MINI REVIEW

Stem cells and colon cancer: The questionable cancer stem cell hypothesis

Cellules souches et cancer du côlon: l’hypothèse ambiguë des cellules souches cancéreuses

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Available online 3 November 2010

Summary The fine-tuning between cell proliferation and differentiation of self-renewing stem cells and pluripotent progenitors in gastric glands and colon epithelial crypts is coordinated by the mechanisms that regulate colon epithelial cell migration and guidance along the crypt axis. This leads to the acquisition of specialized cellular functions and the exfoliation of desquamated senescent and apoptotic epithelial cells at the apical mucosa interface with the gut lumen. Self-renewing stem cells and pluripotent progenitors are involved in the clonal and polyclonal growth of digestive tumors. Several lines of evidence support the existence of a subpopulation of cancer cells with stem cell-like (SCL) phenotypes in solid tumors of breast and digestive system. Consistently, epithelial cancer cell lines in long-term culture are phenotypically and functionally heterogeneous. It is suggested that only a small proportion of transformed cells are clonogenic in vivo and ex vivo to form colonies and to initiate tumor growth in immunodeficient mice. A discrete subpopulation of tumor-initiating SCL cancer cells are highly competent to survive, propagate and spread through the invasive and metastatic cascade. A better understanding of the mechanisms driving the plasticity and pluripotency of stem cells, their derived progenitors and SCL colon cancer initiating cells during tumor progression will open new avenues for the early detection and treatment of local and distant tumors of the digestive tract.

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Introduction

This Review focuses on the relationships between resident stem cells and progenitors involved in the production of normal epithelial cells in the gastrointestinal mucosa, and the emergence of cancer cells with stem cell-like...
(SCL) phenotype and behavior. The biological and clinical significance of the stem cell niche in the digestive mucosa and stem cell visitors originating from the bone marrow and other tissues was also addressed with special reference to tissue repair and neoplasia.

Resident stem cells and progenitors

The gastrointestinal tract is of endodermal origin and presents a general organization of the multipotent stem cell zone apt to maintain the bidirectional migration of digestive cells to the bottom and top of the epithelial units in the gastric isthmus and small intestinal crypts [1,2]. In mice, small intestinal stem cells in the crypts of Lieberkühn, each of which contains 2000 to 3000 cells, undergo up to 1000 divisions in their lifetime [3]. These stem cells divide more slowly than their daughters within the transit cell compartment (Fig. 1A and B). A recent report has identified a new population of Wnt-dependent proliferating stem cells driving the maturation of glandular epithelium in the gastric pylorus and corpus [4]. These multipotent Lgr5-positive stem cells have a restricted and typical localization at the base of the pyloric glands in the adult and were shown to contribute to the regular self-renewal of the glandular units in the pyloric region. About 9% of FACS-sorted Lgr5-positive cells exhibit high clonogenic potential and propensity to generate long-lived organoids that closely resemble pyloric gastric units. Lgr5 encodes the orphan G-protein coupled receptor Gpr49, a biomarker of resident stem cells in the small intestine and colon [5]. In the small intestine four to five Lgr5 positive cells are intercalated with Paneth cells at the crypt base.

In the colon, stem cell progenitors are localized within the stem cell niche of the crypt epithelium and they display intrinsic and acquired anticancer drug resistance in com-

Figure 1 Integration of the genetic, molecular and cellular networks that drive morphogenesis and carcinogenesis of human colon epithelial crypts. (A) On the left is represented an isolated human colon epithelial crypt. (B) Schematic representation of the colon crypt containing self-replicating stem cells in the stem cell niche surrounded by resident (myo)fibroblasts. Committed stem cell - derived progenitors produce differentiated epithelial cells, i.e., enterocytes, mucus-secreting and (neuro)endocrine cells (NEC) that migrate along the crypt. Cell death linked to desquamation of mucosal surface epithelial cells is represented at the apical side of the colon crypt; CIL: colon intraepithelial lymphocyte. (C) Stem cells and epithelial precursors are subjected to carcinogenesis driven by microsatellite, genetic and chromosome instabilities, sporadic mutations and epigenetic defects, hereditary and familial diseases (FAP, HNPCC and other forms), as well as alterations of the Serrated signaling pathways (BRAF mutations, CpG island methylator phenotype, CIMP) which are involved in the progression of colorectal cancer arising from serrated polyps in patients with hyperplastic polyposis syndrome, HPS. Cancer cells and stem cell like (SCL) cancer cells undergoing the epithelial-mesenchymal transitions (EMT) are characterized by loss of the epithelial features (differentiation markers, polarity), acquisition of the mesenchymal phenotype, exacerbation of the invasive and metastatic capacity, and chemoresistance to anticancer drugs. Molecular and cellular interactions between the tumor stroma and mesenchyme lead to critical phenotypes during cancer progression, wound healing and inflammatory situations, such as colitis. Colon stem cells, SCL cancer cells and non SCL cancer cells that undergo EMT are identified by selective molecular biomarkers and are regulated by a series of canonical signaling pathways involved both in normal development and carcinogenesis. (D) On the right, mesenchymal stem cells (MSC), hematopoietic precursors (HP), and endothelial cell precursors (ECP) from the bone marrow and other tissues, as well as circulating tumor cells (CTC), which originate from dormant metastases (according to the self-seeding theory), are selectively recruited to the stroma of inflammatory and neoplastic sites. Local and systemic inflammatory responses modulated by tumor infiltrating immune cells (TIC) are observed.
Stem cells and colon cancer: The questionable cancer stem cell hypothesis

