Biochemical Pharmacology 80 (2010) 423–430

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Apoptosis and cancer stem cells: Implications for apoptosis targeted therapy

Frank A.E. Kruyt [a,*], Jan Jacob Schuringa [b]

[a] Department of Medical Oncology, University Medical Center Groningen, University of Groningen, P.O. Box 30001, 9700RB Groningen, The Netherlands
[b] Department of Hematology, University Medical Center Groningen, University of Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 24 February 2010
Accepted 6 April 2010

Keywords:
Tumor-initiating cells
Resistance
Epithelial–mesenchymal transition
Microenvironment
Anoikis
Plasticity

ABSTRACT

Evidence is accumulating showing that cancer stem cells or tumor-initiating cells are key drivers of tumor formation and progression. Successful therapy must therefore eliminate these cells, which is hampered by their high resistance to commonly used treatment modalities. Thus far, only a limited number of studies have addressed the cancer stem cell killing potential of apoptosis targeted therapies and mechanisms of apoptosis resistance in these cells. Apoptosis resistance may involve inherent cellular mechanisms that may change depending on the differentiations status of stem cells and, on the other hand, extrinsic factors provided by the microenvironment such as secreted survival factors, adhesion-mediated apoptosis resistance and hypoxic conditions. In order to metastasize, cancer stem cells from solid tumors have to break free from their primary epithelial sites and resist cell death activation after detachment (anoikis). The induction of an embryonic genetic program causing the transition from an epithelial to a mesenchymal state (EMT) has been implicated in enhanced migration and metastatic spread of tumor cells and may contribute to apoptosis and anoikis resistance. Considering the plasticity of cancer stem cells the question arises whether a particular apoptosis-inducing strategy will be sufficient for eliminating all the cellular appearances of these cells, also taking into account a varying microenvironment. Here, the different mechanisms of apoptosis resistance that may be encountered in the context of cancer stem cell plasticity described thus far are discussed in relation to the efficacy of apoptosis therapies, such as TRAIL, BCL-2 family and XIAP targeted therapies.

1. Apoptosis targeted therapy

The activation of programmed cell death or apoptosis in tumor cells is a promising strategy for the treatment of cancer [1]. Typically, apoptosis can be triggered by activation of the extrinsic or death receptor pathway or via the intrinsic or mitochondrial pathway. For targeting these pathways in a therapeutic setting predominantly recombinant proteins or agonistic monoclonal antibodies are currently being exploited for activation of the death receptor route. Small-molecule inhibitors or anti-sense approaches are mainly used to target key factors that regulate mitochondrial apoptosis or that are involved in the execution of apoptosis. For example, receptors for the TNF-related apoptosis-inducing ligand (TRAIL) can be targeted by recombinant TRAIL or agonistic antibodies leading to the selective apoptosis activation in tumor cells, leaving normal cells unharmed [2,3]. After binding to TRAIL-R1 (DR4) or -R2 (DR5), apoptosis is triggered by the intracellular formation of a death-inducing signaling complex (DISC) that consists of FAS-associated death domain (FADD) and procaspase-8, causing cleavage and activation of the latter into active caspase-8. Active caspase-8 can either directly cleave and activate executioner caspases, such as caspase-3, or cleave the BCL-2 family member BID resulting in mitochondrial permeabilization and the triggering of caspase-9 that on its turn activates caspase-3 leading to the disassembly of the cell [4]. A well known inhibitor of this route is FLIP (FLICE-inhibitory protein), a catalytically inactive procaspase-8/-10 homologue that after recruitment to the DISC prevents apoptosis activation. The BCL-2 family members are key regulators of mitochondrial apoptosis in which the balance between anti-apoptotic members, such as BCL-2, BCL-XL, BCL-w, MCL-1, the pro-apoptotic members BAX and BAK and the BH3-only proteins including BID, BIM, PUMA and NOXA determines whether mitochondrial pores are formed and apoptosis is activated [5]. Examples of mitochondria-targeted agents are the small-molecule inhibitors ABT-737 and GX15-070 (obatoclax) that target anti-apoptotic members of the BCL-2 family thereby facilitating mitochondria permeabilization [6,7]. The inhibitor of apoptosis (IAP) gene family, in particular X-linked IAP (XIAP) that can bind to and inhibit caspases-9 and -3, is exploited for therapy by making use of anti-sense and small-molecule inhibitor strategies leading to its suppression and thus stimulation of caspase activation [8]. The combined use of these agents with standard therapies or

* Corresponding author. Tel.: +31 50 3615531; fax: +31 50 3614862.
E-mail address: f.a.e.kruyt@onco.umcg.nl (Frank A.E. Kruyt).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.04.010

combinations of these pro-apoptotic agents is usually most effective in preclinical models and currently a number of these pro-apoptotic agents are being evaluated in clinical studies.

The observed tumor selectivity of these treatments is assumed to involve the need of tumor cells to prevent oncogene-dependent apoptosis activation, one of the hallmarks of carcinogenesis. By enhancing anti-apoptosis signaling, tumor cell survival becomes more dependent on anti-apoptotic pathway activation compared to normal cells, thus providing a potential therapeutic window [9]. In contrast however, as generally the case for anticancer therapeutic strategies, the efficacy of apoptosis targeting agents is frequently limited by mechanisms of resistance encountered in tumor cells. The increasingly becoming established cancer stem cell model provides new molecular and cellular insights that can be exploited for the development of better and smarter therapies. In this context, here we will address mechanisms that may be involved in counteracting pro-apoptotic therapies taking into account cancer stem cell plasticity and extrinsic factors that can modulate apoptosis sensitivity.

2. The cancer stem cell model

The conventional model of tumor initiation and progression in which every cell within a tissue has the capacity to generate tumors has been challenged by the identification of tumor-initiating cells or cancer stem cells (CSCs). CSCs likely derive from normal tissue stem cells or early progenitors that already possess self-renewal and unlimited proliferation potential that are also associated with CSCs [10]. Moreover, the long life-span of normal stem cells compared to short-lived differentiated progenitors or terminally differentiated cells would facilitate the accumulation of genetic aberrations which lead to cancer formation [11]. Although these assumptions are currently under investigation and a possible dedifferentiation of more differentiated tissue cells into CSCs cannot be excluded, evidence is accumulating for normal stem cells or early progenitors being at the basis of tumor formation. For example, in rapidly renewing intestinal (small intestine and colon) epithelium normal stem cells have been identified at the bottom of the crypts based on the expression of the Wnt target gene *Lgr5*, also known as *Gpr49*, using knock-in mice models [12]. Disruption of the *APC* (adenomatous poliposis coli) gene leading to the constitutive activation of the Wnt pathway, a well known initiation step in intestinal cancer, was mimicked in mice by selectively deleting *Apc* in the Lgr5-expressing stem cells using an inducible Cre recombinase system. This resulted in the formation of macroscopic adenomas, a first step in malignant transformation of the intestine. Interestingly, when the *Apc* gene was deleted in more differentiated and short-lived progenitors, micoradenomas could be observed that however lacked growth potential and did hardly result in macroscopic adenoma formation [13]. The notion that CSCs may form a small fraction of the total number of tumor cells was illustrated in this intestinal cancer mouse model, where 36 days after tumor-initiation a small fraction of the tumor cells (6.5%) retained Lgr5 expression, the majority (bulk of the tumor) thus representing more differentiated progenitors [13]. Furthermore, in the same mouse model, the notion that normal or cancer SCs would be relatively quiescent and would undergo cell division infrequently was challenged by the observation that the Lgr5-positive cells were cycling almost as fast as their progenitors [14].

