Abstract. – The cancer stem cell hypothesis states that tumors rely exclusively on the continued proliferation of a subset of cancer cells that originated from normal adult stem cells. These cells have two key traits: multipotency, and self-renewal. The prolonged lifespan of stem cells makes them perfect candidates for the accumulation of carcinogenic mutations that would convert them into cancer stem cells (CSCs) no longer responsive to the many regulatory pathways in place that are responsible for tight governance of proliferation and differentiation in normal stem cells. Comprehending what these regulatory pathways are, and how their derailment contributes to oncogenic transformation, can hold the key to finding new strategies to target CSCs in order to effectively treat cancer. Additionally, what environmental factors are involved in promoting or suppressing CSC tumorigenicity requires attention. The possibility that some cancers may have clonal origins in non-stem cell populations that were able to acquire stem cell-like properties, and the lack of complete cell autonomy in carcinogenesis, suggests that the CSC hypothesis is continually evolving. Continued research in this field can shed light on how effective selective elimination of CSCs as opposed to generalized targeting of cancer cells will be in the treatment of cancer.

Key Words: Cancer stem cell (CSC), Self-renewal, Differentiation, progenitor, Wnt-beta-catenin, Notch, Hedgehog, BRCA1, Clonal origin, Niche environment.

Introduction

While the origins of the term “cancer” can be traced back to Hippocrates, the first descriptions of cancer were recorded in Egyptian texts dating back to 3000 BC, annotated as a disease with no treatment. Elucidating the etiology and progression of this disease has been a central pursuit of medical research for centuries since. The urgency to conquer this elusive, lethal disease is apparent in the declaration of the “War On Cancer” and the signing of the National Cancer Act of 1971 in the United States. Landmark discoveries such as the identification of oncogenes in the 1980s have since contributed immensely to our understanding of cancer. However, cancer-related mortality rates have decreased by only 1.5% in the past decade, and approximately 8.2 million deaths annually are reported to result from cancer worldwide. Continued efforts to comprehend what pathways and factors are responsible for triggering carcinogenesis, and what contributes to cancer resistance to therapeutic intervention, are crucial in our battle against this disease. The advent of stem cell biology has led to the discovery of somatic adult stem cells with the unique capacity for self-renewal and for differentiation, the molecular underpinnings of both these characteristics often overlapping with those pathways that are involved in the transformation of the cancer cell. The possibility that many tumors originate from a specific subset of cancer cells with stem cell-like properties, and it is the proliferation of these cells that sustains tumor growth, has gained increasing attention in recent studies. Unraveling the mechanisms by which these cancer stem cells evade regulation and promote cancer progression can lead to the development of novel, potent anti-cancer therapies.

The Cancer Stem Cell Hypothesis

Cancer is thought to arise from cells that accumulate several mutations over the period of their lifespan conferring certain key traits. Dubbed the “hallmarks of cancer”, these characteristics are mainly the ability to maintain growth signaling,
escape pathways impeding proliferation, evasion of apoptosis, acquiring the capacity for limitless replication, triggering angiogenesis and finally gaining invasive capacity. Recently, metabolic adaptation and escape from immune action have also been recognized as fundamental to the evolution of cancer. Importantly, for a cell to be able to acquire the necessary mutations that will promote the development of these characteristics, it would require a span of time that is generally greater than the actual lifespan of most somatic cells. Adult stem cells, unlike other differentiated cells, persist throughout the length of an organism’s adult life, positioning them as likely candidates for carcinogenic transformation. Furthermore, stem cells already possess the ability to proliferate without become senescent and to differentiate into multiple cell types while self-renewing. This can be achieved by either symmetric division to produce two stem cell daughters, or by asymmetric division producing a progenitor fated to become a specific cell type of that tissue, and one stem cell. Albeit that these processes are very tightly regulated, dysregulation of the involved pathways could derail the normal functions of a stem cell, resulting in a cancer stem cell (CSC) capable of uncontrolled division and differentiation into multiple progenitors with varied abilities reminiscent of the origin tissue.

**Normal vs. Cancer Stem Cells**

Ample evidence exists to support the CSC hypothesis. The concept of adult stem cell populations capable of replenishing tissues throughout life was first proven more than half a century ago when McCulloch and Till conducted experiments in which they depleted the bone marrow in mice via exposure to lethal irradiation in mice. They were able to find a population of cells capable of self-renewal that, when grafted into these mice, replenished the depleted bone marrow, now known as hematopoietic stem cells (HSCs). Since this revolutionary discovery, we have observed that stem cells are crucial for the normal functioning of many tissues, such as the lung, skin, skeletal muscle, breast as well as the brain. In adults, the rate of mitosis for most stem cells may not be as rapid and regular as what is observed during fetal development, many stem cell populations remaining in a quiescent state and recruited by external stimuli as necessary. It has been proposed that umbilical cord blood-derived HSCs could be used for the development of HSC based gene therapy programs for gynecological cancers. The most rigorously studied system relying on stem cells is the hematopoietic system, and notably, it was in leukemic cell populations that the first bona fide CSCs were isolated and described. It is important to note, however, that the possibility of tumors growing from undifferentiated cells with unlimited replicative potential was suggested as early as the 1940s, when histopathological analyses of tumor tissues led to the conclusion that many tumors shared traits with embryonic tissues, and that teratocarcinoma consisted of both differentiated and undifferentiated cell types that may all have evolved from cancer cells possessing stem cell properties. These observations were bolstered by studies finding that the replication rates of these seemingly undifferentiated cells was much greater than other cells composing the tumor, and that tumors of a single clonal origin could be produced in mice injected with single undifferentiated, multipotent cells from the original malignancy.

