Stem cells and colorectal carcinogenesis

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Received: October 14th, 2015 – Accepted: January 25th, 2016

Abstract

Colorectal cancer represents an important cause of mortality and morbidity. Unfortunately, the physiopathology is still under study. There are theories about carcinogenesis and it is known that not only a single factor is responsible for the development of a tumor, but several conditions. Stem cells are a promising target for the treatment of colorectal cancer, along with the environment that has an important role.

It has been postulated that mutations within the adult colonic stem cells may induce neoplastic changes. This theory is based on the observation that within a colon cancer, less than 1% of the neoplastic cells have the ability to regenerate the tumor and therefore they are responsible for recurrence. It is important to know that a new way of treatment needs to be found, since these cells are resistant to chemotherapy and radiotherapy.

Keywords: stem cells, colorectal cancer, tumor markers

Introduction

The origin of colorectal cancer stem cells

Hematologists were the first to recognize and use adult stem cells [28,29].

Cancer stem cells were first recognized in acute myeloid leukemia as being the small subset of tumor cells capable of self-renewal, initiation, and maintenance of disease [26].

The normal hematopoietic stem cells accumulated mutations responsible for the transformation into cancer stem cells [37]. Furthermore, the normal hematopoietic stem cells have been used extensively for therapeutic bone marrow transplantation [37]. The similarities between colorectal ontogenesis and carcinogenesis have led some researchers to believe that cancer stem cells arise from either normal adult colonic or remnant foetal stem cells [10,22].

Firstly, both processes produce morphologically similar architectural structures, such as glands. Secondly, markers of gut ontogenesis are found in carcinogenesis but not in normal gut (e.g. cytokeratin 7, nuclear β-catenin) [10].

Thirdly, regulators of gut ontogenesis are overexpressed in colorectal cancers (e.g. Sonic Hedgehog, Notch 1-3 and nuclear β-catenin) [10,11].

To sum up, the normal colonic stem cell appears to be the logical origin for cancer; however, it was not possible to determine this unequivocally.

The cells within the crypt are derived from the stem cells. One of the mitotic cells remains as a stem cell at the bottom of the crypt and another cell is gradually pushed up to the luminal surface of the crypt as an epithelial cell. The cells that reached the uppermost part execute the apoptosis and peel off without replicating or differentiating [1,2,12]. Therefore, any mutations in these cells have essentially no impact on the normal turnover of the mucosa. The cells with damaged DNA (mutated genes) do not cause apoptosis, reach the uppermost part in the crypt, and continue proliferating. This is a pre-cancerous change, aberrant crypt foci (ACF), now being widely used as one of the biomarkers of colon carcinogenesis in chemopreventive experiments [3,4,33,34]. The somatic stem cells reside at the base of the crypts throughout the colonic mucosa. These cells are essential for the normal regeneration of the colonic epithelium. The stem cells reside within a special “niche” which comprises the intestinal sub-epithelial myofibroblasts that tightly control their function. It has been postulated that mutations within these adult colonic stem cells may induce the neoplastic changes. Such cells can then dissociate from the epithelium, travel into the mesenchyme, and thus form invasive cancers. This theory is based on the observation that within a colon cancer, less than 1% of the neoplastic cells have the ability to regenerate the tumor. This group of cells exhibits characteristics of colonic stem cells. Although anti-neoplastic agents can induce remissions by inhibiting the
cell division, the stem cells appear to be remarkably resistant to both standard chemotherapy and radiotherapy. These stem cells may therefore persist after the treatment and form the nucleus for cancer recurrence. Hence, future treatment modalities should focus specifically on controlling the cancer stem cells.

