

## Endogenous anticancer mechanism: differentiation

Miriam Bianchi de Frontin Werneck

Program of Cellular Biology, Brazilian National Institute of Cancer (INCA); Laboratory of Immunopharmacology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, RJ, Brazil

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Tissue homeostasis and cellular dedifferentiation
4. Cancer Stem-Like Cells
  - 4.1. Counter-arguments to the cancer stem cell theory
5. Transcription factors regulating cellular reprogramming and dedifferentiation
  - 5.1. Oct-3/4
  - 5.2. Sox2
  - 5.3. Klf-4
  - 5.4. c-Myc
  - 5.5. Nanog
  - 5.6. p53
6. Therapeutic approaches
  - 6.1. Differentiation therapy
    - 6.1.1. Bone morphogenetic proteins
    - 6.1.2. Retinoic acid
    - 6.1.3. Chromatin remodeling compounds
      - 6.1.3.1. Histone deacetylases
      - 6.1.3.2. DNA methyltransferases
    - 6.1.4. Small non-coding regulatory RNA
7. Perspectives
  - 7.1. A few thoughts on cancer of the lymphoid system and tumor microenvironment
  - 7.2. Concluding remarks
8. Acknowledgements
9. References

## 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

## Endogenous anticancer mechanism: differentiation

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

## Endogenous anticancer mechanism: differentiation

**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<sup>+</sup> 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

## Endogenous anticancer mechanism: differentiation

**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 <sup>1</sup>	Breast ductal cells, Kidney epithelia, Laryngeal squamous cell, Skin	(198, 199), (200), (200), (201, 202)
c-Myc <sup>2</sup>	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, Rhabdomyosarcoma, 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)
$\beta$ -Catenin	Breast, Colon, Liver	(227), (228), (229)

<sup>1</sup> More information in Evans & Liu (76), <sup>2</sup> 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, ultraviolet 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 identified, 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 trophoblasts (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

## Endogenous anticancer mechanism: differentiation

embryonal carcinoma, besides being associated with gastric and pancreatic carcinoma, and transformation following human papillomavirus 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 downregulation 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<sup>Cip1/WAF1</sup>, p57<sup>Kip2</sup> 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  $\beta$ -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<sup>Cip1/WAF1</sup>, but in cases where the expression of p21<sup>Cip1/WAF1</sup> 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<sup>V12</sup>-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 reprogramming 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

## Endogenous anticancer mechanism: differentiation

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<sup>Arf</sup> and p16<sup>Ink4a</sup>, 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 rationale behind this method is to induce differentiation of the more immature tumor populations,

## Endogenous anticancer mechanism: differentiation

**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 <sup>1</sup> , 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-Azacitidine / 5-Aza-2'-deoxycytidine	Myelodysplastic syndrome, Neuroblastoma, Ovarian	(159, 160), (163), (161, 162)

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<sup>+</sup> 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 downregulation 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).

## Endogenous anticancer mechanism: differentiation

### 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

## Endogenous anticancer mechanism: differentiation

**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 downregulated 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

## Endogenous anticancer mechanism: differentiation

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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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

## Endogenous anticancer mechanism: differentiation

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.

### 8. ACKNOWLEDGEMENTS

The author thanks Dr. João P.B. Viola, Dr. Leonardo K. Teixeira, Dr. Guido Lenz and Dr. Patrícia S. de Araujo-Souza for the critical reading of this manuscript. MBFW is supported by a PNPd fellowship from CAPES/FAPERJ and grants from CNPq (483449/2009-1) and INCT-Cancer (573806/2008-0 and 170.026/2008).

### 9. REFERENCES

1. Blacklow, R. S.: Actuarially speaking: an overview of life expectancy. What can we anticipate? *Am.J.Clin.Nutr.*, 86, 1560S-1562S (2007)
2. Jemal, A., R. Siegel, J. Xu & E. Ward: Cancer statistics, 2010. *CA Cancer J Clin*, 60, 277-300 (2010)
3. MS/INCA: Estimativa 2010: incidência de câncer no Brasil. I. N. d. C. R. d. Janeiro, Ed., Gráfica Flama(2009).
4. Hanahan, D. & R. A. Weinberg: The hallmarks of cancer. *Cell*, 100, 57-70 (2000)
5. Poss, K. D.: Advances in understanding tissue regenerative capacity and mechanisms in animals. *Nat Rev Genet*, 11, 710-22 (2010)
6. Sell, S.: Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol*, 51, 1-28 (2004)
7. Wu, Y., R. C. Zhao & E. E. Tredget: Concise review: bone marrow-derived stem/progenitor cells in cutaneous repair and regeneration. *Stem Cells*, 28, 905-15 (2010)
8. Krause, D. S., N. D. Theise, M. I. Collector, O. Henegariu, S. Hwang, R. Gardner, S. Neutzel & S. J. Sharkis: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, 105, 369-77 (2001)
9. Hanahan, D. & R. A. Weinberg: Hallmarks of cancer: the next generation. *Cell*, 144, 646-74 (2011)
10. Frank, N. Y., T. Schatton & M. H. Frank: The therapeutic promise of the cancer stem cell concept. *J Clin Invest*, 120, 41-50 (2010)
11. Hill, R. P. & R. Perris: "Destemming" cancer stem cells. *J Natl Cancer Inst*, 99, 1435-40 (2007)
12. Neumuller, R. A. & J. A. Knoblich: Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. *Genes Dev*, 23, 2675-99 (2009)
13. Powell, A. E., C. Y. Shung, K. W. Saylor, K. A. Mullendorff, J. B. Weiss & M. H. Wong: Lessons from development: A role for asymmetric stem cell division in cancer. *Stem Cell Res*, 4, 3-9 (2010)
14. Furth, J. & M. C. Kahn: The transmission of leukemia in mice with a single cell. *Am J Cancer*, 31, 276-282 (1937)
15. Lapidot, T., C. Sirard, J. Vormoor, B. Murdoch, T. Hoang, J. Caceres-Cortes, M. Minden, B. Paterson, M. A. Caligiuri & J. E. Dick: A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*, 367, 645-648 (1994)
16. Al Hajj, M., M. S. Wicha, A. Benito-Hernandez, S. J. Morrison & M. F. Clarke: Prospective identification of tumorigenic breast cancer cells. *Proc.Natl.Acad.Sci.U.S.A.*, 100, 3983-3988 (2003)
17. Chan, K. S., I. Espinosa, M. Chao, D. Wong, L. Ailles, M. Diehn, H. Gill, J. Presti, Jr., H. Y. Chang, M. van de Rijn, L. Shortliffe & I. L. Weissman: Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc Natl Acad Sci U S A*, 106, 14016-21 (2009)
18. Collins, A. T., P. A. Berry, C. Hyde, M. J. Stower & N. J. Maitland: Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res*, 65, 10946-51 (2005)
19. Hermann, P. C., S. L. Huber, T. Herrler, A. Aicher, J. W. Ellwart, M. Guba, C. J. Bruns & C. Heeschen: Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell*, 1, 313-23 (2007)
20. Hope, K. J., L. Jin & J. E. Dick: Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat.Immunol.*, 5, 738-743 (2004)
21. Li, C., D. G. Heidt, P. Dalerba, C. F. Burant, L. Zhang, V. Adsay, M. Wicha, M. F. Clarke & D. M. Simeone: Identification of pancreatic cancer stem cells. *Cancer Res*, 67, 1030-7 (2007)
22. O'Brien, C. A., A. Pollett, S. Gallinger & J. E. Dick: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*, 445, 106-110 (2007)
23. Patrawala, L., T. Calhoun, R. Schneider-Broussard, H. Li, B. Bhatia, S. Tang, J. G. Reilly, D. Chandra, J. Zhou, K. Claypool, L. Coghlan & D. G. Tang: Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene*, 25, 1696-708 (2006)
24. Prince, M. E., R. Sivanandan, A. Kaczorowski, G. T. Wolf, M. J. Kaplan, P. Dalerba, I. L. Weissman, M. F. Clarke & L. E. Ailles: Identification of a subpopulation of cells with cancer stem cell properties in head and neck

