Mini review

Destabilization of Abnormal Methylation Enzymes: Nature's Way to Eradicate Cancer Stem Cells

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Cancer Stem Cells (CSCs): The Primary Cause of Cancer Treatment Failure

Cancer therapy is dominated by cytotoxic agents that act via mechanisms that damage DNA and activate apoptotic pathways. While cytotoxic agents have been moderately effective to eliminate symptoms and to extend the life span of cancer patients, this approach rarely results in cure. Therefore, cancer related mortality remains as a leading cause of death in most countries.

Over that past decade it has becomes clear that CSCs stand in the way of successful curative results from conventional cytotoxic cell killing to put cancer away. The transition of the tumor to one containing predominantly CSCs is now thought to be a primary cause of treatment failure [1-5]. Many biological characteristics that enable cancer progression are attributable to CSCs, including angiogenesis, metastasis, and drug resistance. CSCs are resistant to both cytotoxic drugs and radiation in part because these cells overexpress ATP binding cassette drug pumps that effectively exclude cytotoxic drugs and have activation of anti-apoptosis programs that negate the pro-apoptotic signals activated by DNA damaging agents [6-9]. CSCs share much in common with normal progenitor stem cells with respect to cell features and biological missions. Their biological missions are to repair and to meet the replacement needs of the organ or tissue. When cancer cells are destroyed by cytotoxic drugs or radiation, CSCs proliferate to replace the dying cancer cells and the tumor regrows. Eventually CSCs become the dominant tumor component and their unchecked growth claims the life of the patient. Therefore, eventual successful cancer therapies must rely on the elimination of not only cancer cells, but also the subpopulation of CSCs. Alternative therapeutic strategies are needed to eradicate CSCs.

Methylation Enzymes (MEs) Function as A Switch to Turn on to Cell Replication and to Turn Off to Terminal Differentiation (TD)

MEs play a critical role on the regulation of cell replication and differentiation. DNA methylation controls the expression of tissue specific genes Racanelli AC, et al. [10], and pre-rRNA ribose methylation controls the production of ribosomes Liau MC et al. [11], which in turn dictates the commitment of cells to initiate replication [12]. If enhanced production of ribosomes is locked in place, it becomes a factor to drive carcinogenesis [13]. Biological methylation is mediated by a ternary enzyme complex consisting of methionine adenosyl transferase (MAT), methyltransferase (MT), and S-adenosylhomocysteine hydrolase (SAHH) [14,15]. These enzymes must be in a ternary enzyme complex to become stable and functional. In the monomeric state, individual enzymes are quickly inactivated. SAHH is the most unstable enzyme, followed by MT, and then MAT. Their stability corresponds very well to their molecular size. MTs in the monomeric state have a greater tendency to be converted into nucleases to trigger apoptosis. The conversion of MTs into nucleases can be prevented by keeping MTs in the dimeric state with SAHH, or by the use of inhibitors of MTs to resist protease modification. SAHH requires a steroid factor to assume a configuration favorable for the formation of a dimeric enzyme complex with MT, which can then associate with MAT to form the ternary enzyme complex. In steroid hormone target tissues, such as prostate and breast, steroid hormones are the stabilizing factor of SAHH. Other tissues require similar steroid factors generated by growth signals to stabilize SAHH [16]. In normal cells, steroid factors are the dominant factors to modulate MEs.

