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REVIEW ARTICLE

The colorectal cancer stem-like cell hypothesis: a pathologist's point of view

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Summary

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide. The partial failure of classic therapeutic options makes scientists to doubt the efficacy of systemic treatments in targeting the essential cell populations and achieving cure as a final goal. Overgrowing data suggest that cancer is a disease closely linked to stem cells (SCs).

It is well known that the first identification of cancer stem-like cells in acute myeloid leukaemia was soon followed by similar results in solid malignancies, including colorectal cancer, and the classic model for colon carcinogenesis supports the development of sudden mutations that will lead to the activation or inactivation of certain oncogenes or tumor suppressors. Thus, this process may go on for years before the first symptoms and the only cells able to withstand for many years, avoid apoptosis and have a high regenerative capacity are the progenitor cells found at the lower part of colon crypts.

A more profound study of the mechanisms and molecular signalling pathways that control the basic characteristics of SCs, such as asymmetrical division or self-renewal, may help comprehend the basic mechanisms of cancer genesis and progression. This will result in the development of new therapeutic agents that may target chemoresistant cell populations and improve the therapeutic results.

In the current review we point out the importance of cancer stem-like cells in colorectal oncology from a pathologist's point of view, stating the obvious correlation between histology, embryology and surgical pathology.

Key words: cancer stem-like cells, colon carcinogenesis, colorectal cancer

Introduction

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide [1-3]. During the past few years, remarkable progress has been made regarding the diagnosis and treatment of this disease due to the translation of the tumor cell biology data into clinical practice. Therapeutic monoclonal antibodies against EGFR, as well as determining the *KRAS* gene status to assess patient response to treatment, are only part of the progress made by modern oncology [4,5]. Despite the development of new therapeutic agents and their interference with classic chemotherapy, the results for advanced-stage colorectal cancer are still scarce [6]. The partial failure of classic therapeutic options makes scientists to doubt the efficacy of systemic treatments in targeting the essential cell populations and achieving cure as a final goal.

Accumulated data suggest that cancer is a disease closely linked to SCs. The first identification of cancer stem cells (CSC) in acute myeloid leukaemia (AML) was soon followed by similar results in solid cancers, including colorectal cancer [7-9]. The classic model for colon carcinogenesis supports the development of sudden mutations that will lead to the activation or inactivation of certain oncogenes or tumor suppressors

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[10,11]. This process may go on for years before the first symptoms, and the only cells able to withstand for many years, avoid apoptosis and have a high regenerative capacity are the progenitor cells found at the lower part of colon crypts [12].

A more profound study of the mechanisms and molecular signalling pathways that control the basic characteristics of stem cells, such as asymmetrical division or self-renewal, may help comprehend the basic mechanisms of cancer genesis and progression. This will result in the development of new therapeutic agents that may target chemoresistant cell populations and improve the therapeutic results [13].

In the current article, we have tried to cover the main aspects of colon tumors appearance and progression based on SCs known properties and the so called cancer stem cells theory.

Stem cells and normal colon structure

Morphology and morphogenesis

The intestinal epithelium originates from the embryonic endoderm, which in turn stems from pluripotent epiblast cells at the onset of gastrulation. The endodermal lining covering the mesoderm and ectoderm undergoes a series of invaginations initiated at the anterior and posterior ends of the embryo, resulting in the formation of a proper gut tube. At this stage, the primitive gut is composed of a uniform layer of cuboidal endodermal cells surrounded by splanchnic mesoderm.. The mesoderm will differentiate into mesenchymal structures (muscle and connective tissue) that will form the large intestine [14].

Once the basic structure is formed, cytodifferentiation occurs along with the gathering in a radial axis, which will establish the final structure of the colon. This structure includes the mucosa formed by unistratified epithelia, lamina propria and muscularis mucosa; submucosa formed by a dense tissue; muscularis propria formed by smooth muscle tissue distributed into two layers; and the peritoneal serosa which partially covers the colon structures. The nervous component that assures the colon mobility is organized into two plexuses formed by cells of the neural crests migrated into the mesenchyme [15].

During embryogenesis, colon formation is under the control of the Wnt signalling pathway. As pluripotent cells differentiate, only a few cells will keep the self-renewal ability and ensure the tissue turn-over during the entire life span. Apart from the mesenchymal component, the epithelial tissue is a very dynamic structure based on extremely robust proliferative compartment, controlled by the special microenvironment also known as the niche. Under the influence of various growth factors and cytokines secreted by the niche, cells proliferate, invaginate, and finally form the colon crypts, the functional unit of the colon [16-l8].

In order to completely comprehend the mechanisms of colon carcinogenesis it is essential to know the crypts' organisation which contains a basal compartment essential for regeneration that contains the SCs, an amplifying compartment, and the mature compartment found at the upper part of the crypt [19,20]. One of these crypts will contain approximately 200 cells that have the ability to differentiate into three directions: mucinous, endocrine and columnar cells [21].