## Endogenous anticancer mechanism: differentiation

- squamous cell carcinoma. *Proc Natl Acad Sci U S A*, 104, 973-8 (2007)
25. Ricci-Vitiani, L., D. G. Lombardi, E. Pilozzi, M. Biffoni, M. Todaro, C. Peschle & R. De Maria: Identification and expansion of human colon-cancer-initiating cells. *Nature*, 445, 111-5 (2007)
26. Schatton, T., G. F. Murphy, N. Y. Frank, K. Yamaura, A. M. Waaga-Gasser, M. Gasser, Q. Zhan, S. Jordan, L. M. Duncan, C. Weishaupt, R. C. Fuhlbrigge, T. S. Kupper, M. H. Sayegh & M. H. Frank: Identification of cells initiating human melanomas. *Nature*, 451, 345-9 (2008)
27. Singh, S. K., C. Hawkins, I. D. Clarke, J. A. Squire, J. Bayani, T. Hide, R. M. Henkelman, M. D. Cusimano & P. B. Dirks: Identification of human brain tumour initiating cells. *Nature*, 432, 396-401 (2004)
28. Suva, M. L., N. Riggi, J. C. Stehle, K. Baumer, S. Tercier, J. M. Joseph, D. Suva, V. Clement, P. Provero, L. Cironi, M. C. Osterheld, L. Guillou & I. Stamenkovic: Identification of cancer stem cells in Ewing's sarcoma. *Cancer Res*, 69, 1776-81 (2009)
29. Tan, B. T., C. Y. Park, L. E. Ailles & I. L. Weissman: The cancer stem cell hypothesis: a work in progress. *Lab Invest*, 86, 1203-7 (2006)
30. Yang, Z. F., D. W. Ho, M. N. Ng, C. K. Lau, W. C. Yu, P. Ngai, P. W. Chu, C. T. Lam, R. T. Poon & S. T. Fan: Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell*, 13, 153-66 (2008)
31. Zhang, S., C. Balch, M. W. Chan, H. C. Lai, D. Matei, J. M. Schilder, P. S. Yan, T. H. Huang & K. P. Nephew: Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res*, 68, 4311-20 (2008)
32. Clarke, M. F., J. E. Dick, P. B. Dirks, C. J. Eaves, C. H. Jamieson, D. L. Jones, J. Visvader, I. L. Weissman & G. M. Wahl: Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res*, 66, 9339-44 (2006)
33. Ricci-Vitiani, L., R. Pallini, M. Biffoni, M. Todaro, G. Invernici, T. Cenci, G. Maira, E. A. Parati, G. Stassi, L. M. Larocca & R. De Maria: Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature*, 468, 824-8 (2010)
34. Wang, R., K. Chadalavada, J. Wilshire, U. Kowalik, K. E. Hovinga, A. Geber, B. Fligelman, M. Leversha, C. Brennan & V. Tabar: Glioblastoma stem-like cells give rise to tumour endothelium. *Nature*, 468, 829-33 (2010)
35. Cohnheim, J.: Ueber entzündung und eiterung. *Path Anat Physiol Klin Med*, 40, 1-79 (1867)
36. Polyak, K. & W. C. Hahn: Roots and stems: stem cells in cancer. *Nat.Med.*, 12, 296-300 (2006)
37. Takahashi, K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda & S. Yamanaka: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131, 861-72 (2007)
38. Takahashi, K. & S. Yamanaka: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126, 663-76 (2006)
39. Maherali, N., R. Sridharan, W. Xie, J. Utikal, S. Eminli, K. Arnold, M. Stadtfeld, R. Yachechko, J. Tchieu, R. Jaenisch, K. Plath & K. Hochedlinger: Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*, 1, 55-70 (2007)
40. Okita, K., T. Ichisaka & S. Yamanaka: Generation of germline-competent induced pluripotent stem cells. *Nature*, 448, 313-7 (2007)
41. Wernig, M., A. Meissner, R. Foreman, T. Brambrink, M. Ku, K. Hochedlinger, B. E. Bernstein & R. Jaenisch: In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*, 448, 318-24 (2007)
42. Kelly, P. N., A. Dakic, J. M. Adams, S. L. Nutt & A. Strasser: Tumor growth need not be driven by rare cancer stem cells. *Science*, 317, 337 (2007)
43. Quintana, E., M. Shackleton, H. R. Foster, D. R. Fullen, M. S. Sabel, T. M. Johnson & S. J. Morrison: Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell*, 18, 510-23 (2010)
44. O'Neil, J. & A. T. Look: Mechanisms of transcription factor deregulation in lymphoid cell transformation. *Oncogene*, 26, 6838-6849 (2007)
45. Palomero, T., W. K. Lim, D. T. Odom, M. L. Sulis, P. J. Real, A. Margolin, K. C. Barnes, J. O'Neil, D. Neuberg, A. P. Weng, J. C. Aster, F. Sigaux, J. Soulier, A. T. Look, R. A. Young, A. Califano & A. A. Ferrando: NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. *Proc.Natl.Acad.Sci.U.S.A.*, 103, 18261-18266 (2006)
46. Palomero, T., D. T. Odom, J. O'Neil, A. A. Ferrando, A. Margolin, D. S. Neuberg, S. S. Winter, R. S. Larson, W. Li, X. S. Liu, R. A. Young & A. T. Look: Transcriptional regulatory networks downstream of TAL1/SCL in T-cell acute lymphoblastic leukemia. *Blood*, 108, 986-992 (2006)
47. Roy, M., W. S. Pear & J. C. Aster: The multifaceted role of Notch in cancer. *Curr.Opin.Genet.Dev.*, 17, 52-59 (2007)
48. Weng, A. P. & J. C. Aster: Multiple niches for Notch in cancer: context is everything. *Curr.Opin.Genet.Dev.*, 14, 48-54 (2004)
49. Weng, A. P., A. A. Ferrando, W. Lee, J. P. Morris, L. B. Silverman, C. Sanchez-Irizarry, S. C. Blacklow, A. T. Look & J. C. Aster: Activating mutations of NOTCH1 in

