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The New Model of Carcinogenesis: The Cancer Stem Cell Hypothesis

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1. Introduction

Cancer has long been seen as a disease that arises from mutations and epigenetic alterations that impair the capacity of any cell within the organism to deregulation its proliferation, avoid apoptosis and metastasis, giving rise to malignant transformation. Progressively, these early cancer cells lead to different generations of daughter cells that accumulate additional mutations, acting in concert to drive the full neoplastic phenotype. Adult stem cells are found in numerous tissues of the body and play an important role in tissue development, replacement and repair. In addition, these cells show long-term replicative potential, together with the capacities of self-renewal and multi-lineage differentiation. These stem cell properties are tightly regulated in normal development, therefore their alteration may be a critical issue for tumorigenesis. The Cancer Stem Cell (CSC) theory arises from the poor probability of short-lived differentiated progenitors or terminally differentiated cells to accumulate mutations that become cancer. On the other side, the normal tissue stem cells or early progenitors that already possess the most important characteristics of cancer are an easy target for the accumulation of genetic aberrations which lead to cancer formation. The new defining model for carcinogenesis, the "cancer stem cell theory" was put forward since 1997, when John Dick and coworkers started a series of pioneering investigations to understand whether the functional hierarchy observed in normal hematopoiesis was conserved in blood tumors (Bonnet & Dick, 1997). According to this model cancer is a stem cell disease that places malignant stem cells at the centre of its tumorigenic activity. Cancer stem cells give rise to undifferentiated cells and terminally differentiated cells, as happens in normal tissue renewal, but the major difference between cancer growth and normal tissue renewal is that whereas normal transit amplifying cells usually differentiate and die, at various levels of differentiation, the cancer transit-amplifying cells fail to differentiate normally and instead accumulate (ie, they undergo maturation arrest), resulting in cancer growth.

Cancer stem cells may be caused by transforming mutations and/or epigenetic events to gain the self-renewal activity and lose some features of differentiation occurring in multi-potential stem cells, tissue-specific stem cells, progenitor cells, mature cells and cancer cells. This theory has been functionally supported by the observation that among all cancer cells within a particular tumor, only a minute cell fraction has the exclusive potential to regenerate the entire tumor cell population. Many groups have extrapolated the cancer stem

cell theory from the haematopoietic system to solid cancers, where using in vitro culture techniques and in vivo transplant models have established evidence of cancer stem cells in colon, pancreas, prostate, brain and breast cancers. Some tumor stem cells, like breast, have upregulated genes which include Notch. Notch signaling has been highlighted as a pathway involved in the development of the breast and is frequently deregulated in invasive breast cancer, as well as other oncogenic pathways like Wnt, Hh and NF-κB. Also studies on haematopoietic cancers show that these important signaling pathways for normal haematopoiesis, are oncogenic, thereby potentially involved in cancer stem cell regulation.

On the other side, the degree of differentiation of a carcinoma depends on the proportion of undifferentiated tumor stem cells. The cancer-derived differentiated cells are not normal, moreover they don't have the potential to develop cancer, so it could be attempt to direct normal differentiation of malignant stem cells and serve as an alternative to cytotoxic therapy. Differentiation therapies are currently underway. To be maximally effective, therapy of cancer must be directed against both the resting stem cells and the proliferating cells of the cancer. Conventional radiation treatment and chemotherapy only kill the actively proliferating cells of the cancer. Successful therapies could be reached if specific stem cell signals are inhibited using gene therapy, while at the same time attacking proliferating cells by conventional radiation treatment or chemotherapy. However, the chemoresistant phenotype of CSCs makes it difficult to increase their sensitivity to anticancer drugs and to decrease the rate of cancer recurrence in patients that is the one of major causes of death all over the world.

This review will focus on the role of stem cell as a target to carcinogenesis, the major oncogenic pathways and finally provides an update of the major chemoresistance related mechanisms of cancer stem cells.

2. The raise of the stem cells

At the dawn of the 20th century, we had recognized that chemicals cause cancer, but we had not yet identified individual cancer-causing molecules, nor did we know their cellular targets. What we lacked was knowledge of the mechanisms by which chemicals cause cancer and the molecular changes that characterize tumor progression. We now are early in a century in which cancer is being investigated at the molecular level, and we have developed technologies that afford unprecedented power to delineate and manipulate altered pathways in cancer cells (Loeb & Harris, 2008). However we still been confronted with the same old kind of questions and realities, Can we harness new insights and technologies to prevent or obliterate human cancers or delay their progression? Can we give more specific molecular-based therapeutic options to a cancer patient? Can we identify the origin of the carcinogenesis process?, defining these questions is a critical step to a complete understanding of carcinogenesis.

In the last 15 years a great advance has been performed trying to understand the mechanisms underlying the origin of the carcinogenesis process. With the concept of stem cells the cancer scenario suffers an extraordinary expansion. Nowadays with the discovery that almost all tissues in the body are able to renovate owing to the presence of Stem Cells (SCs), most of medical research about regeneration therapy involve the study of these cells. Indeed when looking for stem cells in Pubmed there are more than 150,000 papers. Since

1961 with Till and McCulloch initial work, lot of researchers have dealt of isolating and identify stem cells in all tissues (Till & McCulloch, 1961).

Stem Cells are defined principally by its genuine capacity of self-renewal, as a matter of fact in assays for identification of stemness is necessary to demonstrate this property. Some of most popular assays to evaluate functionally are, in the case of hematopoietic stem cells (HSC), the long term repopulating of the entire hematopoietic system of myeloablated animals *in vivo*. These studies have been done by transplanting murine HSC into irradiated, SCID (severe combined immunodeficient) and NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice (Jordan & Lemishka, 1990; Kamel-Reid & Dick J, 1988). Immunophenotypic analysis and functionally *in vitro* are, also, carry out to identify stem cells, for example, HSC are defined by their capacity to initiate and sustain long-term hematopoiesis in liquid cultures in presence of an adherent cell layer formed by stromal elements (Sutherland et al., 1990).

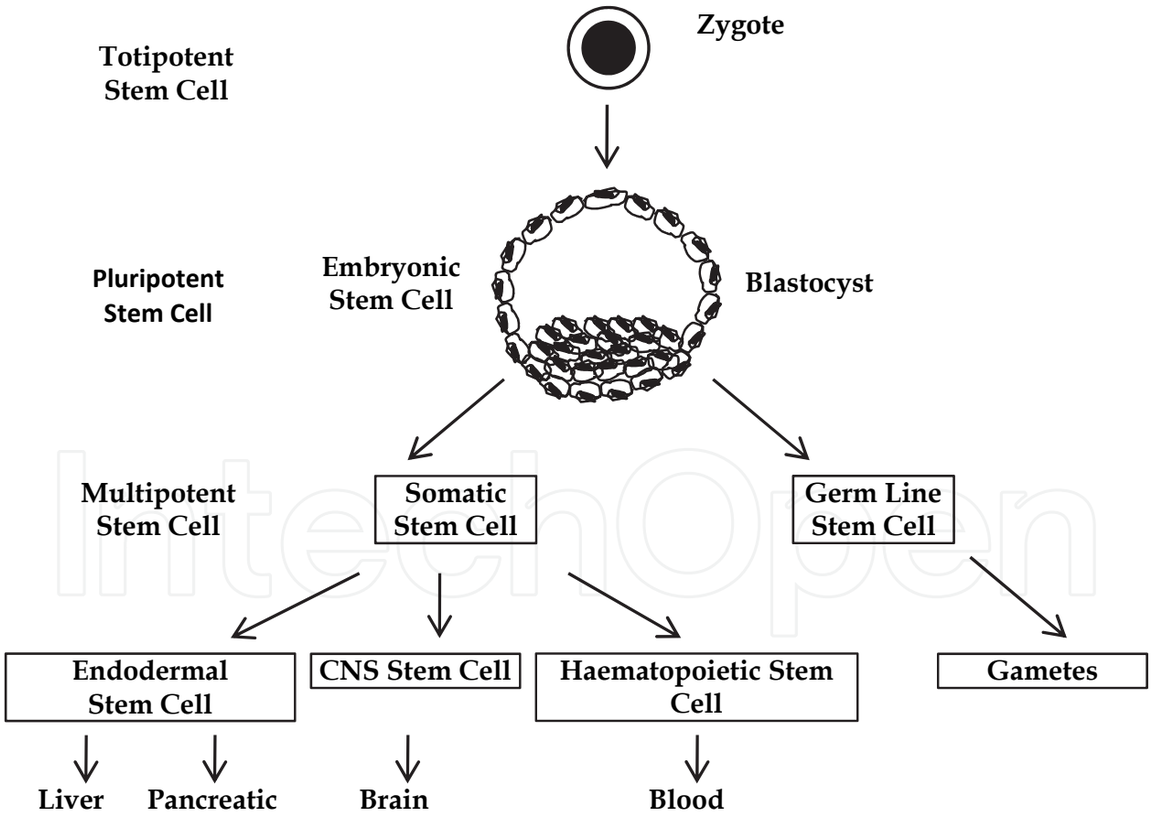
As responsible of the tissue renovation, SCs have an extraordinary expansion potential and an important capacity of differentiation, giving rise to a heterogeneous hierarchical progeny of cells (Mayani, 2003). To produce differentiated cells and at the same time preserve the stem cell pool, stem cells undergo asymmetric or symmetric cell divisions. Asymmetry division refers to two different daughter cells; a stem cell and a progenitor cell capable of differentiating, meanwhile, symmetric cell divisions contribute to regulate cell pool size giving rise to two identical stem cells, or two progenitor cells. In certain cases such as in hematopoiesis, it is likely that both mechanisms operate to preserve the SCs pool and give rise to all the different lineages and maturation stages (Mayani et al., 1993).