The traditional theory for the development of colorectal cancer is that any cell in the mucosa can accumulate genetic mutations and eventually lead to malignant transformation. This is termed as “the somatic” mutation theory of cancer [38]. Nonetheless, more recent evidence is now questioning this belief. There is current interest in the idea that organ-specific stem cells may provide the origins for cancer development. In the bowel, the mucosal stem cells in the base of the colonic crypts may accumulate mutations and hence lead to tumor development. These stem cells are characterized by their capacity to live long and, in their normal state, are endowed with specific abilities such as self-renewal. The normal colonic stem cells generate the colonic mucosa that has an incredible rate of cell production and turnover. Cancer may therefore develop as a result of the alteration of this process through the accumulation of mutations and damage within the controlling stem cells. In the colon, normal adult stem cells reside at the base of the crypt in a “niche” composed of subepithelial intestinal myofibroblasts [23,28]. Here, they proliferate and give rise to the cells that line the colonic crypt. The Unitarian Theory states that “all cell types within the crypt are derived from a single stem cell” [23]. In adulthood, each crypt is monoclonal, that is, one functioning stem cell per crypt, whereas, in the fetus and neonate, the crypts are polyclonal [35]. Cancer stem cells are similar to normal adult stem cells but with the addition of several features that cause the physiologic disarray: angiogenesis, invasion, metastasis, and resistance to apoptosis. Within the cancer, cellular division is driven by internal cellular events regardless of the external stimuli [24]. Similar to the normal adult colonic stem cells, human rectal cancer cells can give rise to all the cell types of the colonic crypt [21]. The realization that only a minority of cells drive tumorigenesis has important implications for the treatment of cancer. Cancer stem cells have a slower rate of division and a greater ability to correct DNA defects than other cells. Therefore, they are more resilient to adjuvant therapy and can promote the evolution of resistant clones of cancer cells. Moreover, if cancer stem cells persist, even if the bulk of the tumor is destroyed by chemotherapy or radiotherapy, the tumor will inevitably recur.

The normal adult stem cells are specific to tissue and organ type. They form only a small fraction of the total cell population and can proliferate over the lifetime of the organism. These relatively undifferentiated and slowly dividing cells are responsible for maintaining tissue integrity and for providing a continuing supply of new cells [6,23]. Their progeny is more differentiated and they divide at a faster rate [23,39]. Adult stem cells have several key features. These include (i) self-renewal; (ii) plasticity; (iii) potential for multilineage differentiation; and (iv) tissue regeneration [6,31]. It is these functional properties, rather than their morphological features, that are of the greatest importance in the identification of adult stem cells [29]. Plasticity is a core feature of stem cells. Adult stem cells from one tissue (e.g. bone marrow) have the ability to engraft into different tissue types (e.g. colon) [10,22]. For example, haemopoetic stem cells can differentiate into a wide range of cell types from all three germ layers [24]. Furthermore, the concept of plasticity may even question the origin of cancerous cells. In a murine model, helicobacter pylori infection stimulated the influx of bone marrow stem cells, which later gave rise to gastric cancer [11]. It is this flexibility in the cells’ behavior that can make them difficult to be characterized.

The stem cell division

The mode of stem cell division is important as it determines the type of progeny and the future cell population numbers. Stem cells are thought to divide slowly [23]. Their division may be either symmetrical or asymmetrical. Symmetric division is the division of a stem cell to produce either two stem cells or two differentiated cells. Symmetric division of colorectal cancer stem cells is essential in achieving exponential numbers of tumor cells [6]. The symmetric division of cancer stem cells into differentiated cells will lead to the lineage extinction and theoretical cancer cure. Asymmetric division is an essential feature of stem cells, as it allows the production of an identical stem cell and a differentiated progenitor cell [6]. It has been postulated that the new stem cell retains the original strand of DNA to minimize DNA replication errors [23]. This has been termed as the “immortal strand hypothesis” [23]. The differentiated progenitor cell divides at a much greater rate than the parent cell and is often called a transit-amplifying cell. These cells divide and differentiate until the fully functional differentiated cell is produced. The asymmetric division allows the maintenance of stable adult stem cell population with simultaneous production of differentiated cells, thus permitting the continuous regeneration of the organs and tissues [31].