Two distinct compartments of proliferating progenitors are operational in colon crypts, the producers of epithelial stem cells with self-renewal properties at the crypt base, and a subset of dividing multipotent progenitor cells escaping from this stem cell factory. These progenitors are characterized by high proliferation rate, migration to the apical side of the epithelial colon crypt, and potential to differentiate into several epithelial lineages. In the adult human colon, the epithelium contains about $10^{11}$ cells, representing the production of $6 \times 10^{14}$ colonicocytes over the lifetime of an individual. Stem and progenitor cell lineages in colon crypts are subjected to epigenetic changes (chromatin structure remodeling, DNA methylation, histone modifications, genomic imprinting), and to chromosomal and microsatellite instability leading to morphogenetic aberrations. The dogma stipulates that stem cells and their derived epithelial cell progenitors in the colon stem cell niche are the targets of the neoplastic transformation. This assumption is based on their continuous active mitosis associated with increased risk of DNA alterations and sequential accumulation of molecular defects inherent to the complex mechanisms of DNA replication and repair, and cell division. For this reason, permanent epithelial cell lines have been established from these immortal resident intestinal cell precursors are subjected to the establishment of permanent epithelial cell lines by vectorization of viral and cellular oncogenes or insertion of non-oncogenic DNA sequences [6—8]. However, the mediators and signals that regulate colon stem cell proliferation, migration and differentiation along the crypt base/apical axis are still poorly understood [9—11]. Stress conditions linked to digestive processes, infections, nutritional restriction, inflammation, luminal pathogens and carcinogens, wounding, adaptation to oxygen and hypoxia are likely to strongly influence these integrated mechanisms at the proliferation-differentiation interface of colon stem cells and progenitors.

Cancer stem cells (CSC) and biomarkers

Recent hypothesis and reports support the notion that transformed stem cells, also designated as cancer-initiating cells, cancer-propagating cells or cancer stem cells, exhibit lower rates of cell division in their niche that allow them to survive after chemotherapy and radiation therapy [12]. CSCs may constitute of rare cancer epithelial cells characterized by the ability to generate new tumors. Thus, these latent CSC might be potentially associated with primary tumor recurrence and metastasis, a new scenario for the Damocles’ sword. However, the relationship between cancer stem cell phenotypes and the emergence of aberrant crypt foci, increased prevalence of bifurcating crypts, development of colon adenoma and in situ carcinoma remains to be explored during carcinogenesis and inflammatory situations, such as ulcerative colitis and Crohn disease [13—15]. Moreover, the validity of their identification at the molecular and cellular levels, and classification as CSC based on functional criteria and expression of biomarkers such as, for instance, CD133 or Lgr5, is still a matter of debate in the literature [16—19]. Such conflict might be explained by the plasticity and versatility of normal epithelial progenitors and cancer cells in terms of gene expression and by the reversible expression of differentiation markers and traits during normal development and carcinogenesis in the digestive tract [20]. Indeed, multi-lineage differentiation capacity and epithelial-mesenchymal transitions (EMT) are features of human embryonic stem cells (ESC) and of normal adult colon stem cells as well [21—24]. EMT contributes to the production of fibroblasts in intestinal fibrosis associated with a model of Crohn colitis and human inflammatory bowel disease [25]. Most importantly, markers of mechanisms of EMT, CSC and circulating tumor cells are highly interconnected. Induction of EMT in normal, immortalized and cancer epithelial cells leads to the expression of stem cell biomarkers and therefore recapitulate overlapping mechanisms and phenotypes that occur both during normal development and cancer [26,27]. Consistently, immortalized adult human colon epithelial progenitor cells express overlapping mesenchymal and stem cell markers, as well as typical colonic epithelial biomarkers in Matrigel [8]. The EMT inducer and E-cadherin repressor ZEB1 favors tumor development by repressing stemness miRNA-200 family members which target pluripotency stem cell factors Sox2 and Klf4, and ZEB1, through a double-negative feedback loop [28,29]. This ZEB1/miRNA-200 feedback loop is considered as a molecular control of cellular plasticity in development, EMT and stemness of cancer cells. The cancer stem cell subpopulation in a given tumor is characterized by high capacity of initiating tumor growth in xenografts [30]. However, the diversification of cancer cell lineages which become positive or negative for stem cell markers during cancer progression and constant propagation in cell culture, may alter positively or negatively their differential potential in tumor take, cancer progression and differentiation [31]. For instance, forced expression of the Wnt pathway target CDX-1 [32] in the CDX-1 deficient colon cancer cell line HCT116 enables lumen formation, a marker of differentiation in vitro. Conversely, changing the levels of the homeobox gene CDX-1 has limited effects on the intestinal architecture, cell renewal, and cell type specification in transgenic mice [33]. Therefore, clonality and growth of these putative CSC in soft agar and xenografts is not representative of their biological context and microenvironment in clinical tumors.

