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Mini-review

Regulation of apoptosis pathways in cancer stem cells

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ABSTRACT

Cancer stem cell are considered to represent a population within the bulk tumor that share many similarities to normal stem cells as far as their capacities to self-renew, differentiate, proliferate and to reconstitute the entire tumor upon serial transplantation are concerned. Since cancer stem cells have been shown to be critical for maintaining tumor growth and have been implicated in treatment resistance and tumor progression, they constitute relevant targets for therapeutic intervention. Indeed, it has been postulated that eradication of cancer stem cells will be pivotal in order to achieve long-term relapse-free survival. However, one of the hallmarks of cancer stem cells is their high resistance to undergo cell death including apoptosis in response to environmental cues or cytotoxic stimuli. Since activation of apoptosis programs in tumor cells underlies the antitumor activity of most currently used cancer therapeutics, it will be critical to develop strategies to overcome the intrinsic resistance to apoptosis of cancer stem cells. Thus, a better understanding of the molecular mechanisms that are responsible for the ability of cancer stem cells to evade apoptosis will likely open new avenues to target this critical pool of cells within the tumor in order to develop more efficient treatment options for patients suffering from cancer.

1. Introduction

According to the cancer stem cell model, human cancers originate in tissue stem or progenitor cells, and only a small fraction of the bulk tumor, i.e. the cancer stem cells (CSCs) have the capacity to proliferate indefinitely [1,2]. There exist many parallels between normal stem cells and cancer-initiating cells, which were first described in acute myeloid leukemia (AML) stem cells in comparison to non-malignant hematopoietic stem and progenitor cells [1]. For example, both of these cell types have the capacity to self-renew and differentiate [3–9]. Furthermore, it is interesting to note that CSCs tend to metastasize to similar organs and tissues much like somatic stem cells migrate to distant tissues. Another shared characteristic feature of both cell types is their resistance to apoptotic stimuli [10–12]. The physiological processes of self-renewal and differentiation are typically disturbed in cancer stem cells resulting in abnormal and uncontrolled differentiation and increased self-renewal capacity [5–9,13]. Thus, any disturbance of the tight control of self-renewal, proliferation, differentiation and cell fate (proliferation versus apoptosis) of tissue stem or progenitor cells may favor the emergence of cancer stem cells and tumorigenesis [2].

Cancer stem cells may originate from a tissue stem or progenitor cell via oncogenic transformation [14]. Alternatively, stem cell pathways may be aberrantly re-activated in a committed progenitor or even more mature cell type, leading to dedifferentiation and the acquisition of stem cell properties [14]. Cancer stem cells are considered to give rise to the clinically observed, phenotypically diverse tumor population consisting of cells displaying varied capacities for abnormal differentiation, uncontrolled proliferation, and evasion of death signals. Therefore, a paradigm is emerging that an effective therapeutic approach against cancers has to take into consideration to target this critical pool of cells. The current review will focus on the different mechanisms of defective apoptotic signaling in CSCs.

2. Cancer stem cells in leukemia and solid cancers

Cancer stem cells have been identified in both hematological malignancies as well as solid tumors. AML has been the first cancer entity in which *bona fide* CSCs have been identified [15]. In addition, most of the initial work on cancer stem cells was performed in leukemia, since the hematopoietic system shares many similarities in the developmental biology and hierarchy during normal hematopoiesis with leukemic stem cells. For example, AML and normal hematopoietic stem cells have in common some phenotypic surface markers (i.e. CD34+ CD38−), pointing to the origin of AML stem cells from hematopoietic stem cells [1,16,17]. However, there is also a cautious note concerning phenotypic surface markers, since there is also evidence that the CD34+ CD38+ AML population harbors leukemia-initiating capacity under certain conditions [18].

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This indicates that additional parameters besides phenotypic markers may be more relevant to identify the leukemia-initiating fraction within the bulk of the disease [18]. Back in the mid-nineties, first evidence was provided that a very small population of CD34+ CD38− acute myeloid leukemia (AML) cells (less than 1%) exhibit the characteristics of stem cells and that only this fraction was able to generate leukemia in immunodeficient mice [19].

Besides AML, chronic myeloid leukemia (CML) represents a well-studied model for the CSC hypothesis [20]. Accordingly, the BCR-ABL oncoprotein has been reported to be expressed at high levels in the most primitive precursor cells including early stem cell progenitors as well as in several cell lineages (i.e. lymphoid and myeloid lineages, and platelets) [20].

Following the initial investigations in hematological malignancies, CSCs have now been identified in a range of solid tumors, including breast, lung, gastric, head and neck, prostate, colon, pancreatic and liver carcinoma, melanoma, multiple myeloma medulloblastoma as well as brain tumors [5–9,21–35].

Transplantation experiments were used to demonstrate the self-renewal potential of CSC following serial transplantation and to test whether phenotypically similar tumors were generated in animal models compared to the initial primary tumor sample [5–9,22,25,30,33,36,37]. In addition to these functional assays, a panel of cell surface markers has been evaluated in order to isolate and characterize CSCs. These comprise among others CD133, CD44, CD24, CD90, epithelial cell adhesion molecule, THY1, ATP-binding cassette B5 (ABCB5) and aldehyde dehydrogenase 1 (ALDH1) [5–9,22,25,30,32,33,36,37].

