Cancer Stem Cells

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Abstract

With recent advents in the field of cancer biology, improved therapeutic responses have been generated. But even after successful therapy, a relapse is inevitable in most cases. Recent advances made by researchers have led to the identification of a subpopulation of cells which can self renew, can differentiate further into various cell types and are tumorigenic. This subpopulation is often classified as “Cancer Stem Cells”. They are so named as they share the basic attributes of self renewal and differentiation, with normal stem cells. The commonly used therapeutic techniques are able to eradicate the bulk of the tumor, but seem to be ineffective against the cancer stem cells. Cancer stem cells were first identified in the case of leukemia and later in solid tumors. Identification of these tumor-initiating cells and elucidating the pathways involved in their regulation holds an important key for development of future therapeutics against cancer.

Introduction

Neoplasm, is an abnormal mass of tissue, which is formed due to the uncontrolled proliferation of cells i.e. neoplasia. A malignant neoplasm is however known as cancer. Cancer, can stay localized or can spread to distant regions in the body (metastasize) through the circulatory system or even the lymphatic system. During last few decades we understand cancer in much detail than ever, but a complete cure is still a distant reality. The current therapeutic approaches are based on the fact that cancer is a proliferation based condition. The first line of treatment is therefore, use of compounds that are anti-proliferative and the cytotoxic drugs, that help in reducing the tumor bulk are accompanied by detrimental side effects. Moreover, the current treatments in some instances can only provide temporary relief with a high risk of disease reoccurrence. What causes a relapse, still remains unanswered and why even after complete eradication, a relapse sometimes become inevitable. We therefore try to understand this paradox in the subsequent pages.

Historical Perspective

Hippocrates, the father of medicine, used Greek words "carcinos" and "carcinoma" to describe tumors, thus calling cancer "Karkinos". Although, Hippocrates had coined the term "cancer", he never discovered cancers. The world's first documented case of cancer was in Egypt, in 1500 BC, where 8 cases of tumor on the breast were documented on papyrus and were treated with a hot instrument known as "the fire drill". Interestingly, it was also recorded that there was no treatment for this disease, except a temporary relief. Farth and Kahn1 showed that a single cell derived from a mouse tumor could give rise to a new tumor in a recipient mouse. The tumor generated in the recipient mouse showed morphological heterogeneity to the parent tumor. Fialkow et al.2 found out that red blood cells, as well as granulocytes from Chronic Myeloid Leukemia (CML) patients were derived from a common cell, i.e. had common ancestry. The term "cancer stem cells" was first used by Park et al. 3. They found a tiny population of multiple myeloma cells in mouse that were responsible for clonogenic growth. Pierce et al.4 in the same year found that highly tumorigenic cells are present in malignant teratocarcinomas, which can further give rise to differentiated, non-tumorigenic cell types. They radio labeled mouse squamous cells carcinoma and observed that undifferentiated areas were the ones which were labeled initially. But, as the time progressed, the well differentiated areas also showed presence of the label. Thus, it was concluded that the well differentiated areas were derived from previously labeled undifferentiated cells. Also, the well differentiated cells did not give rise to any tumors, when transplanted in a suitable host. With these sets of experiments and other emerging data, Pierce proposed the concept of Cancer Stem Cells (CSC) as "a concept of neoplasms, based upon developmental and oncological principles", stating that carcinomas are caricatures of tissue renewal, in that they are composed of a mixture of malignant stem cells, which have a marked capacity for proliferation and a limited capacity for differentiation under normal homeostatic conditions, and of the differentiated, possibly benign, progeny of these malignant cells".
Origin of Cancer Stem Cells

It was now clear that there exists a sub-population of cells that are responsible for relapse of cancer and also responsible for heterogeneity in the cancerous tissue. But how did only a sub-population of cells acquire such characteristics? How did the Cancer Stem Cells originate?

Concept of Clonal Evolution

Nowell et al.6 propounded the concept of "clonal evolution". According to which "Most neoplasms arise from a single cell, and tumor progression results from acquired genetic variability within the original clone allowing sequential selection of more aggressive sub lines". Tumor cell populations are apparently more genetically unstable than normal cells. The acquired genetic instability and associated selection process, most readily recognized cytogenetically, results in advanced human malignancies that were highly individual both karyotypically and biologically. Hence, each person's cancer may require individual specific therapy, and even this may be thwarted by emergence of a genetically variant sub-line resistant to treatment. It pointed towards the fact that clonality and variations occurring in a tumor, may occur due to mitotic errors or different micro environmental pressures, all having a common "unicellular origin". In an experiment mice were injected with the parental cells (B16 melanoma cell line). After few rounds of in vivo selection procedures, it was found that these cells increased the nodules present in the lungs and were found to be more malignant than the original parental cell line7. It was thus determined that highly metastatic cells have a well defined process for tissue invasion, recruitment of lymphocytes, increased angiogenesis and ultimately increased size of the invading tissue. There can be differences between tumors, which can occur due to the difference in origin of the tumor. But, if heterogeneity exists due to the presence of exogenous elements, tumor occurrence should be comparable to the line of origin. Fidler et al. injected some cells into mice, the cells forming different colonies on soft agar were then selected. These were allowed to grow for several cycles and were then compared to the parent cell line. The result obtained showed clones with different degrees of metastasis and tumorigenecity. Therefore, it was proved that metastasis is a well coordinated event and not a random one8-10. The observed findings were in agreement with Nowell's hypothesis of heterogeneous tumor cell lines giving rise to varying populations by pre existing mechanisms, probably due to accumulation of mutations and mitotic errors in the stem cell of origin. The concept of clonal evolution was used by Fearon and Vogelstein11 to explain a model for colon cancer. They showed that there is a sequential pattern of occurrence of mutations in specific cancer genes in the progression of early adenoma to invasive carcinoma. Therefore, this concept was able to explain the increasing malignant nature of tumors 12-13.