Cancer cells displaying the cancer stem cell signature are described to arise from a very rare and relatively quiescent subpopulation that contains one putative cancer stem cell per 60,000 cancer cells [16,30]. CSC and the biomarkers used to enrich for CSCs are under ambivalent stages, as demonstrated, for the CD133 epitope subjected to cell cycle-dependent variations and to regulation by tumor associated signals, such as hypoxia and mTOR signaling [34,35]. Similarly, low oxygen tension prevents differentiation of progenitors in colon epithelial crypts and maintains human ESC in a fully proliferative and pluripotent state during early pregnancy [8,36]. In addition, chemoresistant and invasive colon cancer cells also strongly express putative stemness markers such as Lgr5, CD24, CD26, CD44 and...
CD133 [37–39]. Heterotypic cell-cell fusion also represents a rare phenomenon that supports the emergence of cancer cells with stem cell-like phenotypes (SCL cancer cells) during cancer initiation and diversification. These facts stress the implication of transdifferentiation mechanisms linked to cell lineage switches in the progression of epithelial stem cells and putative latent CSC into invasive cancer cells with acquired latent competence for tumor growth initiation, invasion and spread. Indeed, tumors with neuroendocrine differentiation capacities have been described in committed acid-secreting gastric epithelial progenitors, and transformed prostate epithelium [40,41]. Consistently, cancer cells isolated from human colonic metastases display intestinal stem cell properties, chromosomal instability and plasticity [42]. So the ultimate question is how much stemness is there in the cancer cell phenotype?

It is therefore proposed here that both stem cells and their immediate epithelial cell progenitors are subjected to neoplasia and production of colon epithelial cancer cells with differential and interconvertible SCL and EMT signatures. Consequently, interconversion and intermediacy boundaries between SCL cancer cells and their transformed progenitor counterparts is plausible. The cancer stem cell concept is therefore at the edge between of a transient and reversible state and identity of self-renewing pluripotent progenitors that are produced during tissue regeneration, remodelling and carcinogenesis. In addition, the exact number, localization, and signature of the stem cells in small intestine and colon is currently a matter of controversy [43].

A better understanding of the initial steps and mechanisms driving the neoplasia and transformation of the colon epithelia is a critical prerequisite to define specific targets and effective preventive and therapeutic strategies in experimental and clinical oncology. In clinical oncology, this issue is not a matter of terminology as it concerns the identity, state, and clinical relevance of aggressive cancer cells with SCL properties and characterized by their invasive and metastatic potential, resistance to radiation and to cytotoxic chemotherapy. Pilot studies and therapeutic interventions based on the manipulation and selective elimination of CD133-positive CSC by standard chemotherapy combined with blockade of Sonic Hedgehog and mTOR signaling [45] must be carefully interpreted for extrapolation to clinical trials. Current and future strategies of prevention, diagnosis and treatment of primary and metastatic lesions during cancer progression and recurrence are therefore dependent on the better elucidation of the so far, questionable cancer stem cell hypothesis.