## Endogenous anticancer mechanism: differentiation

- human T cell acute lymphoblastic leukemia. *Science*, 306, 269-271 (2004)
50. Roberts, C. W., M. M. Leroux, M. D. Fleming & S. H. Orkin: Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. *Cancer Cell*, 2, 415-425 (2002)
51. Okamoto, K., H. Okazawa, A. Okuda, M. Sakai, M. Muramatsu & H. Hamada: A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. *Cell*, 60, 461-72 (1990)
52. Rosner, M. H., M. A. Vigano, K. Ozato, P. M. Timmons, F. Poirier, P. W. Rigby & L. M. Staudt: A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. *Nature*, 345, 686-92 (1990)
53. Scholer, H. R., S. Ruppert, N. Suzuki, K. Chowdhury & P. Gruss: New type of POU domain in germ line-specific protein Oct-4. *Nature*, 344, 435-9 (1990)
54. Wang, X. & J. Dai: Concise review: isoforms of OCT4 contribute to the confusing diversity in stem cell biology. *Stem Cells*, 28, 885-93 (2010)
55. Pan, G. J., Z. Y. Chang, H. R. Scholer & D. Pei: Stem cell pluripotency and transcription factor Oct4. *Cell Res*, 12, 321-9 (2002)
56. Boyer, L. A., T. I. Lee, M. F. Cole, S. E. Johnstone, S. S. Levine, J. P. Zucker, M. G. Guenther, R. M. Kumar, H. L. Murray, R. G. Jenner, D. K. Gifford, D. A. Melton, R. Jaenisch & R. A. Young: Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, 122, 947-56 (2005)
57. Chew, J. L., Y. H. Loh, W. Zhang, X. Chen, W. L. Tam, L. S. Yeap, P. Li, Y. S. Ang, B. Lim, P. Robson & H. H. Ng: Reciprocal transcriptional regulation of Pou5f1 and Sox2 via the Oct4/Sox2 complex in embryonic stem cells. *Mol Cell Biol*, 25, 6031-46 (2005)
58. Kuroda, T., M. Tada, H. Kubota, H. Kimura, S. Y. Hatano, H. Suemori, N. Nakatsuji & T. Tada: Octamer and Sox elements are required for transcriptional cis regulation of Nanog gene expression. *Mol Cell Biol*, 25, 2475-85 (2005)
59. Rodda, D. J., J. L. Chew, L. H. Lim, Y. H. Loh, B. Wang, H. H. Ng & P. Robson: Transcriptional regulation of nanog by OCT4 and SOX2. *J Biol Chem*, 280, 24731-7 (2005)
60. Nichols, J., B. Zevnik, K. Anastassiadis, H. Niwa, D. Klewe-Nebenius, I. Chambers, H. Scholer & A. Smith: Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*, 95, 379-91 (1998)
61. Cauffman, G., I. Liebaers, A. Van Steirteghem & H. Van de Velde: POU5F1 isoforms show different expression patterns in human embryonic stem cells and preimplantation embryos. *Stem Cells*, 24, 2685-91 (2006)
62. Lee, J., H. K. Kim, J. Y. Rho, Y. M. Han & J. Kim: The human OCT-4 isoforms differ in their ability to confer self-renewal. *J Biol Chem*, 281, 33554-65 (2006)
63. Lengner, C. J., F. D. Camargo, K. Hochedlinger, G. G. Welstead, S. Zaidi, S. Gokhale, H. R. Scholer, A. Tomilin & R. Jaenisch: Oct4 expression is not required for mouse somatic stem cell self-renewal. *Cell Stem Cell*, 1, 403-15 (2007)
64. Matsuoka, J., M. Yashiro, K. Sakurai, N. Kubo, H. Tanaka, K. Muguruma, T. Sawada, M. Ohira & K. Hirakawa: Role of the Stemness Factors Sox2, Oct3/4, and Nanog in Gastric Carcinoma. *J Surg Res* (2010)
65. Oliver, R. T.: Germ cell cancer. *Curr Opin Oncol*, 11, 236-41 (1999)
66. Wen, J., J. Y. Park, K. H. Park, H. W. Chung, S. Bang, S. W. Park & S. Y. Song: Oct4 and Nanog expression is associated with early stages of pancreatic carcinogenesis. *Pancreas*, 39, 622-6 (2010)
67. Lenz, G.: Transient oncogenes. *Med Hypotheses*, 75, 660-2 (2010)
68. Gubbay, J., J. Collignon, P. Koopman, B. Capel, A. Economou, A. Munsterberg, N. Vivian, P. Goodfellow & R. Lovell-Badge: A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature*, 346, 245-50 (1990)
69. Sinclair, A. H., P. Berta, M. S. Palmer, J. R. Hawkins, B. L. Griffiths, M. J. Smith, J. W. Foster, A. M. Frischauf, R. Lovell-Badge & P. N. Goodfellow: A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, 346, 240-4 (1990)
70. Kiefer, J. C.: Back to basics: Sox genes. *Dev Dyn*, 236, 2356-66 (2007)
71. Avilion, A. A., S. K. Nicolis, L. H. Pevny, L. Perez, N. Vivian & R. Lovell-Badge: Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev*, 17, 126-40 (2003)
72. Gen, Y., K. Yasui, Y. Zen, K. Zen, O. Dohi, M. Endo, K. Tsuji, N. Wakabayashi, Y. Itoh, Y. Naito, M. Taniwaki, Y. Nakanuma, T. Okanoue & T. Yoshikawa: SOX2 identified as a target gene for the amplification at 3q26 that is frequently detected in esophageal squamous cell carcinoma. *Cancer Genet Cytogenet*, 202, 82-93 (2010)
73. Ji, J. & P. S. Zheng: Expression of Sox2 in human cervical carcinogenesis. *Hum Pathol*, 41, 1438-47 (2010)
74. Rodriguez-Pinilla, S. M., D. Sarrío, G. Moreno-Bueno, Y. Rodriguez-Gil, M. A. Martínez, L. Hernandez, D. Hardisson, J. S. Reis-Filho & J. Palacios: Sox2: a possible

## Endogenous anticancer mechanism: differentiation

driver of the basal-like phenotype in sporadic breast cancer. *Mod Pathol*, 20, 474-81 (2007)

75. Sholl, L. M., J. A. Barletta, B. Y. Yeap, L. R. Chirieac & J. L. Hornick: Sox2 protein expression is an independent poor prognostic indicator in stage I lung adenocarcinoma. *Am J Surg Pathol*, 34, 1193-8 (2010)

76. Evans, P. M. & C. Liu: Roles of Krupel-like factor 4 in normal homeostasis, cancer and stem cells. *Acta Biochim Biophys Sin (Shanghai)*, 40, 554-64 (2008)

77. Hinnebusch, B. F., A. Siddique, J. W. Henderson, M. S. Malo, W. Zhang, C. P. Athaide, M. A. Abedrapo, X. Chen, V. W. Yang & R. A. Hodin: Enterocyte differentiation marker intestinal alkaline phosphatase is a target gene of the gut-enriched Kruppel-like factor. *Am J Physiol Gastrointest Liver Physiol*, 286, G23-30 (2004)

78. Zhang, W., D. E. Geiman, J. M. Shields, D. T. Dang, C. S. Mahatan, K. H. Kaestner, J. R. Biggs, A. S. Kraft & V. W. Yang: The gut-enriched Kruppel-like factor (Kruppel-like factor 4) mediates the transactivating effect of p53 on the p21WAF1/Cip1 promoter. *J Biol Chem*, 275, 18391-8 (2000)

79. Zhang, W., X. Chen, Y. Kato, P. M. Evans, S. Yuan, J. Yang, P. G. Rychahou, V. W. Yang, X. He, B. M. Evers & C. Liu: Novel cross talk of Kruppel-like factor 4 and beta-catenin regulates normal intestinal homeostasis and tumor repression. *Mol Cell Biol*, 26, 2055-64 (2006)

80. Rowland, B. D., R. Bernards & D. S. Peeper: The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. *Nat Cell Biol*, 7, 1074-82 (2005)

81. Oster, S. K., C. S. Ho, E. L. Soucie & L. Z. Penn: The myc oncogene: MarvelouslyY Complex. *Adv Cancer Res*, 84, 81-154 (2002)

82. Pelengaris, S., M. Khan & G. I. Evan: Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell*, 109, 321-34 (2002)

83. Nakagawa, M., M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa & S. Yamanaka: Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*, 26, 101-6 (2008)

84. Vita, M. & M. Henriksson: The Myc oncoprotein as a therapeutic target for human cancer. *Semin Cancer Biol*, 16, 318-30 (2006)

85. Yu, J., M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, Slukvin, II & J. A.

Thomson: Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318, 1917-20 (2007)

86. Chambers, I., D. Colby, M. Robertson, J. Nichols, S. Lee, S. Tweedie & A. Smith: Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell*, 113, 643-55 (2003)

87. Mitsui, K., Y. Tokuzawa, H. Itoh, K. Segawa, M. Murakami, K. Takahashi, M. Maruyama, M. Maeda & S. Yamanaka: The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*, 113, 631-42 (2003)

88. Pan, G. & J. A. Thomson: Nanog and transcriptional networks in embryonic stem cell pluripotency. *Cell Res*, 17, 42-9 (2007)

89. Hough, S. R., I. Clements, P. J. Welch & K. A. Wiederholt: Differentiation of mouse embryonic stem cells after RNA interference-mediated silencing of OCT4 and Nanog. *Stem Cells*, 24, 1467-75 (2006)

90. Hyslop, L., M. Stojkovic, L. Armstrong, T. Walter, P. Stojkovic, S. Przyborski, M. Herbert, A. Murdoch, T. Strachan & M. Lako: Downregulation of NANOG induces differentiation of human embryonic stem cells to extraembryonic lineages. *Stem Cells*, 23, 1035-43 (2005)