The role of the environment in relation with the stem cells in colorectal cancer development

The differentiated cells of the colonic mucosa have a short lifespan (of days), whereas normal intestinal stem cells have a long lifespan and therefore more opportunities to accumulate mutations [23]. The Vogelstein adenoma–carcinoma sequence states that mutations accumulate in the cell, leading to malignant transformation. These mutations are accompanied by a phenotypic change in the mucosa. There is now evidence emerging to suggest that the long-lived adult colonic stem cells can accumulate mutations for a prolonged period of time before the phenotypic change becomes apparent [10,21].
The somatic mutation theory of cancer maintains that mutations accumulate within a cell until uncontrolled cellular proliferation occurs, thus giving rise to a cancer [24]. In addition, there is a strong evidence to suggest that the cell’s interactions with its environment are just as important. Therefore, it is essential to understand the concepts of the stem cell niche, the effect of the microenvironment and their role in the causation of cancer. The stem cell niche is the microenvironment that houses the stem cell and controls its activity of self-renewal and generation of tissues [39]. In normally functioning tissues, cells are not self-determining. Their activity is tightly regulated by adjacent cells, extracellular matrix, and soluble factors [31]. In fact, if cells are separated from their normal environment, such as in culture, they lose function and differentiation [31]. The stem cell niche has been shown to have a profound impact on the differentiation of stem cells because of their inherent plasticity. For example, when cultured with myoblasts, neural stem cells differentiate into muscle cells [11].

In the colon, stem cells reside at the base of the crypt. Each is encased by intestinal subepithelial myofibroblasts (ISEMFs) separated only by the basal lamina. The ISEMF forms a syncytium within the lamina propria that extends along the length of the intestinal tract [23,28]. This syncytium receives cholinergic input and encases the surrounding blood vessels [29]. The myofibroblasts extend the cell processes through fenestrations in the basal lamina to achieve direct contact with the epithelial cells [29]. The ISEMF syncytium secretes a variety of cytokines that are important for wound healing and immune cell function [29]. There is evidence to suggest that they control the proliferation and differentiation of the epithelial cells. In addition, they play a role in the electrolyte and water absorption. In pathological states, ISEMFs contribute to fibrosis, desmoplastic reactions, and neoplasia [29].

The manipulation of the microenvironnement through the genetic modification of matrix metalloproteinase’s and stromal cells can lead to the development of cancer [31]. Stromal cells that are unresponsive to transforming the growth factor (TGF)-β allow the epithelial cell growth and invasion [31]. Cancers that arise in the setting of chronic inflammation illustrate the effect of a defective environment. Conversely, a healthy microenvironnement can prevent tumor growth even in the presence of stem cells that carry oncogenic mutations [31]. It is thought that the microenvironnement exerts its effect through epigenetic mechanisms that inhibit or enhance the expression of various genes without altering the DNA sequence. There is only a small subpopulation of cells that have the capacity of generating new primary tumors within any cancer. These cells are termed cancer stem cells primarily because of their ability to self-renew and regenerate tumor tissue. The origin of these cells is unknown. They may arise from normal colonic stem cells, partially differentiated progenitor cells [24] or fully differentiated cells [10]. The last have a relatively short lifespan and thus have little opportunity to accumulate mutations that give rise to a malignant phenotype.

The normal and malignant colorectal stem cells

The identification of normal and malignant colorectal stem cells has always been difficult. Only recently have new methods arisen to aid in their identification and isolation. At this stage, the most important of these has been the identification of surface markers by immunohistochemistry. The presence of these surface markers has facilitated experiments involving disaggregating tumor cells into separate subgroups and testing which subgroup has tumorigenic potential. Other methods of identification include morphological features such as “bell shaped” nuclei and their position at the base of the crypt [22]. In addition, DNA methylation patterns and RNA binding proteins have been used to identify these cells [8,9,20,23].

Tumor markers of colorectal cancer stem cells

CD133 has been shown to be a marker of importance in identifying a population of cells enriched with colorectal cancer stem cells. In an experiment by O’Brien et al., CD133+ colorectal cancer cells were over 200 times more likely to initiate tumor growth than CD133– cells were [19]. When CD133+ cells were injected under the renal capsule of immunodeficient mice, the resultant tumors resembled the parent tumors. Normal colonic mucosa contains a relatively low number of CD133+ cells compared with the malignant mucosa. In a similar study by Ricci-Vitiani et al., it was the CD133+ subgroup of colorectal cancer cells that were found to be tumorigenic [5]. Again, these cells were undifferentiated, rare in normal colonic mucosa and could undergo serial transplantsations with an unlimited growth potential [32]. Importantly, CD133+ cells gave rise to tumors that resembled the parent tumor [5]. The other cell surface markers that may aid in the identification of cellular subpopulations that contain higher densities of colorectal cancer stem cells include CD166 and the EpCAMHIGH/CD44+ phenotype [25]. CD166 is also a mesenchymal stem cell marker but has been associated with poor prognosis in colorectal cancer [25]. EpCAM and CD44 were the first identified as stem cell markers in breast cancer.

