Crucial Role of Interleukin-4 in the Survival of Colon Cancer Stem Cells

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Abstract

Colon tumors may be maintained by a rare fraction of cancer stem-like cells (CSC) that express the cell surface marker CD133. Self-renewing CSCs exhibit relatively greater resistance to clinical cytotoxic therapies and recent work suggests that this resistance may be mediated in part by an autocrine response to the immune cytokine interleukin 4 (IL-4). Blocking IL-4 signaling can sensitize CSCs to apoptotic stimuli and increase the *in vivo* efficacy of cytotoxic therapy. These findings suggest that inhibitors of IL-4 signaling may offer a new therapeutic tool in colon carcinoma. [Cancer Res 2008; 68(11):4022–5]

For many years, colorectal tumors have been described as the proliferation of somatic cells in which multiple genetic alterations had occurred over time (1, 2). As a consequence, every cell within the neoplasm would have the capacity to proliferate extensively and thus have the potential to sustain tumor growth. Different from this notion, emerging data suggests that the capability of initiating and driving tumors, including colon carcinoma, is an exclusive feature of abnormal cells called "cancer stem cells" (CSC) or "tumor-initiating cells", which represent only a small population within the tumor (3, 4).

Although solid evidence is lacking to date, CSCs are thought to derive from self-renewing normal cells that acquire epigenetic and genetic changes required for tumorigenicity, or from proliferative progenitors that reprogram themselves acquiring self-renewal capacity (5). Consequently, CSCs will retain the hallmarks of normal stem cells in being capable of self-renewing, and differentiate into a phenotypically heterogeneous, although aberrant, progeny.

The adult intestinal epithelium has a well-defined structure ordered into crypts and villi, with a hierarchical organization that consists of cells displaying stemness features; rapidly dividing cells, also called "transit-amplifying cells", with little or no stem cells attribute; and differentiated cells, which constitute all the intestinal lineages (6). The intestinal epithelium possesses a high turnover rate and thus epithelial cells with a brief life span. It is therefore more logical to assume that long-lived stem cells or transient-amplifying cells, which undergo a large number of cell divisions, are the source of cells with mutations and epigenetic changes. According to the CSC hypothesis, such mutations would be passed on to the progeny, allowing for progression towards cancer over time and ultimately resulting in a pool of cancer stem cells that feed neoplastic formation (5).

Colon Cancer and Stem Cells

The CSC hypothesis has exciting clinical implications in colorectal cancer. Indeed, it has been speculated that the recurrence of tumors after surgical and/or chemotherapy interventions are due to the resistance of cancer initiating cells to death stimuli. Here, we highlight the current understanding of colon CSCs, and the more recent findings about extrinsic microenvironmental factors that are likely to determine tumor escape from current therapies.

In the context of colorectal carcinoma, the first direct evidence in support of the CSC hypothesis came with the recent finding of self-renewal and tumor-initiating cells with a common and distinct surface-expressed polypeptide, the CD133 transmembrane glycoprotein. Convincing experiments recently conducted by two independent groups showed that only the $CD133^+$ small fraction of cells within a colon carcinoma is capable of initiating tumor outgrowth (7, 8).

In line with these findings, we recently showed that a high number of CD133-depleted cells (2×10^6 $CD133^-$ cells) from colon cancer specimens fails to generate heterotopic tumors in mouse models, whereas as few as 2.5×10^3 $CD133^+$ cells are sufficient to engraft and serially reproduce the original human tumor phenotype (9). Additional supporting data have been obtained *in vitro* under differentiation conditions on Matrigel. In this three-dimensional culture system, only tumorigenic $CD133^+$ cells are able to generate colonies organized in crypt-like structures. Notably, either during *in vitro* or *in vivo* differentiation, these cells display a gradual acquisition of colon epithelial markers, such as CK-20, with the concomitant reduction of CD133 stem cell marker expression. Using conditions previously applied for culturing neurospheres, the $CD133^+$ cells undergo long-term expansion without loss of their ability to reproduce the human original tumor phenotype, thus providing further functional evidence for self-renewal and tumor-initiating capacity.