SCs types generated during development are the fertilized egg, or zygote, which is by definition the "Totipotent" stem cell capable of produce all the different cells of an organism, including those that do not form part of the embryo, such as placenta cells (Alberts et al., 1994, Wobus, 2010). Totipotent stem cells are up to morula (8-cell) stage, each cell produced is identical to the zygote. Then the embryo becomes blastocyst, in which each cell that forms part of the inner cell mass is capable of producing all cells of the embryo itself, but not extraembryonic structures produced only by trophoblast cells that then become placenta. At blastocyst stage, each cell is a "Pluripotent" Embryonic Stem Cell (ESC) with an unlimited proliferation potential (Williams, 1988). Three types of pluripotent stem cells lines with self-renewal capacity and the potential to differentiate into cell types of all three primary germ layers were derived from embryos and fetal stages of mice: embryonic stem (ES) cells were isolated from blastomeres of the early mouse embryo from the 8-cell up to the blastocyst stage (Evans & Kaufman, 1981, Wobus et al., 1991); embryonic germ (EG) cells were isolated from primordial germ cells, the precursor cells of germ cells from 9.5- to 12.5-day fetal stages (Resnick et al., 1992); and embryonic carcinoma (EC) cells have been established from the stem cell population of teratocarcinomas (Figure 1). These three cell types share typical characteristics, such as expression of alkaline phosphatase, the embryonic antigen SSEA-1 (Solter & Knowles, 1978) and the germline-specific transcription factor Oct-4 (Scholer at al., 1989), a short G1 phase of the cell cycle (Rohwedel et al., 1996) and high telomerase activity (Thomson et al., 1998).

A novel source of stem cells is induced pluripotent stem cells (iPSC) and adult multipotent stem cells. iPSC cells were first derived from murine fibroblasts by ectopic expression of

Oct4, Sox2, Klf4 and c-Myc transcription factors, demonstrating that the specialized somatic cells can be reversed into a pluripotent state in vitro (Takahasi & Yamanaka, 2006). Like embryonic stem (ES) cells, iPSC cells are able to self-renew indefinitely and to differentiate into all types of cells in the body. iPSC hold great promise for regenerative medicine, because iPSC avoid immunological rejection but also get away from ethical issues (Chen & Liu, 2009). Since the first report on the derivation of iPSC in 2006, many laboratories all over the world started research on iPSC cells and have made significant progress.

In the other hand, Till and McCulloch (1961) were the first to detect spleen-colony forming units in mouse bone marrow and to realize that these were a kind of stem cell, since that time, stem cell for a number of other adult tissues have been identified. The fundamental role of adult stem cells in a living organism involves maintaining the somatic cell population in tissues in response to cellular injuring or stress, and thus such stem cells are important in maintaining tissue homeostasis in the organism. Adult stem cells are multipotent differentiating into a few cell types, usually limited to the tissue from which they arise, however, a few reports have suggest that have the potential to differentiate to cells other than their tissue of origin (Charbod, 2010; D´lppolito et al., 2004; Jiang et al., 2002) (Figure 1).



Embryogenesis starts with the fertilized oocyte, zygote (totipotent stem cell), which develops into embryonic stem cell (pluripotent stem cell). Somatic and germ line cells represent the multipotent stem cells. CNS: Central Nervous System.

Fig. 1. General Types of Stem Cells.

Stem cells develop within specific microenvironments called "niches" consisting of different cell types and their products, these external elements provide signals that control stem cell behavior by modulating expression and activity of the nuclear transcription factors, gene expression, molecular regulators of cell cycle, epigenetic regulation, etc., (Oh & Kwon, 2010). To avoid aberrant growth or tissue loss, the balance between stem cell proliferation and differentiation must be perfect. In natural conditions, stem cells are found in quiescence and their frequency is very low (Orford & Scadden, 2008). For example in bone marrow, where are located the vast majority of HSC, there are 1 HSC per 1–2 x 10^4 bone marrow cells (i.e., 0.01–0.005% of total bone marrow cells) (Szilvassy & Hoffman, 1995; Thomas et al., 1999), and as we have mentioned previously, the location of HSC within the bone marrow is not random. Most HSC are located within the endosteal region, whereas lineage-committed progenitors and mature cells are distributed away from this region, predominantly in the central marrow region, close to the central marrow vessels (Nilsson et al., 2001).

In the elegant manuscript of the hallmarks of cancer, Hanahan and Weinberg (2011) described a set of events necessary to reach a transformation condition, it was worth-noting for a lot of researchers that some of these events are present in stem cells, because these cells can undergo extensively cell division and has the potential to give rise to both stem cells and cell differentiate into specialized cells, opening the possibility of the participation of stem cells in cancer process. The still unresolved question is how stem cells, which stay in very well maintained niches, interacts with environmental factors that provide them the damage that will became an irreversible deregulated self-renewal? How oncogenes and/or tumor suppressors genes can be compromised in cancer stem cell process?

2.1 Stem cells as target of mutations and transformation

Target cells of transforming mutations is unknown, even in cancer; however there is considerable evidence that certain types of leukemia arise from mutations that accumulate HSC. This proposal is sustained in the fact that cells capable of initiating human Acute Myeloid Leukemia (AML) have a $CD34^+CD38^-$ phenotype in most AML subtypes and thus have similar phenotype to normal HSC. Conversely, $CD34^+CD38^+$ leukemia cells cannot transfer disease, despite the fact that they exhibit leukemic blast phenotype. This suggests that normal HSC rather than committed progenitors are target for leukemic transformation (Reya el at., 2001).

In a work of Miyamoto an colleges (2000), done on HSC from patients in remission, AML1-ETO transcripts which result of the most frequent chromosomal translocation 8;21 were found in a fraction of normal HSC in the marrow. These prospectively isolated HSC and their progeny were not leukemic, indicating that translocation occurred originally in normal HSC and that additional mutation in a subset of these HSC or their progeny subsequently lead to leukemia. Other evidences where clonotypic leukemia-associated chromosomal rearrangements have also been found in $CD34^+CD38^-$ lymphoid (George et al., 2001) and chronic myeloid leukemias (Mauro & Druker, 2001).

Although SCs are often target of genetic events that trigger malignant transformation, in other cases restricted progenitors or even differentiated cells may become transformed. Mouse model in which myeloid leukemia arises from restricted progenitors was created by targeting the expression of transgenes specifically to restricted myeloid progenitors using

the hMRP-8 promoter, this model of leukemia resemble human leukemias in many respects. The enforced expression of anti-apoptotic gene bcl-2 in the myeloid linage leads to a disease that is similar to human chronic myelomonocytic leukemia, including monocytosis, splenomegaly and neutropenia, as the mice age, but rarely these mice develop acute malignancies. To test whether additional mutations are required to synergize with bcl-2 to promote AML, hMRP8-bcl-2 transgenic mice were bred with lpr/lpr Fas-deficient mice. Remarkably, the loss of these two distinct apoptosis pathways led to the development of AML in 15% of the mice (Traver et al., 1998). These mice have an expansion of myeloblast in all haematopoietic tissues, with substantially lowered number of granulocytes in the marrow and blood. As previously described, in the case of spontaneously arising human leukemias it is likely that stem cells accumulate the mutations that are necessary for neoplastic proliferation; however these mutations are expressed in restricted progenitors. That is, mutations that accumulate in stem cells may lead to neoplastic proliferation of primitive progenitors downstream of stem cells. Perhaps the reason why only 15% of mice progress to AML expressing Bcl-2 and lacking Fas is that the progenitors in these mice also must acquire an additional mutation that cause deregulated self-renewal. If a single additional mutation causes transforming events is probably a gain-of-function mutation, such as one that promotes constitutive self-renewal. Due that stabilized β-catenin promote self-renewal of HSCs and other progenitors, Reya and colleges (2001) propose that gain-of-function mutations in β-catenin may, transform deathless pre-malignant cells to cancer cells by promoting proliferation.