Normal colon stem cell markers

Proper studies of colon SCs are quite limited because of a relative lack of specific markers used for their isolation and complete characterisation. Initially, bromodeoxyuridine was used to highlight the SC compartment of various tissues, based on the observation that the division is rare and also the ability to retain labelled DNA for longer time. The progress in understanding the specific biology of a cancer SC has led to the identification of specific markers, mainly surface markers [22].

Mushashi-1 (Msi-1), a protein that binds to RNA, is present in cells with an asymmetrical division-a key feature for SC. The initial identification was in precursor cells found in the sensorial organs of Drosophila melanogaster, but was later proven to be part of the human colon at the base of the crypt [23,24].

By studying the Wnt signaling pathway, Baker et al. have identified leucine-rich repeat G-coupled protein receptor 5 (LGR5) as a marker for cells found at the base of the crypt and able to differentiate *in vitro* into all cell types found in the large intestine structure, as LGR5 is a target of the Wnt pathway in the intestinal SC niche by downregulating Wnt and leading to precocious Paneth cell differentiation in the fetal intestine [25,26].

Such is the case of a recent study that identifies cells expressing Bmi1 which have the ability to give rise to all types of epithelia, but unlike Lgr5+ cells, they are mainly quiescent [27]. Bmi1 regulates SC maintenance and self-renewal as its loss reverses self-renewal induced by β-1 catenin signaling and attenuates the formation of PTEN-deletion-mediated colorectal carcinoma. All these observations have raised the question that more than one cell population with stem-like characteristics exists in the intestine [28].

Resistance to apoptosis is another element of differential diagnosis for SC. Thus, after 24 h of exposure

to ionizing radiation, only a small part of the SC niche is discarded and their descendents are able to undergo mitosis and express DCAMKL-1, a serine-threonine kinase of the CAMK family on the cell membrane. It is worth mentioning that immediately after the exposure to a lethal dose of ionizing radiation DCAMKL-1 cannot be found on the membrane, but it is re-expressed after several days. This suggests the presence of a quiescent cell population [29].

The stem cells niche and the signalling pathways

The intestinal epithelia have the most intense turnover in the adult human body. The proliferation dynamics of the epithelial component is kept, as already mentioned, by the stem and transit-amplifying cell population at the base of the crypt under the control of some key signalling pathways, among Wnt, released by subepithelial fibroblasts, is the most important [30-32].

In the luminal compartment, the cells that emerge are no longer under the influence of Wnt and are more prone to differentiation than to proliferation. Cleavers et al. have demonstrated that the genes activated by Wnt are expressed in the basal part of the crypt in the case of colorectal tumors, while the genes suppressed by Wnt are expressed especially in the upper part of the very same crypt. If the Wnt signaling pathway controls the proliferation of the basal compartment, the differentiation of cells found in the upper part of the crypts is controlled by other pathways such as Notch, BMP or TGF-β [33]. Another class of proteins activated by Wnt with an important role in cell sorting is the Ephrins and their receptors by forming a connection between proliferation and differentiation, ultimately leading to an adequate positioning of the epithelial cells in the crypt [34].

This way, the Wnts released by myofibroblasts are responsible for the organisation of the epithelial component and control the proliferation and differentiation, both in normal and pathological conditions [35-37]. A molecule that plays an essential role in the Wnt signalling pathway and colorectal cancer progression is beta-catenin. In the absence of the signalling pathway activation, beta-catenin is degraded in the proteosomal system by a complex structure formed by APC, CKI, GSK3 and Axin. When Wnt binds to its specific receptors-Frizzled and LRP- the degradation complex is inactivated, beta-catenin accumulates, is translocated to the nucleus, binds to transcription factors of the TCF/LEF superfamily and stimulate proliferation in the basal compartment [38-40].

Stem cells in colon pathology. From dysplasia to adenocarcinoma

Starting the lesion-monoclonal conversion of the crypts

As a result of the observation that colon crypts are monoclonal 3D structures, a new mechanism based on the SC concept might explain its dynamics in normal and pathologic conditions [41].

SCs have the capacity of asymmetric division, so that from one initial cell will appear two identical daughter cells- one that will maintain the stem cell pool and one that will differentiate into a transit amplifying cell [42].

In the vast majority of cases, mitosis of the SCs is asymmetrical but, under the influence of various external stimuli, symmetric division is possible. In this manner, we can have two identical cells that may differentiate or two cells that will maintain the niche [43].

In the first case (two differentiated cells) symmetric division will lead to the extinction of SCs clone. In the second case (two stem cells), the SC clone will replace the extinct cell clones, maintaining the SC pool and leading in time to a monoclonal population of SCs at the base of the crypt [44].