Cancer Stem Cell Concept

According to this, not all cells within a tumor are equal. Broadly, they can be classified into two categories: (i) The differentiated cells, which are rapidly proliferating and form the bulk of the tumor. (ii) The undifferentiated cells that possess the property of self-renewal give rise to differentiated cells as well and perhaps govern tumor longevity. It was demonstrated by Bonnet and Dick14 that cells from Acute Myeloid Leukemia (AML) could be successfully engrafted in immunodeficient mice. CD34+ CD38- cells could only establish successful tumor in immunodeficient mice. In xenograft assays (involves xenotransplantation of sorted cancer cells into immunodeficient mice), they could measure the frequency of the leukemia initiating cells, which was found to be one in a million tumor cells. It was also demonstrated that heterogeneous population of breast cancer cells were found in a breast tumor. Out of the thousands of cells injected, only 100 cells that were CD44+ CD24-/low were tumorigenic in the xenograft assay. With successive cycles, cells of this sub-population gave rise to new cells which were CD44+ / CD24-/low, and also to other non-tumorigenic cells15. Therefore, in both the cases, a small number of cells constituting the sub-population, which can be characterized by the presence of specific markers, were able to transfer the disease into immunodeficient mice. This proves that not all cells present in a tumor are equal. Only the undifferentiated sub-population of cells is required to initiate the formation of a tumor and the well differentiated cells forming the bulk of the tumor are mostly non-tumorigenic.

What Exactly are Cancer Stem Cells (CSC)?

Growing evidence suggests that tumor initiation and maintenance is under control of a small population of...
tumor cells that are different from cells constituting the tumor bulk. These cells have similar biological properties as adult stem cells. Stem cells are crucial for development of tissue and regeneration, while cancer stem cells are required for tumor initiation, maintenance of malignant growth and also regenerating a tumor, i.e., tumor relapse. The discovery of these cells will help to understand which cell and how many cells should be eliminated for an anti-cancer therapy to be successful. In order to consolidate the concept, the "cancer stem cell hypothesis" was postulated which states that "the cancer cell populations have a hierarchical developmental structure in which only a small fraction of cells, that is, the cancer stem cells, have the ability to proliferate indefinitely"16-17. Stem cells with their unique properties are present in all tissues of multi cellular organisms. They have the ability of self-renewal and can also differentiate into all cell types, i.e., of the tissue of origin. These cells have a limitless capacity for cell division, producing at least one daughter cell per cell division which has the same property as the parent cell. Upon division, they also give rise to certain progenitor cells that ultimately differentiate into various components of the tissues and do not have the property of self renewal. These cells are present throughout the lifetime of an organism. Thus, they are easy targets for the accumulation of genetic changes that occur over a period of time and make them immune to cellular functions like apoptosis, i.e., cell death. Cancer stem cells, on the other hand are present in cancerous tissue but, share some of the properties of adult stem cells mentioned above, most importantly, the property of self renewal and tumor growth.

Are Cancer Stem Cells Derived for Normal Stem Cells?

It is now evident that cancer stem cells share some properties as normal adult stem cells, but a crucial question arises, are cancer stem cells always derived from normal adult stem cells that have gone bad? It may be possible, as many cancer stem cells express markers that are generally expressed by normal stem cells. The other possibility is that the finitely dividing stem cell progenitors can accumulate mutations over time and acquire the capacity to self renew. It has also been postulated that well differentiated cells can mutate and acquire stem cell like properties and can thus, be transformed into a cancer stem cell18. These cells are also called as tumorigenic, i.e., tumor-initiating cells . Any cancerous cell, whether it is an adult stem cell or a normal progenitor cell, be considered as a cancer stem cell if it has the properties of stem cells. Cancer stem cells divide infinite number of times and give rise to both, well differentiated cells found in a tumor and to more number of cancer stem cells. Cancer stem cells give rise to cells that have the same properties of their tissue of origin. Tumors thus formed may be morphologically similar to the tissue of origin or may express certain tissue specific genes. However, the difference between normal tissue and cancerous tissue is the loss of homeostatic control mechanisms in the later. Are cancer stem cells so named as they arise from normal stem cells? This might be true in some cases where a stem cell might give rise to a cancer stem cell but, nomenclature cannot be divided on this basis. If this had been the case, then cancer stem cells arising from a non-stem cell origin, would not have been considered as a true cancer stem cells. Therefore, any cell, be it of a stem-cell origin or not, which fulfills the functional requirements, will be considered as a "true cancer stem cell". Any cell which can give rise to a tumor, has the property of self renewal and can maintain this tumor growth indefinitely, will be considered as a "true cancer stem cell". According to this concept targeting of these cancer stem cells should provide everlasting relief to the suffering patients. Normal Stem Cells vs. Cancer Stem Cells It is believed that a cancer stem cell will exhibit all qualitative as well as quantitative properties that are exhibited by normal adult stem cells. This may actually not be the case, most normal adult stem cells behave in accordance with a set of well defined rules, which include a hierarchical developmental process, that is, the parent cell live will give rise to the daughter cells. Stem cell characteristics such as frequency, immune-phenotype, response to intrinsic stimuli etc. are found to be the same in individuals of a given species. All these properties are expressed by normal adult stem cells when in a steady state. Also, the size of a compartment of a particular stem cell is more or less the same in well defined lineages. But in the case of cancer, i.e., in cancer stem cells, these properties may not be the same. That means, the immune-phenotype, the frequency and other biological properties may not be maintained as the cells will not be in a steady state during disease progression. Populations of leukemia cells were analyzed for cell surface markers associated with a particular phenotype, and observed drastic variability from one patient to another. Also, for any given type of cancer stem cell, a range of antigens will be associated. But, the specific expression pattern may be different from one patient
to another. Taken together the normal adult stem cells and cancer stem cells may share some common properties, which is directly dependent on the microenvironment of the cancer stem cells, also known as the cancer stem cell niche, which may ultimately govern the "state of being" of the cancer stem cells.