91. Lin, Y. L., Z. B. Han, F. Y. Xiong, L. Y. Tian, X. J. Wu, S. W. Xue, Y. R. Zhou, J. X. Deng & H. X. Chen: Malignant transformation of 293 cells induced by ectopic expression of human Nanog. *Mol Cell Biochem* (2011)

92. Pan, Y., J. Jiao, C. Zhou, Q. Cheng, Y. Hu & H. Chen: Nanog is highly expressed in ovarian serous cystadenocarcinoma and correlated with clinical stage and pathological grade. *Pathobiology*, 77, 283-8 (2010)

93. Pan, G., J. Li, Y. Zhou, H. Zheng & D. Pei: A negative feedback loop of transcription factors that controls stem cell pluripotency and self-renewal. *FASEB J*, 20, 1730-2 (2006)

94. Papapetrou, E. P., M. J. Tomishima, S. M. Chambers, Y. Mica, E. Reed, J. Menon, V. Tabar, Q. Mo, L. Studer & M. Sadelain: Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proc Natl Acad Sci USA*, 106, 12759-64 (2009)

95. Kawamura, T., J. Suzuki, Y. V. Wang, S. Menendez, L. B. Morera, A. Raya, G. M. Wahl & J. C. Belmonte: Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*, 460, 1140-4 (2009)

96. Qin, H., T. Yu, T. Qing, Y. Liu, Y. Zhao, J. Cai, J. Li, Z. Song, X. Qu, P. Zhou, J. Wu, M. Ding & H. Deng: Regulation of apoptosis and differentiation by p53 in human embryonic stem cells. *J Biol Chem*, 282, 5842-52 (2007)

## Endogenous anticancer mechanism: differentiation

97. Bao, H., J. R. Lyons & C. Zhou: Triple oxygen isotope evidence for elevated CO<sub>2</sub> levels after a Neoproterozoic glaciation. *Nature*, 453, 504-6 (2008)
98. Bao, S., Q. Wu, Z. Li, S. Sathornsumetee, H. Wang, R. E. McLendon, A. B. Hjelmeland & J. N. Rich: Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res*, 68, 6043-8 (2008)
99. Guzman, M. L., R. M. Rossi, S. Neelakantan, X. Li, C. A. Corbett, D. C. Hassane, M. W. Becker, J. M. Bennett, E. Sullivan, J. L. Lachowicz, A. Vaughan, C. J. Sweeney, W. Matthews, M. Carroll, J. L. Liesveld, P. A. Crooks & C. T. Jordan: An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. *Blood*, 110, 4427-35 (2007)
100. Jin, L., E. M. Lee, H. S. Ramshaw, S. J. Busfield, A. G. Peoppl, L. Wilkinson, M. A. Guthridge, D. Thomas, E. F. Barry, A. Boyd, D. P. Gearing, G. Vairo, A. F. Lopez, J. E. Dick & R. B. Lock: Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. *Cell Stem Cell*, 5, 31-42 (2009)
101. Schatton, T., N. Y. Frank & M. H. Frank: Identification and targeting of cancer stem cells. *Bioessays*, 31, 1038-49 (2009)
102. ten Cate, B., M. de Bruyn, Y. Wei, E. Bremer & W. Helfrich: Targeted elimination of leukemia stem cells; a new therapeutic approach in hemato-oncology. *Curr Drug Targets*, 11, 95-110 (2010)
103. Jordan, C. T., M. L. Guzman & M. Noble: Cancer stem cells. *N Engl J Med*, 355, 1253-61 (2006)
104. Rider, C. C. & B. Mulloy: Bone morphogenetic protein and growth differentiation factor cytokine families and their protein antagonists. *Biochem J*, 429, 1-12 (2010)
105. Neumann, J. C., G. L. Chandler, V. A. Damoulis, N. J. Fustino, K. Lillard, L. Looijenga, L. Margraf, D. Rakheja & J. F. Amatruda: Mutation in the type IB bone morphogenetic protein receptor *alk6b* impairs germ-cell differentiation and causes germ-cell tumors in zebrafish. *Proc Natl Acad Sci U S A*, 108, 13153-8 (2011)
106. Piccirillo, S. G., B. A. Reynolds, N. Zanetti, G. Lamorte, E. Binda, G. Broggi, H. Brem, A. Olivi, F. Dimeco & A. L. Vescovi: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature*, 444, 761-5 (2006)
107. Ide, H., T. Yoshida, N. Matsumoto, K. Aoki, Y. Osada, T. Sugimura & M. Terada: Growth regulation of human prostate cancer cells by bone morphogenetic protein-2. *Cancer Res*, 57, 5022-7 (1997)
108. Lombardo, Y., A. Scopelliti, P. Cammareri, M. Todaro, F. Iovino, L. Ricci-Vitiani, G. Gulotta, F. Dieli, R. de Maria & G. Stassi: Bone morphogenetic protein 4 induces differentiation of colorectal cancer stem cells and increases their response to chemotherapy in mice. *Gastroenterology*, 140, 297-309 (2011)
109. Zhao, H., O. Ayrault, F. Zindy, J. H. Kim & M. F. Roussel: Post-transcriptional down-regulation of *Atoh1/Math1* by bone morphogenic proteins suppresses medulloblastoma development. *Genes Dev*, 22, 722-7 (2008)
110. Na, Y. R., S. H. Seok, D. J. Kim, J. H. Han, T. H. Kim, H. Jung, B. H. Lee & J. H. Park: Bone morphogenetic protein 7 induces mesenchymal-to-epithelial transition in melanoma cells, leading to inhibition of metastasis. *Cancer Sci*, 100, 2218-25 (2009)
111. Tang, X. H. & L. J. Gudas: Retinoids, retinoic Acid receptors, and cancer. *Annu Rev Pathol*, 6, 345-64 (2011)
112. Duong, V. & C. Rochette-Egly: The molecular physiology of nuclear retinoic acid receptors. From health to disease. *Biochim Biophys Acta* (2010)
113. Strickland, S. & V. Mahdavi: The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell*, 15, 393-403 (1978)
114. Chen, H., J. M. Dong, Y. Liu & J. F. Chiu: Identification of a cis-acting element in the rat alpha-fetoprotein gene and its specific binding proteins in F9 cells during retinoic acid-induced differentiation. *J Cell Biochem*, 72, 25-34 (1999)
115. Bunaciu, R. P. & A. Yen: Activation of the aryl hydrocarbon receptor AhR promotes retinoic acid-induced differentiation of myeloblastic leukemia cells by restricting expression of the stem cell transcription factor Oct4. *Cancer Res* (2011)
116. Fuchs, E. & H. Green: Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell*, 25, 617-25 (1981)
117. Matthay, K. K., C. P. Reynolds, R. C. Seeger, H. Shimada, E. S. Adkins, D. Haas-Kogan, R. B. Gerbing, W. B. London & J. G. Villablanca: Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. *J Clin Oncol*, 27, 1007-13 (2009)
118. Spanjaard, R. A., M. Ikeda, P. J. Lee, B. Charpentier, W. W. Chin & T. J. Eberlein: Specific activation of retinoic acid receptors (RARs) and retinoid X receptors reveals a unique role for RARgamma in induction of differentiation and apoptosis of S91 melanoma cells. *J Biol Chem*, 272, 18990-9 (1997)
119. Warrell, R. P., Jr., S. R. Frankel, W. H. Miller, Jr., D. A. Scheinberg, L. M. Itri, W. N. Hittelman, R. Vyas, M. Andreeff, A. Tafuri, A. Jakubowski & et al.: Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). *N Engl J Med*, 324, 1385-93 (1991)