It is important to note that these surface markers do not actually identify cancer stem cells but rather a group of tumor cells that are enriched with cells that have “stem cell like” ability. It would appear that less than 1% of the cells sorted for these various surface markers have this ability. Furthermore, because the microenvironnement influences the expression of surface proteins, the process of dissociation of tumor cells performed in these experiments may modify the cell surface expression.
Thus, these results may not be representative of the in vivo situation [32]. Earlier studies frequently state that morphologically normal colon stem cells cannot be distinguished [17]. Gostjeva et al. used a unique method of histological preparation that involved the fixation of a surgical resection within 30 min and the use of thick slices (500 μm) to preserve tissue architecture and nuclear structure [22]. This revealed unique bell-shaped nuclei, of 10–15 μm in height by 7 μm in width. Such nuclei were common in foetal gut, adenomas, and adenocarcinomas but were very rare in normal adult colon. These nuclei were arranged in the foetal gut, in an orderly head-to-toe manner within a tubular syncytium, within which they underwent symmetrical and asymmetrical divisions. In adenocarcinomas, bell-shaped nuclei accounted for approximately 3% of nuclear forms within the central tumor mass. They could be found in large numbers at the bases of malignant crypts but were very rare in normal crypts. The authors believed that these findings supported an embryonic or stem cell origin of colon cancer. They further hypothesized that transformation led to colonic stem cells with juvenile rates of growth, stating that adenomas (preneoplasia) have similar rates of growth as the juvenile colon and could be considered as “foetal outgrowths” [17,22]. When enough mutations have accumulated, the cells acquire the growth rates of embryonic stem cells and go on to form a carcinoma.

In a normal mucosa, the Wnt pathway controls the proliferation and migration within the colonic crypt via the expression of transcription factors and adhesion molecules [23,35]. This pathway is abnormal in colorectal cancers. Patients with familial adenomatous polyposis coli have a malfunctioning APC protein that allows the accumulation of β-catenin in the cytoplasm. β-catenin is normally bound to membranous E-cadherin. When β-catenin enters the nucleus, not only does it trigger the cell cycle, but also it precipitates the loss of membranous E-cadherin and suppresses its expression [6]. Thus, cell–cell contact is reduced, thus permitting the migration of cells from the epithelium to the mesenchyme. This process has been coined as “epithelial to mesenchymal transition” (EMT) [6]. EMT is an essential process in certain physiological circumstances such as embryogenesis and wound healing. In the epithelial cells, the loss of E-cadherin leads to diminished cell–cell contact, allowing the motility and migration of cells [24]. The highest concentration of nuclear β-catenin in colorectal cancers is found at the advancing margin in free tumor cells that have lost E-cadherin expression [6]. The presence of these cells has been associated with metastasis and poor survival [14,15,27]. Lower levels of β-catenin are found in adenomas and within the central tumor [6]. To further enhance the oncogenic potential, nuclear β-catenin increases the expression of survivin, a protein that promotes cellular “proliferation and resistance to apoptosis”. Brabletz et al. proposed that colorectal cancer stem cells at the tumor margin become mobile through their high nuclear β-catenin and subsequent suppression of adhesion molecules [6]. These cells form a “front” that functions as a “germinal layer” and can break away to metastasize to form new colonies of cancer cells [6].

The Lgr 5 gene encodes for a receptor in the Wnt signaling pathway. This gene has been shown to be a sensitive marker for normal adult intestinal stem cells in a mouse model [40,42,43]. Cells that express this gene are restricted to the base of the crypt and are described has having “wedge shaped nuclei”, similar to the “bell shaped nuclei” described by Gostjeva et al. [6,20]. In keeping with the stem cell theory, these cells give rise to all the cells of the mucosa and are self-renewing. Furthermore, they have a greater resistance to radiation-induced apoptosis. Therefore, the Lgr 5 gene, which was first found in colorectal cancer, may also be a marker for colorectal cancer stem cells.