Identification of Cancer Stem Cells

We now know that cancer stem cells are primarily responsible for tumor relapse, but can we identify this subpopulation of cells and distinguish it from the rest of the tumor bulk? With the discovery of cell surface markers and recent advances in cell fluorescence activated cell sorting (FACS), it is now possible to identify the cancer stem cells in a population of malignant tumors.

CD133

Also known as Prominin1, is a member of pentaspan transmembrane glycoprotein. It was first used as a marker for hematopoietic stem cells in neuronal and glial stem cells and other solid tumors. It is specifically found localized in cellular protrusions and was also been found in subpopulations of cancer cells from lungs colon, etc. An increased CD133 expression was observed in primary and metastatic melanoma as compared to melanocytic nevi. It was observed that in the first week of cultivation, CD133+ cells have a higher mitotic index as compared to CD133- population. Also, the expression of CD133 was higher in recurrent glioblastoma tissues than their respective primary tumors. They also found that CD133+ cells from the glioblastoma cell line showed increased expression of proteins like CD90, NESTIN, which are neural precursors and found higher mRNA levels of anti-apoptotic genes. CD133+ cancer stem cells showed more resistance to chemotherapeutic agents as compared to the CD133- cells. The properties that help the tumors to evade therapy, were identified for the very first time in this study. A landmark study demonstrated that CD133+ fraction in the melanoma cells had tumorigenic potential. Magnetically sorted CD133- and CD133+ cells were injected into Non-Obese-Diabetic/Severe-Combined-Immuno Deficiency (NOD/SCID) mice. It was observed that mice injected with CD 133+ cells developed tumors after 40-50 days, while those injected with CD133- cells did not develop any detectable tumors even after 4 months of injection. Using human metastatic melanoma cells, the CD133 cells were down regulated and it resulted in reduced cell motility, slower cell growth, and reduced capacity to metastasize. Just as CD133 cells were to be considered as a marker to identify cancer stem cells, certain experiments showed that CD133- cells were also capable of initiating tumors and could also self renew themselves. It was shown that CD133- cells derived from 6 patients formed tumors, i.e., were tumorigenic when implanted into brains of nude rats. Further analysis of three of these patients revealed that the resulting turnover contained CD133+ cells. Similar results were obtained when working on cells obtained from lung cancer. They observed that CD133+ and CD133- cells had similar properties of colony formation, self renewal, invasion etc. In this paradoxical situation, CD133 can only be considered as an indicator but not as a reliable marker for identifying cancer stem cells. It can be considered as marker for a type of cancer but they not a universal marker for identification of cancer stem cell.

ABCB5

A chemoresistance mediator ABCB5, was found to be expressed on cells that were tumor initiating and capable of self renewal. Expression of ABCB5 in tumor cells was found to be related with clinical melanoma progression. When xenotransplanted, ABCB5+ melanoma cells were found to be more tumorigenic than ABCB5- cells. Also ABCB5- population of cells gave rise to only ABCB5- cells and showed no further differentiation. Whereas, ABCB5+ population of cells generated both ABCB5+ and ABCB5- sub-populations. An anti-ABCB5 monoclonal antibody was also tested in nude mice. They observed that the anti-ABCB5 antibody inhibited the growth of initial as well as that of established tumors by causing Anti-body Dependent Cell-Mediated Cytotoxicity (ADCC) in ABCB5+ cells. Previously, a human melanoma sub-population was identified which was co-expressing ABCB1, ABCB5 and ABCB2. These cells showed anchorage independent growth than the negative fraction and higher self renewal capacity. They had also identified tumorigenic human melanoma cells by the expression of ABCG 2, which was co-expressed with CD133 marker.

CD34

Pioneering evidence for the existence of Leukemic Stem Cells (LSC) was first put forward by using FACS. They xenotransplanted CD34+ AML cells into NOD/SCID mice and observed that CD34+ CD38- fraction showed tumorigenecity in the transplanted mice. Whereas, both the CD34+ CD38+ and CD34- fractions were found to be non-tumorigenic. They also
found that the engrafted leukemic cells had the ability to self renew as they were successfully transplanted into secondary recipients. Therefore, these approaches, namely xenotransplantation and then serial transplantation are widely accepted procedures for the identification of cancer stem cells.