The great value of mouse models in determining the cellular origin of cancer and to study stem cell biology was also demonstrated for highly infiltrative and incurable malignant astrocytomas/gliomas. Inactivation of the tumor suppressors *p53*, *Nf1*, and *Pten* in normal neural stem/progenitor cells and not in differentiated glial cells was able to give rise to high-grade astrocytomas that displayed infiltration and multilineage differentiation [15].

In patient-derived tumor material obtained from different hematological and solid tumor types evidence for the existence of CSCs is accumulating [16–18]. Not surprisingly, first in hematological malignancies making use of the extensive knowledge available on hematopoietic stem cells (HSC) and differentiated cell lineages in this system. In acute myeloid leukemia (AML) the cell surface markers $CD34^+/CD38^-$ that are the same markers used for the isolation of HSC, were found to enrich for AML cells with leukemia initiating capacity when transplanted in immunodeficient (NOD/SCID) mice [19]. In solid tumors, including glioblastoma, breast cancer, colon cancer, lung cancer, ovarian cancer, CSC fractions have been enriched making use of single or multiple cell surface markers, such as $CD133^+$ (glioblastoma, colon cancer, lung cancer, ovarian cancer), $CD44^+/CD24^{low}$ (breast cancer), $CD133^+$ or $CD44^+/CD117^+$ (ovarian cancer) [17,18]. High multidrug resistance (MDR) pump activity associated with high expression of the ATP-binding cassette B5 (ABCB5) or ABCG2/Bcrp1, but also elevated aldehyde dehydrogenease 1 (ALDH1) activity, are thought to be more common markers for (cancer) stem cells and are also being exploited for the enrichment of CSCs [20,21]. There is a broad consensus that stem cell assays are essential for confirming the stem cell features of candidate CSCs enriched by cell surface markers, high MDR or ALDH1 activity [22]. Tumor-initiating, self-renewal and lineage capacity should be demonstrated by (serial) transplantation assays in (immunodeficient) mice and/or *in vitro* assays such as colony-formation, spheroid growth and label-retention assays. However, the stem cell properties of tumor cells will be greatly influenced by the environment to which cells are exposed and therefore a major limitation in this field is the lack of well-established models [23]. Regarding the prevalence of CSCs within a tumor, this is not necessarily a small cell fraction but might vary in size depending on tumor type. Also, variation in CSC prevalence might exist between individual tumors of the same type, and tumors consisting of nearly 100% CSC have been reported [24,25].

The markers used for enriching CSCs appear not to detect all CSCs within a tumor. For example, the use of CD133 as a CSC marker is debated since also CD133 negative cell populations have been shown to have stem cell features [26]. Furthermore, CD133 may enrich for SCs but also progenitor cells as was illustrated in mice intestine where CD133 stained both SCs and a proportion of progenitor cells whereas Lgr5 was selective for only SCs [27]. Thus, research efforts are continuing to identify more accurate CSC markers. For example, recently using a label-retention approach normal mammary SCs were isolated from cultured mammospheres based on their quiescent nature, and cell surface markers $CD49F^+/DLL1^{high}/DNER^{high}$ could be deducted to enrich for breast CSCs [28]. This is different from the $CD44^+/CD24^{low}$ cells have been found earlier to enrich for breast cancer SCs [29]. Novel markers could be either more precise in detecting CSC or could select for different CSC populations within the same tumor, possibilities that need further investigation.

The CSC model has lead to several predictions that make CSCs prime suspects to be investigated in the context of resistance to therapy and metastatic disease. Therapies in general may kill mainly non-CSC that have a limited proliferative potential and more drug sensitive characteristics whereas the remaining resistant CSCs will soon re-establish tumor growth and cause relapse from therapy [30]. Increasing research efforts are directed at the identification of novel therapeutic approaches to target CSCs, such as ones directed against the Wnt, Notch and Hedgehog pathways known to be involved in the regulation of growth and self-renewal properties of CSCs [31].

In the CSC model, CSCs with tumor-proliferation capacity that escape from the primary tumor and settle at secondary sites within the body are thought to be mainly responsible for metastatic disease. During this process the CSCs encounter different cellular surroundings (microenvironments) that may affect sensitivity to apoptosis-inducing therapies by exposing the CSCs to different cocktails of cell survival modulating soluble factors and/or cellular interactions. Recently, a genetic program that is activated in epithelial cells at certain stages of embryonal development that facilitates the release of cells from epithelial layers and increases cellular motility, named epithelial–mesenchymal transition (EMT) has been implicated in metastasis [32,33]. The high plasticity of CSCs and their exposure to changing microenvironments is likely to play an important role in their susceptibility to apoptosis targeting agents. In this context, here we will describe the available evidence obtained for apoptosis resistance in CSCs and implications for apoptosis targeted therapies.

3. Inherent apoptosis resistance in CSCs

Cancer stem cells, like normal tissue stem cells, are thought to be apoptosis resistant in order to secure the production of progeny. Although as yet not extensively studied, this could be caused by intracellular mechanisms that lead to blockades in the apoptotic pathways. Evidence for this in solid tumors predominantly has been provided for brain tumors, i.e. glioblastoma (GB). Apoptosis resistance was illustrated in $CD133^+$ GBSCs isolated from glioblastoma patients in comparison to $CD133^-$, non-CSC fractions by enhanced resistance to different chemotherapeutic agents that was associated amongst others with higher levels of expression of several anti-apoptotic mRNAs, including BCL-2, BCL-XL, IAPs and FLIP [34]. More direct evidence for apoptosis resistance in GBSC has been obtained by showing that TRAIL resistance in $CD133^+$ GBSCs compared to $CD133^-$ cells is related to the suppression of caspase-8 expression as a result of promoter methylation of the *CASP8* gene [35]. The DNA demethylation agent 5-Aza-2′-deoxycytidine could restore caspase-8 expression that was, however, not sufficient for making the GBSC sensitive for TRAIL. No differences in cFLIP expression were found between $CD133^+$ en $CD133^-$ cells, making a role for this protein in resistance unlikely. Also a proteome array consisting of 30 apoptosis-related proteins did not reveal additional clues for resistance and therefore the precise mechanism of TRAIL resistance remains elusive [35].