2.2 Evidences about the existence of Cancer Stem Cells (CSC)

The first's evidences about the existence of Cancer Stem Cells were documented for leukemia and multiple myeloma reporting that only small subset of cancer cells is capable of extensive proliferation. When leukemic cells were transplanted in vivo, only 1-4% of cells could form spleen colonies (Bruce & Van der Gaag, 1963; Wodinsky et al., 1967). Even when mouse myeloma cells were obtained from ascites, separated from normal hematopoietic cells and put in clonal in vitro colony forming assay, only 1 in 10,000 to 1 in 100 cancer cells were able to form colonies (Park et al., 1971). The clonogenic leukemic cells were described as leukemic stem cells because the differences in clonogenicity among the leukemia cells mirrored the differences among normal haematopoietic cells. Bonnet and Dick (1997) show that human Acute Myeloid Leukemia (AML) stem cells could be identified prospectively and purified as $CD34^{+}CD38^{-}$ cells form patient samples. Despite the fact that these cells represent only 0.2% of variable proportion of AML cells, they were the only cells with the capacity of transferring AML from human patients to NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice in the vast majority of cases. For solid tumors, also has been shown that the cells are phenotypically heterogeneous and that only a small proportion of cells are clonogenic in vivo and in culture; for example, in lung cancer, only 1 in 1000 to 1 in 5000; ovarian cancer and neuroblastoma cells were found to form colonies in soft agar. Only in terms of leukemic stem cells, these findings led to the hypothesis that only a few cancer cells are actually tumorigenic and that these tumorigenic cells could be considered as cancer stem cells (CSC).

In most human breast cancer, only a very small fraction of the tumor clone defined as $CD44^{+}$,CD24neg/low and representing 11-35% of total cancer cells, is able to sustain tumor

grow when xenografted in NOD/SCID mice (Al-Hajj et al., 2003). The CSC working model has also been proved in brain tumors, Galli et al., (2004) succeeded to isolate and propagated neurospheres from human glioblastoma, which are highly enriched in long-term cell renewing multi-linage-differentiating and tumor-initiating cells. Another example for demonstrating the existence of CSC was found in rapidly renewing intestinal cells (small intestine and colon). This epithelium is full with normal stem cells localized at the bottom of the crypts and expressing the Wnt target gene Lgr5. Stem cells that were disrupted of the APC (Adenomatous Polyposis Coli) gene were leading to constitutive activation of the Wnt pathway, a well known initiation step in intestinal cancer. The result was the formation of microscopic adenomas, a first step in malignant transformation of the intestine. Interestingly, when the APC gene was deleted in more differentiated and short-lived progenitors, microadenoma could be observed that however lacked growth potential and did hardly result in microscopic adenoma formation (Lapham et al., 2009).

2.3 Theory of cancer stem cell

In cancer biology has long recognized that tumor is complex collections of aberrant cells, each of which ultimately derives from a single antecessor, the cell of origin that describes their clonal source. The process by which the cell of origin develops into an ever-expanding, heterogeneous tumor that retains many features of its parent tissue remains mysterious. In this classical carcinogenesis model, a somatic differentiated cell had to be reprogrammed or "dedifferentiated" to regain immortalization properties of cancer. The limited life span and proliferation capacity of the differentiated cell should be induced to an irreversible change that enables the cell to get the ability to have unlimited proliferation (Trosko, 2009); then after, this initiated cell have to survive long enough to acquire the hallmarks of cancer. Recently the cancer stem cell theory has re-emerged as a compelling model to explain both the clonality and some of the heterogeneity of tumors. It postulate that each tumor contains a subset of cells, cancer stem cells, that are unique responsible for tumor growth, heterogeneity and metastasis. These cells specialized; possess important features, self-renewal and differentiation potential, that together pointing out them from the remainder of the tumor cells (Rothenberg and Clarke, 2009).

The cancer stem cell theory proposes that tumors have a cellular hierarchy that is a caricature of their normal tissue counterpart because they reflect the pluripotency of the originally transformed cell.

Two observations lead to this theory, first most tumors arise from a single cell, but not all the cells in a tumor are identical this is also known as tumor heterogeneity (Park et al., 1971). A widely held belief in cancer biology is that all cellular heterogeneity found in tumors may be attributed to genomic instability and the selection for cells that can adapt to the tumor microenvironment. However recent evidence strongly supports that CSC also plays a major role in tumor heterogeneity (Lobo et al., 2007).

The second observation was build came from studies that demonstrated that a large number of cancer cells were required to growth a tumor (Bruce & Van der Gaag, 1963; Hamburger & Salmon, 1977). Two formal possibilities could explain this observation: either all cancer cells had a low probability of proliferating extensively or most cancer cells were unable to

proliferate extensively and only a small, definable subset of cells was consistently clonogenic.

In both cases, some of the cancer cell heterogeneity would arise a result of environmental differences within the tumor and continuing mutagenesis. The essential difference between these possibilities is the prediction, according to the second possibility that whatever the environment or mutational status of the cells, only a small phenotipically distinct subset of cancer cells has the ability to proliferate extensively or form a new tumor.

The cancer stem theory postulate that only some tumor cells, cancer stem cells, are tumorigenic. When they divide, these malignant cells self-renew, and also give rise to the non-self-renewing cells that go on to elaborate the heterogeneous cells within a cancer. In this sense, CSC´s both drive tumor growth, and also initiate the execution of a developmental program. Cancer stem cells thus utilize pre-existing developmental hierarchies, and give rise to some of the cellular heterogeneity seen in such tumors.

After proposal of cancer stem theory, the term "cancer stem cell" is an operative definition that does not necessarily connote a developmental relationship to normal stem cells; instead, it merely states that a subset of cells within a tumor can self-renew and elaborate tumor heterogeneity. Recently, the definition depends on the assay of self-renewal and tumorigenicity. Also, the cancer stem cell theory does not imply what percentage of cells within a tumor are cancer stem cells. Some very aggressive tumors may have a high percentage of CSC´s (Kelly et al., 2007, Kennedy et al., 2007), and chemotherapeutic treatments may increase the frequency of CSC´s in a tumor (Dylla et al., 2008); however neither of these observations affects the operative definition. Is important to mention that stochastic model and the cancer stem are not mutually exclusive. Since they are malignant cells, CSC´s likely exhibit genomic instability, and have a mutator phenotype (Hannahan & Weinberg, 2000). Thus, as they divide and give rise to both tumorigenic and non-tumorigenic progeny subclones of CSC´s may develop.

2.4 Proposal of cancer stem cell model integration into the stochastic model

It is believed that the initiation step is caused by a mutation or any other irreversible event, in which the single normal cell acquire an advantage proliferation capacity and/or to avoid apoptosis. Given the natural characteristics of stem cells, the initiation process could be more easily carry out if the cell has per se the capacity of self-renewal, then to complete the step, only the deregulation of these mechanisms would be needed, a good example of this is the aberrant activation of the Wnt signaling pathway in many cancer types and especially those of the gastrointestinal tract. Indeed, Wnt signaling activity was shown to designate colon CSCs (de Sousa et al., 2011).

On the other side, promoting conditions can be wound healing, surgery, cell death, inflammatory agents, growth factor hormones, and mutagenic or non-mutagenic chemicals that stimulate proliferation, then, the initiated cell must be clonally expanded, process which also can be facilitated by self-renewal and great proliferation properties of stem cells. It is important to highlight that in most of the tumors it has been seen blocked differentiation, so the initiated cell would not terminally differentiate and neither die by apoptosis (Piscaglia et al., 2007). Indeed, if we begin the transformation with a stem cell rather than with a differentiated cell, the block of differentiation process would be easier that reprogramming

or "dedifferentiation, consequently, in promotion step the adult stem cells inhibits its ability to complete differentiation under normal conditions and the symmetric division is preferred, meanwhile any other normal stem cell would divide preferentially in the asymmetric way to favor differentiation, and could die by apoptosis or suffer senescence if any damage is not resolved (Reviewed in Trosko, 2009)(Figure 2).