From these latter studies, a consequent question is whether all the $CD133^+$ cells constitute the CSCs, or whether $CD133^+$ is a feature of a fraction of cells that include stem cells and more differentiated progenitor cells. Following the principle of serial dilution, O'Brien and colleagues observed that not all the $CD133^+$ cells in a tumor contain the colon cancer–initiating potential. Accordingly, recent experiments describe a minority subset of stem-like $CD44^+/EpCAM^{high}/CD166^+$ cells contained in the $CD133^+$

Note: M.G. Francipane and M. Perez Alea contributed equally to this work.

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tumor cell population, and propose the cell surface glycoprotein CD44 and the mesenchymal stem cell marker CD166 as stronger markers of colon CSCs (10).

Also of interest are the studies that focus on the biology of intestinal adult stem cells, within which cancer may initiate. In normal stem cells, the balance between stemness and differentiation is tightly controlled by several signaling pathways which, if deregulated, could support carcinogenesis. Among these, Notch, Hedgehog, and Wnt transduction signals are likely to be critically associated with the tumorigenesis of a variety of tumors, including colon cancer (11). Specifically, the mammalian RNA-binding protein Musashi-1 (Msi-1) and the macromolecule Lgr5 (GPR49), have been highlighted as potential stem cell markers in the intestinal epithelium. Our more current data show that Msi-1 is abundantly expressed in colon CSCs, which suggests that it could play an active role in driving tumorigenesis as well (12). Similarly, Lgr5 protein expression has been shown to be highly restricted to the cycling columnar cells at the crypt base, and because these cells have the ability to generate all the epithelial lineages, they represent, with high probability, the adult stem cells (6).

Combining these studies, it is highly unlikely that a single protein marker can provide unequivocal identification of colon CSCs. Consequently, ongoing efforts should be made to determine the combinations of stemness markers that could clearly define the tumor-initiating cell. This would facilitate early disease detection and prognosis and would allow for the development of more effective therapeutics targeting CSCs.

Interleukin 4–Mediated Death Resistance and Implications for Novel Therapies

Although the molecular identification of colon CSCs specialized in maintaining tumor mass is essential, it is also important to discern their response to therapy and their role in disease recurrence and metastasis. In a recent report, we showed that the subpopulation of $CD133^{+}$ colon CSCs is more resistant to conventional chemotherapeutic drugs and to putative innovative therapies such as tumor necrosis factor–related apoptosis inducing ligand compared with the majority of epithelial cells within the tumor (9). This selective resistance was also observed in CSCs