In normal neural stem/progenitor cells (NPCs) apoptosis resistance has also been examined. These cells are present in the developing and adult central nervous system where they can either self-renew or differentiate into three main cell types, neurons, astrocytes and oligodendrocytes. Apoptosis plays a key role during the development of the central nervous system as well as in brain maturation [36]. A lack of caspase-8 expression was found to be responsible for resistance to death receptor-induced apoptosis in adult and embryonic NPCs in response to recombinant TRAIL, anti-CD95/Fas and TNFα [37]. Evidence was presented that during differentiation of the NPCs *in vitro* caspase-8 levels increased that correlated with sensitivity to death receptor-induced apoptosis. However, further analyses of the mechanism of resistance in NPCs showed that in addition to a lack of caspase-8 expression also high expression levels of the DISC inhibitory protein PED/PEA-15 was involved, which decreased during differentiation. In another study using human fetal cortical NPCs, despite the detected high levels of TRAIL-R2 in these cells, TRAIL failed to trigger apoptosis caused by low levels of caspase-8 mRNA and protein [38]. In addition, the IAP member cIAP-1 also contributed to TRAIL resistance in these NPCs and following its RNA interference-mediated silencing sensitivity to apoptosis could be restored. Interestingly, an alternative role for death receptor signaling by CD93/FASL was demonstrated in neural stem cells leading to stem cell survival and neuronal specification involving Src/PI3K/AKT/mTOR signaling [39].

Studies with transgenic mice and in particular knock-out mice in which Bcl-2 family members and caspases-9 and -3 were deleted have shown the importance of the intrinsic/mitochondrial route in apoptotic decision making in NPCs and progeny, which is essential for proper neuronal development (for review see [36,40]). For example, deletion of the pro-apoptotic Bcl-2 (in mice) members Bax and Bak in double knock-out mice were found to regulate the number of NPCs, but on the other hand were not required for apoptosis activation after neurotoxic injury [41]. Targeting of BCL-2 family members in glioblastoma with the small-molecule BH3 mimetic ABT-737, which mainly inhibits BCL-2 and BCL-XL, displayed proliferation inhibition and apoptosis activation in non-GBSC fractions whereas GBSCs were resistant. This could be attributed to high levels of another anti-apoptotic BCL-2 member, MCL-1, and its down-regulation by RNA interference re-sensitized these cells [42].

Small-molecule XIAP inhibitors have also been tested for possible therapeutic efficacy in preclinical GBSC models. Radiotherapy-resistant glioblastoma cells, including the GBSC fraction, could be sensitized for γ-irradiation by combined XIAP inhibitor treatment that resulted in enhanced caspases activation in the GB cells, whereas no toxicity was observed in normal rat neurons or glial cells [43].

The functioning of the apoptotic machinery and sensitivity to apoptosis-inducing treatments has been explored in leukemia [44], however, only a relative small number of studies have examined efficacy in the stem cell compartment. In $CD34^+$ hematopoietic stem/progenitor cells resistance to CD95/FASL-mediated apoptosis was observed that was associated with lack of expression of the normal caspase-8 mRNAs and instead high expression of a smaller splice variant, named caspase-8L, was detected. This transcript encodes a caspase-8 variant that can be recruited to the DISC but fails to activate the caspase cascade [45]. Moreover leukemic blasts from acute myelogenous leukemia (AML) patients only expressed this variant and it was postulated that this protection against CD95/FASL apoptosis contributed to leukemic development. The effect of TRAIL alone or in combination with the PI3/Akt survival signaling pathway inhibitor perifosine has been investigated in $CD34^+$ AML patient and healthy donor derived cells. In particular combined drug treatment resulted in reduction of clonagenic activity in patient samples displaying active Akt, and no effects on normal $CD34^+$ cells were reported [46]. Sensitization for TRAIL was mediated by perifosine-induced upregulation of TRAIL-R2 and down-regulation of cFLIP and XIAP. Targeting anti-apoptotic BCL-2 family members with ABT-737 was also demonstrated to have a potent killing effect in the majority of tested stem cells derived from AML patients (4 out of 6) and leaving HSCs from healthy donors unharmed [47].

In addition, TRAIL signaling has been examined in CD133 positive and negative Jurkat (T-lymphoma) and MCF7 (breast cancer) cells [48]. Although the CSC properties of the cells were not experimentally confirmed, CD133 expressing cells were reported resistant to TRAIL-induced apoptosis as a result of high cFLIP levels and down-regulation of cFLIP caused re-sensitization. In contrast to the reports above showing death receptor ligand resistance in CSC fractions, one study has reported TRAIL sensitivity in chemotherapy-resistant colon cancer cell line-derived cells obtained by FACS sorting based on high-drug efflux activity (side-population cells). Elevated levels of TRAIL-R1 expression were suggested to be involved TRAIL sensitivity although stem cell properties of the side-population cells were not confirmed in this model [49].

For a summary of the reported mechanisms of inherent apoptosis resistance described above see Table 1.

Table 1
Mechanisms of inherent apoptosis resistance in normal and cancer stem cells and sensitivity to apoptosis target therapies.

Cell type	Mechanism of apoptosis resistance	Apoptosis targeted therapy resistance	Sensitization strategy	References
Neural progenitor cells (NPC)	Caspase-8 ↓ PED/PEA15 ↑ Caspase-8 ↓ cIAP-1 ↑	DR ligands TRAIL	Differentiation Actinomycin D cIAP-1 RNAi	Ricci-Vitiani et al. [37] Peng et al. [38]
Hematopoietic stem cells (HSC)	No caspase-8 caspase-8L ↑	FasL	–	Mohr et al. [45]
Glioblastoma stem cell (GBSC)	cFLIP ↑ BCL-2/Bcl-XL ↑ XIAP ↑ Caspase-8 ↓ MCL-1 ↑ XIAP ↑	Not determined TRAIL ABT-737 γ-Radiation	– 5-Aza-2′-deoxycytidine[a] MCL-1 RNAi XIAP inhibitors[b]	Liu et al. [34] Capper et al. [35] Tagscherer et al. [42] Vellanki et al. [43]
Leukemic stem cells	No caspase-8 caspase-8L ↑ cFLIP ↑ XIAP ↑ MCL-1 ↑ BCL-2-p	FasL TRAIL ABT-737[c]	– Perifosine (Akt inhibitor) Inhibition of ERK signaling MCL-1 RNAi	Mohr et al. [45] Tazzari et al. [46] Konopleva et al. [47]

[a] Demethylation of *CASP8* gene restored caspase-8 expression, but is not sufficient for apoptosis sensitization.
[b] Both primary cultured glioblastoma cells and GBSCs were sensitized, and no toxicity was seen in nonmalignant neuronal cells.
[c] 4 out of 6 AMLSCs samples were sensitive.