**CD44**

With the development of experimental techniques like the xenograft assay, FACS etc., researchers have been able to identify the tumorigenic stem cells in solid tumors. In the case of human breast carcinoma, cells expressing the CD44 and CD24 markers were isolated. These markers are heterogeneously expressed in tumor cells. These isolated cells were then engrafted in the mammary pads of NOD/SCID mice and it was observed that only the CD44+ CD24- fraction initiated tumor formation, i.e., were tumorigenic. On the contrary, a 100 times more number of cells from the CD44+ CD24+ or CD44- fraction(s) did not initiate tumor formation, i.e., were non-tumorigenic. The engrafted turnover, however, maintained the immunophenotypic heterogeneity with the parental cell line as it contained CD44+ CO24- as well as CD44+ CD 24+ and CD44- cells. Further on, these engrafted tumor cells were successfully transplanted in secondary recipient mice and provided substantial proof for self-renewal.

**A2B5**

A2B5 is a cell surface ganglioside that is found on the neural precursor cells present in the brain of an adult human showed a fraction of Neural Stem Cells (NSC) isolated from the sub ventricular zone of the human embryo are A2B5+. A2B5+ cells were found to be capable of creating intracranial tumors. However, A2B5 population of were non-tumorigenic. It was also found that A2B5+ / CD133+ and A2B5+/CD133- cell populations were tumorigenic and capable of neurosphere formation. It can therefore be concluded that above markers can aid identification of cancer stem only to a certain extent. We can faithfully sort out marker negative and marker positive populations, but we cannot be sure that the isolated markers will act as universal cancer stem cell markers. Markers that have been identified are more or less confined to the specific tissue of origin. Further research on better identification of cancer stem cells may provide us a better insight into the number of cancer stem cells that are needed to be removed to prevent tumor relapse.

**Basic Attributes of Cancer**

**Stem Cells**

**ARE CANCER STEM CELLS RARE?**

Xenotransplantation is the only technique that can help in validation of a prospective cancer stem cell fraction. Usually, thousands of cells are injected into the recipient organism, which ultimately results in tumor growth. It has always been reasoned that successful tumor transplantation confirms the presence of the tumorigenic cancer stem cell. Since the discovery of cancer stem cells in Acute Myeloid Leukemia, it is believed that cancer stem cells are indeed a rare population. While trying to study cells initiating human melanomas, a frequency of one in a million was obtained. Experiments done on human melanoma cells revealed that tumorigenic cells are rare in NOD/SCID mice, only if monitoring for a short period of time. By using NOD/SCID IL-2Rnull mice that lack the activity of Natural Killer (NK) cells along with Matrigel (a mix of growth factors and structural proteins) in place of the NOD/SCID mice, the frequency of these cells was drastically increased when observed for a longer duration. On injecting unsorted melanoma cells, 27% were found to be tumorigenic. Experiments were carried out on syngenic rodents, that gave rise to spontaneous tumors. They found that, the number of cells required to transplant tumor cells depends on the type of cells and the site of transplantation in the recipient mice. Further studies were carried out on leukemias originating in mice. They transplanted these cells into a suitable recipient and observed a very high frequency of the tumorigenic cancer stem cells, i.e., one cell in ten. In agreement with the results obtained from earlier experiments, it was postulated that a low number of tumor-initiating cells observed in xenotransplantation experiments may be due to the fact that only very few cells are able to adapt in the new microenvironment. To conclude, cancer stem cell population has been found to be rare in some cases and not so rare in others. But, there is increasing evidence that the human microenvironment cannot be replicated. The stem cell niche, that is utmost essential for tumor growth cannot be reconstituted. Factors like these may govern the existence of a sub-population of cancer stem cells in a tumor.

**ARE CANCER STEM CELLS RESISTANT TO THERAPY?**

Cancer stem cells have come to be known as a sub-population of cells that cause tumor relapse as they can evade the effects of chemotherapeutic agents. The question that arises is how do they evade...
therapy? It is believed that cancer stem cells do so by exhibiting certain properties, which include - dormancy, high expression of anti-apoptotic proteins, high expression of ABC pumps, etc. Experiments were carried out on the CD44high and CD24low fraction of breast cancer stem cells and they were found to be resistant to conventional chemotherapy37. On the other hand, cancer stem cells were found to be resistant to ionizing radiations38. It was also found out that leukemic stem cells that are resistant to the chemotherapeutic drug imanitib, are the ones that sustain Chronic Myeloid Leukemia (CML)39. Patients undergoing neo-adjuvant chemotherapy were also studied37. They observed that marker negative cells were killed by chemotherapy which decreased tumor bulk. But, marker positive population of cells appeared to be resistant as their relative numbers were found to be increasing in the residual tumor. Similarly, experiments were carried out on T-lineage acute lymphoblastic leukemia (T-ALL)40. They had identified the tumor-initiating cells in T-ALL and found them to be persistent even after treatment with dexamethasone. Cancer cells that are resistant to therapy may have intrinsic difference when compared with other cancer cells that are not resistant. There is also a possibility that a certain population of cells might have been missed by the current mode of therapy. As we all know by now that cancer cells accumulate mutations over a period of time. This may just explain the development of resistance in leukemic cancer cells which were earlier not resistant. Clearly, in depth knowledge is required to understand the mechanisms adopted by the cancer stem cells by which they can evade therapy.

DO CANCER STEM CELLS EXHIBIT DORMANCY?