## Endogenous anticancer mechanism: differentiation

120. Touma, S. E., J. S. Goldberg, P. Moench, X. Guo, S. K. Tickoo, L. J. Gudas & D. M. Nanus: Retinoic acid and the histone deacetylase inhibitor trichostatin a inhibit the proliferation of human renal cell carcinoma in a xenograft tumor model. *Clin Cancer Res*, 11, 3558-66 (2005)
121. Cras, A., D. Darsin-Bettinger, N. Balitrand, B. Cassinat, A. Soulie, M. E. Toubert, L. Delva & C. Chomienne: Epigenetic patterns of the retinoic acid receptor beta2 promoter in retinoic acid-resistant thyroid cancer cells. *Oncogene*, 26, 4018-24 (2007)
122. Farias, E. F., K. Petrie, B. Leibovitch, J. Murtagh, M. B. Chornet, T. Schenk, A. Zelent & S. Waxman: Interference with Sin3 function induces epigenetic reprogramming and differentiation in breast cancer cells. *Proc Natl Acad Sci U S A*, 107, 11811-6 (2010)
123. Tavares, T. S., D. Nanus, X. J. Yang & L. J. Gudas: Gene microarray analysis of human renal cell carcinoma: the effects of HDAC inhibition and retinoid treatment. *Cancer Biol Ther*, 7, 1607-18 (2008)
124. Higuchi, T., M. Kizaki & M. Omine: Induction of differentiation of retinoic acid-resistant acute promyelocytic leukemia cells by the combination of all-trans retinoic acid and granulocyte colony-stimulating factor. *Leuk Res*, 28, 525-32 (2004)
125. Cimino, G., F. Lo-Coco, S. Fenu, L. Travaglini, E. Finolezzi, M. Mancini, M. Nanni, A. Careddu, F. Fazi, F. Padula, R. Fiorini, M. A. Spiriti, M. C. Petti, A. Venditti, S. Amadori, F. Mandelli, P. G. Pelicci & C. Nervi: Sequential valproic acid/all-trans retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukemia. *Cancer Res*, 66, 8903-11 (2006)
126. Garcia-Manero, G., H. M. Kantarjian, B. Sanchez-Gonzalez, H. Yang, G. Rosner, S. Verstovsek, M. Rytting, W. G. Wierda, F. Ravandi, C. Koller, L. Xiao, S. Faderl, Z. Estrov, J. Cortes, S. O'Brien, E. Estey, C. Bueso-Ramos, J. Fiorentino, E. Jabbour & J. P. Issa: Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. *Blood*, 108, 3271-9 (2006)
127. de The, H. & Z. Chen: Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. *Nat Rev Cancer*, 10, 775-83 (2010)
128. Lessard, J. A. & G. R. Crabtree: Chromatin regulatory mechanisms in pluripotency. *Annu Rev Cell Dev Biol*, 26, 503-32 (2010)
129. Ringrose, L. & R. Paro: Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet*, 38, 413-43 (2004)
130. Bochar, D. A., L. Wang, H. Beniya, A. Kinev, Y. Xue, W. S. Lane, W. Wang, F. Kashanchi & R. Shiekhattar: BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell*, 102, 257-65 (2000)
131. Cheng, S. W., K. P. Davies, E. Yung, R. J. Beltran, J. Yu & G. V. Kalpana: c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. *Nat Genet*, 22, 102-5 (1999)
132. Genovese, C., D. Trani, M. Caputi & P. P. Claudio: Cell cycle control and beyond: emerging roles for the retinoblastoma gene family. *Oncogene*, 25, 5201-5209 (2006)
133. Giacinti, C. & A. Giordano: RB and cell cycle progression. *Oncogene*, 25, 5220-5227 (2006)
134. Guidi, C. J., R. Mudhasani, K. Hoover, A. Koff, I. Leav, A. N. Imbalzano & S. N. Jones: Functional interaction of the retinoblastoma and Ini1/Snf5 tumor suppressors in cell growth and pituitary tumorigenesis. *Cancer Res*, 66, 8076-8082 (2006)
135. Khidr, L. & P. L. Chen: RB, the conductor that orchestrates life, death and differentiation. *Oncogene*, 25, 5210-5219 (2006)
136. Nagl, N. G., Jr., D. R. Zweitzig, B. Thimmapaya, G. R. Beck, Jr. & E. Moran: The c-myc gene is a direct target of mammalian SWI/SNF-related complexes during differentiation-associated cell cycle arrest. *Cancer Res*, 66, 1289-1293 (2006)
137. Wang, G. G., C. D. Allis & P. Chi: Chromatin remodeling and cancer, Part II: ATP-dependent chromatin remodeling. *Trends Mol.Med.*, 13, 373-380 (2007)
138. Wang, G. G., C. D. Allis & P. Chi: Chromatin remodeling and cancer, Part I: Covalent histone modifications. *Trends Mol Med*, 13, 363-72 (2007)
139. Botrugno, O. A., F. Santoro & S. Minucci: Histone deacetylase inhibitors as a new weapon in the arsenal of differentiation therapies of cancer. *Cancer Lett*, 280, 134-44 (2009)
140. Jones, P. A. & S. B. Baylin: The epigenomics of cancer. *Cell*, 128, 683-92 (2007)
141. Leder, A., S. Orkin & P. Leder: Differentiation of erythroleukemic cells in the presence of inhibitors of DNA synthesis. *Science*, 190, 893-4 (1975)
142. Tanaka, M., J. Levy, M. Terada, R. Breslow, R. A. Rifkind & P. A. Marks: Induction of erythroid differentiation in murine virus infected erythroleukemia cells by highly polar compounds. *Proc Natl Acad Sci U S A*, 72, 1003-6 (1975)
143. Riggs, M. G., R. G. Whittaker, J. R. Neumann & V. M. Ingram: n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature*, 268, 462-4 (1977)
144. Cousens, L. S., D. Gallwitz & B. M. Alberts: Different accessibilities in chromatin to histone acetylase. *J Biol Chem*, 254, 1716-23 (1979)