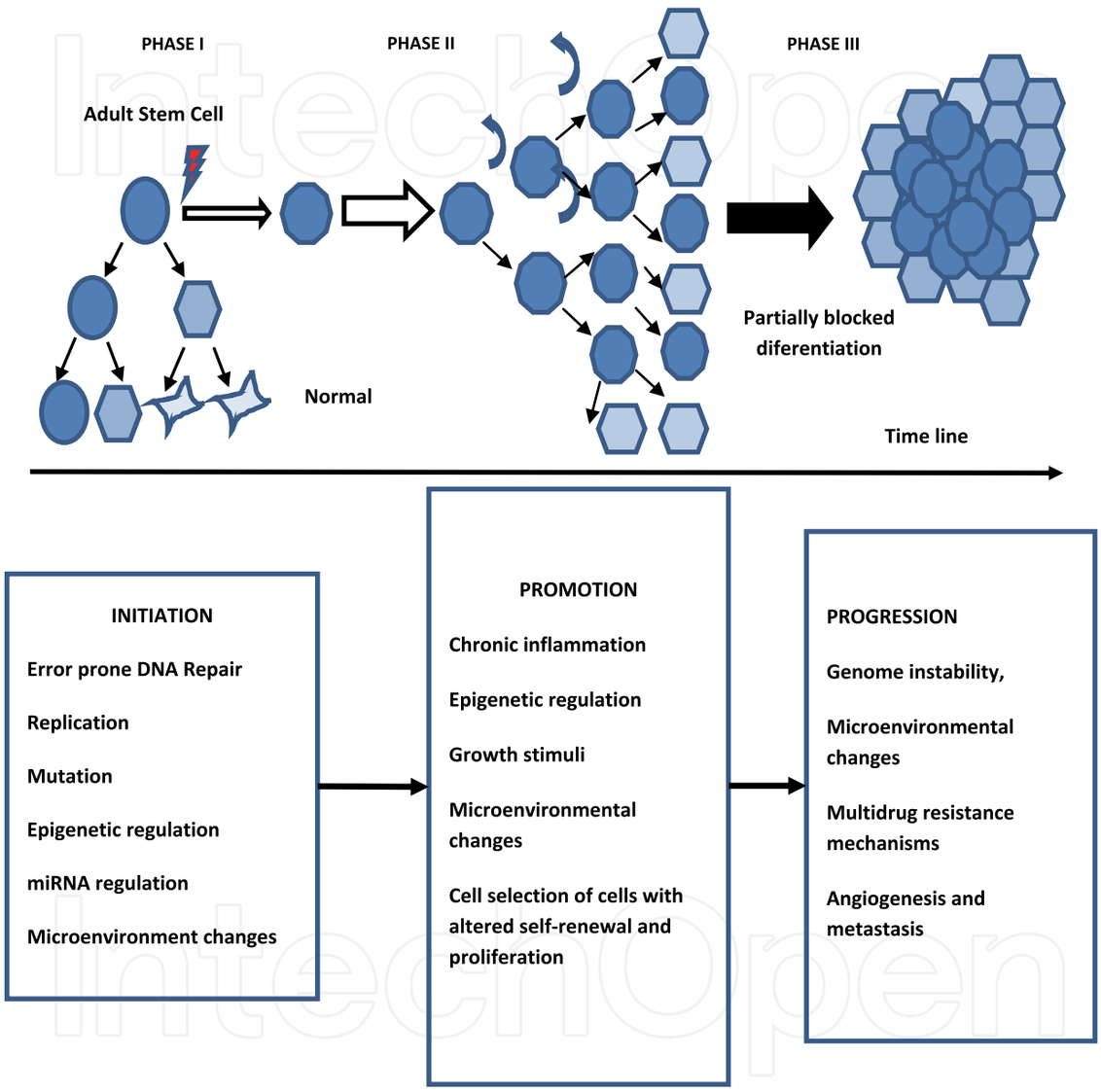


Fig. 2. Integration of the Cancer Stem Cell model into the stochastic model of carcinogenesis.

The stochastic model of carcinogenesis process consists of 3 principal steps. The first one is the initiation process, which had to irreversibly convert a normal somatic differentiated cell to a premalignant state. The second step is the promotion stage, where the single initiated cell is clonally expanded by a mitogenic process, and is unable to die, even if the microenvironmental conditions favorite apoptosis. The last step, the progression, has traditionally seen as the process when the tumor completes its malignant transformation and become metastatic. Cancer stem cells may be caused by transforming mutations and/or

epigenetic events, to gain the self-renewal activity and lose some features of differentiation occurring in multi-potential stem cells, or progenitor cells. This model has been functionally supported by the observation that among all cancer cells within a particular tumor, only a minute cell fraction has the exclusive potential to regenerate the entire tumor cell population. Many groups have extrapolated the cancer stem cell model from the hematopoietic system to solid cancers, using in vitro culture techniques and in vivo transplant models; they have established evidence of cancer stem cells in colon, spleen, prostate, brain and breast cancers.

Finally, when the expanded initiated cells accrue sufficient alterations to become growth stimulus independent, and resistant to growth inhibitors and apoptosis, to have unlimited replicative potential and invasive and metastatic phenotypes, then the progression phase has been achieved (Trosko et al., 2004). About stem cells, some of these have *per se* the properties unlimited replicative potential, as wells as the homing capacity, passing inadvertently all over the organism, and also plasticity, to change their phenotype (Figure 2). Given is well known these processes also, enable metastasis, a program involved in various steps of embryonic morphogenesis and wound healing called ''epithelial-mesenchymal transition'' (EMT), has been recently implicated in metastases. Cells which suffer EMT acquire stem cells phenotype and the abilities to invade, to resist apoptosis, and to disseminate.

2.5 Evidences that not all cancer cells are tumorigenic

Under the CSC´s model, only a subset of cells is capable to self-renew and generate at least part of a tumor´s developmental heterogeneity, other malignant cells within the tumor should be non-tumorigenic. A number of independent observations, both in the clinic and basic research level needs to take in account to be clear that tumor are heterogeneous cell populations.

The most startling experiments in cancer biology are of Southam and Brunschwig in 1966. At that time, physicians had realized that malignant cells could be found circulating in the blood or lymphatic of cancer patients, but only small percentage of these seemed to give rise to metastatic tumors. Southam and Brunschwig sought to test this observation directly by harvesting tumor cells of patients with various disseminated or unresectable cancer, dissociating tumor fragments into single cell suspensions, and then subcutaneously autotransplanting varying number of cells to roughly determine how many tumor cells were needed to generate a new tumor (Southam et al., 1966). In spite of the deep ethical and methodological flaws, this study did suggest that, using their methods, only a subset of cells within a tumor is tumorigenic. Similar findings have being documented in different cancer patients, patients with malignant ascites, breast cancer (Braun et al., 2005, Riethdorf et al., 2008), and liver cancer. In the case of cancer patients with malignant ascites who have implantation of peritoneo as procedure which help alleviate abdominal distention, but also has the unavoidable effect of shunting huge number of tumor cells into systemic circulation, disseminated cancer is a rare event (Campioni et al., 1986, Tarin et al., 1984). A related clinical example is the recognition that viable breast cancer cells have been found in the bone marrow of a large percentage of patients. Although in many patients the presence of these cells correlates with an increased probability of overt metastasis and a worse overall prognosis, the vast majority of these cells either remain dormant for years or are simply not

tumorigenic (Braun et al., 2005; Riethdorf et al., 2008). Another supportive example is the observation that percutaneous biopsy of breast and liver tumors frequently leads to the disruption of the tumor and the deposition of thousands of malignant cells along the biopsy track, yet even with large bore needles, the rate of tumor seeding of the biopsy track is relatively low (Ryd et al., 1993; Eriksson etal., 1984; Smith, 1984; Diaz et al., 1999). Several possibilities could explain this discrepancy, including the idea that only a fraction of the tumor cells are tumorigenic.

Along these lines, in many different animal models of cancer (both with hematologic malignancies and solid tumors) a large number of tumor cells must be transplanted into a recipient animal in order to generate a tumor. Similarly, only a minority of tumors cells is able to proliferate in vitro and generate colonies (Hamburger & Salmon, 1977a; Hill and Milas, 1989; Cho et al., 2008).

2.6 Return of some tumorigenic cells to nonmalignant cells

The Cancer Stem Cells theory implies that tumor heterogeneity is, at least in part, due to the execution of a developmental program in the progeny of cancer stem cells. As defined, cancer stem cells should be immature, multipotent, and able to give raises both tumorigenic and non-tumorigenic cells. This has been shown in the case of mouse teratocarcinoma, a malignant germ cell tumor that can give rise to differentiated cell types of all three germ layers. When this tumor develops, it will metastasize and kill the host within a matter of weeks. Single cells, when injected subcutaneously into recipient mice, cause tumor to form. However, when single genetically-marked teratocarcinoma cells are isolated and injected into a normal mouse blastocyst, the progeny of the teratocarcinoma cells contribute functioning terminally-differentiated post-mitotic cells to multiple different tissues without inducing more tumors (Mintz & Illmensee, 1975). Importantly, among cancer, teratocarcinomas are not unique in their ability to execute a program of differentiation. Experiments with a transplantable squamous cell cancer in rats showed that tumor growth is driven mainly by immature, proliferating cells that subsequently develop into differentiated cells that, when transplanted, lose their tumorigenicity (Pierce & Wallace, 1971). Also, supportive data in humans comes from the genetic analysis of leukemic clone can also be seen in mature, well-differentiated cells of various lineages. This suggests that the cancer is driven by immature hematopoietic cells that retain the capability of generating mature, noncancerous progeny (Fialkow, 1972; Fialkow et al., 1977).