In cancer cells and telomerase expressing primitive stem cells, such as embryonic stem cells and progenitor stem cells, MAT is associated with telomerase (hTERT) Liau MC et al. [17], which promotes increased stability of the ME complex. The association of MAT^L, which is the normal isozyme of MAT, with telomerase changes the kinetic property of MAT^L and the regulation of MEs. K_m values of MAT^L and MAT^{LT}, the telomerase associated cancer isozyme, are 3 μM and 20 μM methionine, respectively, and those of $SAHH^L$ and $SAHH^{LT}$ are 0.3 μM and 2 μM adenosine, respectively [14,15,17]. The increased K_m value of MAT^{LT} suggests that MEs of cancer cells have elevated levels of bound S-adenosylmethionine (AdoMet). According to Prudova A, et al. [18], the binding of AdoMet to a protein could protect that protein against protease digestion. It appears then that the increased pool size of AdoMet in cancer cells contributes to the stability and activity of MEs to promote malignant growth. Chiba P, et al. [19] found that the pool sizes of AdoMet and S-adenosylhomocysteine (AdoHcy) shrunk greatly when cancer cells were induced to undergo TD. This finding strongly supports our hypothesis that the association of telomerase with MEs greatly increases the stability and the activity of MEs of cancer cells, thereby suppressing the hypomethylation of nucleic acids necessary for cells to undergo TD [15,20]. Thus, it is very clear that abnormal MEs play a critical role in the evolution and progression of cancer.

Abnormal MEs in Cancer

The association of telomerase with MEs locks MEs in an extremely stable and active state. Their increased enzyme function expands the methyl-group pool, blocking cell differentiation. In addition, telomerase activity is enhanced when it resides in the ME complex, enabling increased telomere maintenance and limitless proliferative capacity.

There is another way to achieve DNA demethylation to bypass the differentiation blockade created by abnormal MEs. Ten-eleven translocation protein (Tet) dioxygenases carries out oxidation of 5-methylcytidine (5-mC) to generate 5-hydroxymethycytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5-caC) [21-24]. Five-caC is finally replaced by cytosine by thymine DNA glycosylase and base excision repair [25,26]. Five-hmC is the stable intermediate in the oxidative demethylation of 5-mC [27].

Three Tet dioxygenases have been identified. Tet 1 preferentially acts on 5-mC located at transcriptional start sites, whereas Tet 2 preferentially acts on 5-mC located in the CpG rich domains in the gene body [28]. Tet 3 is expressed at very high level in oocytes and zygotes, but rapidly declines at the two-cell stage. Tet 3 is responsible for the elimination of paternal 5-mC in fertilized oocytes [29]. Tet enzymes are very active in embryonic stem cells to direct embryonic lineage differentiation [30, 31]. These enzymes are frequently mutated to become dysfunctional or silenced in cancer cells [32-35]. The expression and function of Tet enzymes marks a critical difference between cancer cells and primitive normal stem cells. Blockade of differentiation is apparently a normal process to build up cell mass for the development of fetus in the case of embryonic stem cells, or for wound healing in the case of progenitor stem cells. Cancer cells lose the mechanism to break through the blockade of differentiation created by abnormal MEs. Consequently destabilization of abnormal MEs is an attractive option to induce TD of cancer cells.

Destabilization of Abnormal MEs as an Effective Approach for Cancer Therapy

Blockade of differentiation by abnormal MEs may play a more fundamental role in CSCs than the activation of oncogenes or inactivation of suppressor genes. After all, oncogenes and suppressor genes are cell cycle regulatory genes, which have important roles to play when cell are replicating. They have no role to play when replicating cells are diverted to terminally differentiated cells. In one stroke, destabilization of abnormal MEs can induce terminal differentiation to wipe out all damages created by oncogenes and mutated tumor suppressor genes, which have attracted so much attention in the cancer field.

The successful therapy of acute promyelocytic leukemia with all-trans-retinoic acid (ATRA) must be considered as the best example of differentiation therapy in cancer, which was accomplished by destabilization of abnormal MEs. The therapy yields a stunning complete response rate of ~90% [36]. Remissions, however, are transient. Most patients relapse within a year [37,38]. The combination of ATRA and arsenic trioxide produces a more satisfactory, long lasting remission [38]. ATRA is a differentiation inducer (DI) and arsenic trioxide is a differentiation helper inducer (DHI) [16]. DIs are chemicals capable of eliminating the association of telomerase with abnormal MEs, leading to their destabilization and cellular differentiation [39]. DHIs are inhibitors of the individual enzymes of the ternary MEs [40]. DHIs by themselves at very high concentrations can also achieve significant induction of TD. At concentrations inactive as DIs, DHIs can greatly potentiate the activity of DIs. Their helping role in modulating the ME complex is a key feature of DHIs. DIs are much more effective than DHIs to induce TD. DIs alone, however, cannot push all cancer cells to complete TD. But in the presence of DHIs, complete TD can be accomplished [16]. Therefore, it is essential to have both DI and DHI agents in combination to make a perfect therapeutic for the induction of TD. Arsenic trioxide is very toxic. It is also a potent carcinogen [41]. It is advisable to replace arsenic trioxide with the more effective and less toxic DHIs we have described [16, 42].

