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Endogenous anticancer mechanism: differentiation

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1. ABSTRACT

It has been recently shown that within heterogeneous tumor masses a small population of less differentiated transformed cells has the ability to self-renew and regenerate the bulk of the tumor. Their similarities with normal stem cells in terms of gene expression patterns, proliferative capacity and surface markers rendered them the name of cancer stem-like cells (CSC), and these are thought to be the tumor initiating cells (TIC). Their limited susceptibility to classical anti-tumor therapy help explain the high incidence of cancer-treatment relapses observed in selected malignancies. Much effort is being directed towards the understanding of factors that maintain CSC survival and their self-renewal capacity, with the goal that these same signaling pathways can be harnessed for treatments that aim at inducing CSC differentiation. This review will discuss the CSC theory, its implications, potential signaling pathways responsible for maintaining their undifferentiated and pluripotent states, and new venues being explored to target these cells in modern cancer therapy.

2. INTRODUCTION

For the past fifty years cancer has been the second deadliest disease in the US (1, 2). Within the Brazilian population similar statistics apply, cancer was responsible for 17% of deaths in 2007 and 0.5 million new cases are estimated for 2011 (3). Insights that translate into new approaches for cancer prevention and therapy have come from efforts in understanding tumor development, growth and propagation both from an intrinsic perspective and in the context of tumor/host cross-talk. Hanahan and Weinberg (2000) enumerated six essential alterations in cell physiology which, if combined, can lead to cellular transformation and tumor development (4); i.e. self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion/metastasis. From these characteristics, four depend exclusively on changes to the cellular physiology such that the cell becomes independent or refractory to environmental cues meant to keep the system at check and prevent overt growth of dysfunctional tissue. Among the

signaling pathways contributing to the transformation process, many are involved in early steps of tissue development and are part of the gene expression signature of less differentiated cell types. In addition to these observations, it has been recently shown that within some heterogeneous tumors a population of less differentiated transformed cells called cancer stem-like cells (CSC) is capable of giving rise to the bulk of the tumor, and may be the culprit of a high incidence of cancer-treatment relapses in these cases. This review will discuss the CSC theory, its implications, the signaling pathways responsible for maintaining their undifferentiated and pluripotent states, and the new venues being explored to target these cells in modern anti-cancer therapy.

3. TISSUE HOMEOSTASIS AND CELLULAR DEDIFFERENTIATION

Differentiation of cells within a tissue is generally associated with acquisition of specialized functions at the expense of their proliferative capacity and pluripotency. Chromatin remodeling and the expression of tissue-specific transcription factors progressively limit the diversity of cell types a given cell can give rise to. Interactions with neighboring cells and homeostatic signals trigger pathways that actively inhibit cellular growth and proliferation, promoting tissue-specific function and cell survival. However, every day specialized cells from adult tissue die and need to be replaced to maintain the organism's homeostasis. Moreover, if the tissue is injured, a large number of cells is mobilized in order to fill in for those displaced by the insult. In both cases, cellular proliferation and differentiation need to occur for proper tissue recovery and minimal scarring. It has been shown that cells adjacent to the site of injury are capable of recovering their capacity to migrate and proliferate, and in this way participate in the process of tissue maintenance, healing and growth (5). Further contributing to this process, populations of tissue-specific stem cells migrate to the site of injury where, through orchestrated proliferation and differentiation, replace the damaged tissue repairing the injury. It was originally proposed by Cohnheim that stem cells originated in the bone marrow would have the ability to participate in the healing process of diverse tissue types (6). Through the recognition of specific stimuli, they would migrate from the blood stream into the parenchyma and differentiate according to specific tissue factors (7). It is still debated if bone marrow-derived cells are capable of differentiating into several different tissue types, as single-cell *in vivo* transfers suggest (8), or if it is the fusion of hematopoietic stem cells with differentiated cells that gives rise to regenerating tissue (6). Nevertheless, we now know that resident stem cells, called reserve stem cells, exist and are present in different tissues. They proliferate very seldom but respond readily upon cell loss. Resident stem cells can give rise to all cell types of the tissue they belong to and have been identified in bone marrow, both in the hematopoietic and stromal populations, the intestines, bronchial lining and liver (6), but are expected to be present in every tissue of the body.

The potential for cells to acquire a transformed phenotype, meaning, to proliferate with minimum stimulus and lose sensitivity to growth arrest or apoptosis-inducing signals, is given by the sum of mutations genetically inherited and acquired by exposure to genotoxic stress from the environment. These alterations ultimately lead to abnormal expression or function of proteins involved in the control of proliferation and cell death. The presence of tissue stem cells provides a great benefit to the organism, since it facilitates and quickens local tissue repair. However, it is also a liability if carcinogenesis is to be considered. In the context of cancer, a population with the capacity to self-renew, proliferate and give rise to cells with a more differentiated phenotype has also been described. Cancer stem-like cells pose a great challenge in the clinical setting due to their indolent and less-differentiated characteristics.

4. CANCER STEM-LIKE CELLS

Transformation has been linked to a process by which cells regain the ability to extensively proliferate and self-renew, and in the process lose their tissue-specific function (4, 9). In that line of thought, the heterogeneity of tumors, composed of transformed cells at various stages of differentiation, suggested that the accumulation of mutations generated cells progressively worse in maintaining genome integrity, therefore favoring the further accumulation of mutations. The step-wise loss of control of the processes of cellular proliferation and death, added to a gained independence of survival signals from the environment and the ability of manipulating its niche towards a tumor-promoting state, leads to the establishment and growth of complex tumor structures. However, xenotransplant experiments using human tumors have shown that only some cells derived from the tumor bulk have the capacity to propagate the tumor in its entirety when transferred into immunocompromised mice (10). This subpopulation of tumor cells capable of giving rise to all the distinct cell types of a tumor upon transfer was initially named CSC. Questions have been raised about the proper definition of these cells, and if they would also have the capacity of initiating the tumor, and therefore deserve the name of Tumor Initiating Cells (TIC), or if they are generated during tumor progression and would be limited to propagating but not initiating cancer. Moreover, some authors argue that the capacity to asymmetrically divide, an important regulatory mechanism of stem cells, has not been shown for CSC, defending the use of the more restricted term TIC (11). Others have shown that even though asymmetric division is an important characteristic of stem cells, tumor suppressor genes participate on its homeostasis, and therefore the abnormal expression or absence of tumor suppressor genes like adenomatous polyposis coli (APC) or p63 may directly contribute to stem cell transformation (12, 13). In this review, we will use the term CSC when referring to the tumor population capable of initiating cancer and giving rise to the bulk of the tumor upon transplantation. The origin of CSC, e.g. bulk tumor cells that acquire the capacity to self-renew or transformed tissue-specific stem-like cells, is debated upon and has not yet been clearly shown.