3. Pathways involved in stem cell deregulation

Cell signaling involved in cancer had been extensively studied in the last decades; several pathways had been involved in this process. Many of the molecular mechanisms underlying tumorigenesis in cancer and self renewal in stem cells have been elucidated in the past decade, but with the introduction of the cancer stem cell theory, the carcinogenesis field began to change the form to look at the pathways involved in this process.

A subtle change in the conceptualization of the illness had been developed in the last years, from our first cancer definition as a cell proliferation problem to an acquirement of stem-like phenotype passing through a dead incapacity problem, the form to deal with this process had been change enormously. Cancer cells have the ability to divide indefinitely and spread

to different parts of the body during metastasis. At the other hand embryonic stem cells can self renew and, through differentiation to somatic cells, provide the building blocks of the human body (Dressen & Brinvanlou, 2007). Then it is possible to hypothesizer that certain highly conserved genes may contain evolutionary-conserved self-renewal machinery, and this machinery (at least in part), must be present in both normal and cancer stem cells. Day by day, the study of stemnes genes take relevance in the understanding the circuitry of cancer cells.

Among of the pathways that have been involved in the stemnes phenotype, seven of them seem to be constantly present: cascades of signaling involve Signal Transducers and Activators of Transcription (STAT), Fibroblast Growth Factor (FGF), Tumor Growth Factor (TGF), WNT, Phosphatidylinositol 3-kinase PI3K/AKT, Notch and Hedgehog signaling pathways. Furthermore, the stemnes phenotype is the result of a very well orchestrated and unique gene expression pattern characterized by the activation of some genes and the repression of others. Then, elucidation of the stemnes phenotype is based primarily on the identification of the transcription factors involved in regulating gene expression of the stemnes state (Cavaleri & Schöler, 2009). Among them Oct-4, Sox2, Nanog, NF-kB and recently miRNAs has been involved in this process, for that, in the present section we will discuss their role in carcinogenesis.

3.1 Jak/STAT signaling

Cells can communicate with each other through the secretion of cytokines. After binding their receptors, receptor-associated Janus Kinases (JAKS), as well as interacting Signal Transducers and Activators of Transcription (STATs) through tyrosine phosphorylation form homodimers, shuttle to the nucleus and participate in transcriptional regulation of a variety of genes (Darnell, 2002, 2005). STATs are also activated in response to growth promoting factors such as Epidermal Growth Factor (EGF) or Platelet-Derived Growth Factor (PDGF). Identification of STAT3 as a determinant of ES renewal came from Smith´s and Yokota´s laboratories at the end of 90's, where demonstrated that STAT3 docking sites are essential in mediating transmission of the signal in self-renewing ES cells. Thus, the JAK/STAT pathway plays an important role in mediating cell fates, such as apoptosis, differentiation and proliferation, in response to growth promoting factors and cytokines (Matsuda et al., 1999; Smith, 2001).

Deregulated JAK/STAT signaling can contribute directly and indirectly to tumorigenesis. Mutations, fusions, and/or amplification of JAK/STAT signaling components, such as the HER2/neu- in mammary and stomach carcinomas, or Epidermal Growth Factor-Receptor (EGF-R) in breast, brain and stomach tumors, can confer hypersensitivity to mitogenic signals and promote proliferation (Slamon, et al., 1987; Yarden, & Ullrich, 1988).

Additionally, STAT3 is constitutively activated in several major human carcinomas. Also, STAT3 is persistently active in over 50% of lung and breast tumors and more than 95% of head and neck cancers (Darnell, 2005).

3.2 FGF signaling

Twenty-two FGF family orthologs are conserved among mammalian genomes. FGF dimmers bind to FGF receptors with extracellular immunoglobulin-like domain and

cytoplasmic tyrosine kinase domain. FGF signals are transduced through FGF receptors to activated PKC signaling cascade and the $Ca2^{+}$- mediated NFAT signaling cascade. FGF signals are also transduced through FGF receptors and FRS2/FSR3-SHP2-GRB2 docking protein to the SOS-RAS-RAF-MEK-ERK signaling cascade and GAB1/GAB2-PI3K-PKD-AKT signaling cascade. The RAS-ERK had been implicated in cell growth, differentiation and cancer, while PI3K-AKT signaling cascade in the cell survival, cell fate determination and also in cancer.

3.3 TGFβ signaling

The TGFβ superfamily includes nearly 30 proteins in mammals, e.g. TGFβ, activins, nodal GDF, and BMPs. Smad 1/5/8 transduce signals from Bmps, and GDFs ligands, whereas Smad 2/3 transduce signals from TGFβ, activins and nodal. Upon activation by phosphorylation and association with a common Smad4, the receptor-activated Smads translocate to the nucleus to regulate gene expression in concert with other transcription factors (Cavaleri and Schöler, 2009). It has long been appreciated that the TGF-β pathway plays a crucial role during embryonic development. Several lines of evidence suggest that TGF-β signaling is also involved in sustaining the undifferentiated state in hESC. Activin / Nodal branch of TGF-β signaling is necessary to sustain pluripotency. In contrast the BMP branch of TGF-β signaling appears to play the opposite role (Dressen & Brivanlou, 2007). In conclusion, extensive studies established the TGF-β signaling pathway as a major regulator during embryonic development. Mutations or downregulation of TGF-β receptors, inactivation of SMAD4 or p15INK4B can be found in a variety of cancers. BMP2 is dramatically overexpressed in 98% of lung carcinomas (Langenfeld et al., 2003; Langenfeld et al., 2006). TGF-β signaling can also enhance malignancy of epithelial tumors by stimulating metastasis (Hartwell et al., 2006; Yang et al., 2004; Zavadil & Bottinger, 2005).

3.4 WNT signaling

WNT/β- catenin pathway, is associated with many kind of cancers, secreted Wnt ligands bind to frizzled receptors and activate a cascade important in development. WNT signaling increases the expression of HoxB4 and Notch-1 genes. Both of these proteins are implicated in the specification and /or self-renewal of HSCs (Reya et al., 2003). In addition, the Wnt/β-catenin pathway is involved in the maintenance of the normal epithelial cells and in regenerative responses during tissue repair (Reguart et al., 2005).

Wnt signaling has been implicated in blood diseases and colon carcinoma. Activating mutations of β-catenin or inactivating mutations of the adenomatosis polyposis coli (APC) gene, which targets β-catenin for degradation, occur in a large percentage of colon cancers (Kolligs et al., 1999). In CML, β-catenin accumulates in granulocyte-macrophage progenitor cells when CML progresses to blast crisis (Jamieson et al., 2004). β-catenin accumulation has also been associated with breast cancer, melanoma, sarcoma, myeloid leukemia, multiple myeloma, brain tumors and in the other hand, mutations in β-catenin are also observed in endometrial, prostate and hepatocellular carcinomas (Reguart et al, 2005).

3.5 PI3K/AKT signaling

Phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway responds to a variety signals, like hormonal receptors, transmembrane tyrosine kinase-linked receptors (RTK) and

intracellular factors and has been implicated in the regulation of several processes such as cellular proliferation, cell death and cytoskeletal rearrangements (Hennessy et al., 2005). Activation of PI3K occurs through interaction with various activating proteins such as protein kinase C (PKC), RHO, RAC, mutated RAS, SRC and leads to activation of phosphatidylinositol-3,4,5-biphosphate (PIP3). PI3K/AKT signaling is counteracted by PTEN (and SHIP1, SHIP2), which dephosphorylates PIP3. In the other hand AKT protein kinase interacts with Phophoinositide Kinase 1 (PDK1), and regulates directly or indirectly a number of downstream targets, such as NF-κB, BAD, pro-caspase 9, MDM2, p53 and GSK3.

Deregulated PI3K/AKT signaling has been observed in various cancers. Mutations in the PI3K/AKT pathway inhibitor and tumor suppressor PTEN has been found in glioblastomas, lung carcinomas and melanomas whereas AKT overexpression or overactivation has been found in breast, ovarian, thyroid and a variety of other cancers (Vivanco & Sawyers, 2002).

3.6 Notch signaling pathway

DLL and Jag families are transmembrane-type ligands for the Notch family receptors (Radtke & Raj, 2003). Ligand binding induces the cleavage of the Notch family members by metalloprotease and γ–secretase to release Notch intracellular domain (NICD) for the interaction with CSL or NF-kB transcription factors.