**Clonal Origins of Cancer**

That tumors evolving from a single clonal origin could be created in vivo provided strong support for the CSC hypothesis, and the concept that not all cells in a tumor are critical for sustaining cancer growth. However, it was the discovery of shared clonal characteristics of cancer cells that could be traced back to a stem cell origin that truly strengthened the relevance of this model. The Philadelphia chromosome, causing a fusion between the genes BCR and ABL (BCR-ABL), was found to be consistently associated with chronic myeloid leukemia (CML), and was furthermore found in all the non-lymphoid hematopoietic cells of CML patients. This was the first genetic data confirming that cancers result from the expansion of a single mutated cell, and that the original cell was likely a stem cell. The BCR-ABL fusion augments its tyrosine kinase activity and renders it unresponsive to negative regulation, allowing it to constitutively undermine the tumor suppressor activity of PTEN and down-regulate apoptosis. Increasing the activity of PTEN may indeed prove to be an effective target for the design of novel anti-cancer treatments.

Another study providing evidence for the clonal origins of cancer involved assessment of heterozygosity for the glucose-6-phosphate dehydrogenase enzyme in female patients with CML. The patients in this study were all heterozygous
for G6PD, such that all somatic cells would either express one or the other isoform but with a generally equal pattern of expression across cells. However, all the hematopoietic cell types isolated from the patients showed expression of only one isoform, suggesting that all these cells evolved from a single precursor.

The loss of BRCA1 expression is associated with poor prognosis and increased malignancy or breast cancer. Loss of heterozygosity resulting in loss of wilde-type BRCA1 was also detected in breast cancer cells that likely evolved from a CSC and were of the same clonal lineage. BRCA1 is crucial for repair of double-strand breaks in DNA, and its loss could allow for the accumulation of carcinogenic mutations. Recently, it was observed that loss of BRCA1 promoted de-differentiation of leukemic cancer stem cells, allowing for the prolonged, uncontrolled self-renewal of these cells. These observations suggest that mutations enhancing the replicative potential of stem cells, and releasing them from built-in mechanisms of control such as DNA repair, may lead to the formation of cancer stem cells that can sustain tumorigenesis.

**Intrinsic Mechanisms of Transformation**

Many of the mutations detected in CSCs occur in those signaling pathways that are central to the proper regulation of normal stem cell proliferation, differentiation and growth. One of the major pathways involved in the regulation of growth and apoptosis is the Wnt-β-catenin pathway. Wnt is a paracrine or autocrine factor that signals the transcription of key genes required to promote stem cell renewal. Wnt binds to its receptor Frizzled on the extracellular surface, leading to sequestration of Dishevelled and release β-catenin to form the APC-Axin-GSK3 complex. This will prevent its degradation by the proteasome, allowing it to accumulate in the cytosol and translocate to the nucleus where it participates in the transcriptional activation of genes such as TCF/LEF. The aberrant increase in nuclear β-catenin levels due to mutations in the Wnt signaling pathway have been associated with the transformation of normal hematopoietic stem cells, liver stem cells and colon stem cells. Inhibiting β-catenin activity may be an effective means by which to target the CSC population in the treatment of a variety of cancers.

Notch signaling has been demonstrated to promote the self-renewal of stem cells. The Notch genes code single transmembrane receptors that are activated via interaction at the extracellular domain with Delta/Jagged. Binding of Delta/Jagged signals cleavage of Notch’s extracellular domain by γ-secretase, leading to the release of the Notch intracellular domain (IC-Notch). IC-Notch can then translocate to the nucleus, bind with the transcription factor CSL, and activate expression of genes involved in self-renewal. Notch1 mutations allowing increased IC-Notch accumulation in the nucleus or decreased the proteasomal degradation of IC-Notch were observed in patients with chronic and acute lymphoblastic leukemia. Furthermore, increased Notch signaling activity has been detected in breast CSCs. Inhibiting Notch signaling could, therefore, be an effective strategy to target the CSC population, eradicating those cells crucial for maintenance of tumorigenesis.