In pathology, if a mutation occurs (such as the APC), SCs will have a proliferative advantage and these cells will replace the old ones in a process called niche succession [45]. These progenitors have this mutation encrypted in their genome and will fill the entire niche, so that a monoclonal conversion of the crypt appears [46]. The time period this process takes place is called clonally stabilisation time of the crypt and normally it may last up to 40 years [47].

Developing the lesion-generating adenomas

The development of the normal colon mucosa is achieved through duplication of the crypt by fission. In rare conditions it may appear in adults in the case of various pathological conditions such as ulcerative colitis or tumors, and represents an important regenerative mechanism [48,49].

Studies carried out in mice have shown that the crypt volume has a gaussian distribution and the one that initiate the entire process has a bigger volume. This observation has led to the concept of crypt cycle, a very slow process in adult tissues [50].

In the colon, an adenoma is considered to be a premalignant condition. The genetic alteration known to initiate this pathological condition is a somatic mutation found at the level of the tumor suppressor gene APC,

identified in over 80% of all colorectal cancers. APC encodes a large protein that is part of the Wnt signalling pathway and the deregulation of this pathway lead to an increased proliferative activity in the SC pool [46].

If a mutation occurs in the APC gene, through the processes of niche succession and clonally conversion previously described the formation of a crypt that contains only APC +/- cells may arise. Even if this crypt shows a normal histological structure under a light microscope, its crypt cycle is accelerated [45]. This is confirmed by the fact that the mucosa of individuals with familial adenomatous polyposis (FAP), carriers of the APC mutation, has an increased crypt turnover in comparison with normal ones. Thus, crypts will contain only APC +/- cells, have a high proliferative potential and also a high probability to acquire a second mutation on another allele [51].

Once the other allele suffers the very same mutation, the niche succession process occurs again, followed by the monoclonal conversion of the crypt that will have mainly APC-/- cells, genetically enough to produce an adenoma. This way, we will have a crypt composed mainly of dysplastic cells surrounded by normal crypts corresponding to the first structure identified in optic microscopy, called monocryptic adenoma [52]. In time, the cells that carry APC or KRAS mutations are able to proliferate, change stromal cells secretion and proliferation, in order to form new niches and give rise to macroscopic structures - adenomatous polyps [53,54].

Generating colorectal cancer - mutations, niche involvement and epithelial-to-mesenchymal transition

The malignant transformation of the preneoplastic conditions in the colon lead to the appearance of cell populations characterised by an increased proliferative potential, resistance to apoptosis, increased invasion capacity and metastatic potential [55-57].

Increased mitotic rate in the proliferative compartment of colorectal tumors is correlated with the acquisition of novel mutations as a result of genetic instability. If the APC mutation is considered essential for the initial phase, the loss of p53, K-RAS activation, genome hypomethylation or the hypermethylation of specific promoters are just a few of the genetic events necessary for the continuation of tumor growth [58-60]. A key role in the proliferation seems to go to the nuclear localisation of beta-catenin. Immunohistochemistry studies showed that beta-catenin has a non-homogeneous nuclear expression in colorectal cancers. This so-called beta-catenin paradox may be explained by the cancer stem cell theory, i.e. only a small subset of cells responsible for tumorigenic potential are positive at the nuclear level [61-63].

Nevertheless, the proliferation is not homogeneous, cell populations being heterogeneous in tumors. Today tumor cells heterogeneity may be partially explained by two theoretical models. The stochastic model assumes the existence of an initially homogeneous cell population subjected to both internal and external pressure, generating different clones as a response. In the case of this model, we can consider that cells acquire a functional phenotype (or stemness status), that may be obtained by any cell if certain conditions are fulfilled. Recent observations have shown that in colorectal cancers the progenitor compartment is enlarged, mostly due to the Wnt-mediated interference [64,65]. Vermeulen et al. have shown that during tumor development the fibroblasts that form the niche have Wnt increased signalling activity and can instruct more differentiated tumor epithelial cells to acquire a SC status [63].

The hierarchical model assumes that the tumor is organised similar to normal tissues, with undifferentiated SCs able to generate mature progenitors. This model is closer related to the cancer SC hypothesis in regards to the crypt architecture that includes the keeping of the previously described structures to a certain extent [66,67].

In the case of colorectal cancers, both models may be described as being compatible to the cancer SC model. The hierarchical model includes the conservation to a certain extent of the cryptic aspect in colorectal cancers, but the possibility of cell recruitment to a dynamic proliferative compartment according to external stromal signals is similar to the stochastic model.