The hypothesis that one small population within the tumor mass gives rise to the bulk of tumor was first

Table 1. Distribution of some stem cell-related CSC antigens

Stem Cell Marker	Tumor	Ref.
CD24	Pancreatic	(21)
CD34	AML	(195)
CD44	Bladder, Breast, Colon, Head and Neck, Ovarian, Pancreatic	(17), (16), (196), (24), (31), (21)
CD47	Bladder	(17)
CD133	CNS/Glioma, Colon, Ewing, Pancreatic	(27), (22, 25), (28), (19)

raised by Furth and Kahn in 1937 (14), but these cells were only isolated and characterized in 1994 from acute myeloid leukemia patients by the group of John Dick (15). Since then, populations of CSC have been isolated, adoptively transferred and characterized in hematopoietic, breast, brain, colon, pancreatic, prostate, head & neck, bladder, liver and ovary tumors, melanoma and sarcoma masses (16-31). The ability to give rise to the bulk of tumors upon transfer into a susceptible host has been mapped to tumor cells bearing surface markers associated with immature and stem cells, as shown for acute myelogenous leukemia CSC that express CD34, CSC from brain/glioma, colon, prostate, pancreatic and sarcoma that express CD133, and CSC from prostate, bladder, colon, breast, head & neck and ovarian that express CD44 (Table 1). Indeed, these CSC share with normal stem cells the capacity to self-renew and further differentiate, as mentioned above (10, 32). Based on the work of several independent groups, CSC have been defined as tumor-derived cells that self-renew and upon serial transplantation *in vivo* are able to recapitulate the original tumor in its entirety and continuously grow (32). Interestingly, recent work has shown that the contribution of CSC to tumor growth is not limited to tumor cells, bulk or CSC. CD133$^+$ glioma CSC also differentiate into endothelial cells that go on to form the tumor vasculature (33, 34), fulfilling one of the hallmarks of cancer in an unanticipated way (4). If CSC can give rise to other tumor-associated tissue remains to be shown.

The origin of CSC in different tumors is an open question in the field. Whereas some researchers believe they arise from mutations in cells that already have stem qualities, others consider that CSCs can be generated by dedifferentiating events (32). Since tumors are complex structures and very different depending upon characteristics of the tissue where they grow, different tissues may favor one or another originating event, and therefore the confirmation of one hypothesis does not negate the other. It has been suggested by Cohnheim over a century ago that bone marrow-derived circulating stem cells can infiltrate normal tissue and participate on its repair upon injury (6, 35). The concept behind this idea is that blood-born stem cells are capable of infiltrating any tissue in the organism, and following cues present in the site, differentiate towards the niche where they are present. Therefore, if these cells were to bear transforming mutations that render them refractory to growth control, they could seed tumors anywhere in the host (36). New findings have demonstrated that stem cells from sources besides the bone marrow can contribute to tumorigenesis, as seen for residual cells left from early embryonic stages that can delay differentiation and give rise to cancer, usually within the first years of life. This is the case of Wilms tumors, which is common in children younger than 8 years old, and neuroblastomas, among others (6). In addition, it is now known that bone marrow is not the only source of adult stem cells, and other tissues like fat and gut are seeded with tissue-specific stem cells. Cohnheim's theory can therefore be applied to these cell types, and some authors speculate that these are indeed the source of CSC in most cases (6). In these cases, tumors arise from mutations genetically inherited and/or derived from genotoxic stress accumulated by embryonic or stem-like cells, and possibly through asymmetric division generate the bulk of a heterogeneous cancer. Unless the source of differentiated cells is targeted, therapy will be limited and inefficient, as discussed later.

In addition to the idea that tumors arise from mutations to cells that already have the capacity to proliferate, self-renew and generate more differentiated daughter cells, there is the hypothesis that differentiated adult cells may give rise to cancer upon accumulation of transforming mutations, following a step of dedifferentiation. We now know that the forced expression of only four distinct transcription factors, i.e. Oct4, Sox2, Klf4 and c-Myc, in fully differentiated mature cells can lead to utmost dedifferentiation, generating induced Pluripotent Stem (iPS) cells (37, 38). iPS cells have capacities of embryonic stem cells and contribute to the formation of whole embryos upon injection into blastocysts (39-41). It can be therefore extrapolated that mutations to somatic cells that alter the expression of these genes can promote a transformation-prone dedifferentiated state, and the generation of pluripotent cells, as discussed below. Overall, independent of the origin of CSC, their role in propagating certain tumors is well established and in identifying and characterizing these cells new treatment options may become available.

4.1. Counter-arguments to the cancer stem cell theory

Despite the suggestive data, several questions remain on how broadly the CSC theory can be applied. CSC have often been described as a small subpopulation within a heterogeneous tumor mass, which holds the capacity to regenerate the tumor in its complexity upon transfer into a new host. However, data on both solid and lymphoid tumors have demonstrated that, at least in some types of cancer, a high frequency of tumor-propagating cells can be detected upon transfer of limited numbers of tumor cells into immunocompromised mice. Moreover, these tumor-propagating cells could not be identified by a specific marker. That is the case of melanoma and T and B cell lymphomas (42, 43). Kelly *et al.* (42) suggested that the population of tumor propagating cells, at least within lymphoid tumors, may be greater than anticipated by xenotransplant experiments. Different from observations in cases of chronic myelogenous leukemia, breast and brain tumors, where only large numbers of tumor cells transplant the tumor due to the low frequency of CSC, few B or T cell lymphomas were sufficient to generate tumor in syngenei

Table 2. Transcription factors associated with pluripotency and transformation

Gene	Associated Tumor Type	Ref.
Oct3/4	Embryonal, Gastric, Pancreatic, Uterus (HPV)	(51), (64), (66), (197)
Sox2	Breast, Cervical, Esophagus, Gastric, Lung	(74), (73), (72), (64), (75)
Klf-4[1]	Breast ductal cells, Kidney epithelia, Laryngeal squamous cell, Skin	(198, 199), (200), (200), (201, 202)
c-Myc[2]	Acute lymphocytic leukemia, B-cell lymphocytic leukemia, Burkitt's lymphoma, Diffuse large cell lymphoma, Multiple myeloma, Plasma cell leukemia, Bladder, Breast, Colon, Gastric, Glioma, Liver, Medulloblastoma, Melanoma, Neuroblastoma, Ovarian, Prostate, Renal clear cell carcinoma, Retinoblastoma, Rhabdosarcoma, Small-cell lung carcinoma	(203), (203, 204), (204, 205), (205), (206), (206), (207), (208), (209, 210), (211), (212), (213), (214, 215), (216), (217), (218, 219), (220, 221), (222), (223), (224, 225), (226)
Nanog	Ovarian	(92)
β-Catenin	Breast, Colon, Liver	(227), (228), (229)