The effect of chemotherapy and radiotherapy on malignant stem cells

Apart from surgery, the mainstay of anticancer therapies is to rapidly target the dividing cells. Regarding the stem cell model for cancer, these therapies would target the transit-amplifying cells and differentiated cells that form more than 99% of the tumor. The cancer stem cells that can initiate new tumors are relatively slow cycling and are therefore less affected by these therapies. Furthermore, the elaboration of multidrug-resistant proteins by stem cells adds another layer of protection [23]. These proteins may assist in transporting chemotherapeutic agents out of the cytosol and in increasing the resistance to apoptosis [24]. Therefore, rather than targeting rapidly dividing cells, drugs that promote the terminal division may deplete the stem cell component of the tumor and lead to an eventual tumor eradication. An example of this is seen in hematological malignancies where retinoic acid can drive stem cell differentiation and therefore improve survival [10,24].

In addition to the resistance to chemotherapy, cancer stem cells are frequently resistant to standard radiotherapy regimes. In glioblastoma multiform (GBM), CD133+ cells have been identified as potential stem cells. In this setting, Bao et al. demonstrated that the CD133+ cells are resistant to radiotherapy because of improved DNA repair mechanisms [18]. This radioresistance can be reduced by drugs that shorten the pause in the cell cycle, thus preventing DNA repair [18]. Radiotherapy for GBM is associated with an increase in the proportion of CD133+ cells [18]. This may help explain their inevitable recurrence and the corresponding 2% 5-year survival rate for GBM [13]. Similarly, in the setting of rectal cancer, radiotherapy can frequently reduce the size of the tumor or even lead to a complete pathological response, but, without surgery, the risk of recurrence approaches 80% [16]. Thus, it is tempting to conclude that residual rectal
cancer stem cells are responsible for locoregional recurrence. In support of this idea, it has been shown that the expression of CD44v6 (a stem cell marker) is associated with higher rates of locoregional recurrence of rectal cancer [41].

Conclusions

With our evolving understanding of colorectal cancer stem cells, it is now easier to appreciate why the current systemic therapies only induce partial or incomplete remission. The surgical excision is currently the only effective management strategy we have against this group of cells. Therefore, there is a pressing need to develop new therapies that can target this unique subpopulation of cancer cells. Such treatments would assist in both eradicating the disease and maintaining a longer duration of remission. Unfortunately, stem cells form a core unit in the human body, and trying to selectively target them is, at present, exceedingly difficult. Similarly, as appealing as these theories might be, they still need to be scientifically validated in human models.

The stem cells in colorectal cancer are believed to be uniquely endowed with the capacity to renew themselves [7, 26, 30, 36, 37]. By definition, single colorectal cancer stem cells can lodge in a permissive site, such as the liver, and produce a metastasis. Currently, it is not possible to isolate individual colorectal cancer stem cells, although certain cell-surface proteins (e.g., CD133, CD44, CD166, and aldehyde dehydrogenase1) are promising markers. Normal stem cells that reside in the colonic crypt rely on adhesive and soluble stromal-epithelial interactions to maintain division and differentiation. The extent of alterations in these regulatory mechanisms in colorectal cancer stem cells is a promising area of investigations, since agents that control the growth of colorectal cancer stem cells could theoretically be used for cancer prevention and treatment. The family of growth factors and events such as the regulation of the nuclear localization of beta-catenin seem to be central to normal homeostasis in intestine stem cells; mutations in the components of these pathways leading to the development of colorectal cancer. A paradigm of abnormal stem cell biology is illustrated by patients with FAP, who have mutations in the adenomatous polyposis coli (APC) gene. The wild-type protein encoded by this gene is important in the prevention of mass beta-catenin accumulation in the nucleus and the subsequent over transcription of cell cycle proteins; these being the basic mechanisms behind stem cell regulation in the gut and their role in the natural history of tumor progression.

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