## Endogenous anticancer mechanism: differentiation

145. Andreeff, M., R. Stone, J. Michaeli, C. W. Young, W. P. Tong, H. Sogoloff, T. Ervin, D. Kufe, R. A. Rifkind & P. A. Marks: Hexamethylene bisacetamide in myelodysplastic syndrome and acute myelogenous leukemia: a phase II clinical trial with a differentiation-inducing agent. *Blood*, 80, 2604-9 (1992)
146. Richon, V. M., S. Emiliani, E. Verdin, Y. Webb, R. Breslow, R. A. Rifkind & P. A. Marks: A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc Natl Acad Sci U S A*, 95, 3003-7 (1998)
147. Yoshida, M., M. Kijima, M. Akita & T. Beppu: Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem*, 265, 17174-9 (1990)
148. Mann, B. S., J. R. Johnson, K. He, R. Sridhara, S. Abraham, B. P. Booth, L. Verbois, D. E. Morse, J. M. Jee, S. Pope, R. S. Harpanhalli, R. Dagher, A. Farrell, R. Justice & R. Pazdur: Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. *Clin Cancer Res*, 13, 2318-22 (2007)
149. Olsen, E. A., Y. H. Kim, T. M. Kuzel, T. R. Pacheco, F. M. Foss, S. Parker, S. R. Frankel, C. Chen, J. L. Ricker, J. M. Arduino & M. Duvic: Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol*, 25, 3109-15 (2007)
150. Richon, V. M.: Cancer biology: mechanism of antitumour action of vorinostat (suberoylanilide hydroxamic acid), a novel histone deacetylase inhibitor. *British Journal of Cancer*, 95, S2-S6 (2006)
151. Lin, H. Y., C. S. Chen, S. P. Lin & J. R. Weng: Targeting histone deacetylase in cancer therapy. *Med Res Rev*, 26, 397-413 (2006)
152. Tan, J., S. Cang, Y. Ma, R. L. Petrillo & D. Liu: Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. *J Hematol Oncol*, 3, 5 (2010)
153. Kelly, T. K., D. D. De Carvalho & P. A. Jones: Epigenetic modifications as therapeutic targets. *Nat Biotechnol*, 28, 1069-1078 (2010)
154. Lister, R., M. Pelizzola, R. H. Dowen, R. D. Hawkins, G. Hon, J. Tonti-Filippini, J. R. Nery, L. Lee, Z. Ye, Q. M. Ngo, L. Edsall, J. Antosiewicz-Bourget, R. Stewart, V. Ruotti, A. H. Millar, J. A. Thomson, B. Ren & J. R. Ecker: Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, 462, 315-22 (2009)
155. Bogdanovic, O. & G. J. Veenstra: DNA methylation and methyl-CpG binding proteins: developmental requirements and function. *Chromosoma*, 118, 549-65 (2009)
156. Toyota, M., N. Ahuja, M. Ohe-Toyota, J. G. Herman, S. B. Baylin & J. P. Issa: CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A*, 96, 8681-6 (1999)
157. Teodoridis, J. M., C. Hardie & R. Brown: CpG island methylator phenotype (CIMP) in cancer: causes and implications. *Cancer Lett*, 268, 177-86 (2008)
158. Shen, L., H. Kantarjian, Y. Guo, E. Lin, J. Shan, X. Huang, D. Berry, S. Ahmed, W. Zhu, S. Pierce, Y. Kondo, Y. Oki, J. Jelinek, H. Saba, E. Estey & J. P. Issa: DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. *J Clin Oncol*, 28, 605-13 (2010)
159. Kaminskas, E., A. T. Farrell, Y. C. Wang, R. Sridhara & R. Pazdur: FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist*, 10, 176-82 (2005)
160. Kantarjian, H., J. P. Issa, C. S. Rosenfeld, J. M. Bennett, M. Albitar, J. DiPersio, V. Klimek, J. Slack, C. de Castro, F. Ravandi, R. Helmer, 3rd, L. Shen, S. D. Nimer, R. Leavitt, A. Raza & H. Saba: Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer*, 106, 1794-803 (2006)
161. Fang, F., C. Balch, J. Schilder, T. Breen, S. Zhang, C. Shen, L. Li, C. Kulesavage, A. J. Snyder, K. P. Nephew & D. E. Matei: A phase I and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. *Cancer*, 116, 4043-53 (2010)
162. Fu, S., W. Hu, R. Iyer, J. J. Kavanagh, R. L. Coleman, C. F. Levenback, A. K. Sood, J. K. Wolf, D. M. Gershenson, M. Markman, B. T. Hennessy, R. Kurzrock & R. C. Bast, Jr.: Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. *Cancer* (2010)
163. George, R. E., J. M. Lahti, P. C. Adamson, K. Zhu, D. Finkelstein, A. M. Ingle, J. M. Reid, M. Krailo, D. Neubergh, S. M. Blaney & L. Diller: Phase I study of decitabine with doxorubicin and cyclophosphamide in children with neuroblastoma and other solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer*, 55, 629-38 (2010)
164. Stathis, A., S. Hotte, E. X. Chen, H. Hirte, A. Oza, P. Moretto, S. Webster, A. Laughlin, L. A. Stayner, S. McGill, L. Wang, W. J. Zhang, I. Espinoza-Delago, J. L. Holleran, M. J. Egorin & L. L. Siu: Phase I study of decitabine in combination with vorinostat in patients with advanced solid tumors and non-Hodgkin's lymphomas. *Clin Cancer Res* (2011)
165. Kaiser, J.: Epigenetic drugs take on cancer. *Science*, 330, 576-8 (2010)

## Endogenous anticancer mechanism: differentiation

166. Mishra, P. J. & G. Merlino: MicroRNA reexpression as differentiation therapy in cancer. *J Clin Invest*, 119, 2119-23 (2009)
167. Farazi, T. A., J. I. Spitzer, P. Morozov & T. Tuschl: miRNAs in human cancer. *J Pathol*, 223, 102-15 (2011)
168. Foley, N. H., I. Bray, K. M. Watters, S. Das, K. Bryan, T. Bernas, J. H. Prehn & R. L. Stallings: MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through targeting of nuclear receptor corepressor 2. *Cell Death Differ* (2011)
169. Taulli, R., F. Bersani, V. Foglizzo, A. Linari, E. Vigna, M. Ladanyi, T. Tuschl & C. Ponzetto: The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. *J Clin Invest*, 119, 2366-78 (2009)
170. Adams, B. D., D. M. Cowee & B. A. White: The role of miR-206 in the epidermal growth factor (EGF) induced repression of estrogen receptor-alpha (ERalpha) signaling and a luminal phenotype in MCF-7 breast cancer cells. *Mol Endocrinol*, 23, 1215-30 (2009)
171. Datta, J., H. Kutay, M. W. Nasser, G. J. Nuovo, B. Wang, S. Majumder, C. G. Liu, S. Volinia, C. M. Croce, T. D. Schmittgen, K. Ghoshal & S. T. Jacob: Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res*, 68, 5049-58 (2008)
172. Nasser, M. W., J. Datta, G. Nuovo, H. Kutay, T. Motiwala, S. Majumder, B. Wang, S. Suster, S. T. Jacob & K. Ghoshal: Down-regulation of micro-RNA-1 (miR-1) in lung cancer. Suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1. *J Biol Chem*, 283, 33394-405 (2008)
173. Silber, J., D. A. Lim, C. Petritsch, A. I. Persson, A. K. Maunakea, M. Yu, S. R. Vandenberg, D. G. Ginzinger, C. D. James, J. F. Costello, G. Bergers, W. A. Weiss, A. Alvarez-Buylla & J. G. Hodgson: miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. *BMC Med*, 6, 14 (2008)
174. Hermeking, H.: The miR-34 family in cancer and apoptosis. *Cell Death Differ*, 17, 193-9 (2010)
175. Sotillo, E., T. Laver, H. Mellert, J. M. Schelter, M. A. Cleary, S. McMahon & A. Thomas-Tikhonenko: Myc overexpression brings out unexpected antiapoptotic effects of miR-34a. *Oncogene* (2011)
176. Markiewicz, M. A., I. Brown & T. F. Gajewski: Death of peripheral CD8+ T cells in the absence of MHC class I is Fas-dependent and not blocked by Bcl-xL. *Eur.J.Immunol.*, 33, 2917-2926 (2003)
177. Pestano, G. A., Y. Zhou, L. A. Trimble, J. Daley, G. F. Weber & H. Cantor: Inactivation of misselected CD8 T cells by CD8 gene methylation and cell death. *Science*, 284, 1187-1191 (1999)
178. Chang, J. T., V. R. Palanivel, I. Kinjyo, F. Schambach, A. M. Intlekofer, A. Banerjee, S. A. Longworth, K. E. Vinup, P. Mrass, J. Oliaro, N. Killeen, J. S. Orange, S. M. Russell, W. Weninger & S. L. Reiner: Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science*, 315, 1687-91 (2007)
179. Luckey, C. J., D. Bhattacharya, A. W. Goldrath, I. L. Weissman, C. Benoist & D. Mathis: Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *Proc.Natl.Acad.Sci.U.S.A.*, 103, 3304-3309 (2006)
180. Wang, X., M. B. Werneck, B. G. Wilson, H. J. Kim, M. J. Kluk, C. S. Thom, J. W. Wischhusen, J. A. Evans, J. L. Jesneck, P. Nguyen, C. G. Sansam, H. Cantor & C. W. Roberts: TCR-dependent transformation of mature memory phenotype T cells in mice. *J Clin Invest* (2011)
181. Dunn, G. P., A. T. Bruce, K. C. Sheehan, V. Shankaran, R. Uppaluri, J. D. Bui, M. S. Diamond, C. M. Koebel, C. Arthur, J. M. White & R. D. Schreiber: A critical function for type I interferons in cancer immunoeediting. *Nat.Immunol.*, 6, 722-729 (2005)
182. Shankaran, V., H. Ikeda, A. T. Bruce, J. M. White, P. E. Swanson, L. J. Old & R. D. Schreiber: IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature*, 410, 1107-1111 (2001)
183. Smyth, M. J., K. Y. Thia, S. E. Street, E. Cretney, J. A. Trapani, M. Taniguchi, T. Kawano, S. B. Pelikan, N. Y. Crowe & D. I. Godfrey: Differential tumor surveillance by natural killer (NK) and NKT cells. *J.Exp.Med.*, 191, 661-668 (2000)
184. Smyth, M. J., K. Y. Thia, S. E. Street, D. MacGregor, D. I. Godfrey & J. A. Trapani: Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. *J.Exp.Med.*, 192, 755-760 (2000)
185. Swann, J. B. & M. J. Smyth: Immune surveillance of tumors. *J.Clin.Invest*, 117, 1137-1146 (2007)
186. van den Broek, M. E., D. Kagi, F. Ossendorp, R. Toes, S. Vamvakas, W. K. Lutz, C. J. Melief, R. M. Zinkernagel & H. Hengartner: Decreased tumor surveillance in perforin-deficient mice. *J.Exp.Med.*, 184, 1781-1790 (1996)
187. Werneck, M. B., G. Lugo-Villarino, E. S. Hwang, H. Cantor & L. H. Glimcher: T-bet plays a key role in NK-mediated control of melanoma metastatic disease. *J Immunol*, 180, 8004-10 (2008)
188. Willimsky, G. & T. Blankenstein: Sporadic immunogenic tumours avoid destruction by inducing T-cell tolerance. *Nature*, 437, 141-146 (2005)