[1] More information in Evans & Liu (76), [2] More information in Vita & Henriksson (84)

secondary hosts (42). This has later been shown for melanoma tumor cells of diverse stages isolated from the patient's primary cutaneous (II and III) tumor or metastatic (III and IV) sites, as well as for xenografts passaged in mice (43). These observations have raised two distinct possibilities. Being critical of the models used to study CSC and its characteristics, xenotransplant of human tumor cells into immunocompromised mice may mislead the interpretation of some recent findings. It is discussed that the low frequency of cells capable of transplanting the bulk of tumor may be so due to assay conditions, or species-specific requirements for the establishment of a tumor-prone niche, and not particularly due to rarity of the CSC population (42, 43). Nevertheless, the origin of CSC is still uncertain, and the possibility of bulk tumor cells going through a process of de-differentiation, by which some of these cells are able to give rise to CSC in the tumor host, remains to be tested. Even though specific markers separating the CSC-prone cell population from bulk tumor cells have not been found by Quintana *et al.* (43), the observation that the tumorigenic capacity of cells from each tumor is inversely correlated to the growth rate of the new tumor mass suggests that the tumor propagating cells are functionally distinct from other cells that compose the tumor, in that they grow in a slower pace, as described for CSCs.

5. TRANSCRIPTION FACTORS REGULATING CELLULAR REPROGRAMMING AND DEDIFFERENTIATION

Genetic alterations may arise randomly during the process of genome replication, or be induced by exogenous factors, e.g. viral infection, chemicals, ultra-violet light and ionizing radiation. Most of these "mistakes" are resolved by the cellular repair machinery and go unnoticed throughout the life of an individual. However, some mutations at genes associated with DNA repair, cell cycle check-points or survival compromise the identification and response to further mutations, which are perpetuated by DNA replication. Cellular transformation may then occur due to overexpression of oncogenes or inactivation of tumor suppressor genes induced as a consequence of these mutations. Interestingly, many of the genes responsible for cellular transformation are important players in the maintenance of precursor cells, or involved with key signaling events in the initial steps of differentiation. As examples, known pathways altered in leukemias and lymphomas involve overexpression of Notch, which signaling is essential for the commitment of common lymphoid progenitor cells towards the T cell lineage in the thymus (44-49), or Snf5 downmodulation, which expression is essential for double negative to double positive transition during T cell thymic development, as will be further discussed (50).

As mentioned above, four transcription factors deserve special attention given their role in the development of diverse cell types and capacity to revert differentiated, mature murine and human fibroblasts back to pluripotency, namely Oct4, Sox2, Klf4 and c-Myc (37, 38). Oct4 and Klf4 were essential for the appearance of iPS cell colonies after transduction of murine embryonic fibroblasts (MEFs), Sox2 increased the frequency of those colonies, contributed to the acquisition of an ES cell morphology and was essential for pluripotency *in vivo*, whereas c-Myc contributed to their ES cell morphology in culture (37, 38). Interestingly, overexpression of these transcription factors has been associated with cellular transformation in diverse types of human cancers (Table 2).

5.1. Oct-3/4

Oct-4 (octamer-binding transcription factor 4, also known as POU5F1, Oct3, Oct3/4, OTF3 or OTF4) is a member of the POU family of transcription factors (51-53), for which three different alternative splicing variants have been indentified, Oct4A, Oct4B and Oct4B1 (54). Until recently no distinction was made between the different Oct4 isoforms (54), and Oct4 was generally described as a nuclear protein important for maintaining the pluripotent state of blastomeres, of cells from the inner cell mass of blastocysts and of adult germ cells (55). When heterodimerized with Sox2, Oct4 is able to bind to conserved DNA elements and control the expression of several genes associated with the stem cell phenotype, including itself, Sox2 and Nanog (56-59). Oct4-deficient embryos arrest at the blastocyst stage due to differentiation of its inner cell mass towards throphoblasts (60). Oct4B protein, however, has a different pattern of expression and subcellular localization, being present in mouse embryo cells from the four-cell stage onward, always in the cytoplasm, what suggests it is not working as a transcription factor (54). Indeed, Oct4B does not contribute to the maintenance of the pluripotent state (61, 62). The expression of Oct4 by somatic cells is controversial. Even though several groups have reported its expression in diverse tissue types, the levels detected are generally low, and the presence of several Oct4 pseudo genes complicates detection of functional transcripts (54, 63). In the context of tumors, overexpression of Oct4 is an important marker for

embryonal carcinoma, besides being associated with gastric and pancreatic carcinoma, and transformation following human papilomavirus infection (6, 51, 64-66) (Table 2). Furthermore, it has been suggested that Oct4 is a transient oncogene in prostate cancer, being necessary for the generation of prostate CSC but not for their maintenance (67). Though this hypothesis needs further testing, it raises the possibility that oncogenes essential in the process of cellular transformation may not be readily detectable in bulk tumor cells, but nevertheless a good target when aiming at CSC (67). When it comes to ascribing tumorigenic properties to specific Oct4 isoforms, recent work suggests that Oct4A is associated with transformation of embryonal cells, whereas somatic tumor cells tend to overexpress Oct4B or the pseudogene, Oct4P1 (54).

5.2. Sox2

Sox2 is part of the Sry-related box (Sox) family of transcription factors, a member of the high mobility group superfamily, so called due to its first member's importance in male sex determination (sex-determining region Y) (68, 69). The murine sox family can be further divided into nine sub-groups (SoxA-H, including SoxB1 and SoxB2), which may function as gene expression activators, repressors or regulatory subunits. Sox2 is a member of the SoxB1 sub-group, acts by activating gene transcription (70), and plays an important role during various stages of mammalian development and cellular specification (71). It is essential for embryo implantation and the development of neural structures, but its expression maintains the neuronal progenitor state, therefore dowregulation of Sox2 and the other SoxB1 transcription factors (Sox1 and Sox3) is essential for neuronal differentiation (70). Sox2 can be found overexpressed in diverse types of human tumors, among them lung adenocarcinomas, gastric, esophagus, stomach, cervical and breast cancers (64, 72-75) (Table 2).