Cancer stem cells are believed to share some of the attributes of normal adult stem cells. Stem cells are known to work behind the scenes and differentiate into various cell types, i.e., break their dormancy, in an "as in when demand" scenario. Similarly, Dormancy was found to be a fundamental attribute of leukaemia stem cells41. On the other hand, mammary gland stem cells were found to have a derived a gene signature for these dormant, normal stem cells42. When they applied this gene signature to tumor cells from the same tissue of origin, they found that it correlated with cancer stem cell behavior. Therefore, it may prove to be practical to check the status of dormancy of a cancer stem cell before xenotransplantation. This attribute of cancer stem cells explains their ability to retain DNA labels for a longer period of time when compared to their well differentiated progeny. It may also explain the tendency of cancer stem cells to evade the anti-proliferative effects of chemotherapy. Dormancy may also be responsible for certain cells being transported to different sites in the body (metastasis) and generating a tumor relapse even after many years of preventive surgery.

PATHWAYS INVOLVING CANCER STEM CELLS:

As cancer stem cells have similar properties with normal adult stem cells, they seem to have connected pathways that regulate their development. Recent advances in the field of cancer biology have demonstrated the presence of many genes and pathways that are involved in the regulation of cancer stem cells. The signaling pathways regulating cancer stem cells are Notch, Sonic Hedgehog, BCI-2, Wnt/ b-catenin and Bmi-1. These pivotal pathways may be deregulated in the case of cancer stem cells.

Notch

The Notch signaling pathway is essential for the regulation of many developmental processes and maintenance of adult tissues. It was shown that the Notch signaling pathway up regulates the breast tissue development43. But when deregulated, it gives rise to invasive breast cancer. It was also demonstrated that the Notch signaling pathway increases the incidences of self renewal in mammary stem cells44. It also acts on early progenitor cells and promotes their proliferation. The above mentioned activity was found to be completing inhibited by Notch 4 antibody or a g-secretase inhibitor. These results suggest that any disturbance in the normal Notch pathway will alter the self renewal property of cancer stem cells and may ultimately lead to a malignant neoplastic growth45.

Sonic Hedgehog

The basic hedgehog signaling plays a significant role in carcinogenesis as it is involved in the self renewal of cancer stem cells. It was observed that along with a combination of growth factors, populations enriched for adult stem cells exhibited increased self renewal ability in response to sonic hedgehog signaling in vitro46-47. It was also observed that many cancers like basal cell carcinoma arise from mutations that abruptly activate the sonic hedgehog signaling pathway48. They also observed, that in the absence of the sonic hedgehog signaling pathway, stem cells from the ovary cannot proliferate as stem cells. Whereas, increased hedgehog signaling produced stem cells in excess. This suggests that deregulated self renewal ability might alter hedgehog signaling, which may increase the possibility of generation of numerous stem cells.

BCI-2
Work on BCI-2 has been going on for many years due to its role as a proto-oncogene with in cancer cells. As found in many cancers, BCI-2 is over-expressing which ultimately prevents apoptosis. It was also observed that, apart from prevention of apoptosis due to the over-expression of the BCI-2 proto-oncogene, there is an increase in the number of stem cells in vivo. It was also suggested that apoptosis plays a crucial role in the maintenance of the stem cell microenvironment. Therefore, BCI-2 pathway plays a significant role in the regulation of cancer stem cells as it is found to be over-expressed in most cancers, and ultimately preventing cell death, i.e., apoptosis.

**Wnt/ß-Catenin**

There has been a lot of study on this pathway that is involved in the regulation of self renewal and oncogenesis in different tissues. Once the Wnt receptor is activated, b-catenin and other proteins of the Wnt gene family accumulate in the cytoplasm. They are eventually transported into the nucleus. This transport of b-catenin into the nucleus activates genes that are associated with the property of self renewal. An increase in the frequency of stem cells was observed when activated b-catenin is over-expressed51. Experiments were carried out on CML cells and it was observed that as b-catenin levels increased in the nuclei of granulocyte macrophage progenitors, the self renewal ability and the leukemic potential of these cells was greatly enhanced52. Therefore, deregulated Wnt/b-catenin pathway within cancer stem cells may be linked with their increased ability to self renew.

**Bmi-1**

Similar to previously mentioned pathways, Bmi-1 signaling pathway plays a significant role in the self renewal of cancer stem cells. The expression of Bmi-1 was studied in neural stem cells53. Bmi-1 expression was also observed in proliferating progenitor cells but was not seen in the differentiated cells. This suggests that Bmi-1 plays an important role in the self-renewal of adult stem cells. Bmi-1 was found to play a definitive role in cancer, when they observed that Bmi-1 promotes the formation of lymphomas54-55. Bmi-1 signaling was studied in human breast cancer stem cells that were CD44+ CD24-/low Lin- 56. Moreover, they found Bmi-1 plays an important role in mammosphere size and mammosphere initiating cell number. Therefore, Bmi-1 regulates self renewal in normal stem cells as well as in cancer stem cells present in the mammary glands. The above results suggest that deregulation of the Bmi-1 signaling pathway may be associated with the acquisition of self renewal properties in cancer stem cells. There may be similar signaling pathways for regulation of especially self-renewal in cancer stem cells and in normal adult stem cells, but presence of certain differences cannot be ruled out in the case of cancer stem cells. Therefore, such differences can be targeted and cancer stem cells can be eliminated without harming the normal stem cells.