4. Extrinsic factors modulating apoptosis in CSCs

4.1. Soluble factors and apoptosis in the microenvironment

Microenvironmental cells that nurture stem cells and enable them to perform their physiological functions in tissue homeostasis form the stem cell niche. Niche cells are thought to shield stem cells from environmental insults, differentiation inducers and apoptotic stimuli [50]. In a similar way a niche may exist for CSCs that may specify amongst other their self-renewing properties although, as has been hypothesized, CSCs possibly are less dependent on the niche than normal SCs as a result of the accumulation of intrinsic mutations leading to altered niche-independent proliferation properties [51,52]. A relative limited number of studies have addressed the existence of a CSC niche, in particular for leukemic SCs and brain tumor SCs (for reviews see [53,54]). For example, bone marrow endothelial cells in the microenvironment of both normal and leukemic SCs were shown to be required for their homing and engraftment, and factors in the microenvironment were found to promote the survival of AML cells causing resistance to chemotherapy [55,56]. In brain tumors, $CD133^+$ CSCs were shown to localize and bind to vascular endothelial cells that stimulated tumor formation in mice models, which has been taken as evidence for the existence of a vascular niche for brain CSCs [57].

Extrinsic factors such as cytokines, chemokines and signals involving cellular adhesion can be produced by a niche and together with developmental regulatory signaling molecules, such as Hedgehog (Hh), Wnt, Notch and bone morphogenetic proteins (BMP) they have been shown to participate in the control of (C)SC renewal and differentiation. For example, Notch signaling was found to improve the survival of mouse embryonic NPCs that was mediated by an upregulation of Bcl-2 and Mcl-1; RNA interference silencing of these anti-apoptotic genes prevented Notch-dependent survival [58]. Therapeutic targeting of Notch signaling by γ-secretase inhibitors was recently shown to deplete GBSCs and prevent the growth of neurospheres *in vitro* and in xenografts in mice by a mechanism involving the inhibition of Akt survival signaling [59]. More direct connections between Notch signaling and the apoptotic core machinery in tumor cells have also been reported. In Jurkat T lymphoblastoid cells activation of Notch signaling was shown to block mitochondrial and death receptor-mediated apoptosis by increased expression of BCL-2, BCL-XL, cIAP-2 and cFLIP causing chemo and TRAIL resistance [60]. In malignant melanoma cells blocking of Notch signaling resulted in the accumulation of the pro-apoptotic BCL-2 member NOXA that stimulated the activation of apoptosis in a p53-independent way [61].

The Wnt pathway has been shown to inhibit apoptosis signaling and in immature pre-B cell lines activation of Wnt signaling could block TRAIL-induced apoptosis via a not completely elucidated mechanism that at least partially involved TRAIL receptor down-regulation and ERK1/2 and NF-κB signaling [62]. In human colorectal cancer cells TRAIL resistance was linked to Wnt/β-catenin-dependent expression of osteoprotegerin (OPG), a soluble decoy receptor for TRAIL [63]. Recently, the anti-apoptotic BCL-2 member BCL-w was identified as a target gene of the Wnt pathway providing a link with inhibition of mitochondrial apoptosis [64].

Other secreted factors have also been implicated in apoptosis resistance in CSCs. The autocrine production of IL-4 by $CD133^+$ colon CSCs appeared to contribute to protection against apoptosis induced by chemotherapeutics and TRAIL. The use of IL4 neutralizing antibodies sensitized for apoptosis by down-regulation of cFLIP, PED and BCL-XL [65].

A variety of stimuli can activate the NF-κB pathway such as cytokines and stress signals that control many cellular processes including inflammation, the immune response, cell growth and apoptosis. Growth factors and cytokines present in the microenvironment and also hypoxic conditions can induce NF-κB that subsequently leads amongst others to the transcriptional activation of anti-apoptotic targets genes [66]. Moreover, NF-κB is often constitutively activated in hematological and solid tumor cells, which has been associated with genetic aberrations in *NFκB* or *IκB* genes, the latter encoding specific inhibitors of NF-κB [66,67]. The role of NF-κB signaling in normal and malignant stem cells has been explored in a relative small number of studies and particularly in hematological systems. For example, in normal leukemic stem cells (AML $CD34^+$), but not in normal stem cells ($CD34^+$) NF-κB was found to be constitutively activated and to promote survival [68–70]. Treatment with the proteosome inhibitor MG-123 and the sesquiterpine lactone parthenolide PTL, which impair NF-κB signaling, induce apoptosis in AML $CD34^+$ cells [68,70]. The fact that PTL impaired the engraftment potential of AML $CD34^+$ cells, but not of normal $CD34^+$ cells, suggests that not only leukemic progeny but also leukemic stem cells might be effectively targeted using this approach [71]. Recently, a novel aminoparthenolide, DMAPT (LC1) was identified as a novel anti-leukemic agent via its inhibitory effects on NF-κB [72]. Critical targets downstream of NF-κB that mediate the anti-apoptotic signals in leukemic stem cells still need to be determined.

4.2. Adhesion-based apoptosis regulation

Apart from soluble factors in the microenvironment also direct interactions between tumor cells and/or normal cells, and/

or extracellular components/matrix are known to contribute to apoptosis resistance in cancer and may obstruct therapeutic efficacy. Pathways that are associated with adhesion-mediated apoptosis resistance in tumor cells appear to frequently converge in activation of the MAPK/ERK and PI3K/Akt pathways [73]. The MAPK/ERK pathway can directly modulate the apoptotic machinery by for example phosphorylation and degradation of the pro-apoptotic BCL-2 family member BIM [74]. ERK also has been shown to affect the expression of BCL-2, BCL-XL and MCL-1 causing their down-regulation upon ERK inhibition [75]. PI3K/AKT signaling can stimulate survival by phosphorylation-dependent inhibition of pro-apoptotic BAD and caspase-9, or indirectly by suppressing the transcription of BIM, PUMA and NOXA [76]. In another study, inhibition of both ECM–cell and cell–cell interactions in glioblastoma cell lines was required for sensitization for amongst others TRAIL and CD95/FASL-induced apoptosis [77]. In $CD133^+$ GBSCs, including ones obtained from patient specimens, RNA interference-mediated down-regulation of the neuronal cell adhesion molecule L1CAM was found to prevent neurosphere formation and to induce apoptosis, which correlated with reduced tumor formation in a xenograft mice model. However, the mechanism underlying apoptosis activation was not further studied [78]. Adhesion-dependent apoptosis suppression has been reported for HSCs [79], and the disruption of interactions between leukemic and vascular cells via VCAM-1 was found to induce mitochondrial apoptosis [80]. Interestingly, targeting the leukemia microenviroment by inhibition CXCR4 using AMD3465 has been shown to enhance the sensitivity towards the FLT3 inhibitor sorafenib, suggesting that SDF1α/CXCR4 interactions between leukemic stem cells and the bone marrow niche can contribute to resistance against therapy-induced apoptosis [81].