5.3. Klf-4

Klf-4 is a member of the Krüppel-like factor, a family of transcription factors involved in both activation and repression of gene expression (76). It is expressed in later stages of embryonic development, the endothelia, gut, skin, lung, testis, thymus, cornea, cardiac myocytes and lymphocytes, controlling the expression of proteins that participate in development, cellular differentiation, proliferation and apoptosis. Due to the diversity of genes it targets, Klf4 can function as an oncogene or tumor suppressor protein in a cell-specific manner. In cells of the colon epithelia, Klf4 functions as the latter, inducing the expression of $p21^{Cip1/WAF1}$, $p57^{Kip2}$ and the enterocyte differentiation marker intestinal alkaline phosphatase (77, 78), while inhibiting that of cyclins D1, D2, E and B1 (76). Moreover, Klf4 interacts with and blocks the activity of β-catenin (79), a known player in the development of polyposis and colorectal cancer (discussed below). Indeed, loss of KLF4 expression is correlated with human colorectal cancer and large adenomas in mouse models, while KLF4 superexpression leads to reduced growth of intestinal cancer in xenograph experiments (76). Overall, Klf4 functions as a tumor suppressor protein in intestinal epithelia, and its loss is associated with esophageal and bladder cancer, non-small-cell lung carcinoma and leukemia (76). However, Klf4 inhibits p53 expression, phenotype usually overcome by the induced expression of $p21^{Cip1/WAF1}$, but in cases where the expression of $p21^{Cip1/WAF1}$ is repressed by other factors, the effect of Klf4 on p53 may become apparent and cellular transformation be promoted. This mechanism, at place in primary fibroblasts where the Ras^{V12}-induced senescence is reverted to transformation by the overexpression of KLF4 (80), may be relevant to other tumors as well. Indeed, KLF4 is found overexpressed and is associated with the transformed phenotype in cancers of the skin, kidney epithelia, laryngeal squamous cell and breast ductal cells, functioning as an oncogene in these tissues (76) (Table 2). Therefore the role of Klf4 in the induction of pluripotency may be dependent on one or more of the other factors, with c-Myc being a likely candidate (76).

5.4. c-Myc

c-Myc is a transcription factor that regulates the expression of genes involved in cellular proliferation, apoptosis, cell growth and differentiation in response to signaling provided by growth factors and adhesion molecules (81). Overexpression of c-Myc alone leads to apoptosis, however, if counterbalanced by a survival factor, like the expression of pro-survival members of the Bcl-2 family, its overexpression will lead to cell cycle entry and ultimately cellular transformation and tumor growth (82). It has been speculated that, in the iPS cocktail, this role is being played by Klf4, and that the pro-apoptotic effect of c-Myc and the induction of cell cycle arrest by Klf4 are being balanced by each others presence (76). Experimental overexpression of c-Myc leads to the development of teratomas, which despite being a hallmark of pluripotency, can be an important side effect to organisms both generated by and injected with iPS cells overexpressing this transcription factor (37, 38, 40, 83, 84). Indeed, increased c-Myc expression is associated with cancer development in virtually every human tissue (Table 2), its translocation to the immunoglobulin enhancer being a hallmark of Burkitt´s lymphoma (84). Even though the induction of a pluripotent state can be dissociated from carcinogenesis by the omission of c-Myc in the iPS cell cocktail (83), a significant reduction in the efficiency of the reprograming process is observed, highlighting the contribution of this oncogene to the propagation and overall pluripotency of iPS cells.

5.5. Nanog

Human fibroblasts were shown to revert back to a pluripotent state by the overexpression of Nanog and Lin28, instead of Klf4 and c-Myc, in association with Oct4 and Sox2 (85). Indeed, even with the ectopic expression of the originally identified factors, use of Nanog as a selection marker for embryonic pluripotency instead of the initially used Fbx15, generated cells phenotypically closer to ES cells. These new iPS cells displayed similar DNA methylation and gene expression patterns to ES cells and were capable of contributing to adult animals after injection into blastocysts (39-41), suggesting that endogenous Nanog expression was essential for full reversion to pluripotency. Whereas Oct4, Sox2 and Nanog are essential for

pluripotent stem cell induction, Lin28 major contribution was to enhance the frequency of clones generated (85).

Nanog is a homeoprotein with homology to the NK2 gene family, originally described based on its ability to maintain embryonic stem cells *in vitro* and promote its self-renewal capacity in the absence of the cytokine leukemia inhibitory factor (LIF) (86, 87). It is expressed in the inner cell mass (ICM) and epiblast during early embryonic development, and by embryonic stem, embryonic germ and embryonic carcinoma pluripotent cell lines (88). As development progresses Nanog expression is lost, a necessary step for the generation of increasingly differentiated tissue, such that Nanog expression is not found in adult cells (88). Reduction in Nanog expression is necessary and sufficient to induce cellular differentiation of both mouse and human ES cells *in vitro* (89, 90), and its overexpression leads to cellular transformation as shown for HEK293 cells (91). Indeed, Nanog expression is positively correlated with progression of some solid tumors, including ovarian cancer where more expression of Nanog is associated with higher stage and grade of the disease (92) (Table 2). Further work demonstrated that it controls the expression of Oct4, the suggested mechanism for Nanog's pro-stem cell function (93).

It is important to point out that continuous expression of the Yamanaka factors does not seem to be required for maintenance of iPS cells or their pluripotent capacity. A recent report studying the stoichiometry and duration of expression of these genes in transduced human embryonic fibroblast demonstrated that their transient expression is sufficient to generate iPS capable of differentiating into endoderm and mesoderm (94). These results suggest that the transient expression of dedifferentiating factors by mature cells may be sufficient for the early steps of transformation to take place (67). Their lack of expression in fully-grown tumor masses could lead to an underestimation of the impact and role of those factors in the development of human cancer (67).

5.6. p53

Conversely, as oncogenes are associated with the induction of a pluripotent state, proteins that counteract transformation, such as tumor suppressor genes, may hinder iPS generation. Indeed, the tumor suppressor p53 reduces the efficiency of iPS induction and tissue types that express lower levels of p53 are more easily reprogrammed to originate iPS cells (95). Interestingly, ectopic expression of the iPS-generating factors Klf4, Oct4 and Sox2 leads to up-regulation of p53 and its target gene p21. Impaired or reduced p53 expression through knock out or knock down approaches lead to the generation of iPS cells with higher efficiency than in control cells. Indeed, p53 is capable of binding to the promoter regions of *oct4* and *nanog* and reduce their expression (96). Similar results were obtained with reduced expression of p21, $p19^{Arf}$ and $p16^{Ink4a}$, suggesting that the p53 and Rb signaling pathways contribute to cellular homeostasis preventing dedifferentiation of mature cells (95). These observations suggest that the transformed and pluripotent phenotypes share the use of genes involved in early stages of cellular development, and can be impaired by genes that promote cellular differentiation and control proliferation.