Based on the concept that stem cells and CSCs have in common several major characteristics, including unlimited self-renewal capacity, it is also assumed that similarities in key signaling pathways also exist. For example, several developmental pathways that have been implicated during embryogenesis and in self-renewal of normal tissue stem and progenitor cells have recently been linked to the regulation of CSC functions, including the Hedgehog, Wnt or Notch pathway [38–40].

3. Apoptosis signaling pathways

Two key apoptosis signaling pathways have been delineated that eventually result in the activation of caspases that act as common death effector molecules of apoptotic cell death [41]. The death receptor (extrinsic) pathway is activated in response to cross-linking of transmembrane cell surface receptors of the death receptor family, including CD95 and TRAIL receptors [42]. This leads to the assembly of a multi-protein complex at activated death receptors that drives caspase-8 activation [42]. Once activated, caspase-8 either directly cleaves and activates effector caspase-3 or, alternatively, processes Bid into the active fragment tBid, which translocates to mitochondrial membranes to initiate mitochondrial outer membrane permeabilization.

The mitochondria (intrinsic) pathway can be engaged by a large variety of intracellular stimuli, for example cellular stress conditions [43]. Mitochondrial outer membrane permeabilization results in the release of apoptogenic proteins such as cytochrome c or Smac from the mitochondrial intermembrane space into the cytosol [43]. This leads to complex formation of cytochrome c, Apaf-1 and caspase-9 to build the apoptosome or alternatively to neutralization of 'Inhibitor of apoptosis' (IAP) proteins by Smac [44]. Apoptosis signaling networks are tightly controlled for example, by pro- and anti-apoptotic members of the Bcl-2 protein family [45].

CSCs typically harbor intrinsic or acquired defects and/or inefficient signaling in either the extrinsic or intrinsic pathway of apoptosis as discussed in further details below.

4. Apoptosis and cancer stem cells

In addition to mechanisms that control proliferation, differentiation and self-renewal of CSCs, cell death pathways including apoptosis have in recent years been implicated in the control of CSCs. Evasion of apoptosis will likely contribute to the survival of CSCs, since programmed cell death by apoptosis represents a key cellular failsafe mechanism to protect against oncogenic events [46]. This implies that CSCs depend on compensatory mechanisms to escape the induction of apoptosis upon oncogenic transformation. Otherwise, they would not be able to survive and self-renew especially in hostile microenvironments under environmental stress conditions. Inactivation of apoptosis signaling pathways also helps CSCs to evade the cytotoxic activity of most anticancer therapies, since most therapies that are currently used for the treatment of cancers largely depend on intact apoptosis programs for their antitumor efficacy.

4.1. Death receptors and CSCs

Death receptors belong to the TNF receptor superfamily that can initiate cell death upon appropriate stimulation, for example upon binding of their cognate ligands [47]. They all harbor a characteristic intracellular domain called the death domain that transduces the cell death signal from the plasma membrane to intracellular signaling molecules [47]. TRAIL/Apo-2L belongs to the TNF family of death receptor ligands that trigger apoptotic cell death upon binding to one of its two agonistic receptors on the cell surface [42].

Glioma cells with stem cell features such as CD133 expression and neurosphere formation, have been reported to harbor only low levels of caspase-8 mRNA and protein [48]. This was at least in part due to hypermethylation of the caspase-8 promoter [48]. However, restoration of caspase-8 expression by demethylation agents failed to overcome TRAIL resistance [48], suggesting that additional factors contribute to the intrinsic resistance of glioma stem cells towards TRAIL. In addition, heterogeneity of glioblastoma cancer stem cells in the genomic status of caspase-8, the expression of caspase-8 as well as the sensitivity to TRAIL-induced apoptosis has recently been reported [49].

Also, FLIP has been implicated in the resistance of cancer stem cells towards TRAIL. Cancer stem cells with high expression of CD133 were described to harbor high expression levels of FLIP and to display resistance to TRAIL-induced apoptosis compared to cancer cells with low CD133 expression levels [32,50]. Of note, downregulation of FLIP by RNA interference resulted in sensitization of the cancer stem cells population to TRAIL-mediated apoptosis [50], supporting the notion that high FLIP expression contributes to the resistance of cancer stem cells to TRAIL. In support of this notion, treatment of breast cancer stem cells with TRAIL and concomitant knockdown of cFLIP selectively targeted the functional breast cancer stem cells pool [51]. Elimination of stem cell renewal resulted in a marked reduction in primary tumors and in an almost complete suppression of metastases following transplantation [51]. This indicates that combined therapy with TRAIL and FLIP knockdown reduces the breast cancer stem cell pool from breast cancer cell lines, thereby suppressing metastatic disease progression in breast cancer.

In contrast to these studies showing that cancer stem cells exhibit resistance to TRAIL-induced apoptosis, a higher sensitivity of cancer stem cells towards the death receptor ligand TRAIL has also been described. Accordingly, the side population (SP) within the human colon cancer cell line SW480 was shown to express much higher levels of the agonistic TRAIL receptor 1 as compared to the non-SP cells [52]. Also, SP cells turned out to be more sensitive to TRAIL-induced apoptosis than non-SP cells [52]. Higher lev-