Notch signals are transduced to the canonical CSL-NICD signaling cascade and the non-canonical NICD-NF-kB signaling cascade. HES1, HES5, HEY1 and 2 and HEYL genes, encoding transcriptional repressor with bHLH and orange domains, are target genes of the canonical Notch signaling cascade. The notch signaling activation have been implicated in gastric cancer resulting in the maintenance of stem or progenitor cells through the inhibition of epithelial differentiation (Katoh, 2007).

3.7 Hedgehog signaling pathway

Sonic hedgehog (SSh), Indian hedgehog (IHh) and Desert hedgehog (DHh) are members of the hedgehog (Hh) family of secreted signaling proteins having diverse function during vertebrate development. Hh signaling also functions postembryonically in tissue homeostasis through effects on stem or progenitors cells. Inappropriate activity of the Hh pathway have been linked in pancreatic, skin, brain and gastric cancer and tumor types that arise sporadically or in genetically predisposed individuals. SHh signaling is launched by binding with the transmembrane protein Patched (PTCH) resulting in the loss of the PTCH activity and consequent phosphorylation and post-transcriptional stabilization of smoothened (SMO) protein. Pathway activation via SMO thus can occur either by Hh protein stimulation or through loss of PTCH activity (Ruiz et al., 2002; Tang et al., 2011).

3.8 Regulation factors involved in cancer stem cells gene expression

3.8.1 Oct-4

The Oct3/4 gene, a POU family transcription factor Oct3/4 or Oct4, (also referred to as Pou5f1), was first found in ovulated oocytes, mouse pre-implantation embryos, ectoderm of the gastrula (but not in other germ layers) and primordial germ cells, as well as in embryonic stem cells but not in their differentiated daughters (Rosner et al., 1990).

Nowadays is well known that Oct-4 is also expressed in Adult Stem Cells, where it normally start the terminal differentiation process and when Oct4 gene activity is down-regulated, differentiation of both stem cells and embryonal carcinoma cells occurred (Tai et al., 2004). In addition, it has been shown, that the success or failure of cloning depends on expression of this gene, during reprogramming of the genome of a nucleus transferred to an enucleated oocyte (Boiani et al., 2002). Seemingly in contrast, the Oct4 gene has also been shown to be expressed in some human tumor cells but not in normal somatic tissues.

Also, Oct-4 provides adult stem cells the property to sell-renew and proliferate, so when differentiation process takes place the Oct-4 expression is lost. In Tai´s work (2004), when the human breast epithelial, spleen and liver stem cells were induced to differentiate, Oct4 expression markedly diminished, but when a stem cell is initiated by up-regulation of Oct-4, the cell is able to differentiate and maintain the transcription factor expression and consequently the ability to self-renew occurred. Using antibodies and PCR primers against Oct-4, the group of Trosko, tested human breast, liver, spleen, kidney, mesenchyme and gastric stem cells, as wells as, the cancer cell lines HeLa and MCF-7 and human, dog and rat tumors for evidencing the Oct4 expression. They found that adult human stem cells, immortalized non-tumorigenic cells and all tumor cells tested express Oct-4, but not the differentiated cells (Tai et al., 2005). In addition, with these results, they conclude that adult cells expressing the Oct4 gene could be the target cells for initiation of the carcinogenic process, because they were able to isolate non-tumorigenic, but immortalized clones that exhibited phenotypic markers similar to the original stem cell and, after X-ray irradiation, they could also isolate weakly tumorigenic clones which could be rendered highly tumorigenic after transfection with the c-erb B-2/neu oncogene.

3.8.2 Sox2

Sox2 belongs to the Sry-related HMG box-containing family of proteins that binds to the minor groove of –DNA through the 79 amino-acid HMG domain. Sox2 is a major stemness factor. Indeed, it is a critical transcription regulator of the normal stem cell phenotype of ESCs, with a restricted number of partners, including Oct-4 and Nanog. It controls self-renewal and differentiation processes through coordinated transcriptional programs. As forced Oct4 expression induces pluripotency in Sox2-null cells, a group of researchers concluded that the primary role of Sox2 in induced pluripotent stem cells is controlling Oct4 expression, and they perpetuate their own expression when expressed concurrently. More recent studies indicated that Sox2 exits in the nuclei of ES cells and acts as a transcriptional factor to maintain the unique characters such as clonogenicity, pluripotency, and self-renewal of them (Masui et al., 2007).

A number of links were recently found between Sox2 and cancer; it has been intensively investigated and was found to contribute to the establishment of lung, prostate and spleen cancer (Saigusa et al., 2009, Sanada et al., 2006, Rodriguez-Pinilla et al., 2007).

3.8.3 Nanog

In 2003 Chambers and Mitsui reported the identification of Nanog as a new member of the embryonic stem cell stage. Nanog is a gene expressed in ESCs and is a key factor in maintaining pluripotency. Nanog is thought to function in concert with other factors such as pou5f1 and Sox2 to establish ESC identity. Oct4 and Sox32 control Nanog transcription by

binding to this element in both mouse and human ES cells. Overexpression of Nanog in mouse embryonic stem cells causes them to self-renew in the absence of leukemia inhibitory factor. In the absence of Nanog, mouse embryonic stem cells differentiate into visceral/parietal endoderm (Chambers et al., 2003, Mitsui et al., 2003). Loss of Nanog function causes differentiation of mouse embryonic stem cells into other cell types (Lin et al., 2005). Gene knockdown of Nanog promotes differentiation, thereby demonstrating a role for these factors in human embryonic stem cell self-renewal (Zaehres et al., 2005).

Somatic cells respond to DNA damage by activating p53, which causes cell cycle arrest or apoptosis. ES cells, under high levels of DNA damage, lack the p53 dependent G1 arrest and cells undergo apoptosis. However, at low levels of DNA damage, it has been shown that p53 binds to the promoter of Nanog and suppresses its expression in mouse embryonic stem cells. p53 can thus induce differentiation of embryonic stem cells into other cell types which undergo efficient p53-dependent cell-cycle arrest and apoptosis (Lin et al., 2005). Nanog protein has been implicated in several types of cancer such as: bladder, colorectal, gastric, prostate and oral squamous cell carcinoma (Chiou et al., 2008).

3.8.4 NF-kB

Nuclear factor of kB (NF-kB) is a transcription factor involved in the inflammatory and innate immune responses (Lin & Karin, 2007). The activation of NF-kB occurs as it is transported from the cytoplasm to the nucleus upon degradation of the inhibitory subunit. In the nucleus, it binds to specific kB sites on the DNA and mediates the expression of mostly genes involved in the cellular response to stress (Ghosh & Karin, 2002). The REL family proteins of NF-kB form various homo and heterodimers, and their activity is regulated by two main pathways. The canonical NF-kB activation applies to dimers that are composed of RELA, c-REL, and p50, which are held captive in the cytoplasm by specific inhibitors that are known as the inhibitor of kB (IkB) proteins. This pathway is normally triggered in response to microbial and viral infections and exposure to proinflammatory cytokines and physical and chemical stresses. Cellular stresses such as ionizing radiation and chemotherapeutic agents also activate NF-kB (Karin & Ben-Neriah, 2000).

The first link between NF-κB and cancer was when the subunit p50 was identified as a member of the REL family, which is also the family of the famous oncoprotein v-Rel, of the REL retrovirus (REV-T) (Gilmore et al., 2004). Activation of NF-kB is a tightly regulated event, in tumor cells, different types of molecular alterations may result in an impaired regulation of NF-kB activation. In such cases, NF-kB becomes constitutively activated, which leads to deregulated expression of NF-kB controlled genes. According to Hanahan and Weinberg in the hallmarks of cancer NF-kB is able to induce all of cellular alterations that become cancer (Hanahan & Weinberg, 2011).

3.8.5 MiRNA Regulation

Micro RNAs are small, 19−22 nucleotide (nt) long, non-coding RNAs that inhibit gene expression at the posttranscriptional level. The mRNA/miRNA duplex then inhibits translation either through a (mRNA 5') cap-dependent mechanism affecting initiation or through increased degradation of the mRNA. Given the frequency with which miRNA target motifs are conserved within 3'UTRs, it is estimated that 20 to 30% of all human genes

are targets of miRNAs, and that for each miRNA hundreds of genes exist that carry conserved sequence motifs within the 3'UTR (Peter, 2009).

miRNAs have been shown to regulate embryonic development (Stefani and Slack, 2008). Upregulation of miRNAs is required for various differentiation processes in fact, little is known with respect to mechanisms by which miRNA function in controlling the developmental potential of ES cells. However, it is still largely unknown how ES cell-specific transcription factors and miRNAs work together (Bar et al., 2008; Calabrese et al., 2007; Mineno et al., 2006).