**Further Evidences**

### I. In Leukemia:

Leukemia is a cancer of the blood or bone marrow characterized by the un-regulated increase of the leukocytes. Leukemias are treated with chemotherapeutic agents like imatinib, sometimes with radiation therapy and even with bone marrow transplantation in some cases. But in most cases, a relapse is inevitable. Cancer stem cells, the culprit for tumor relapse, were first purified in leukemias by Bonnet & Dick 14.

(a) A mathematical model was developed to show how leukemic stem cells respond to therapy in the case of CML. CML is a type of cancer of the blood, in which two genes encoding the BCR and ABL proteins are spliced together as a result of a mutation. The BCR-ABL fusion protein so formed is expressed in the bone marrow of the affected patients. ABL is an enzyme that is required for normal cell growth. But, when ABL is fused with BCR, it is over-expressed which causes leukemia, i.e., overproduction of White Blood Cells (WBC). The drug imatinib is a very potent chemotherapeutic agent that is used in the treatment of CML. It inhibits the activity of the BCR-ABL fusion protein and hence regulates the production of leukocytes. The response to the drug can be tracked by measuring the BCR-ABL gene transcript levels. But, imatinib treatment is not a complete cure for the disease and there have been cases where tumors develop resistance to the drug due to the acquisition of new mutations in the ABL enzymes. The mathematical model was based on the normal blood cell development and includes cells at four different developmental levels. The first level includes the stem cells, i.e., the immature cells that can self renew themselves. The second level includes the leukemic progenitor cells, which cannot self-renew and have already started to develop. The third level includes the differentiated leukemic cells, which have already started to develop into a particular cell type. The fourth and the final level include the terminally differentiated cells that are the fully mature blood cells. The models were then tested against imatinib, the changes being monitored by measuring the BCR-ABL transcript levels in chronic myeloid leukemia patients. Initially, there...
was a rapid reduction in the BCR-ABL gene transcript level, i.e., around 5% per day, which was in correspondence with the model that the terminally differentiated and the differentiated cells would decrease first. Subsequently, the decrease in the gene transcript level was slow, i.e., around 0.8% per day, which corresponded to a decrease in the leukemic progenitor cells. However, the stem cell compartment was found to be preserved. After stopping the therapy with imatinib or development of resistance to the drug, the level of BCR-ABL transcripts rose slowly in correspondence to an increase in the progenitor cell population, which is eventually fuelled by the leukemic stem cell population. Thereafter, there is a rapid increase in the level of BCR-ABL transcripts which corresponds to tumor relapse. Thereafter it is clear that the leukemic stem cell population was not eradicated, which resulted in a tumor relapse.

This model can further be utilized for testing new drugs against chronic myeloid leukemia and especially against those drugs that can completely wipe out the leukemic stem cell population.

(b) A set of historical experiments were carried out, with the help of which they could explain two very important findings – presence of leukemic stem cells and the possibility of progenitor cells being transformed into stem cells58. The experiments were carried out on myeloid progenitor cells that are produced by the blood forming stem cells. These cells have already started to specialize and have no self renewal ability. They showed that some fusion proteins, like those including the Mixed Lineage Leukemia (MLL) gene, can induce the expression of certain specific stem cell genes in myeloid progenitor cells. This makes the cell capable of self renewal and turns them into tumorigenic cancer stem cells. The MLL-AF9 protein was expressed in Interleukin (IL) – 7R- Lin- ScA-1- c-Kit+ CD34+ Fcg RII/IIIhigh Granulocyte Macrophage Progenitors (GMP). These were then transplanted into sub-lethally irradiated syngenic mice. Within a period of 80 days, the mice transplanted with MLL-AFP transformed Granulocyte Macrophage Progenitors developed AML, which was found to be similar to the other leukemia models. Moreover, the frequency of leukemia initiating cells was calculated. Mice that were injected with 500 or more leukemic cells died of AML. Mice that were injected with 100 cells, half of them died due to the disease. Mice that were injected with 20 cells only, all of them survived. The frequency of leukemia initiating cells was found to be 1 in 150 cells. They also suggested that the stem cells renewal program is hierarchical as a specific set of genes are immediately activated by MLL-AF9 expression, which includes members of the Hox gene family.

II. In the Case of Solid Tumors: Melanomas The term solid tumor is used to distinguish between a localized mass of tissue and leukemia, that takes on the fluid properties of the organ it affects – blood. Melanoma on the other hand is a solid malignant tumor of the melanin producing cells, i.e., melanocytes, which are predominantly found in the skin. A subpopulation of Malignant Melanoma Initiating Cells (MMIC) was identified28. These cells are defined by the expression of the chemoresistance mediator ABCB5 that acts as a molecular marker. To determine the ability of marker positive and marker negative to initiate tumor formation, they were xenotransplanted into NOD/SCID mice. The observed that only 1 out of 23 mice injected with the highest cell dose of ABCB5 melanoma cells generated a tumor, whereas 7 out of 23 mice that were injected with an unsorted cell population, generated tumors. Interestingly, all mice injected with the highest cell dose of ABCB5+, generated a tumor. Moreover, ABCB5+ cell population also generated a tumor in secondary recipient mice. These results indicate that the MMIC frequency is enriched in the subpopulation of melanoma cells defined by ABCB5 and also demonstrates their ability for self-renewal and differentiation. To find out further that, MMIC population is also required for tumorigenicity, selective killing of this fraction was carried out. Selective killing of this subset was carried out by administering an anti-ABCB5 monoclonal antibody. Nude mice were used in these xenograft models, in place of NOD/SCID mice, as they are capable of killing tumor cell populations by Antibody Dependent Cell Mediated Cytotoxicity (ADCC). After the anti-ABCB5 antibody treatment, it was observed that tumor growth was significantly hampered as compared to the untreated mice.