Stem cell pathways and regulators

It is now well accepted that molecular alterations of the Wnt pathways are involved in the initiation of sporadic colon cancers [46–48]. As shown in Fig. 1, colon stem cells in their niche are positive for several biomarkers and targets of the Wnt signaling pathways namely Lgr5, the lineage regulator ASCL2, and the antiapoptotic mediator survivin, as well as for CD133, CD44, CXCR4, the E-cadherin gene repres-
sor SLUG (which is involved in EMT, cancer cell invasion and anticancer drug resistance), and the detoxifying enzyme Aldehyde Dehydrogenase 1 (ADH1) [49–54]. The basic helix-loop-helix transcription factor Achaete Scute-like 2 (ASCL2) is crucial for the maintenance of Lgr5 positive stem cells in the intestine [55]. In contrast, the stem cell and cancer stem cell marker CD133 seems devoid of functional significance in tumor initiation and progression [56]. The majority of these colon stem cell and cancer cell biomarkers and players are expressed also in adult stem cells and putative cancer stem cell in breast, liver, pancreas and prostate cancers [39,57–60]. In addition, multiple local stemness factors act positively or negatively on the stem cell niche survival and self-renewal as well as on the maintenance and divergence of normal epithelial precursor cells towards cellular pluripotency, tumor growth, recurrence, metastasis and drug resistance (Fig. 1). These autocrine, paracrine and intracrine regulators include i) the pathways using Wnt, Keratinocyte Growth Factor (KGF), Interleukin-4 (IL-4), Aurora-A kinase, Notch, Hedgehog, Transforming Growth Factor beta (TGFβ) family members including Bone Morphogenic Protein (BMP) and Nodal; ii) the phosphatase and tensin homologue PTEN and controlled pathways such as the signaling axis of the EGF-IGF family of growth factors detected in both gastric epithelial lineage progenitors and colon progenitors; iii) neuroendocrine factors, Integrins, Collagen type I; iv) CXCR4 and related Chemokine receptors and agonists, hypoxia, Fibroblast Growth Factors (FGFs) as well as Ephrin-B1 and its receptor tyrosine kinases Eph-B2 and -B3; v) miRNAs that target critical oncogenic and chemoresistance pathways such as EZF, a transcription factor and cell cycle regulator controlled by the miRNA cluster 17-92 [10,11,36,54,60–72]. Increasing evidence indicates that miRNAs contribute to the self-renewal and pluripotency of adult and ESC, and to the reprogramming of somatic cells into induced pluripotent stem cells [28,73–76]. Consistently, most of these effectors, which are implicated in stem cell regulation, are also critical for tissue regeneration and repair [67,77].

Stem cell visitors in colon mucosa and tumors

Stem cell visitors surrounding human colon crypts in the stem cell niche and infiltrating growing tumors include local mesenchymal stromal cells and distant foreign visitors. The stem cell niche, which is localized at the base of the crypt, is infiltrated by local myofibroblasts (Fig. 1). Pericytroph myofibroblasts and also tumor-associated (myo)fibroblasts are thought to produce several factors that regulate basal stem cells in a paracrine manner, including activators or inhibitors of the Wnt, Notch, BMP, TGFβ, and Hepatocyte Growth Factor (HGF)/Met pathways [78–80]. Thus, stem cells and cancer cells in growing tumors are submitted to direct and indirect interactions with a complex stromal population of invasive tumor-resident and tumor-derived mesenchymal stromal cells. Subepithelial myofibroblasts subjacent to the basement membrane and muscularis mucosa are acting as effectors of the stromal reaction.

During tumor-induced local angiogenesis, growing tumors are progressively invaded by immune cells, endothelial cells and circulating blood cells, including macrophages and neu-
Stem cells and colon cancer: The questionable cancer stem cell hypothesis

Mesenchymal stem cells (MSC) are multipotent progenitors of mesoderm, endoderm (hepatocytes), and ectoderm (neural precursors). Derivatives of MSCs provide the rationale and expectations to manipulate these pluripotent lineages for ex vivo expansion and preserving the epithelial barrier in colitis and during cancer treatment [86, 94, 95]. Engineered mesenchymal stem cells administered via topical and systemic routes are now considered as suitable vectors to deliver healing or killing drugs and cytokines, prodrugs and cytotoxic compounds, neutralizing miniantibodies, bispecific diabodies, growth and survival factors, and to initiate immune, promorphogenic or cell death responses [88, 96–99].