4.3. Hypoxia

Low oxygen levels (hypoxia) associated with SC niches have also been identified as an important factor in the regulation of differentiation and cell death of normal and CSCs and have been recognized as a main cause for resistance to cancer therapeutics (for reviews see [53,82]). The family of hypoxia-inducible transcription factors (HIFs), most notably HIF-1α, plays a key role in cellular adaptation to hypoxia by modulating a range of processes, such as angiogenesis, energy metabolism and cell survival [83]. The direct effect of hypoxia on apoptosis activation in CSCs has as yet not been extensively studied. In a similar way as found in tumor cell culture models, hypoxia-induced apoptosis resistance in CSCs may involve the upregulation of HIF-1α target genes that can regulate the intrinsic apoptotic pathway, including anti-apoptotic MCL-1 and BCL-XL [84,85]. Low oxygen levels can result in decreased rates of intracellular production of reactive oxygen species (ROS) that are also balanced by free radical scavenger systems. Hematopoietic stem cells, NPCs and recently also mammary epithelial normal and malignant SCs have been shown to contain lower ROS levels than their more mature progeny [86]. ROS have dual roles in cancer; on one hand they have an oncogenic effect related to their oxidative DNA damaging activity, and on the other hand ROS can mediate various signaling cascades including the activation of apoptosis that may have a tumor suppressive effect [87]. For example, CD95/FASL death receptor activation was shown to stimulate NADPH oxidase resulting in increased ROS levels and subsequently enhanced caspase-8 activation and apoptosis [88]. Elevated levels of ROS have also been shown to enhance TRAIL-induced apoptosis by upregulation of TRAIL-R2, down-regulation of cFLIP and BCL-2 [89,90]. Thus low ROS levels in (C)SCs may have a suppressive effect on apoptosis activation.

5. Anoikis, epithelial–mesenchymal transition and apoptosis resistance

The CSC model suggests that CSCs with high tumor-forming potential are likely key players in metastatic disease. Metastasis is a multistep process involving dissemination from the primary tumor, invasion of neighboring tissues and entry into circulation in order to establish secondary tumors at distant sites. The different stages in this process pose hurdles for the primary tumor cells thought to make this process highly inefficient [91]. For example, cells that detach from epithelial layers usually undergo apoptosis, named anoikis, as a result of loss of cell–cell and/or cell–extra cellular matrix (ECM)-dependent pro-survival signaling. Tumor cells that originate from epithelia have to suppress anoikis activation and need to acquire anchorage-independent growth capacity in order to disseminate and metastasize. The property of CSCs *in vitro* to growth in non-adherent conditions indicates that they are anoikis resistant, although the precise mechanism of resistance has not been examined directly in this cell population. In tumor cells in general, anoikis can be mediated by both death receptor and mitochondrial-mediated apoptosis and blockades in these routes have been linked to anoikis resistance in malignant cells [92]. Small-molecule inhibitors of cFLIP or its RNA interference-mediated down-regulation caused anoikis sensitization in prostate cancer cells identifying cFLIP as an important resistance factor [93]. Caspase-8 appears to play a pivotal role by mediating both death receptor-induced anoikis as well as death receptor independent anoikis involving interactions with unligated integrins that are able to recruit caspase-8 to the membrane leading to activation as was found in neuroblastoma [94]. In addition, down-regulation of caspase-8 enhanced tumor cell motility and metastasis demonstrating its function as a metastasis suppressor gene. In colorectal cancer cells TRAIL-R2/DR5 was reported as a mediator of anoikis [95]. Members of the BCL-2 family are also involved in regulating anoikis with for example stabilization of MCL-1 or degradation of pro-apoptotic BIM being able to suppress anoikis in oncogene (v-src)-transformed fibroblasts [96]. In the same study the direct down-regulation of MCL-1 by short-hairpin (sh) RNAs or indirectly via PI3K/Akt pathway inhibition leading to increased MCL-1 degradation restored anoikis. Furthermore, the caspase inhibitor XIAP was reported to suppress anoikis and small-molecule antagonists reduced metastasis formation in a prostate cancer mouse model [97].

The increased migratory and invasive phenotype of tumor cells may be facilitated by the activation of epithelial–mesenchymal transition (EMT), a process that has been originally described during embryonal development [32,33]. The reverse, mesenchymal–epithelial transition (MET), can take place at secondary tumor sites in order to establish distant tumors. The relationship between EMT and CSCs is not fully understood. CSCs may be in particular prone to EMT and on the other hand tumor cells that undergo EMT have been found to obtain and display stem cell like characteristics in breast cancer [98]. Several stimuli that trigger EMT activation have been described, including growth factors (transforming growth factor-β (TGFβ), Wnt, Notch), tumor–stroma interactions and hypoxia, in which epigenetic mechanisms and non-coding micro-RNAs play important roles in the mesenchymal reprogramming [33]. Epithelial tumor cells undergoing EMT obtain mesenchymal markers and lose epithelial markers, of which loss of the adhesion protein E-cadherin is a major event leading to disruption of intercellular contacts, allowing cells to detach from the surrounding cells. Transcriptional repressors such as Snail and Slug are important mediators of EMT and suppress the expression of, amongst others, E-cadherin [32] In cell culture models EMT can be triggered by shRNA-mediated silencing of E-cadherin and was shown to promote invasiveness and anoikis resistance in

immortalized breast epithelial cells [99]. The mechanistic relationship between EMT and anoikis resistance has not been extensively investigated as yet, however some evidence has been presented that links activation of Snail and Slug with resistance to p53-mediated apoptosis. This was shown in HSCs that were rescued from irradiation-induced apoptosis by p53-dependent transcriptional activation of Slug that on its turn suppressed the transcription of the pro-apoptotic Bcl-2 (mouse) member Puma in mice models [100]. In stem-like ovarian cancer cells both Snail and Slug were found to repress amongst other genes involved in p53-dependent apoptotic signaling, including *BCL-2*, *BID*, *PUMA* and *CASP9* [101]. Thus EMT inducers have pleiotropic effects that, in addition to inducing a mesenchymal state, also modulate migration and cell survival capacities of cells.

6. Conclusions and perspectives

The CSC model is under ongoing construction [102], but already provides important novel biological insights in tumor formation and progression and gives a basis for understanding tumor cell plasticity and dynamics. The therapeutic consequences are also rapidly emerging, not only by the notion that CSCs need to be eradicated for successful therapy, but also by acknowledging the cellular heterogeneity within tumors and changing microenvironments that are associated with variable sensitivities to treatments. Clearly the success of therapies, in particular those that directly target apoptosis will depend on the status of the apoptotic machinery.

Apoptosis resistance has been reported in normal and cancer stem cells from neuronal or hematopoietic origin whereas, based on the limited available data differentiated progenitors were found to be sensitive (see also Table 1). Furthermore, the transition of epithelial tumor cells to cells with mesenchymal features also affects apoptosis sensitivity and has been particularly linked to anoikis resistance. The role of the microenvironment in the maintenance of stem cell properties has been identified and soluble factors and cellular adhesion have been found to modulate apoptosis sensitivity by inducing signals that can affect the expression of key proteins in the apoptotic machinery. Moreover, changing microenvironments and hypoxic conditions that CSCs encounter during tumor progression may result in different settings of the apoptotic machinery and thus different sensitivities to therapeutics. In Fig. 1, the variability in apoptosis sensitivity in the context of CSC plasticity is depicted in a simplified way. CSCs with an inherent high level of apoptosis resistance can produce different more differentiated progenitor tumor cells or transit amplifying tumor cell lineages that are generally more apoptosis sensitive, although variability in microenvironments will also affect apoptosis susceptibility in different subsets of these tumor cells. In addition, different levels or mechanisms of apoptosis resistance in CSCs may occur caused by variations in mircroenvironments following migration/invasion of CSCs, which can be associated with EMT resulting CSCs with mesenchymal features that display anoikis resistance.

