cancer

The breast cancer-associated Chk2 defects include loss of Chk2 expression (left) and abnormal activation (right). Sections of formaldehyde-fixed normal and cancerous tissue were immunostained with antibodies to total Chk2 (left) and Chk2 phosphorylated by ATM on threonine 68 (right). *e*, epthelium; *s*, stroma; *t*, tumor tissue.

and p53 do operate in a linear pathway in response to DSBs, cancer cells defective in Chk2 are still under selective pressure to inactivate p53, a concept consistent with the observed concomitant mutations of *p53* and *Chk2* in some tumors (Bell et al., 1999; Falck et al., 2001b; Sullivan et al., 2002; Reddy et al., 2002).

Checkpoint kinases as potential therapeutic targets

The common, possibly universal occurrence of checkpoint defects distinguishes cancer cells from normal cells, thereby providing a potential target for therapeutic intervention. One strategy, tested with a moderately positive outcome in various cancer models in cell culture, has been to inhibit the checkpoint signaling to enhance the impact of concomitant treatment with DNA-damaging drugs or radiation (reviewed in Bartek et al., 2001; Dixon and Norbury, 2002). The rationale behind such selective sensitization of cancer cells is that, unlike normal cells with a full arsenal of checkpoint responses, tumors defective in some checkpoint(s) could be deprived of their remaining checkpoint pathway(s), and consequently driven into cell death due to accumulation of excessive DNA damage. So far, the major scenario explored for such experiments has been to target p53-deficient cancers that lack the G1 checkpoint and to inhibit the checkpoint kinases known to participate in the G2 checkpoint, such as ATM/ATR (e.g., by caffeine) or Chk1 (by chemical inhibitors such as UCN-01). Despite the fact that the applicability of UCN-01 in the clinic seems limited due to sequestration of the inhibitor by plasma proteins (reviewed in Dixon and Norbury, 2002), new small molecule inhibitors of Chk1, possibly even more specific than UCN-01, are emerging (Sørensen et al., 2003), and this strategy to sensitize tumors is clearly worth exploring further.

A conceptually distinct approach is to selectively enhance cell death in cancer cells that often express higher than normal levels of the proapoptotic E2F1 transcription factor, itself a substrate of Chk2 (Stevens et al., 2003; see Figure 2). The first study following this strategy, based on tumor-selective suprathreshold stabilization of E2F1 by β-lapachone, produced encouraging results (Li et al., 2003), and clinical trials along this route should follow. Given the wide range of genetic defects in individual tumors, exploring alternative strategies to modulate checkpoint responses in cancer therapy is clearly warranted, and such interference may eventually require tailor-made decisions based on profiling of each tumor for defects in the relevant pathways.

Finally, while most experiments in this direction, as well as the initial clinical trials using UCN-01, target Chk1, the intriguing phenotypes of Chk2-deficient mice may inspire analogous attempts with chemical inhibitors of Chk2. Thus, given the resistance of multiple cell types lacking Chk2 to radiation-induced apoptosis (Hirao et al., 2002; Takai et al., 2002), perhaps chemical inhibition of Chk2 during radiation might protect sensitive tissues such as lymphocytes or intestinal epithelium from the side effects of radiotherapy or drugs that cause DSBs. The critical issue here would be to identify suitable inhibitors of Chk2 and test whether this strategy could be applied without increasing the incidence of tumors.

Emerging roles: Chk1 a "workhorse," Chk2 an "amplifier" of checkpoint responses

Considering their overlapping substrate specificities on the one hand and differential requirements for embryogenesis and viability and different roles in oncogenesis on the other, simple models of redundant or parallel functions performed by Chk1 and Chk2 in responses to genotoxic stress seem very unlikely. Instead, their emerging biological mission is one of mutual complementation and intimate cooperation, a partnership where Chk1 operates as a workhorse, while Chk2 contributes decisively yet only under circumstances that cause DSBs. This concept can perhaps best be illustrated by the involvement of Chk1 and Chk2 in regulation of the protooncogenic human Cdc25A phosphatase in response to ionizing radiation, a mechanism which requires a series of phosphorylation events performed jointly in cooperation by Chk1 and Chk2 (Sørensen et al., 2003). Chk1 alone targets the same four residues of Cdc25A even in normal unperturbed cell cycles (Sørensen et al., 2003), and this function of Chk1 is required to regulate the physiological turnover of Cdc25A, and thereby normal cell cycle transitions. This workhorse function of Chk1 is also a prerequisite for the DNA damage-induced accelerated degradation of Cdc25A (Zhao et al., 2002; Sørensen et al., 2003), a ubiquitin/proteasome-mediated process which silences Cdc25A. The resulting inhibition of the G1/S- and G2/M-promoting cyclin-dependent kinases leads to promptly deployed cell cycle delays in G1 (Mailand et al., 2000), in S (Falck et al., 2001), as well as in G2 (Mailand et al., 2002) phases of the cell cycle. Conceptually, the essential role of Chk1 in the maintenance of unperturbed S phase events shows one example of why *Chk1* is an essential gene which cannot be substituted even by a functionally overlapping kinase such as Chk2. Together with the lethal consequences of Chk1 deficiency in mammals, this model could also explain the paucity of cancer-associated mutations of Chk1.

In contrast, the nonessential Chk2 kinase contributes to the maintenance of genomic integrity only conditionally, and partly in a redundant fashion, to some extent replacable by the essen-

tial workhorse Chk1, and possibly even by other kinases such as cTAK or Plk3. These features are consistent with Chk2 being dispensible for embryonic development and viability, and with the observed low-penetrance tumor suppressor function in both human and mouse tumorigenesis. In the latter model of Chk2-deficient mice, such a tumor suppressor role of Chk2 is manifest mainly under treatment of the mice with external carcinogens, i.e., under conditions when the otherwise "dormant" tumor suppressor function of this checkpoint amplifier is challenged by the increased burden of genotoxic stress. To follow the example quoted above for Chk1, one such situation demanding Chk2 function is the generation of DSBs in S phase, a particularly vulnerable period of the cell cycle, when Chk2 increases the rate of phosphate exchange on Cdc25A residues targeted by Chk1 (Sørensen et al., 2003), and thereby amplifies the "housekeeping" function of Chk1.

There are still a large number of issues to be addressed before the current knowledge about Chk1 and Chk2 can be applied for the benefit of cancer patients. Among the immediate tasks is the search for additional substrates of these kinases, better mechanistic understanding of their integration within the checkpoint network, and a more comprehensive assessment of the extent and clinical as well as epidemiological significance of cancer-associated aberrations of Chk2 particularly. Last but not least, isolation of new small molecule inhibitors of Chk1 and Chk2 and design and validation of novel strategies of checkpoint modulation, combined with the traditional radiation and chemotherapy modalities, hold promise for improved treatment of cancer.

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