However, efficacy and safety limitations of ectopic mesenchymal stem cells and ESC derivatives in clinical therapy are linked to the risk of genomic instability, spontaneous chromosome changes, and malignant transformation after their propagation and amplification in cell culture and following systemic and topical administration and long-term persistence in blood and target organs [2, 100–103]. As expected, this instability seems to be associated with relatively rare mutations but the risk must be taken into account. These ethical and biosafety limitations are reminiscent of the natural history of sporadic cancers during aging. Thus, in case of stem cell and mesenchymal stem cell therapies, the individual genomic instability and genetic alterations of mesenchymal stem cell populations in each patient should be reconsidered. Indeed, it was speculated that the probable primordial cells for the emergence of gastrointestinal stromal tumors (GIST) might be the primitive mesenchymal cells [104]. Conversely, it is possible that some GIST may arise from irreversible EMT in epithelial cancer cells. Moreover, uncontrolled growth and differentiation phenotypes of transduced mesenchymal stem cells might cause deleterious damages related to the multipotency and plasticity of these cell lineages in vitro and in vivo [105]. Other reports support the possibility that transplanted mesenchymal stem cells might contribute to carcinogenesis, metastasis and elevation of the tumor mass in colon and prostate cancer [2, 106, 107]. It is therefore likely that these ectopic mesenchymal stem cells provide additional trophic and survival factors to resident cancer cells in primary tumors and metastases. Thus, administration of mesenchymal stem cells may favor the reintiation and development of primary tumor sites and dormant metastases. It is therefore anticipated that engineered mesenchymal stem cells expressing a security suicide gene will help to control the fate and destruction of these therapeutic biovectors following recovery and healing of injured colonic tissues.

On the other hand, therapeutics that target chemoresistant stem cells and SCL cancer cells are based on the expression of selective stem cells markers and specific targeting of symmetric stem cell division to decrease the exponential clonal growth of colon tumors [37, 53, 108]. Hence, protection of the resident normal stem cells that often express the same markers in the digestive tract and in other tissues, is a critical requirement for the reliability and safety of this approach. Strategies directed on signaling pathways inherent to SCL cancer cells are limited by the presence of similar networks in normal stem cell populations in several tissues. Safe therapy to target SCL cancer cells and progenitors should avoid collateral damages in normal stem cell populations [104].

Conclusive remarks and expectations

The plasticity and permanent adaptation of cancer cells to intrinsic defects are related to oncogenic, epigenetic and post-genetic events, clonal evolution, diversification and aging. These mechanisms support the versatile genotype and phenotype of the transformed stem cell progenitors in...
primary tumors during cancer progression and treatment. The extrinsic pressure of the tumor stroma microenvironment and the immune system are also playing critical roles in tumor initiation and the emergence of metastatic cancer cells. Invasive cancer cells escape from growing tumors initiating hypoxia, cell death, quiescence and senescence signals. Necrosis, oxidative and metabolic stress and autophagy malfunction are also described in colon cancer [109]. Applying the stem cell and induced pluripotent stem cell technology and transplantation and cancer vaccine in the field of inflammation and cancer treatment in the digestive tract will bring major advances for innovative developments and clinical trials [110—112]. Despite the intense research and expectations linked to the development of embryonic and adult stem cells for their promising therapeutic applications in regenerative medicine, future investigations should consider increasing examples of their limitation in experimental and clinical oncology. Regarding the genetic and molecular diversity of the cancer pathways that initiate sporadic and hereditary colon cancers [46,113—117], further identification and characterization of the SCL cancer cells during colon cancer progression will help to define more effective strategies to target selectively or simultaneously SCL and non SCL cancer cells in solid neoplasms. It is plausible that these cancer cell populations are interdependent for their mutual growth and survival in early-stage tumors. They might engage reciprocal and dynamic signalling crosstalks via transcrip tronic and reversible quiescence factors and chemokines involved in tumor maintenance, dormance, evolution, and drug resistance. These SCL and non SCL cancer cells may represent the ultimate signature of multiple and reversible states of intermediary cell populations balancing between self-renewal, reciprocal conversion, EMT transitions, differentiation and «identity». With the tumor stroma and mesenchymal stem cell populations, they can contribute differentially to tumor growth, cheemosresistance and metastasis. Therefore, we cannot exclude the possibility that selective ablation of stem cell-like cancer cells contribute to the paradoxal emergence of aggressive tumors arising from non SLC cancer cells. Focusing targeted therapy on the minority subpopulations of SCL cancer cells may compromise the management of cancer patients. Identification, reprogramming, and elimination of resident stem cell progenitors engaged in neoplasia is therefore the next critical challenge to progress in this field, beside the possible benefits of a safety stem cell therapy for colon cancer patients.

Conflicts of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was supported by INSERM, IPSEN, and ARC no.3765. In the memory of Pr. Martin Rodbell. There is no conflict of interest with this review. Due to space limitations, several relevant papers in the stem cell field were not quoted.


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