Yet another critical pathway required for the promotion of stem cell proliferation is the Sonic Hedgehog (Shh) pathway (Figure 1). Shh signaling is known to activate proliferation of neural, hematopoietic as well as lung stem cells, and mutations enhancing Shh signaling are known to lead to the transformation of normal adult stem cells into CSCs. Shh is a paracrine factor that is activated by cleavage and N-terminal modification by addition of a cholesterol group. Shh will bind its receptor, Patched-1, and in doing so relieve inhibition of Smoothened. Patched-1 regularly pumps oxysterols out of the cytosol-Shh binding prevents this function, allowing oxysterols to accumulate and activate Smoothened. Smoothened signaling will then allow activation of proliferative genes via the action of GLI transcription factors. Targeting of Shh signaling could also lead to the development of novel, promising cancer treatments (Figure 1).

**Extrinsic Factors Regulating CSCs**

Understanding the intracellular pathways important for normal stem cell function, and whether their improper regulation resulting from mutation events could contribute to the formation of CSCs is imperative if we are to develop new strategies specifically targeting CSCs, which are required and sufficient for tumor growth. However, increasing evidence suggests that the extracellular environment contributes significantly to the evolution of CSCs. Hematopoietic stem cells interact considerably with the bone marrow niche (Figure 2). Osteoblast expression of N-cadherin has been demonstrated to control stem cell number, and expression of N-cadherin as
the cell types they interact with play a major role in carcinogenic potential. Yet another example of environmental factors determining the fate of carcinogenesis is the finding that colon tumor cells incapable of individually developing tumors, when isolated and cultured in the presence of myofibroblasts secreting hepatocyte growth factor were suddenly able to trigger tumor growth. Secretion of the growth factor promoted the Wnt pathway in the colon tumor cells, which was previously down-regulated. The importance of the extracellular environment in promoting or negating cancer formation, regardless of intrinsic signaling, suggests that through CSCs and the cell types they interact with play a major role in carcinogenic potential. Yet another example of environmental factors determining the fate of carcinogenesis is the finding that colon tumor cells incapable of individually developing tumors, when isolated and cultured in the presence of myofibroblasts secreting hepatocyte growth factor were suddenly able to trigger tumor growth. Secretion of the growth factor promoted the Wnt pathway in the colon tumor cells, which was previously down-regulated. The importance of the extracellular environment in promoting or negating cancer formation, regardless of intrinsic signaling, suggests that through CSCs and

**Figure 1.** The Shh signaling pathway. Binding of the Sonic hedgehog (Shh) ligand to its receptor Patched-1 relieves Patched-1 mediated inhibition of Smoothened. Activated Smoothened is then capable of triggering the actions of the transcription factor Gli, which is usually targeted for proteolysis. Gli will turn on the transcriptional program favoring proliferative signals in the stem cell.
Cancer stem cells: targeting tumors at the source

formation via mutation of normal stem cells may indeed lead to the generation of a tumorigenic subset of cancer cells, how the disease evolves may rely on several factors and an accurate model of cancer progression may be more complex.

The Evolving Model of CSC Origin

The CSC hypothesis has revolutionized our perspective on the pathogenesis of cancer and raised questions as to the most effective strategies for drug development against cancer. However, careful tracing of the origin of the cancer-generating cell is required to truly assess how effectively this hypothesis can be applied in understanding the etiology of certain cancers. Several instances of cancers originating not from a stem cell that acquired transformative mutations, but from differentiated, replication-limited progenitors, have been documented. In CML, the original tumor is sustained by classical CSCs, however over the course of the disease, it is the differentiated granulocyte-macrophage progenitors and B-lymphoid progenitors that accumulate new mutations that confer upon them even greater tumorigenic potential, resulting in an even more aggressive cancer clone that supercedes the CSCs. It has also been observed that introducing expression of the oncogenic MLL-AF9 fusion gene in mouse granulocyte-macrophage

Figure 2. Interactions of hematopoietic stem cells with the osteoblast niche. Osteoblasts create an environment that maintains the quiescent state of hematopoietic stem cells (HSCs). Key molecular mechanisms involved in this process include production of angiopoietin-1 (Ang-1) by the osteoblast, which binds to Tie2 receptors on the stem cell surface and activate p21, which maintains quiescence. Additionally, anchoring of the HSCs to the osteoblast via N-cadherin interaction will inhibit β-catenin translocation to the nucleus and prevent activation of self-renewal genes.
progenitors can create a cell population capable of tumorigenesis in spite of the fact that these cells retain their progenitor immunophenotype and are not classical CSCs\textsuperscript{55}. Although the concept of cancer stem cells as the necessary and sufficient cancer cell subtype for tumor sustenance is a powerful model of carcinogenesis and may lead to the efficient eradication of tumors, it is critical that this model be scrutinized in the light of evidence to suggest that alternative mechanisms, such as environmental influence or acquisition of “stemness” by differentiated cells, may be at play.

Conclusions

More than four decades after we declared a war against cancer, we have progressed considerably in our understanding of this disease but continue to unearth new questions that require elucidation. Understanding the pathways de-regulated in CSCs, and how they can be targeted to develop new therapies, is a promising future strategy to fight cancer. Clinical studies testing the effectiveness of eradicating CSCs specifically will be needed to ultimately determine whether the cancer stem cell hypothesis correctly predicted the ideal anti-cancer target.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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