## Endogenous anticancer mechanism: differentiation

189. Werneck, M. B., A. Vieira-de-Abreu, R. Chammas & J. P. Viola: NFAT1 transcription factor is central in the regulation of tissue microenvironment for tumor metastasis. *Cancer Immunol Immunother* (2011)
190. de Visser, K. E., A. Eichten & L. M. Coussens: Paradoxical roles of the immune system during cancer development. *Nat.Rev.Cancer*, 6, 24-37 (2006)
191. Lima, L. G., R. Chammas, R. Q. Monteiro, M. E. Moreira & M. A. Barcinski: Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer Lett*, 283, 168-75 (2009)
192. Qian, B. Z. & J. W. Pollard: Macrophage diversity enhances tumor progression and metastasis. *Cell*, 141, 39-51 (2010)
193. Mintz, B. & K. Illmensee: Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc Natl Acad Sci U S A*, 72, 3585-9 (1975)
194. Papaioannou, V. E., M. W. McBurney, R. L. Gardner & M. J. Evans: Fate of teratocarcinoma cells injected into early mouse embryos. *Nature*, 258, 70-73 (1975)
195. Bonnet, D. & J. E. Dick: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*, 3, 730-7 (1997)
196. Dalerba, P., S. J. Dylla, I. K. Park, R. Liu, X. Wang, R. W. Cho, T. Hoey, A. Gurney, E. H. Huang, D. M. Simeone, A. A. Shelton, G. Parmiani, C. Castelli & M. F. Clarke: Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A*, 104, 10158-63 (2007)
197. Brehm, A., K. Ohbo, W. Zwerschke, V. Botquin, P. Jansen-Durr & H. R. Scholer: Synergism with germ line transcription factor Oct-4: viral oncoproteins share the ability to mimic a stem cell-specific activity. *Mol Cell Biol*, 19, 2635-43 (1999)
198. Foster, K. W., A. R. Frost, P. McKie-Bell, C. Y. Lin, J. A. Engler, W. E. Grizzle & J. M. Ruppert: Increase of GSK3 $\beta$  messenger RNA and protein expression during progression of breast cancer. *Cancer Res*, 60, 6488-95 (2000)
199. Pandya, A. Y., L. I. Talley, A. R. Frost, T. J. Fitzgerald, V. Trivedi, M. Chakravarthy, D. C. Chhieng, W. E. Grizzle, J. A. Engler, H. Krontiras, K. I. Bland, A. F. LoBuglio, S. M. Lobo-Ruppert & J. M. Ruppert: Nuclear localization of KLF4 is associated with an aggressive phenotype in early-stage breast cancer. *Clin Cancer Res*, 10, 2709-19 (2004)
200. Foster, K. W., S. Ren, I. D. Louro, S. M. Lobo-Ruppert, P. McKie-Bell, W. Grizzle, M. R. Hayes, T. R. Broker, L. T. Chow & J. M. Ruppert: Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GSK3 $\beta$ . *Cell Growth Differ*, 10, 423-34 (1999)
201. Foster, K. W., Z. Liu, C. D. Nail, X. Li, T. J. Fitzgerald, S. K. Bailey, A. R. Frost, I. D. Louro, T. M. Townes, A. J. Paterson, J. E. Kudlow, S. M. Lobo-Ruppert & J. M. Ruppert: Induction of KLF4 in basal keratinocytes blocks the proliferation-differentiation switch and initiates squamous epithelial dysplasia. *Oncogene*, 24, 1491-500 (2005)
202. Huang, C. C., Z. Liu, X. Li, S. K. Bailey, C. D. Nail, K. W. Foster, A. R. Frost, J. M. Ruppert & S. M. Lobo-Ruppert: KLF4 and PCNA identify stages of tumor initiation in a conditional model of cutaneous squamous epithelial neoplasia. *Cancer Biol Ther*, 4, 1401-8 (2005)
203. Miranda Peralta, E. I., Y. Valles Ayoub, L. Hernandez Mendoza, L. M. Rangel Ramirez, A. Castrejon Rojas, J. Collazo-Jaloma, M. Gutierrez Romero, R. Gonzalez Constance & P. Gariglio Vidal: [MYC protein and proteins antigenically related with MYC in acute lymphoblastic leukemia]. *Rev Invest Clin*, 43, 139-45 (1991)
204. Burmeister, T., S. Schwartz, H. A. Horst, H. Rieder, N. Gokbuget, D. Hoelzer & E. Thiel: Molecular heterogeneity of sporadic adult Burkitt-type leukemia/lymphoma as revealed by PCR and cytogenetics: correlation with morphology, immunology and clinical features. *Leukemia*, 19, 1391-8 (2005)
205. Frost, M., J. Newell, M. A. Lones, S. R. Tripp, M. S. Cairo & S. L. Perkins: Comparative immunohistochemical analysis of pediatric Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Clin Pathol*, 121, 384-92 (2004)
206. Avet-Loiseau, H., F. Gerson, F. Magrangeas, S. Minvielle, J. L. Harousseau & R. Bataille: Rearrangements of the c-myc oncogene are present in 15% of primary human multiple myeloma tumors. *Blood*, 98, 3082-6 (2001)
207. Sardi, I., M. Dal Canto, R. Bartoletti, R. Guazzelli, F. Travaglini & E. Montali: Molecular genetic alterations of c-myc oncogene in superficial and locally advanced bladder cancer. *Eur Urol*, 33, 424-30 (1998)
208. Mizukami, Y., A. Nonomura, T. Takizawa, M. Noguchi, T. Michigishi, S. Nakamura & T. Ishizaki: N-myc protein expression in human breast carcinoma: prognostic implications. *Anticancer Res*, 15, 2899-905 (1995)
209. Finley, G. G., N. T. Schulz, S. A. Hill, J. R. Geiser, J. M. Pipas & A. I. Meisler: Expression of the myc gene family in different stages of human colorectal cancer. *Oncogene*, 4, 963-71 (1989)
210. Smith, D. R., T. Myint & H. S. Goh: Overexpression of the c-myc proto-oncogene in colorectal carcinoma. *Br J Cancer*, 68, 407-13 (1993)