When taking together the currently available reports describing inherent and microenvironment-dependent apoptosis resistance in CSCs, a picture emerges in which either the extrinsic or intrinsic apoptotic pathways, or both routes, can be impaired and that apoptosis susceptibility varies depending on differentiation status and exposure to pro-survival stimuli from the microenvironment. A direct consequence of this is that for example death receptor-targeted agents will be ineffective when caspase-8 expression is lacking, or FLIP or anti-apoptotic BCL-2 family members are overexpressed, as was reported in both normal and cancer SCs. In addition, it is evident that when using BCL-2 family targeting agents it is important to identify and inhibit the anti-apoptotic BCL-2 member that is causing resistance to mitochondrial apoptosis in the CSC compartment. Thus, different apoptosis targeting agents will be needed to effectively eradicate both CSCs and their more differentiated progenitors, in which the greatest challenge is to kill inherent and microenvironment-dictated apoptosis resistant CSCs. Also sensitizing strategies could be applied using combinations that can relieve distinct apoptosis blockades, or by inducing differentiation in the CSC compartment resulting in more apoptosis-prone progenitor cancer cells. The targeting of survival-promoting mechanisms in the microenvironment also appears to be a promising approach, including the disruption of niche–CSC interactions that may result in enhanced therapy/apoptosis sensitivity. The ultimate challenge remains to ensure that normal stem cells will not be harmed. The CSC model provides a framework to start exploring the specific requirements for sensitizing CSCs for apoptosis, which may vary for individual tumor types, continuously taking into account that pathways and mechanisms that are essential for normal stem cell homeostasis are left intact.

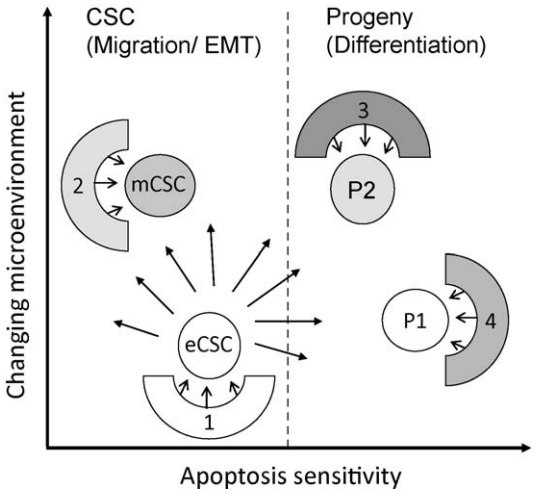

Fig. 1. Inherent- and microenvironment-dependent modulation of apoptosis sensitivity in the context of the CSC model. Schematic representation showing the dynamic nature of apoptosis sensitivity that may vary depending on the differentiation status of the tumor cells and an altering microenvironment (numbered 1–4). Inherent apoptosis resistance in CSCs may be caused by mutations/modifications at the genetic or epigenetic level. In addition, also interactions with the microenvironment (soluble factors, cell–cell and/or cell–ECM interactions (adhesion), hypoxia, represented by arrows) can activate signaling pathways that contribute to apoptosis resistance in CSCs (microenvronment 1). CSCs that migrate and invade surrounding tissues and enter circulation during metastasis run into novel microenvironments that may cause a further increase in apoptosis resistance, represented by microenvironment 2. In tumors from epithelial origin epithelial CSCs (eCSC) may undergo epithelial to mesenchymal transition (EMT) leading to the occurrence of CSC with mesenchymal features (mCSC) that have been associated with anoikis resistance and the onset of metastatic disease. CSC-derived more differentiated progenitors (P1 and P2) may be more susceptible to apoptosis and differences in microenvironments (3 and 4) may further modulate apoptosis sensitivity. The changing status of apoptosis susceptibility that is associated with blockades in death receptor and/or mitochondrial apoptosis pathways will require the use of different strategies to efficiently eradicate both CSCs and progeny in a background of a variable microenvironment. See text for more details.

Acknowledgements

FK is supported by grant VU 2006-3567 from the Dutch Cancer Society and TIPharma T3.112. JJS is supported by grants from the Dutch Cancer Society (RUG 2009-4411, RUG 2009-4275, RUG 2009-4566) and The Netherlands Organization for Scientific Research (NWO-VIDI 916.560.82).