DHIs alone can be very effective for cancer therapy too. Imatinib mesylate is the standard of care for chronic myeloid leukemia [43]. The therapeutic efficacy of imatinib mesylate on chronic myeloid leukemia is almost as good as ATRA plus arsenic trioxide on APL, and remissions are long lasting. This is another example of how targeting and destabilizing abnormal MEs can be an effective cancer therapy. We have demonstrated that signal transduction inhibitors (STIs) such as imatinib mesylate were excellent DHIs [16]. DHIs, however, are not very effective at achieving induction of TD by themselves. DIs are produced endogenously Liau MC et al. [42], but cancer patients tend to lose their DIs via renal losses. Higher

levels of immune related cytokines in cancer patients leads to leaky nephron vascular tufts that fail to retain the lower molecular weight DIs. Exogenous replacement of DIs, however, can compensate for the deficiency of natural DIs to achieve effective therapy.

Phenylbutyrate was our initial finding of an agent with DHI activity. However, this agent was only moderately active, requiring mM concentrations to function [40]. Nevertheless, it has demonstrated therapeutic efficacy on often untreatable brain tumors [44,45]. It's benefits were enhanced when used in combination with STIs [46,47], which were effective in μM concentrations [16]. It is remarkable that an ordinary chemical such as phenylbutyrate could in some cases cure primary glioblastoma brain tumors. This suggests that focusing on abnormal MEs as a target of CSCs presents a real opportunity to improve cancer outcomes. DHIs are easily available and relatively non-toxic. DHIs, such as dietary polyphenols, are frequently suggested for chemoprevention of cancer [48-51].

Destabilization of Abnormal MEs to eradicate CSCs

In 1987, Liau MC et al. [52] introduced the concept of chemosurveillance as a natural defense mechanism against cancer. This hypothesis was based on the observation that healthy people could maintain a steady level of hydrophobic metabolites in their plasma, whereas cancer patients tended to show deficiency of such metabolites due to excessive urinary excretion [53]. Among such metabolites were chemicals active as DIs and DHIs [39,40]. The implication is that healthy people have enough DIs and DHIs in their circulation to keep a check on the evolution of cancer cells, whereas in cancer patients a lack of DIs and DHIs depresses their capacity to stop the replication of cancer cells. The evolution of cancer in the case of myelodysplastic syndrome (MDS) strongly supports the validity of this hypothesis.

MDS often starts with a display of an immunological disorder [54], which prompts the local production of inflammatory cytokines. Among such cytokines, TNF is the critical factor related to the development of MDS [55]. It causes excessive apoptosis of bone marrow stem cells, severely affecting the ability of the patient to produce hematopoietic cells such as erythrocytes, platelets, and neutrophils. TNF is also named cachectin, because of its causation of cachexia, defined as weight loss and muscle wasting unresponsive to nutrient intake, a syndrome commonly shared by terminal cancer patients. A characteristic disorder of cachexia is the excessive urinary excretion of low molecular weight metabolites because of vascular hyperpermeability caused by TNF [56,57]. As a consequence, chemosurveillance normally operating in healthy people to keep progenitor cells in check becomes dysfunctional, allowing progenitor stem cells to build up in order to replenish unipotent stem cells wiped out by TNF. The high level of telomerase in the peripheral and bone marrow leukocytes in MDS patients is an indication of the widespread multiplication of progenitor stem cells [58,59]. During the course of MDS progression, mutations affecting the enzyme functions of Tet 2, DNMT 3A, IDH 1/2, AXL 1, EXH 2, and RNA splicing enzymes are frequently observed [60-64], which may play significant roles on the evolution of progenitor stem cells

to become CSCs [65]. As anemia in MDS patients becomes worse, chromosomal abnormalities such as translocations and deletions characteristic of cancer cells arise and accelerate replication, eventually pushing MDS patients to progress to AML [66-69].