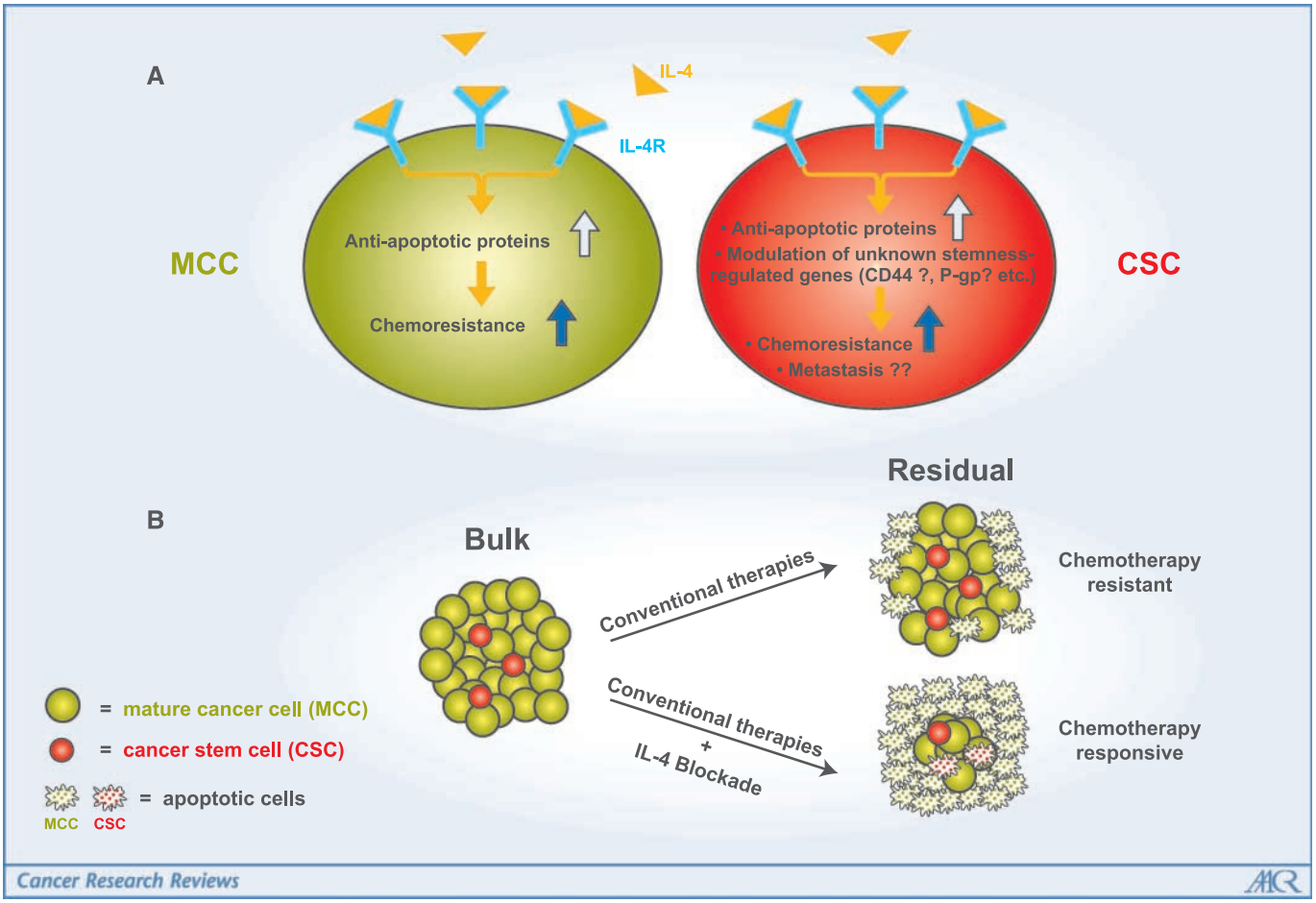

Figure 1. Model of IL-4–mediated death resistance in colon mature cancer cells (*MCC*) and cancer stem cells (*CSC*). The autocrine and paracrine effects of IL-4 render both MCCs and CSCs refractory to anticancer drugs in part due to an increase of antiapoptotic proteins. The differential sensitivity of MCCs versus CSCs could be attributed to the IL-4 target molecules that probably differ in the two cellular groups. In the population of CSCs, apart from establishing prosurvival signals, IL-4 could lead to the expression of ATP-dependent drug efflux pumps, considered major components of multidrug resistance, such as P-glycoprotein, and of proteins involved in the metastatic process such as CD44 (*A*). Consequently, CSCs would remain viable after therapy, and consequently, be responsible for the minimum residual disease. Conversely, when conventional therapies are combined with IL-4 blockers, both MCCs and CSCs are effectively killed, rendering the tumor unable to maintain itself, and thus, to grow back (*B*).

in vivo. These studies highlight the importance of refractoriness of CSCs in colorectal tumors, and provide further evidence in support of the idea that tumor-initiating cells are highly resistant to cytotoxic cancer therapies.