References

[1] Reed JC. Drug insight: cancer therapy strategies based on restoration of endogenous cell death mechanisms. Nat Clin Pract Oncol 2006;3(7):388–98.
[2] Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. Nat Rev Drug Discov 2008;7(12):1001–12.
[3] Kruyt FA. TRAIL and cancer therapy. Cancer Lett 2008;263(1):14–25.
[4] Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 1999;15:269–90.
[5] Brunelle JK, Letai A. Control of mitochondrial apoptosis by the Bcl-2 family. J Cell Sci 2009;122(Pt 4):437–41.
[6] Shore GC, Viallet J. Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. Hematology Am Soc Hematol Educ Program 2005;226–30.
[7] Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435(7042):677–81.
[8] LaCasse EC, Mahoney DJ, Cheung HH, Plenchette S, Baird S, Korneluk RG. IAP-Oncogene 2008;27(48):6252–75.
[9] Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature 2004;432(7015):307–15.
[10] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105–11.
[11] Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. Annu Rev Med 2007;58:267–84.
[12] Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003–7.
[13] Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009;457:608–11.
[14] Barker N, van de Wetering M, Clevers H. The intestinal stem cell. Genes Dev 2008;22:1856–64.
[15] Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell 2009;15:45–56. Erratum in: Cancer Cell 2009;15(3):240.
[16] Wang JC, Dick JE. Cancer stem cells: lessons from leukemia. Trends Cell Biol 2005;15(9):494–501.
[17] Ailles LE, Weissman IL. Cancer stem cells in solid tumours. Curr Opin Biotechnol 2007;18(5):460–6.
[18] Cho RW, Clarke MF. Recent advances in cancer stem cells. Curr Opin Genet Dev 2008;18(1):48–53.
[19] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3(7):730–7.
[20] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5(4):275–84.
[21] Douville J, Beaulieu R, Balicki D. ALDH1 as a functional marker of cancer stem and progenitor cells. Stem Cells Dev 2009;18:17–25.
[22] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006;66(19):9339–44.
[23] Hill RP. Identifying cancer stem cells in solid tumors: case not proven. Cancer Res 2006;66(4):1891–5.
[24] Kennedy JA, Barabé F, Poeppl AG, Wang JC, Dick JE. Comment on "Tumor growth need not be driven by rare cancer stem cells". Science 2007; 318(5857):1722.
[25] Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature 2008; 456(7222):593–8.
[26] Bidlingmaier S, Zhu X, Liu B. The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. J Mol Med 2008;86(9):1025–32.
[27] Snippert HJ, van Es JH, van den Born M, Begthel H, Stange DE, Barker N, et al. Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine. Gastroenterology 2009;136(7). 2187–94.
[28] Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 2010;140(1):62–73.
[29] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003;100(7):3983–8. Erratum in: Proc Natl Acad Sci USA 2003;100(11) 6890..
[30] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414(6859):105–11.
[31] Hombach-Klonisch S, Paranjothy T, Wiechec E, Pocar P, Mustafa T, Seifert A, et al. Cancer stem cells as targets for cancer therapy: selected cancers as examples. Arch Immunol Ther Exp (Warsz) 2008;56(3):165–80.
[32] Thiery JP, Sleeman JP. Complex networks orchestrate epithelial–mesenchymal transitions. Nat Rev Mol Cell Biol 2006;7(2):131–42.
[33] Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009; 9(4):265–73.
[34] Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67.
[35] Capper D, Gaiser T, Hartmann C, Habel A, Mueller W, Herold-Mende C, et al. Stem-cell-like glioma cells are resistant to TRAIL/Apo2L and exhibit down-regulation of caspase-8 by promoter methylation. Acta Neuropathol 2009; 117(4):445–56.
[36] De Zio D, Giunta L, Corvaro M, Ferraro E, Cecconi F. Expanding roles of programmed cell death in mammalian neurodevelopment. Semin Cell Dev Biol 2005;16(2):281–94.
[37] Ricci-Vitiani L, Pedini F, Mollinari C, Condorelli G, Bonci D, Bez A, et al. Absence of caspase 8 and high expression of PED protect primitive neural cells from cell death. J Exp Med 2004;200(10):1257–66.
[38] Peng H, Huang Y, Duan Z, Erdmann N, Xu D, Herek S, et al. Cellular IAP1 regulates TRAIL-induced apoptosis in human fetal cortical neural progenitor cells. J Neurosci Res 2005;82(3):295–305.
[39] Corsini NS, Sancho-Martinez I, Laudenklos S, Glagow D, Kumar S, Letellier E, et al. The death receptor CD95 activates adult neural stem cells for working memory formation and brain repair. Cell Stem Cell 2009;5(2):178–90.
[40] Johnsen JI, Kogner P, Albihn A, Hendriksson MA. Embryonal neural tumours and cell death. Apoptosis 2009;14(4):424–38.
[41] Lindsten T, Golden JA, Zong WX, Minarcik J, Harris MH, Thompson CB. The proapoptotic activities of Bax and Bak limit the size of the neural stem cell pool. J Neurosci 2003;23(35):11112–9.
[42] Tagscherer KE, Fassl A, Campos B, Farhadi M, Kraemer A, Böck BC, et al. Apoptosis-based treatment of glioblastomas with ABT-737, a novel small molecule inhibitor of Bcl-2 family proteins. Oncogene 2008;27(52):6646–56.
[43] Vellanki SH, Grabrucker A, Liebau S, Proepper C, Eramo A, Braun V, et al. Small-molecule XIAP inhibitors enhance gamma-irradiation-induced apoptosis in glioblastoma. Neoplasia 2009;11(8):743–52.
[44] Del Poeta G, Bruno A, Del Principe MI, Venditti A, Maurillo L, Buccisano F, et al. Deregulation of the mitochondrial apoptotic machinery and development of molecular targeted drugs in acute myeloid leukemia. Curr Cancer Drug Targets 2008;8(3):207–22.
[45] Mohr A, Zwacka RM, Jarmy G, Büneker C, Schrezenmeier H, Döhner K, et al. Caspase-8L expression protects CD34+ hematopoietic progenitor cells and leukemic cells from CD95-mediated apoptosis. Oncogene 2005;24(14): 2421–9.
[46] Tazzari PL, Tabellini G, Ricci F, Papa V, Bortul R, Chiarini F, et al. Synergistic proapoptotic activity of recombinant TRAIL plus the Akt inhibitor Perifosine in acute myelogenous leukemia cells. Cancer Res 2008;68(22):9394–403.
[47] Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006;10(5):375–88.
[48] Zoblova R, McDermott L, Stantic M, Prokopova K, Dong LF, Neuzil J. CD133-positive cells are resistant to TRAIL due to up-regulation of FLIP. Biochem Biophys Res Commun 2008;373(4):567–71.
[49] Sussman RT, Ricci MS, Hart LS, Sun SY, El-Deiry WS. Chemotherapy-resistant side-population of colon cancer cells has a higher sensitivity to TRAIL than the non-SP, a higher expression of c-Myc and TRAIL-receptor DR4. Cancer Biol Ther 2007;6(9):1490–5.
[50] Moore KA, Lemischka IR. Stem cells and their niches. Science 2006; 311(5769):1880–5.
[51] Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. Cancer Res 2006;66(9):4553–7. Erratum in: Cancer Res 2006;66(12):6458..
[52] Sneddon JB, Werb Z. Location, location, location: the cancer stem cell niche. Cell Stem Cell 2007;1(6):607–11.
[53] Konopleva M, Tabe Y, Zeng Z, Andreeff M. Therapeutic targeting of microenvironmental interactions in leukemia: mechanisms and approaches. Drug Resist Updat 2009;12(4–5):103–13.
[54] Iwasaki H, Suda T. Cancer stem cells and their niche. Cancer Sci 2009; 100(7):1166–72.
[55] Sipkins DA, Wei X, Wu JW, Runnels JM, Côté D, Means TK, et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature 2005;435(7044):969–73.
[56] De Toni F, Racaud-Sultan C, Chicanne G, Mas VM, Cariven C, Mesange F, et al. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. Oncogene 2006;25(22):3113–22.
[57] Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11(1): 69–82.
[58] Oishi K, Kamakura S, Isazawa Y, Yoshimatsu T, Kuida K, Nakafuku M, et al. Notch promotes survival of neural precursor cells via mechanisms distinct from those regulating neurogenesis. Dev Biol 2004;276(1):172–84.
[59] Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 2006;66(15):7445–52.
[60] Sade H, Krishna S, Sarin A. The anti-apoptotic effect of Notch-1 requires p56lck-dependent, Akt/PKB-mediated signaling in T cells. J Biol Chem 2004;279(4):2937–44.
[61] Qin JZ, Stennett L, Bacon P, Bodner B, Hendrix MJ, Seftor RE, et al. p53-independent NOXA induction overcomes apoptotic resistance of malignant melanomas. Mol Cancer Ther 2004;3(8):895–902.
[62] Doubravská L, Símová S, Cermak L, Valenta T, Korínek V, Andera L. Wnt-expressing rat embryonic fibroblasts suppress Apo2L/TRAIL-induced apoptosis of human leukemia cells. Apoptosis 2008;13(4):573–87.
[63] De Toni EN, Thieme SE, Herbst A, Behrens A, Stieber P, Jung A, et al. OPG is regulated by beta-catenin and mediates resistance to TRAIL-induced apoptosis in colon cancer. Clin Cancer Res 2008;14(15):4713–8.