## Endogenous anticancer mechanism: differentiation

211. Hara, T., A. Ooi, M. Kobayashi, M. Mai, K. Yanagihara & I. Nakanishi: Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. *Lab Invest*, 78, 1143-53 (1998)
212. Shervington, A., N. Cruickshanks, H. Wright, R. Atkinson-Dell, R. Lea, G. Roberts & L. Shervington: Glioma: what is the role of c-Myc, hsp90 and telomerase? *Mol Cell Biochem*, 283, 1-9 (2006)
213. Kawate, S., T. Fukusato, S. Ohwada, A. Watanuki & Y. Morishita: Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic features, proliferative activity and p53 overexpression. *Oncology*, 57, 157-63 (1999)
214. Eberhart, C. G., J. Kratz, Y. Wang, K. Summers, D. Stearns, K. Cohen, C. V. Dang & P. C. Burger: Histopathological and molecular prognostic markers in medulloblastoma: c-myc, N-myc, TrkC, and anaplasia. *J Neuropathol Exp Neurol*, 63, 441-9 (2004)
215. Rouah, E., D. R. Wilson, D. L. Armstrong & G. J. Darlington: N-myc amplification and neuronal differentiation in human primitive neuroectodermal tumors of the central nervous system. *Cancer Res*, 49, 1797-801 (1989)
216. Treszl, A., R. Adany, Z. Rakosy, L. Kardos, A. Begany, K. Gilde & M. Balazs: Extra copies of c-myc are more pronounced in nodular melanomas than in superficial spreading melanomas as revealed by fluorescence in situ hybridisation. *Cytometry B Clin Cytom*, 60, 37-46 (2004)
217. Brodeur, G. M., R. C. Seeger, M. Schwab, H. E. Varmus & J. M. Bishop: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science*, 224, 1121-4 (1984)
218. Baker, V. V., M. P. Borst, D. Dixon, K. D. Hatch, H. M. Shingleton & D. Miller: c-myc amplification in ovarian cancer. *Gynecol Oncol*, 38, 340-2 (1990)
219. Wu, R., L. Lin, D. G. Beer, L. H. Ellenson, B. J. Lamb, J. M. Rouillard, R. Kuick, S. Hanash, D. R. Schwartz, E. R. Fearon & K. R. Cho: Amplification and overexpression of the L-MYC proto-oncogene in ovarian carcinomas. *Am J Pathol*, 162, 1603-10 (2003)
220. Buttyan, R., I. S. Sawczuk, M. C. Benson, J. D. Siegal & C. A. Olsson: Enhanced expression of the c-myc protooncogene in high-grade human prostate cancers. *Prostate*, 11, 327-37 (1987)
221. Fleming, W. H., A. Hamel, R. MacDonald, E. Ramsey, N. M. Pettigrew, B. Johnston, J. G. Dodd & R. J. Matusik: Expression of the c-myc protooncogene in human prostatic carcinoma and benign prostatic hyperplasia. *Cancer Res*, 46, 1535-8 (1986)
222. Kozma, L., I. Kiss, A. Nagy, S. Szakall & I. Ember: Investigation of c-myc and K-ras amplification in renal clear cell adenocarcinoma. *Cancer Lett*, 111, 127-31 (1997)
223. Lee, W. H., A. L. Murphree & W. F. Benedict: Expression and amplification of the N-myc gene in primary retinoblastoma. *Nature*, 309, 458-60 (1984)
224. Dias, P., P. Kumar, H. B. Marsden, H. R. Gattamaneni, J. Heighway & S. Kumar: N-myc gene is amplified in alveolar rhabdomyosarcomas (RMS) but not in embryonal RMS. *Int J Cancer*, 45, 593-6 (1990)
225. Toffolatti, L., E. Frascella, V. Ninfo, C. Gambini, M. Forni, M. Carli & A. Rosolen: MYCN expression in human rhabdomyosarcoma cell lines and tumour samples. *J Pathol*, 196, 450-8 (2002)
226. Lui, W. O., D. M. Tanenbaum & C. Larsson: High level amplification of 1p32-33 and 2p22-24 in small cell lung carcinomas. *Int J Oncol*, 19, 451-7 (2001)
227. Imbert, A., R. Eelkema, S. Jordan, H. Feiner & P. Cowin: Delta N89 beta-catenin induces precocious development, differentiation, and neoplasia in mammary gland. *J Cell Biol*, 153, 555-68 (2001)
228. Kinzler, K. W. & B. Vogelstein: Lessons from hereditary colorectal cancer. *Cell*, 87, 159-70 (1996)
229. Anna, C. H., M. Iida, R. C. Sills & T. R. Devereux: Expression of potential beta-catenin targets, cyclin D1, c-Jun, c-Myc, E-cadherin, and EGFR in chemically induced hepatocellular neoplasms from B6C3F1 mice. *Toxicol Appl Pharmacol*, 190, 135-45 (2003)
230. Kirschbaum, M., P. Frankel, L. Popplewell, J. Zain, M. Delioukina, V. Pullarkat, D. Matsuoka, B. Pulone, A. J. Rotter, I. Espinoza-Delgado, A. Nademane, S. J. Forman, D. Gandara & E. Newman: Phase II Study of Vorinostat for Treatment of Relapsed or Refractory Indolent Non-Hodgkin's Lymphoma and Mantle Cell Lymphoma. *J Clin Oncol*, 29, 1198-203 (2011)
231. Galanis, E., K. A. Jaeckle, M. J. Maurer, J. M. Reid, M. M. Ames, J. S. Hardwick, J. F. Reilly, A. Loboda, M. Nebozhyn, V. R. Fantin, V. M. Richon, B. Scheithauer, C. Giannini, P. J. Flynn, D. F. Moore, Jr., J. Zwiebel & J. C. Buckner: Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. *J Clin Oncol*, 27, 2052-8 (2009)
232. Blumenschein, G. R., Jr., M. S. Kies, V. A. Papadimitrakopoulou, C. Lu, A. J. Kumar, J. L. Ricker, J. H. Chiao, C. Chen & S. R. Frankel: Phase II trial of the histone deacetylase inhibitor vorinostat (Zolinza, suberoylanilide hydroxamic acid, SAHA) in patients with recurrent and/or metastatic head and neck cancer. *Invest New Drugs*, 26, 81-7 (2008)
233. Crump, M., B. Coiffier, E. D. Jacobsen, L. Sun, J. L. Ricker, H. Xie, S. R. Frankel, S. S. Randolph & B. D.

## Endogenous anticancer mechanism: differentiation

Cheson: Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid) in relapsed diffuse large-B-cell lymphoma. *Ann Oncol*, 19, 964-9 (2008)

234. Bradley, D., D. Rathkopf, R. Dunn, W. M. Stadler, G. Liu, D. C. Smith, R. Pili, J. Zwiebel, H. Scher & M. Hussain: Vorinostat in advanced prostate cancer patients progressing on prior chemotherapy (National Cancer Institute Trial 6862): trial results and interleukin-6 analysis: a study by the Department of Defense Prostate Cancer Clinical Trial Consortium and University of Chicago Phase 2 Consortium. *Cancer*, 115, 5541-9 (2009)

235. Luu, T. H., R. J. Morgan, L. Leong, D. Lim, M. McNamara, J. Portnow, P. Frankel, D. D. Smith, J. H. Doroshow, C. Wong, A. Aparicio, D. R. Gandara & G. Somlo: A phase II trial of vorinostat (suberoylanilide hydroxamic acid) in metastatic breast cancer: a California Cancer Consortium study. *Clin Cancer Res*, 14, 7138-42 (2008)

236. Vansteenkiste, J., E. Van Cutsem, H. Dumez, C. Chen, J. L. Ricker, S. S. Randolph & P. Schoffski: Early phase II trial of oral vorinostat in relapsed or refractory breast, colorectal, or non-small cell lung cancer. *Invest New Drugs*, 26, 483-8 (2008)

237. Traynor, A. M., S. Dubey, J. C. Eickhoff, J. M. Kolesar, K. Schell, M. S. Huie, D. L. Groteluschen, S. M. Marcotte, C. M. Hallahan, H. R. Weeks, G. Wilding, I. Espinoza-Delgado & J. H. Schiller: Vorinostat (NSC# 701852) in patients with relapsed non-small cell lung cancer: a Wisconsin Oncology Network phase II study. *J Thorac Oncol*, 4, 522-6 (2009)

238. Modesitt, S. C., M. Sill, J. S. Hoffman & D. P. Bender: A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol*, 109, 182-6 (2008)

239. Stadler, W. M., K. Margolin, S. Ferber, W. McCulloch & J. A. Thompson: A phase II study of depsipeptide in refractory metastatic renal cell cancer. *Clin Genitourin Cancer*, 5, 57-60 (2006)

**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: hematopoietic stem cell.

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

**Send correspondence to:** Miriam B.F. Werneck; Instituto Nacional de Cancer (INCA); Rua Andre Cavalcanti 37, 5º andar; Centro, Rio de Janeiro, RJ; Brasil 22031-050, Tel: 55213207 6534, Fax: 55213207 6587, E-mail: meiwerneck@gmail.com