Accumulating evidence shows that CSCs, in general, contribute to chemoresistance through the enhanced activity of multidrug transporters, expression of high levels of antiapoptotic proteins, and other molecular mechanisms that alter the normal balance between proliferation and cell death (13). In addition to these intrinsic factors, microenvironmental soluble molecules, including growth factors and cytokines, can significantly contribute to the refractoriness of CSCs. Chemokines produced by inflammatory cells have powerful effects on tumor development, regulating the growth, migration, and differentiation of all cell types of the tumor microenvironment. Perhaps the best indication for the significance of inflammatory cytokines during neoplastic progression comes from the strong association of chronic inflammation and colon cancer. Interleukin 4 (IL-4) and IL-6–producing Th_2 lymphocytes are significantly elevated in patients with colorectal cancer, as compared with healthy individuals. Conversely, the low incidence of colon tumor recurrence is positively correlated with the presence of markers for Th_1 polarization (14).

Over the past few years, another source of cytokines has emerged as critically important, the epithelial tumor cell itself. Several experimental data from *in vitro* and *in vivo* experiments have shown that autocrine production of IL-4 for example, by cancer cells from breast, thyroid, colon, and lung is an important negative regulator of apoptosis, conferring resistance to death receptors and chemotherapy-induced cell death (15, 16). Furthermore, autocrine IL-4 in pancreatic tumors may suppress cancer-directed immunosurveillance, facilitating tumor growth and metastasis (17). Work by our group has recently provided evidence that death-resistant colon CSCs also release IL-4, which installs a death-resistant phenotype. Blocking IL-4 with either a neutralizing antibody or a mutated, inhibitory form of IL-4 (IL-4DM), strongly sensitizes not only mature cancer cells (MCC), but also CSCs to chemotherapeutic drugs. The increased chemosensitivity is associated with the reduction of prosurvival molecules, which are often up-regulated in colon carcinoma. These results were validated in immunodeficient mice in which the efficacy of chemotherapy was significantly enhanced and more sustained over time when conventional drugs were combined with IL-4DM or IL-4 neutralizing antibody. Interestingly, cells that underwent apoptosis *in vivo* as a result of IL-4 blockade were not limited to MCCs, which is the case in the absence of IL-4 blockade, but the $CD133^+$ CSCs were also included (9).

Our results sustain the role of IL-4 as an autocrine survival signal for both MCCs and CSCs. However, only a differential function of IL-4 could explain the different sensitivities displayed by CSCs versus MCCs. Even if not yet proven, IL-4 could interact with signaling pathways that are selectively activated in CSCs, and thus, could be associated with stemness features which probably result in a higher resistance to death stimuli. Consequently, CSC-derived IL-4 might result in the up-regulation of factors able to increase their aggressiveness, such as P-glycoprotein and putative stem marker CD44. Indeed, IL-4 and IL-13 have already been shown to be potent inducers of the CD44 exon variants v3 and v6, which are apparently associated with aggressive tumors in colonic epithelium (18). Similarly, P-glycoprotein, a membrane efflux pump localized in epithelial cells of the small and large intestine, is likely involved in the metastatic dissemination of colon cancer cells (19). IL-4 could therefore represent an interesting link between cell survival and stemness (Fig. 1).

As mentioned above, the predominant presence of IL-4 cytokine in the colon tumor microenvironment could additionally install an immunosuppressive state that facilitates cancer growth by avoiding immune recognition (14, 17). Of note, anti–IL-4 therapeutics have already been tested in clinical trials of respiratory diseases, and no adverse effects were observed (20, 21). Extension towards treatment of colon carcinoma may therefore follow quickly.

On the basis of the data presented here, the development of therapeutic strategies that target colon CSCs and/or their microenvironmental niche would facilitate apoptotic death signals over proliferative effects. Following this rationale, the IL-4/IL-4R axis could be regarded as a powerful target for colon anticancer therapeutics. Nonetheless, additional investigations are needed in order to better understand IL-4–activated signaling pathways in the CSC population of colon and other tumors, which will facilitate clinical protocols to overcome common epithelial cancer types that express IL-4.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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