A strong link between miRNA deregulation and human cancer has been established. A comparison of miRNA expression in normal and tumor tissues demonstrated global changes in miRNA expression in various human malignancies. In addition, mapping of 186 human miRNA genes has revealed that they are frequently located at fragile sites and other cancer associated chromosomal regions. Consequently miRNAs have been demonstrated to act either as oncogenes or tumor suppressors (He et al., 2007; Akao et al., 2006; Zhang et al., 2006; Calin et al., 2004).

4. Cancer stem cells as a target for chemotherapy: The problem of resistance

Despite all the new knowledge and advances in treatment, cancer remains one of the most common causes of death all over the world. Failure in treatment is the principal cause of the mortality, and it could be due to the cancer ability to recur and spread after initial therapies. Relapses may be caused in part to the existence of CSCs. Thus, CSCs are regarded as the root of cancer origin and recurrence. New therapeutic approaches targeting these malignant cells have become the topic of ongoing research. However, the chemoresistant phenotype of CSCs makes difficult to increase their sensitivity to anticancer drugs and to decrease the rate of cancer recurrence in patients.

4.1 Mechanisms of resistance to traditional chemotherapy

The trouble of chemotherapy resistance has been present since 1945, when Gilman and co-workers introduced chemotherapy into clinical practice at the end of the Second World War. They used nitrogen mustard to treat a patient with advanced malignant lymphoma, after an initial regression of the disease, a second course of therapy was given, however a lesser therapeutic effect was observed and after the third treatment, the tumor no longer responded to the agent (Goodman et al., 1946).

The drug resistance can be due to diverse factors: pharmacokinetics, such as inadequate access of the drug to the tumor, inadequate infusion rate and inadequate route of delivery (Garattini, 2007). Another important factor is drug metabolism which can inactivate and efflux the drug. The cytochrome P450 enzymes, a multigene family of constitutive and inducible haemo-containing oxidative enzymes from the liver, play an important role in the metabolism of a diverse range of xenobiotics and are often over expressed in a variety of solid tumors, in which they can contribute to drug resistance. The third factor are the membrane proteins such as solute carriers channels and ATP-binding cassette (ABC) transporters; they can facilitate the drug efflux from the cell, in fact colon and liver cancer have an intrinsic drug resistance, due to the function of these type of transporters, which are already highly expressed in the healthy tissues (Gottesman et al., 2002, 2006).

Mutation or over expression of the drug's target is another mechanism of resistance, for example the case of protein BCR-ABL in CML. The chimaeric BCR-ABL protein is a constitutively active protein tyrosine kinase with an important role in the regulation of cell growth (Melo & Barnes, 2007). Imatinib mesylate (formerly STI571; Gleevec, Novartis, Basel, Switzerland) is a potent and highly specific competitive inhibitor of the BCR-ABL tyrosine kinase. Initially, it had a high rate of cytogenetic and hematologic responses in patients with chronic-phase CML, in whom previous therapy had failed, and actually, its use has revolutionized the management and clinical expectations of CML patients. Unfortunately, not long after its initial use, resistance to Imatinib was demonstrated in CML patients.

Approximately 50% of imatinib-resistant CML patients carry a resistance-associated point mutation in BCR-ABL, which interferes with imatinib binding, and more than 50 different resistance-associated point mutations in BCR-ABL have been seen (Gorre et al., 2001). Also BCR-ABL gene amplification or over expression at the mRNA and protein levels has been detected in clinical samples (Hochhaus et al., 2002). The BCR–ABL fusion oncogene has also been implicated in NF-kB activation, cell survival, and tumorigenesis in human leukemias (Reuther et al., 1998).

As mentioned above, resistance appears not only to traditional chemotherapy but also to targeted therapies such as tamoxifen, which targets the estrogen receptor (ER) in breast cancer (Ali & Coombes, 2002); Imatinib, which targets the constitutively active kinase BCR-ABL important role in the regulation of cell growth (Melo & Barnes, 2007) in CML.

In addition to p53 mutations which inactive the tumor suppressor, p53 pathway can also be inactivated in wild-type p53- carrying tumors, via indirect mechanisms such as MDM2/MDMX amplification. In fact, most wild-type p53 types of cancer harbor alternative genetic alterations such as mutations in APC in colon cancer, BRCA1 and BRCA2 in breast cancer, and B-RAF in melanoma (Soussi & Wiman, 2007).

4.2 The cancer stem cell and drug resistance

The failure to eradicate cancer may be as fundamental as a misidentification of the target. Antitumor treatments designed and selected for broad cytotoxic activity may kill mostly of cancer cells and induce regression of the tumor; however according to the CSC model, cancer stem cells will re-establish tumor growth and cause relapse from therapy. In other words, therapeutic approaches that do not eradicate the CSC compartment are likely to achieve little success (Reya et al., 2001). In fact, the whole drug resistance concept has been revised incorporating the CSC paradigm, and recognizing of stem cells in different tissues, can help to distinguish the degree of damage tolerance and multipotentiality and then translate it into differential drug susceptibilities depending on the tissue of origin (Donnenberg & Donnenberg, 2005).

Current views favor the model that cancer stem cells are innately resistant to chemotherapy through their relative quiescence, their enhanced capacity for DNA repair, decreased entry into apoptosis, and ABC transporters expression. The inherently slow cycling rate of CSCs has been hypothesized to provide them inherent defense against most traditional chemotherapeutic regents, which are antiproliferative and most effectively target the fastest dividing cancer cells (Graham et al., 2002). On the other side, the problem of resistance can be more complex, the expression and activity of ABC transporters is one of the most

important markers for stem cells and cancer stem cells, they have high multidrug resistance (MDR) pump activity associated with high expression of the ATP-binding cassette family of proteins such as ABCB1 (P-glycoprotein) and/or ABCG2 (Breast Cancer Resistance Protein-1, BCRP1) (Wu & Alman, 2008).

The well-known, side-population (SP) phenotype is due to the expression of ABCG2 and has been detected in both normal and AML hematopoietic stem cells. In fact, expression of ABCG2 and Hoechst 33342 effluxes are two of the best markers of these cells, interestingly that expression is often turned off during differentiation to progenitor and mature blood cells (Kim et al., 2002). Imatinib is both an ABCG2 substrate, and inhibitor, making it susceptible to efflux by stem cells that express this transporter (Burguer et al., 2004; Houghton et al., 2004).

Imatinib and Gefitinib are, also, both direct and downstream inactivators of ABCG2 and, therefore, serve as candidates to reverse cancer stem cell chemoresistance and potentially target cancer stem cells (An & Ongkeko, 2009). Nevertheless, the level of contribution of ABC efflux chemoresistance and their inhibition remains controversial in clinical trials (Leonard et al., 2003). Unfortunately, there are lots of reasons that make, the ABC transporters, an incomplete and dangerous targeted to finish with the chemotherapy resistance. The expression of ABCG2 by microvessel endothelium of the brain suggests that ABCG2 plays an important role in maintaining the Blood Brain Barrier BBB. Thus, anti-ABCG2 therapy that successfully eliminates ABCG2+ CSCs may lead to adverse neurotoxic effects (Cooray et al., 2002). On the other side, it has been proposed that selective pressure imposed by chemotherapy leads to both mutation and secondary genetic changes, including MDR upregulation in the bulky tumor (Donnenberg, & Donnenberg, 2005).

The redundancy of the individual transporters within the MDR phenotype together with several other resistance-related proteins expressed in solid tumors (eg, glutathione S-transferase, metallothionin, O6-alkylguanine-DNA-alkyltransferase, thymidylate synthase, dihydrofolate reductase, heat shock proteins), may contribute to the failure when inhibiting only the MDR proteins.

4.3 Novel targets against cancer stem cells

In a very recent work for characterization of cancer stem cells in CML, it has been demonstrated that TKI (tyrosine kinase inhibitors) don't function with CSC of CML. First, efflux Hoechst dye, express CD34, lack CD38 and cytokine-non-responsive cells were isolated, and then these cells had been shown to regenerate bcr-abl-positive haemopoiesis in immunocompromised mice upon transplantation. CML stem cells express very high levels of functional wild-type bcr-abl; however, no kinase domain mutations were detected in the stem cell population. FTIs (farnesyl transferase inhibitors) have activity against CML. BMS-214662 was the most effective of these and induced apoptosis of phenotypically and functionally defined CML stem cells in vitro, as a single agent and in combination with IM or Dasatinib. In association with apoptosis, there was activation of caspase 8 and caspase 3, inhibition of the MAPK pathway, IAP-1 (inhibitor of apoptosis protein-1), NF-kB and iNOS (inducible nitric oxide synthase) (Jorgensen & Holyoake, 2011). Fumitremorgin C (FTC) is derived from the fermentation broth of *Aspergillus fumigates* and was the first specific ABCG2 inhibitor. FTC is a potent inhibitor of ABCG2 and has been shown to reverse

mitoxantrone-resistance in selected cancer cell lines (NeRabindran et al., 1998). Moreover, neurotoxicity has precluded the clinical use of FTC. Inhibitors of ABCB1 are already in clinical use, some of these compounds also effectively target ABCG2, including Elacridar (GF120918) and Tariquidar (XR9576) (Robey et al., 2004, 2007).