6. THERAPEUTIC APPROACHES

Until recently anti-tumor therapy was aimed at the most prominent tumor populations, and had the goal of reducing the cancer mass, if not to completely destroy it, to at least reduce its size such that surgical removal of the solid tumor could be attempted. However, the large number of relapses in patients that had responded well to therapy challenged the efficacy of the methods and asked for new treatment protocols. With the characterization of CSC, a plausible explanation for at least some of the relapsing patients appeared, with the possibility that the resilience of these cells throughout treatment was responsible for the re-growth of the tumor. Since most therapies disregarded small populations showing low levels of proliferation, generally the case of CSC, those often remained untouched, or worse, accumulated mutations induced by the treatment itself, only to slowly expand and create a new complex tumor mass. Indeed, CSC from diverse tumor types have been shown resistant to current anti-tumor therapy, either by the aforementioned low level of proliferation, increased expression of resistance factors such as DNA damage repair enzymes or by the expression of extrusion channels that provide multiple drug resistance (10, 97). Until recently it was uncertain if the targeted destruction of CSC would truly impact tumor growth, given the difficulty of finding molecular targets that could selectively act on the CSC population and not the bulk of tumor. Even though an ideal treatment protocol should aim at both populations, it remained to be shown if the theory raised by CSC discovery was real. Proof-of-principal experiments now show that the specific targeting of CSC upon transfer of human tumors into immunodeficient host mice significantly reduces tumor growth of melanoma, hepatocellular carcinoma, glioma, bladder cancer and leukemia (17, 26, 30, 98-100). Therefore, investing in the development of therapy that target CSC to be associated with depletion of bulk tumor cells holds good promises. Nowadays, two major lines of therapy are being pursued: 1-targeting CSC-specific surface molecules or signaling pathways to selectively take out these cells; 2-using compounds that induce the differentiation of CSC, and therefore render them susceptible to other therapies currently in use. The former is not the focus of this review, so for a detailed discussion on modern antibody and small molecule-based CSC therapy refer to recent reviews (101, 102). Nevertheless, it is important to mention the promising pre-clinical data obtained by targeting the surface molecule CD34 in acute myelogenous leukemia patients, CD133 in xenotransplant experiments using brain, colon, prostate, pancreatic and sarcoma human cells, and how inhibition of NFkappaB *in vivo* retarded tumor growth (10, 32, 103). All these molecules were identified as markers for CSC of each specific tissue, and the reduction in tumor growth was associated with targeting this population.

6.1. Differentiation therapy

The rational behind this method is to induce differentiation of the more immature tumor populations,

Table 3. Differentiation-inducing therapeutic approaches

Differentiation Drug / Compound	Target Tumor	Ref.
BMPs	Androgen-sensitive Prostate, Colorectal, Glioma, Medulloblastoma, Melanoma	(107), (108), (106), (109), (110)
RA/ATRA	APL[1], Embryonal, Keratinocytes, Kidney, Melanoma, Neuroblastoma, Teratoma, Thyroid	(115, 119), (113), (116), (120), (118), (117), (113), (121)
HDAC inhibitor - Vorinostat	Cutaneous T-cell lymphoma, Non-Hodgkin's lymphoma, Mantle cell lymphoma, Glioblastoma multiforme, Head & Neck, Diffuse large-B-cell lymphoma, Prostate, Breast, Non-small cell lung carcinoma, Colorectal, Ovarian, Renal	(148, 149), (230), (230), (231), (232), (233), (234), (235, 236), (236, 237), (236), (238), (239)
5-Azacytidine / 5-Aza-2'-deoxycytidine	Myelodysplastic syndrome, Neuroblastoma, Ovarian	(159, 160), (163), (161, 162)

[1] Acute promyelocytic leukemia

since those are the ones that propagate cancer. The critical characteristics of CSC are their ability to self-renew and give rise to all populations of the tumor bulk, properties shared with tissue-specific stem cells. In inducing their differentiation, it is expected that these cells will become susceptible to modern anti-tumor therapy, and lose their ability to reconstitute the tumor at later times, avoiding patient relapse (Table 3).

6.1.1. Bone morphogenetic proteins

Tumor-specific differentiating agents have been described and show promise for clinical use. That is the case of bone morphogenetic proteins (BMPs), cytokines of the TGF-beta superfamily initially described for their positive role in bone formation through the induction of osteoblasts differentiation, but later shown to influence the morphogenesis of diverse types of tissues (104). Impaired BMP signaling by the absence of the type Ib BMP receptor Alk6b in zebrafish leads to impaired germ-cell differentiation and tumorigenesis, and its reduced is associated with these types of tumors in humans (105). *In vitro* treatment of $CD133^+$ human glioblastomas CSC with BMPs, specifically BMP4 and BMP2, led to cellular differentiation and consequent reduced *in vitro* proliferation and clonogenic formation, as well as reduced *in vivo* growth of these cells upon xenotransplants (106). Similar results were observed for androgen-sensitive prostate cancer cells, medulloblastoma and colorectal cancer where BMPs reduced cell proliferation *in vitro*, while forced BMP4 expression by medulloblastoma or colorectal cancer cells hindered tumor growth *in vivo* (107-109). Furthermore, treatment of melanoma cells with BMP7 led to mesenchymal-epithelial transition, reduced migration and enhanced chemotherapy susceptibility (110).

6.1.2. Retinoic acid

A more general method initially used and most studied is treatment of tumor cells and patients with retinoic acid (RA) or all-trans retinoic acid (ATRA). RA is generated by the metabolization of ingested vitamin A, travels the blood associated with serum retinol-binding protein (RBP4), and is internalized by cells through the surface receptor Stra6 (111). RA signals mostly through the interaction with a heterodimer between the nuclear retinoic acid receptors (RAR)-alpha, -beta or -gamma, and retinoic X receptors (RXR)-alpha, -beta or -gamma. The RAR/RXR heterodimers act as transcription factors binding to RA response elements (RAREs) present in the promoter sequence of the genes they regulate. In the absence of ligand, RAR/RXR remain bound to RAREs, however they recruit chromatin remodeling factors that render these loci closed and therefore inhibit the expression of their target genes. The interaction with RA or ATRA leads to a conformational change of the receptors, dissociation of this repressive complex and recruitment of proteins that increase locus accessibility and promotes gene expression (112).