[64] Lapham A, Adams JE, Paterson A, Lee M, Brimmell M, Packham G. The Bcl-w promoter is activated by beta-catenin/TCF4 in human colorectal carcinoma cells. Gene 2009;432(1–2):112–7.
[65] Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell 2007;1(4):389–402.
[66] Prasad S, Ravindran J, Aggarwal BB. NF-kappaB and cancer: how intimate is this relationship. Mol Cell Biochem 2010;336:25–37.
[67] Turco MC, Romano MF, Petrella A, Bisogni R, Tassone P, Venuta S. NF-kappaB/Rel-mediated regulation of apoptosis in hematologic malignancies and normal hematopoietic progenitors. Leukemia 2004;18(1):11–7.
[68] Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, et al. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 2001;98(8):2301–7.
[69] Birkenkamp KU, Geugien M, Schepers H, Westra J, Lemmink HH, Vellenga E. Constitutive NF-kappaB DNA-binding activity in AML is frequently mediated by a Ras/PI3-K/PKB-dependent pathway. Leukemia 2004;18(1):103–12.
[70] Schepers H, Eggen BJ, Schuringa JJ, Vellenga E. Constitutive activation of NF-kappa B is not sufficient to disturb normal steady-state hematopoiesis. Haematologica 2006;91(12):1710–1.
[71] Guzman ML, Rossi RM, Karnischky L, Li X, Peterson DR, Howard DS, et al. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood 2005;105(11):4163–9.
[72] Neelakantan S, Nasim S, Guzman ML, Jordan CT, Crooks PA. Aminoparthenolides as novel anti-leukemic agents: discovery of the NF-kappaB inhibitor, DMAPT (LC-1). Bioorg Med Chem Lett 2009;19(15):4346–9.
[73] Westhoff MA, Fulda S. Adhesion-mediated apoptosis resistance in cancer. Drug Resist Updat 2009;12(4–5):127–36.
[74] Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ. Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. J Biol Chem 2003;278(21).
[75] Boucher MJ, Morisset J, Vachon PH, Reed JC, Lainé J, Rivard N. MEK/ERK signaling pathway regulates the expression of Bcl-2, Bcl-X(L), and Mcl-1 and promotes survival of human pancreatic cancer cells. J Cell Biochem 2000;79(3):355–69.
[76] Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129(7):1261–74.
[77] Westhoff MA, Zhou S, Bachem MG, Debatin KM, Fulda S. Identification of a novel switch in the dominant forms of cell adhesion-mediated drug resistance in glioblastoma cells. Oncogene 2008;27(39):5169–81.
[78] Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res 2008;68(15):6043–8.
[79] Wang MW, Consoli U, Lane CM, Durett A, Lauppe MJ, Champlin R, et al. Rescue from apoptosis in early (CD34-selected) versus late (non-CD34-selected) human hematopoietic cells by very late antigen 4- and vascular cell adhesion molecule (VCAM) 1-dependent adhesion to bone marrow stromal cells. Cell Growth Differ 1998;9(2):105–12.
[80] Petit I, Karajannis MA, Vincent L, Young L, Butler J, Hooper AT, et al. The microtubule-targeting agent CA4P regresses leukemic xenografts by disrupting interaction with vascular cells and mitochondrial-dependent cell death. Blood 2008;111(4):1951–61.
[81] Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood 2009;113(24):6215–24.
[82] Panchision DM. The role of oxygen in regulating neural stem cells in development and disease. J Cell Physiol 2009;220(3):562–8.
[83] Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3(10):721–32.
[84] Shroff EH, Snyder C, Chandel NS. Role of Bcl-2 family members in anoxia induced cell death. Cell Cycle 2007;6(7):807–9.
[85] Chen N, Chen X, Huang R, Zeng H, Gong J, Meng W, et al. BCL-xL is a target gene regulated by hypoxia-inducible factor-1{alpha}. J Biol Chem 2009;284(15):10004–12.
[86] Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009;458(7239):780–3.
[87] Wang J, Yi J. Cancer cell killing via ROS: to increase or decrease, that is the question. Cancer Biol Ther 2008;7(12):1875–84.
[88] Reinehr R, Becker S, Eberle A, Grether-Beck S, Häussinger D. Involvement of NADPH oxidase isoforms and Src family kinases in CD95-dependent hepatocyte apoptosis. J Biol Chem 2005;280(29):27179–94.
[89] Kim YH, Jung EM, Lee TJ, Kim SH, Choi YH, Park JW, et al. Rosiglitazone promotes tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by reactive oxygen species-mediated up-regulation of death receptor 5 and down-regulation of c-FLIP. Free Radic Biol Med 2008;44(6):1055–68.
[90] Kim S, Lee TJ, Leem J, Choi KS, Park JW, Kwon TK. Sanguinarine-induced apoptosis: generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. J Cell Biochem 2008;104(3):895–907.
[91] Eccles SA, Welch DR. Metastasis: recent discoveries and novel treatment strategies. Lancet 2007;369(9574):1742–57.
[92] Simpson CD, Anyiwe K, Schimmer AD. Anoikis resistance and tumor metastasis. Cancer Lett 2008;272(2):177–85.
[93] Mawji IA, Simpson CD, Gronda M, Williams MA, Hurren R, Henderson CJ, et al. A chemical screen identifies anisomycin as an anoikis sensitizer that functions by decreasing FLIP protein synthesis. Cancer Res 2007;67(17):8307–15.
[94] Stupack DG, Teitz T, Potter MD, Mikolon D, Houghton PJ, Kidd VJ, et al. Potentiation of neuroblastoma metastasis by loss of caspase-8. Nature 2006;439(7072):95–9.
[95] Laguinge LM, Samara RN, Wang W, El-Deiry WS, Corner G, Augenlicht L, et al. DR5 receptor mediates anoikis in human colorectal carcinoma cell lines. Cancer Res 2008;68(3):909–17.
[96] Woods NT, Yamaguchi H, Lee FY, Bhalla KN, Wang HG. Anoikis, initiated by Mcl-1 degradation and Bim induction, is deregulated during oncogenesis. Cancer Res 2007;67(22):10744–52.
[97] Berezovskaya O, Schimmer AD, Glinskii AB, Pinilla C, Hoffman RM, Reed JC, et al. Increased expression of apoptosis inhibitor protein XIAP contributes to anoikis resistance of circulating human prostate cancer metastasis precursor cells. Cancer Res 2005;65(6):2378–86.
[98] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell 2008;133(4):704–15.
[99] Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res 2008;68(10):3645–54.
[100] Wu WS, Heinrichs S, Xu D, Garrison SP, Zambetti GP, Adams JM, et al. Slug antagonizes p53-mediated apoptosis of hematopoietic progenitors by repressing puma. Cell 2005;123(4):641–53.
[101] Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, et al. Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells 2009;27(9):2059–68.
[102] Marotta LL, Polyak K. Cancer stem cells: a model in the making. Curr Opin Genet Dev 2009 Feb;19(1):44–50.