Another field of research in the resistance is directed at the identification of pathways known to be involved in the regulation of growth and self-renewal properties of CSCs, such as the Wnt, Notch and Hedgehog (Hombach-Klonisch et al., 2008).

4.4 Role of NF-kB in chemotherapy resistance

The role of the canonical NF-κB pathway in mammary tumorigenesis was investigated using a transgenic (TG) mouse expressing a dominant-negative inhibitor of kappaB (IkappaB alpha(SR (S32A/S36A))). TG and control mice were subjected to a chemical carcinogenesis protocol. Hyperkeratinized squamous metaplasias, were found in both TG and control mice. p65/RelA- and NF-κB DNA-binding activity were detected in mammary luminal lesions, but rarely in squamous metaplasias. Analysis of NF-κB family proteins and target genes using microarray data from a cohort of human mammary tumors revealed the expression of a canonical NF-κB pathway, but not non-canonical pathway proteins in HER2+ luminal cancers. HER2+ tumors also showed differential regulation of specific NF-κB target genes relative to basal and ER+ luminal cancers. Isolation of mammary cell populations enriched for stem and progenitor cells by fluorescence-activated cell sorting and with the help of an NF-κB-EGFP reporter mouse, demonstrated that luminal progenitors contain activated NF-κB whereas the mammary stem cell-enriched population does not. Together these data suggest that the canonical NF-κB pathway is active in normal luminal progenitor cells before transformation and is required for the formation of mammary luminal-type epithelial neoplasias (Pratt et al., 2009).

As we have reviewed in this study, the NF-κB pathway is a major source of pro-inflammatory cytokines, which may contribute to cancer chemoresistance. Constitutive NF-κB activity has been observed in ovarian cancer stem cells (OCSCs). Leizar and coworkers used Eriocalyxin B (EriB) for the inhibition of NfκB and induction of cell death. OCSCs and mature ovarian cancer cells (mOCCs) were treated with increasing concentrations of EriB, and then, cell viability was measured, caspase activity and cytokine levels were quantified. EriB decreased the percent of viable cells in all cultures tested with GI(50) of 0.5-1 μm after 48 hrs of treatment and induced cell death by inhibition of NF-κB activity, EriB produced decreased cytokine levels and activation of caspases, too. Down-regulation of XIAP and sensitizing of OCSCs to TNFα and FasL-mediated cell death were others effects of EriB (Leizer et al., 2011).

In a very recent work, with the fact that Disulfiram is a specific inhibitor of ALDHs and, therefore, it may also be an inhibitor of Breast Cancer Stem Cells (BCSCs), Wang's group used Disulfiram (DS)/copper and inhibited BCSCs and enhanced cytotoxicity of paclitaxel (PAC) in Breast Cancer (BC) cell lines: MCF7, MDA-MB-231 and T47D. The constitutive NF-kB activity in BC cell lines was inhibited by DS/Cu. Combination index isobologram analysis demonstrated a synergistic effect between DS/Cu and PAC. The increased Bax and Bcl2 protein expression ratio indicated that intrinsic apoptotic pathway may be involved in DS/Cu-induced apoptosis, and this may be caused by simultaneous induction of ROS and inhibition of NF-kB (Yamamoto, et al., 2011).

4.5 Novel cytotoxic compounds for CSCs through the inhibition of NF-kB pathway and its effectors

Actually series of compounds have been tested as inhibitors of Cancer Stem Cells growth and their potent cytotoxic effects on these cells. Exploring the mechanism of action, most of these compounds target the NF-kB pathway or some of the genes regulated by the transcription factor.

CDF (difluorinated-curcumin), a novel analog of the dietary ingredient of curcumin, was examined in combination with 5-fluorouracil and oxaliplatin (5-FU + Ox), the mainstay of colon cancer chemotherapeutic, as a treatment for eliminating colon CSCs. Through multiple and simply methodologies that include real-time RT-PCR, Western blot, MTT assay, caspase-3 activity, colonosphere formation, Hoechst-33342 dye exclusion and NF-κB-ELISA, it was observed that CDF together with 5-FU + Ox were more potent than curcumin in reducing CD44 and CD166 in chemo-resistant colon cancer cells, accompanied by inhibition of growth, induction of apoptosis and disintegration of colonospheres. These changes were associated with down-regulation of the membrane transporter ABCG2 and attenuation of EGFR, IGF-1R, and NF-κB signaling consistent with inactivation of β-catenin, COX-2, c-Myc and Bcl-xL and activation of the pro-apoptotic Bax (Kanwar et al., 2011).

Emerging evidence suggests that highly treatment-resistant tumor-initiating cells (TICs) play a central role in the pathogenesis of pancreatic cancer. Tumor necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL) is considered to be a novel anticancer agent; however, recent studies have shown that many pancreatic cancer cells are resistant to apoptosis induction by TRAIL due to NF-kB signalling. Several chemopreventive agents are able to inhibit NF-kB, for example the broccoli compound sulforaphane. This compound prevents metastasis in clinical studies. Kallifatidis G and coworkers isolated TICs (CD44(+)/CD24(-), CD44(+)/CD24(+) or CD44(+)/CD133(+)) and grew in immunodeficient mice. Specific binding of transcriptionally active cRel-containing NF-kB complexes in TICs was observed. Sulforaphane prevented NF-kB binding, downregulated apoptosis inhibitors and induced apoptosis, together with prevention of clonogenicity. In a xenograft model, sulforaphane strongly blocked tumor growth and angiogenesis, while combination with TRAIL had an additive effect without cytotoxicity in normal cells (Kallifatidis et al., 2009).

5. Conclusions

One of the most important functions in stem cell biology is the regulation of self-renewal. Self-renewal is required by stem cells so that they may last a lifetime, and all stem cells must self-renew and regulate the balance between self-renewal and differentiation. Many pathways that were primarily described in cancer may also regulate normal stem cell development. Among them Oct4 and Nanog, are essential regulators of early development and ES identity. Disruption of either of these factors causes loss of pluripotency. The HMG-box transcription factor, Sox2, heterodimerize with Oct4 in ES cells and regulates expression of Oct4. Each binds to its own promoter and to the promoter of the other two genes. In ES cells Oct4, Sox2 and Nanog act in concert to maintain the pluripotent state. These three transcription factors co-occupy promoters of hundreds of genes, activating some genes and silencing others, in a coordinated fashion, that together promote ES cell self-renewal, however a deregulation of this well orchestrated regulation could generate special conditions that favorites the cancer establishment.

Then there is a very tiny line that differentiates Stem cells and Cancer Stem Cells. However in terms of ill- understanding process our conception of cancer, through the cancer stem cells theory, is changing very fast. The acquisition of stemness like phenotype seems to be involved in several steps of carcinogenesis, then the knowledge generated behind this phenotype could benefits several areas from cell biology to clinical application. Promotion of survival and drug resistance, are the most relevant aspects for a clinical treatment succeed. Gaining a better insight into the mechanisms of stem cell resistance to chemotherapy and the develop of new therapeutic strategies at the molecular level will lead to an effective treatment strategy for preventing the emergence of chemo-resistant cancer cells by eliminating CSCs.

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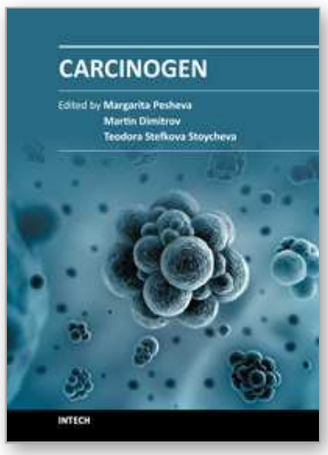

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During the last decades, cancer diseases have increased all over the world. The low quality of food and strong pollution of environment are the main prerequisites for carcinogenesis. The main problem for scientists is to find strategy for prevention of cancer diseases. Therefore, the information about the models for studying carcinogenesis and mutagens which appear during cooking, environmental pollutants, and tests for specific detection of carcinogens is particularly important. The book "Carcinogen" is intended for biologists, researchers, students in medical sciences and professionals interested in associated areas.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

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