RA induces cellular differentiation, as initially shown in cell lines derived from embryonal carcinoma (113), through the upregulation of genes that promote differentiation, like AFP (114), and downregulation of pluripotency-associated ones like Oct4 or telomerase (115). RA signaling can also lead to cell cycle arrest at the G1 stage through the dowregulation of cyclin D1 by inducing protein degradation and reducing mRNA synthesis, with consequent reduction of the phosphorylation of retinoblastoma (Rb) protein, leading to an inactivation of E2F and deficient upregulation of cyclin E and CDKs (111). In the treatment of cancer, RA is able to induce cellular differentiation of keratinocytes, teratocarcinoma cells, acute promyelocytic leukemia (APL), melanoma cells and some stages of neuroblastoma *in vitro* (113, 116-119). In the clinical side, these results correlated with some success, which is often achieved by combination of RA with other treatment protocols to overcome retinoid resistance. Many tumors display mutations or altered chromatin remodeling patterns such that their cells do not express RA receptors, being therefore refractory to RA therapy alone (111, 112). Pre-clinical studies showed that combination of RA with inhibitors of histone deacetylases (HDAC) restored the expression of RARbeta2 by human renal cancer in xenografts, and consequently induced tumor growth inhibition (120), data similar to that observed for breast, thyroid and renal cancer cells (121-123), and to concomitant treatment of leukemia cells with RA and G-CSF (124). On the bed side, combination of HDAC inhibitors and retinoid administration showed good results in the treatment of leukemia patients (125, 126), and combinations between RA and arsenic trioxide shows promising results towards a cure (127). It is important to point out that when talking about clinical outcomes, it is not possible to subscribe all the tumor-static effects of RA treatment to the induction of differentiation of CSC, since the halt in cellular proliferation or induction of apoptosis in cells other than CSCs may also contribute to the success of these therapies (111). Moreover, in some cases the treatment with RA may have a tumor-promoting effect, as observed for hepatocellular carcinoma where either by overexpression of RARgamma, its altered subcellular localization or the lack of RARalpha corepressor protein, RA signaling leads to tumor growth instead of arrest (112).

6.1.3. Chromatin remodeling compounds

The stem cell state is generally associated with the activity of transcription and chromatin remodeling factors that lead to silencing of gene expression. This is the case of genes from the polycomb group of proteins and members of the Swi/SNF family of chromatin remodeling factors (128, 129). The deregulation of these factors is associated with tumorigenesis in several types of human cancer, and classical oncogenes and tumor suppressors like the Rb protein, c-Myc and BRCA1 directly interact with chromatin remodeling complexes, i.e. HDAC and/or Swi/Snf, an essential step in the regulation of certain target genes (130-136). Several different modifications of DNA and histones have been described to guide the chromatin control of gene expression, which include but are not limited to covalent histone modifications with the addition of methyl, acetyl or phosphate groups; utilization of histone variants substituting the classical H2A, H2B, H3 or H4 (e.g. H2AX, H2A.Z); DNA methylation and ATP-dependent chromatin remodeling. Each of these modifications can be correlated with cancer progression given their role in gene activation or silencing and DNA repair (137, 138). A different line of therapy being increasingly adopted in the past few years relies on the use of inhibitors of HDAC and of DNA methyltransferases, and aims at inducing the differentiation of cells in which chromatin remodeling is misregulated through the reactivation of silenced genes (139, 140).

6.1.3.1. Histone deacetylases

The differentiating properties of the HDAC inhibitor butyric acid was first described for erythroleukemia cells *in vitro* prior to the true understanding of the biochemical changes induced by this compound (141, 142). In 1977, the modifications to histones following treatment with butyric acid were first documented (143), but was not until 1979 that its target was defined as HDAC (144). Already in 1992, the amphipathic compound hexamethylene bisacetamide, not yet identified as an HDAC inhibitor, was used in the clinics as treatment for hematological cancers with the promise that, as in the pre-clinical studies, induction of tumor cell differentiation would lead to cancer remission (145). Indeed, the results were promising but the onset of thrombocytopenia as a side effect to the treatment asked for new, more effective drugs (139, 145). The identification of butyrate as an inhibitor of HDAC was followed by the description of trichostatin A, isolated from *Streptomyces hygroscopicus*, and the synthetic suberoylanilide hydroxamic acid (SAHA) as more effective, second-generation HDAC inhibitors (146, 147). In 2006 the first HDAC inhibitor, Vorinostat, was approved by the FDA for the treatment of cutaneous T-cell lymphoma (148, 149). Further studies indeed demonstrated HDAC to be over- or misexpressed in gastric, prostate, colon and hematological human malignancies (150), and to play a broad regulatory role by modulating the function of not only histones but also tubulin, p53 and heat shock protein 90, among others, besides regulating transcription upon interaction with oncogenes (151). The field of epigenetic drugs has gone a long way since then. Even though it has become clear that combination therapy is more efficient than treatment with HDAC inhibitors alone, more specific, new generation compounds are now available (139). There are currently over 80 active clinical trials testing 11 HDAC inhibitors of four different classes (e.g. hydroxamates like SAHA, cyclic peptides, aliphatic acid including Valproic acid, and benzamides), for their impact in hematological and solid tumors (152).

6.1.3.2. DNA methyltransferases

Besides reducing histone deacetylation, another line of therapeutics aims at reverting gene silencing by inhibiting DNA methyltransferases (DNMTs). DNA methylation is carried out by specific DNMTs on cytosine residues most commonly found in CpG islands, present in the promoter region of about 60% of all human genes (153). The regulation of CpG methylation in promoter cytosines that do not compose islands is less studied, but seems related to the regulation of tissue-specific gene expression (140). Recent studies on ES cells have shown that cytosines in the trinucleotides CHH and CHG (where H = A, C or T) can also be methylated, a property lost upon cellular differentiation but regained in iPS cells (154). The impact of these pluripotency-specific types of methylation is still not fully understood, but leaves the question if they are also present in CSC and can be a potential target in anti-tumor therapy. Cytosine methylation generates a binding site for methyl-CpG binding domain proteins (MBDs) and methyl-CpG binding zinc-finger proteins of the Kaiso family, and subsequent recruitment of HDACs, nucleosomal remodeling complex (NuRD) and Swi/Snf proteins that contribute to the compaction of the target loci and silencing of gene expression (155). Physiologically, DNA methylation is essential for eukaryotic development, through its modulation of gene expression, imprinting upon cellular proliferation, X chromosome inactivation and suppression of repetitive genome elements (153). Given its broad reach, as mentioned above virtually all promoters in the human genome have CpG rich regions, whether in CpG islands or not, which may be targeted for methylation, alterations in the methylation machinery may lead to global deregulated gene expression and pathology as seen in some cases of diabetes, lupus, asthma and several neurological disorders (153). In the context of cancer, tumor-specific patterns of promoter CpG island methylation have been identified in colorectal cancer (156), and later studies showed a correlation between the "CpG island methylator phenotype" (CIMP) and progression of diverse types of tumors including gastric, lung, liver, ovarian and leukemias (157). Moreover, several reports have demonstrated that the tumor's CIMP can be correlated with its response to specific treatments in patient cohorts, giving prognostic relevance to the cancer methylation profile. Indeed, the methylation frequency is in an inverse correlation with overall survival of myelodysplastic syndrome patients (158). Currently, 5-Azacytidine and 5-Aza-2'-deoxycytidine, which act as hypomethylating agents by inhibiting DNMT, have been approved for clinical use in myelodysplastic syndrome (159, 160), and new phase I clinical trials are testing the efficacy of these drugs in solid tumors like neuroblastoma, epithelial ovarian cancer and other solid tumors (161-164). At the end of 2010 the American Association for Cancer Research together with