Vidaza and decitabine are the two hypomethylating agents approved for the therapy of MDS in the USA. CDA-2 is the hypomethylating agent approved for the therapy of cancer in China [70], which was first described and characterize by Ming C. Liau [71]. Azacitidine and decitabine achieve DNA hypomethylation by promoting covalent bond formation between DNMT and the azacytosine base incorporated into DNA to titrate out DNMT Santi DV, et al. [72], whereas CDA-2 achieves DNA hypomethylation by converting abnormal MEs into normal enzymes via components active as DIs and DHIs [15,39]. An abbreviated clinical trial of CDA-2 for MDS was conducted on 117 patients in China. Based on two cycles of treatment protocols, CDA-2 yielded a slightly better therapeutic efficacy under cytological evaluation, and a markedly better therapeutic efficacy under hematological improvement evaluation in comparison to vidaza and decitabine [73,74]. It had a superior therapeutic effect and was devoid of serious toxic side effects, whereas vidaza and decitabine are proven carcinogens [75,76], and damaging to DNA [77]. It has been reported that vidaza was very toxic to embryonic cells, particularly against cardiomyocytes [78].

The evolution of CSCs from progenitor stem cells in the case of MDS is a clear-cut illustration of the widely accepted two hit theory of carcinogenesis proposed by Knudson [79]. The expression of telomerase to lock MEs into an abnormally active state represents the first hit on progenitor stem cells. The second hit arises from knock out of Tet enzymes, resulting in progenitor stem cells transforming into CSCs. This is more problematic if it occurs in the presence of depressed chemo surveillance needed to halt the replication of CSCs. The dysfunction of chemo surveillance in the case of MDS is caused by immunological disorders. Immune surveillance does not seem to play an essential role to prevent the formation of CSCs from progenitor stem cells. After all, CSCs and progenitor stem cells are almost indistinguishable. We hypothesize that the sequence of events in the evolution of CSCs into MDS starts from underlying immunological disorders and increased levels of cytokines like TNF that lead to diminished levels of circulating DIs and DHIs, a state permissive for the emergence of CSCs. Immune surveillance and immune checkpoints do have an important role to play in the prevention of cancer, which is to prevent excess release of immune related cytokines, such as TNF. Chemo surveillance plays a more direct role to prevent gene silencing and CSC development. Nature has it own way to offer the best solution for cancer prevention. Destabilization of abnormal MEs is nature's choice for prevention.

Destabilization of abnormal MEs is an effective approach to combat cancer. It turns cancer cells into non-dividing cells which may eventually undergo senescence. Therapy that targets abnormal MEs may be preferred for hematological cancers versus solid tumors. The therapeutic end point of hematological cancers is

based on the disappearance of cancer cells, whereas the therapeutic end point of solid tumor therapy is tumor regression. CSCs initially make up a minority of the solid tumor population, such that stimulating their differentiation may not impact on tumor size. Disappearance of the circulating CSCs via detection of specific cell surface markers is a more appropriate end point for the assessment of therapeutic effect on CSCs.

Summary

Cancer cannot be cured unless CSCs are also completely eliminated. CSCs do not respond to chemotherapy and radiotherapy. Therefore, alternative therapies must come into play to target CSCs. Induction of differentiation via destabilization of abnormal MEs presents a new target in the war on CSCs. This may be the nature's choice to keep progenitor cells from evolving into CSCs.

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Conflict of interest

We declare that there is no conflict of interest.

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