Inconsistencies and Challenges

As the cancer stem cell model is gaining worldwide acceptance, there is a debate over the properties that defines this subpopulation. There are many inconsistencies in the identification of cancer stem cells, which suggests misinterpretation of experimental results. There are no gold standards to accurately define a cancer stem cell, most of them being highly restricted to the tissue of origin. The most widely used technique to find out the tumorigenicity of cancer stem cells is the xenotransplantation assay. An important question that arises is, how can the human
microenvironment be regenerated in mice? For a successful xenograft assay, the human tumorigenic cells should adapt to the microenvironment of mice. This again raises an important question that, whether it is the ability of the cancer cells to adapt to the new environment or their tumorigenic capabilities that drives the xenograft assay17. It was observed that both lymphoma cells, with or without the stem cell like characteristics were able to initiate tumor formation in the recipient mice36. These findings led them to conclude that previous xenotransplantation assays may be underestimating the population of tumor initiating cells. This inconsistency may be more pronounced in the case of solid tumors as they require the support of endothelial cells, fibroblast cells, paracrine factors etc. for establishing a tumor. Therefore, they would have to recruit mouse stromal and endothelial cells for establishment of a tumor. Previously, it had been shown that the same fraction of tumorigenic cells formed a tumor at one site but not the other, i.e., tumor establishment depends on the site of tumor transplantation35. The cancer stem cell hypothesis believes that only the cancer stem cells have the property of self renewal and the rest of the tumor cells will ultimately die without them. Also, one of the assumption of this hypothesis is that the genetic events that drive tumor progression, may only occur in cancer stem cells as other cell populations are assumed to be evolutionarily dead. This hypothesis also assumes stability in the tumor cells and overlooks the possibility that other cancer or non-cancer cells can also acquire this ability17. There are many inconsistencies in this concept, but it still appears to be the most convincing explanation for tumor relapse.

Clinical Relevance

Malignant plasma cells form the bulk of the tumor in the case of multiple myeloma. Experimental results suggested that a sub-population of less differentiated cancer stem cells is the one that gives rise to the myeloma plasma cells59. These cells resembled memory B-cells and were found to possess the property of self renewal and were tumorigenic in vitro. The chemotherapeutic agents lenalidomide and bortezomib, successfully eliminate the myeloma plasma cells but could not eliminate the myeloma cancer stem cells60. This pattern seemed to be equivalent to be equivalent removing a "dandelion". Destroying the dandelion will only destroy the visible part of the weed, but the unseen "root" can cause a regrowth is not eliminated. Chemotherapeutic agents like rituximab and alemtuzumab selectively attack the myeloma cancer stem cells in vitro and not the myeloma plasma cells. Therefore, this treatment appears to be removing the root of the dandelion, which will eventually kill the whole weed61-62. With taking into consideration the above mentioned findings, myeloma cancer stem cells were targeted with rituximab and cyclophosphamide was used along with rituximab to debulk the tumor and to provide conditioning for stem cell transplantation63. It was found that cyclophosphamide treatment did not go as per plan but the population of myeloma cancer stem cells decreased significantly with rituximab treatment followed by progression free survival. It was observed that, rituximab did not kill the cancer stem cells but was detected on their surface. The data observed in the myeloma patients, suggested "clinical relevance" for cancer stem cells for the very first time64.

Potential Therapeutic Targets

The cancer stem cell concept is now widely accepted. If proven to be true, it will have a significant effect on cancer treatment. The timely identification of cancer stem cells will provide a potent target for chemotherapy. In most cases, even timely and successful treatment may lead to a tumor relapse. In such cases, anticancer therapies only help patients to lead a longer life, without eliminating the “root of the problem”- the cancer stem cells. Most of today’s anti-cancer therapies are very specific, like targeting BCR-ABL fusion proteins in the case of CML. Chemotherapeutic agents like Rituximab show limited or no result in the case of lymphomas. The inherent property of stem cells dormancy, may limit the access of chemotherapeutic agents. Also, high expression of membrane efflux pumps like the ABC transporters may also aid in cancer stem cells to avoid therapy. But, targeting of the transporters may prove to be an important route of therapy. It has been suggested that properties common to both, normal stem cells and cancer stem cells, may pave the way for the development of novel cancer therapies61. Many signaling pathways that are required for the growth and maintenance of normal stem cells, also play a vital role in the functioning of cancer stem cells. Inhibition of signaling pathways like Notch65 and hedgehog66 showed promising results. In the case of Notch signaling path, inhibition is carried out by small molecule inhibitors of g-secretase. With the introduction of g-secretase inhibitors (GSI), the cleavage of Notch receptor ceases and it remains bound to the cell membrane. As the intracellular domain is stopped from being transported into the
nucleus, the Notch signaling pathway is stopped. An increase in glioma cell survival on increasing the Notch signaling was observed. In the case of the hedgehog signaling pathway, after binding of the ligand to the patched receptor, the protein Smoothened is activated. Neurosphere cultures were treated with cyclopamine, which is a smoothened inhibitor, stopped sphere formation and better radiation therapy. It was also demonstrated that by treatment with cyclopamine, the tumor volume in intracranial neurosphere xenografts decreased.