Table 4. Role of selected mIR in tumorigenesis

mIR	Gene	Ref.
Tumor Suppressor mIRs	mIR-1, mIR-34a, mIR-124, mIR-137, mIR-260	(166, 169), (174), (173), (173), (169, 170)
Oncogenic mIRs	mIR-10a/b, mIR-34a	(168), (175)

Hollywood's initiative Stand Up for Cancer has funded the first phase II clinical trial to test DNA demethylating drugs in solid cancers, granting more than 9 million dollars over a 3-year period towards a task force headed by Stephen Baylin and Peter Jones, leaders in the field (165). This is a demonstration of the increasing clinical relevance of modulation of DNA methylation for the progression of cancer and the expert's hope for future treatments.

6.1.4. Small non-coding regulatory RNA

Finally, a new strategy that is being explored for therapy but is still in its initial steps is the use of small, non-coding regulatory RNA sequences (microRNA; miRNA) in trying to revert the stem cell properties and induce differentiation of tumor propagating cells (166). Alterations in the miRNA profile has been described for several tumors, and are involved in the modulation of apoptosis, cellular proliferation and tumor metastasis, in addition to differentiation and maintenance of stem cell properties (167). miRNA can be classified as oncogenic or tumor suppressor if found respectively upregulated or dowregulated in transformed cells, even if their functional target has not yet been identified (167). A few preclinical studies have already described specific miRNAs involved in differentiation of tumor cells and suggested their use in anti-cancer therapy. Notably, miR-10a/b, miR-260, miR-1, miR-124, miR137 and miR-34a deserve a more detailed discussion due to their role as tumor suppressor or oncogenes (Table 4).

miR-10a/b contributes to transformation of neuroblastoma cells. If miR-10a/b expression is downregulated or if its target NCOR2 (nuclear receptor corepressor 2) is rendered resistant, differentiation of neuroblastoma cells is induced (168).

miR-260 plays the opposite role, contributing to tumor suppression in rhabdomyosarcoma (RMS) and the breast cancer cell line MCF-7. In the former, it downmodulates the expression of the MET tyrosine-kinase receptor, important in the pathogenesis of RMS. Overexpression of miR-260 leads to reduced *in vitro* and *in vivo* growth of tumor cells through induction of a differentiated expression profile in both embryonal and alveolar RMS (169). In the latter, its expression following EGFR signaling downmodulates the expression of estrogen receptor and its responsive genes, inhibiting cell growth and promoting apoptosis (170).

miR-1 expression is reduced in RMS cell lines and its re-expression leads to a halt in anchorage-independent growth and differentiation of embryonal and alveolar RMS cells *in vitro*, an effect ascribed to its targeting of MET receptors similarly to miR-260 (169). Moreover, miR-1 is expressed by normal human liver and bronchial epithelium cells but found downmodulated in hepatocellular carcinoma and lung cancer (166). In human hepatocellular carcinoma cells, its function has been mapped to the downmodulation of fork-head P1 (FOXP1), HDAC4 and MET, and most interestingly, the arrest in proliferation and increased apoptosis seen upon treatment of cells with 5-Azacytidine can be ascribed to the induced re-expression of miR-1 and subsequent downmodulation of its targets (171). In human lung cancer cells, overexpression of miR-1 leads to a reduction in cellular proliferation, clonogenic capacity, anchorage-independent growth and migration, which correlates with reduced *in vivo* growth and the downregulation of FOXP1, HDAC4, MET and the proto-oncogene serine/threonine kinase Pim-1. Treatment of lung cancer cells with the HDAC inhibitor trichostatin A led to upregulation of miR-1 expression, and miR-1 expression rendered these cells more susceptible to doxorubicin-induced apoptosis due to enhanced caspase 9, 3 and 7 activation and reduction in the Bcl-2 family member Mcl-1 (172).

miR-124 and miR-137 expression lead to differentiation of brain tumor CSC, inhibiting growth of glioblastoma and astrocytoma (173). Similarly to miR-1, treatment of glioblastoma cells with the differentiating agent 5-Aza-2'-deoxycytidine lead to upregulation of miR-137 (173).

The caveat of the "miRNA re-expression therapy" approach is that, like for many protein-coding genes, a gene product may have cell-specific functions, which sometimes are antagonistic and dependent on interacting partners (i.e. Klf4, as discussed above). This is observed for miR-34a, initially described as a tumor suppressor miRNA due to its upregulation following p53 activation (174), but recently described to have oncogenic properties when overexpressed in the presence of c-Myc (175). Situations like these should be kept in mind when devising new treatment strategies, either by improving delivery or controlling miRNA expression in the targeted cells.

7. PERSPECTIVES

We know from diverse lines of evidence the power of intrinsic pathways of cellular transformation. Over- or misexpression of proto-oncogenes, global gene silencing through misregulated chromatin remodeling proteins, reduction in expression of tumor suppression factors, altered response to paracrine growth or inhibitory factors, all contribute to the transformation of a target cell. Here we discussed how the recently characterized CSC reinforced new and old therapies that try to take advantage of differentiation pathways in the treatment of cancer. New strategies are focusing on more global therapeutic approaches, taking advantage of epigenetic and microRNA regulatory pathways to induce expression of differentiation-associated genetic signatures. Rather than aiming at the creation of new monotherapies, these approaches hold the

promise of inducing CSC differentiation and rendering them susceptible to currently used drugs.