The proliferative activity is joined by the ability to invade the surrounding tissues and a key role is given to the epithelial to mesenchymal transition (EMT). A crucial process in embryogenesis, but also in the forming of tissues and organs during morphogenesis, EMT contributes to tumoral progression through various mechanisms. In colorectal cancer, EMT occurs in the invasion front and results in the generation of cells with an increased metastatic potential, joined by the acquisition of specific SC markers [68].

TGF-β activation favors EMT and inhibits apoptosis and thus the cell lines that are resistant to oxaliplatin express various markers characteristic both for EMT and stemness, besides from having a characteristic mesenchymal appearance [69]. The metastatic potential that characterizes malignant cells involves the transient modulation of the epithelial phenotype. EMT is a reversible process and necessary for metastasis. In fact, the EMT-mesenchymal to epithelial transition (MET) hypothesis is formulated by analysing the progression process of colorectal cancers and the formation of hepatic metastasis [70].

Initially described as a mechanism for normal SC

trafficking, the SCDF1α-CXCR4 axis has also been proven to be involved in colon cancer stem cell dissemination. As a result, several studies that have tried to block colorectal cancers have successfully reduced the metastatic potential and opened new opportunities in the treatment of advanced-stage cancers [71-73].

Colon cancer stem cells markers

One of the first markers identified by flow cytometry is CD133, also known as Prominin, a glycoprotein on the surface of the cell membrane with a possible role in cell proliferation [74, 75]. In order to highlight the tumorigenic potential of CD133+ cells, these cells were implanted into NOD/SCID mice and the observation that, in comparison with CD133- cells, tumor appeared in the mice treated with CD133+ cells [8]. When mice, previously injected with CD133+ cells and treated later with oxaliplatin, showed further tumor increase, suggesting that cancer cells gained further chemoresistance [76]. Some studies have even proven a link between CD133 in tumors and the clinical outcome of patients [77,78].

Another potential marker is ALDH1, an enzyme that turns retinol to retinoic acid and thus protecting cells from the oxidative stress [79]. This way, cells live longer. When ALDH+ cells are injected into NOD/SCID mice have the capacity to generate tumors *in vivo*. It has been observed that during tumor progression in mice injected with CD133+/ALDH1+ cells, these two markers helped identify a subpopulation of cells with stem-like characteristics [80].

Lgr5 is also reported as a marker in colon cancer, but maintenance of the expression of some markers during malignant transformation will determine which cells will acquire characteristics similar to normal SCs [26]. Other described markers involved in colon cancer progression are CD44, CD24, CD29, CD166 or EpCAM [81]. Some data from various clinical studies also suggest the possible involvement of embryonic markers such as Oct ¾ or SOX2 in tumor cell biology, but their validation is necessary in future publications [78].

Clinical perspectives

Two main protocols are used in the clinic for the treatment of patients diagnosed with colorectal cancer and hepatic metastasis: FOLFIRI (5-FU, leucovorin and irinotecan) and FOLFOX (5-FU, leucovorin and oxaliplatin). These regimens are used to reduce the size of metastasis in order to increase surgical resectability. New targeting agents are added to block signalling pathways and include bevacizumab, a monoclonal antibody that blocks VEGF, and cetuximab, a monoclonal antibody that blocks EGFR [82].

Even though combinations of these treatments have resulted in increased overall survival, there is no cure for metastatic colorectal cancer because a small population of stem-like cancer cells manage to elude systemic treatments. One therapeutic option is forcing these cells to differentiate. Sialomycin, a potassium ionophore, has successfully managed to reduce the number of CD44 high/CD24 low cells in breast cancer while paclitaxel has the exact opposite effect. The suggested mechanism is linked to potassium channels and the interference with the EMT transition [83].

Plerixafor, also known as AMD3100, blocks the binding of CXCR4 to CXCL12 and results in a limitation of the calcium flux induced by SDF1α during distant dissemination. But further, more detailed studies of the colon cell biology, both normal and tumoral, are needed in order to improve the identification of targets responsible for the tumoral process and progress and help create new therapeutic plans.

Concluding remarks

An ever increasing body of data sheds light on the role of some cells with stem-like characteristics in the genesis and progression of malignant tumors. Even so, studies performed so far have failed to offer a complete picture of the mechanisms through which such cells drive the tumoral process. Still it is obvious that more data is needed for a complete comprehension of the alterations that appear in cancers.

The dynamic processes that characterise changes of the colon architecture in the context of genetic instability, make it difficult to correctly identify a phenotypically and spatially moving target in order to eliminate resistant tumor cells.

In this article, we have tried to explain the main mechanisms that lead to colorectal cancer development, based on the current knowledge on SCs and cancer SCs hypothesis. Future characterisation of possible subpopulations of stem-like cells that have different properties, including proliferation and metastasis, but also the modulation of the tumoral stroma through the help of genetically modified mesenchymal SCs, are only a few of the directions worth pursuing to improve the outcome of patients.

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