Therefore, inhibiting the Notch and Hedgehog signaling pathways may pave a new path for cancer therapies. Another important therapeutic target is the stage of differentiation at which the disease arises. The normal cell cycle checkpoints protect the normal stem cells from cellular damage. It was postulated that CML, arises from myeloid stem cells which are considered as low grade stem cells. These in turn are not considered as the most primitive hematopoietic stem cells. It has been proposed that the more primitive normal stem cells should replenish the normal progenitor repertoire if targeted therapy eliminated the CML stem cells and the normal stem cells. Another therapeutic target which may provide tumor selectivity is telomerase. Telomerase is required by the normal stem cells to prevent the shortening of the telomeres post replication. Due to the presence of relatively long telomeres, normal functioning of the cell can be maintained even in the absence of telomerase. In the case of most malignancies, uninterrupted telomerase may be essential to stabilize the short telomeres present in the cancer stem cells. Mice, INK4a/−/− 73 or APC min 74 in which predisposed cancer cells were observed, were crossed with telomerase knock-out mice, which significantly lowered the incidence of cancer. Hence, the difference in telomere length between normal and cancer stem cells, provides for a potential therapeutic target. The efficacy of treatment against the targets should be monitored only after debulking the tumor, which is only possible transiently.

Conclusions and Future Prospects

The current therapeutic techniques target the cancer cells that form the bulk of the tumor, thus do not provide a lifelong cure as chances of a relapse always haunt the patient. The emerging data suggests that the culprits for relapse are the cancer stem cells. This subpopulation of cells has the ability to self renew and are tumorigenic in nature. Thus, targeting of this subpopulation is required, provided the theory is proved. The consortium of researchers worldwide is still speculative of the theory supporting the existence of cancer stem cells. But with ongoing research and the data collected, the existence of this subpopulation of cancer stem cells has been proven. Developing new therapies against the cancer stem cells show a promising future. Although, selectively targeted therapy against the cancer stem cells might not show the expected response of eradication of the tumor cells immediately. Once the root of the weed is cut, it takes time for the whole plant to wilt and ultimately die. Therapy involving the targeting of the shared pathways between the normal and cancer stem cells has proven to be useful. Although there is no gold standard for the identification of cancer stem cells, the tissue specific cell surface markers have made the identification process easier. There are many inconsistencies present in the model which need to be resolved. Development of standardized protocols, for the identification of CSC and advanced therapy is the demand of the hour. Also, the presence of inherent genomic instability may drive the well differentiated cancer cells to acquire the stem-cell like properties. Therefore, a cocktail of drugs is required that will be effective in eradicating all the subpopulations of cancer stem cells. It was suggested three types of challenges faced by the researchers. Firstly, we must ascertain the evolutionary evidence within the tumor, i.e., if a parent-offspring relationship exists. If cancer stem cells are the ones giving rise to the bulk of the populations, their eradication will be useful. Secondly, standardized experimental systems should be established. The most commonly used xenotransplantation assay cannot tell that the tumor microenvironment can be replicated or not. Will these cells behave in the same way in the human system? Questions like these need to be answered. Lastly, the current therapies which prove to be a clinical end point, should be revised to assess the role of the cancer stem cells in disease progression. It can thus be concluded by saying, “disease knows no barrier – be it geographical or religious”. Understanding the basic biology of this subpopulation of cancer stem cells and advanced therapeutics, seems to be the only ray of hope for millions affected by this disease worldwide, which may not only treat but completely cure them.

References


63. Huff CA, Wang Q, Rogers K, Jung M, Borrello IM, Jones RJ et al. Correlation of clonogenic cancer stem...


Illustrations

Illustration 1

Figure 1: In normal development (a) stem cells (blue) divide into progenitor cells (green) which then give rise to well differentiated cells (orange). In carcinogenesis, mutations can transform stem cells into cancer stem cells which then divide incessantly. These mutations can occur in the cancer stem cells (b), in the progenitor cells (c) as well as in the well differentiated cells (d).
Illustration 2

Figure 2: (a) The mathematical model of CML developed by Michor et al, divides the cells into four basic categories, i.e., the cancer stem cells (green) which can self renew and also give rise to other types of cells, the progenitor cells (light blue), the differentiated cells (yellow) and the terminally differentiated cells (pink). (b) On treatment with imatinib, the BCR-ABL gene transcript levels decreased which corresponded with the rapid reduction in the differentiated and the terminally differentiated cell population. (c) On further treatment with imatinib, the BCR-ABL gene transcript levels decreased further in accordance with the reduction in the progenitor cell population. (d) On stopping the imatinib treatment or the development of resistance to the drug, there was a slow increase in the gene transcript levels corresponding to the increase in the progenitor cell population. (e) Further on the progenitors give rise to the differentiated cells population, i.e. results in a relapse of the disease.
Illustration 3

Table 1: Marker positive as well as marker negative populations have been found to be tumorigenic.

<table>
<thead>
<tr>
<th>MARKER</th>
<th>INDICATOR FOR</th>
<th>MARKER +/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34</td>
<td>Leukemias</td>
<td>+</td>
</tr>
<tr>
<td>CD44</td>
<td>Breast, Liver, Pancreatic malignancies</td>
<td>+</td>
</tr>
<tr>
<td>CD133*</td>
<td>Brain, Liver, Lung malignancies, Melanomas</td>
<td>+/-</td>
</tr>
<tr>
<td>A2B5</td>
<td>Brain malignancies</td>
<td>+</td>
</tr>
<tr>
<td>ABCB5</td>
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