7.1. Thoughts on cancers of the lymphoid system and tumor microenvironment

In most tissues, differentiation is associated with the loss of pluripotency, proliferative capacity and self-renewal. As discussed thus far, the generation of CSC and tumor-propagating cells requires those to revert back to a de-differentiated state and in that way generate the bulk of the tumor. The lymphoid system is unique in that differentiation is naturally associated with acquisition of these so-called stem characteristics. As shown for $CD8^+$ T cells, mature naïve T lymphocytes depend on intermittent recognition of major histocompatibility complex molecules presenting self-peptides for survival (176, 177), but maintain the capacity to extensively proliferate and further differentiate upon encounter with their cognate antigen. When exposed to this antigen under favorable conditions, peripheral mature $CD8^+$ T lymphocytes differentiate into effector or memory cells by asymmetric division, such that effector cells are short lived and memory cells acquire the ability to self-renewal and further differentiate upon new stimuli (178). Indeed, memory $CD8^+$ T cells share the genetic signature of hematopoietic stem cells (HSC), expressing genes associated with both longevity and self-renewal (179). These characteristics suggest that memory T cells, and memory B cells alike, would be good targets for transformation and could easily generate tumor cells with the characteristics of CSC. Indeed, memory $CD8^+$ T cells are prone to transformation in the absence of the tumor suppressor and chromatin remodeling factor Snf5, in a manner that they still depend on TCR signaling for expansion (180). These data suggest that, in some cases, rather than simply inducing differentiation a successful therapeutic protocol would require skewing the differentiating cells towards specific short-lived subpopulations.

Beyond the scope of this review but worth of attention is the role the tumor microenvironment plays in the process of establishment and growth of the tumor masses. Transformed cells can be flagged and destroyed by the organism through the initiation of an anti-tumor response, a process that has been fully appreciated when looking at tumor development and progression in immunocompromised individuals, mice and humans (181-188). Moreover, stromal and parenchyma tissue which may include endothelial cells also play an important role in sustaining the growth of transformed cells *in vivo* (189). They both secrete and respond to factors produced by the cancer cell (190-192), establishing a tumor-promoting cancer microenvironment and often sustaining the tumor-propagating CSC. The specific factors contributing to CSC homeostasis provided by the tumor niche have not yet been identified, but there is clear evidence that a healthy microenvironment, at least in the context of teratomas and embryonic development, can reprogram teratogenic cells transferred into blastocysts and promote the development of normal animals from these otherwise tumorigenic cells (193, 194). It is true that transplantable teratogenic cells differ from several other tumor types in that they do not bear chromosomal abnormalities. However, it is likely that among the factors secreted by the tumor-associated cells, stimuli that maintain the undifferentiated state of CSC are also present, and nullifying their action may facilitate differentiation therapies that target the intrinsic factors herein discussed. Modern therapy calls for the combination of different approaches that target both CSC, tumor microenvironment and the bulk of tumor cells, all in seek of long term remission, and if possible, a cure.

7.2. Concluding remarks

Overall, a cell or tissue is considered transformed when it maintains the ability to survive and often gain the ability to proliferate independently of the cues provided by the organism. Cells only perform their physiological roles when they are not within the cell cycle, therefore while proliferating they do not contribute to the processes important for the organism´s survival. During differentiation, cells exit the cell cycle and express tissue specific genes and proteins that, more than characterizing them as a particular tissue, confer the ability to perform a very specific function. Invariably, through the accumulation of mutations in diverse pathways discussed throughout this series of reviews, the cell loses the capacity to exit the cell cycle and therefore, to differentiate into an organ-specific cell. To state that differentiation is an evolutionarily selected way to avoid cellular transformation would underestimate the benefit of generating specialized organs and tissues for the survival of multicellular organisms. Nevertheless, terminally differentiated cells are not capable of reentering the cell cycle and therefore this is a safe way of maintaining the integrity of an organism. The problem comes from the plasticity that exists, and is so essential for life itself, that is the capacity to heal and regenerate. Because organs can be injured and need repair, many cell types are equipped such that they allow a certain level of de-differentiation or transition into a more proliferative, less specialized state in physiological conditions. Alternatively, as discussed, a class of tissue specific stem cells may supply organs with this pool of cells that responds readily to healing stimuli. Either way, it is the inability to achieve differentiation once the stimulus is gone, perpetuated by the accumulation of mutations that mimic these signals or impede the perception of their absence, which culminates with cellular transformation and tumorigenesis. Corroborating this opinion, most if not all genes described to date to participate on or induce transformation are essential for the process of differentiation or regulate genes that act on this process. Therefore, as it is clear for many other physiological processes, cellular differentiation was not selected as such but has an important impact on limiting transformation.

As discussed here, there are many anti-tumor therapies that take advantage of the differentiation-inducing capacity of specific compounds. The plethora of tissue and tumor types that can be targeted by generic molecules like RA or some chromatin remodeling agents turns out to be their benefit but also their Achilles' heel, since it leads to devastating side effects in patients. However, the concept that tumorigenesis is associated with an altered differentiation capacity and therefore a less differentiated

phenotype, which can be counteracted by forced cellular differentiation has been clearly demonstrated and new venues searching for more specific differentiating agents are worthy of being pursued.

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Abbreviations: CSC: cancer stem cell; TIC: tumor initiating cell; APC: adenomatous polyposis coli; CD: cluster of differentiation; iPS cell: induced pluripotent stem cell; MEF: murine embryonic fibroblast; ES cell: embryonic stem cell; Oct: octamer-binding transcription factor; Sox: Sry-related box; Klf: Kruppel-like factor; LIF: leukemia inhibitory factor; ICM: inner cell mass; NFkappaB: nuclear factor kappa B; BMP: bone morphogenic protein; TGF: transforming growth factor; RA: retinoic acid; ATRA: all-trans retinoic acid; RAR: retinoic acid receptor; RXR: retinoic X receptor; RAREs: RA response elements; Rb: retinoblastoma; CDK: cyclin-dependent kinase; APL: acute promyelocytic leukemia; HDAC: histone deacetylase; G-CSF: granulocyte colony stimulating factor; Swi/SNF: switch/sucrose non-fermenting; SAHA: synthetic suberoylanilide hydroxamic acid; DNMT: DNA methyltransferase; MBD: methyl-CpG binding domain; NuRD: nucleosomal remodeling complex; CIMP: CpG island methylator phenotype; miR: micro RNA; NCOR: nuclear receptor corepressor; RMS: rhabdomyosarcoma; EGFR: epidermal growth factor receptor; HSC: hematopoetic stem cell.

Key Words: Differentiation, Cancer, Cancer Stem Cells, Tumor-Inducing Cells, Induced Pluripotent Cells, Review

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