We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

8

What do We Know About Cancer Stem Cells? Utilizing Colon Cancer as an Example

Benhaim Leonor, Labonte Melissa and Lenz Heinz-Joseph
University of southern California, Norris Comprehensive Cancer Center, Department of medical oncology. Los Angeles, California, USA

1. Introduction

Cancer is a heterogeneous disease that begins locally before spreading and developing extended metastasis. The initial tumor initiation and development is the results of a cells dysfunction which occurs when accumulated genetic abnormalities transform a normal cell into a tumoral cell with the ability of self-renewal and proliferation. While the exact chronology of this evolution remains unknown, two major models of tumor cell initiation have been proposed (figure 1) (Wang and Dick, 2005):

In the stochastic model, each cell contains a low but similar probability to acquire the necessary genetic mutations resulting in the capacity of proliferation and survival. During the life of the cell, accidentals genetics modifications or mutations may occur and result in the acquisition of the self-renewal potential and thus the ability to sustain neoplasic growth. Then, throughout clonal evolution, cells are subjected to further genetic variations resulting in a heterogeneous tumor. In this model, the relative genetic instability of tumoral cells accounts for the cellular heterogeneity.

In the Stem cell model, each cell has a different probability of acquiring a specific tumoral phenotype. As a result, beyond a simple monoclonal expansion of transformed cells, tumors are more likely considered as a complex tissue where tumor initiation and growth is driven by a minority of tumor cells. This population of initiating cells or cancer stem cells (CSC) is functionally distinct and exhibit specific activated pathways compared to the bulk cells. CSC's display specific properties such as the capability of self-renewal, asymmetric cell division, and capacity to differentiate. The progeny cells - which composed the bulk part of the tumor - undergo specific genetic or epigenetic changes resulting in their limited capacity to divide and survive. In this model, these genetic changes are not rules by accidental genetic events but more likely driven by specific regulations pathways. While the origin of these CSC remains unknown, it is hypothesized that they derive from normal tissue stem cells, or from partially differentiated progenitor cells acquiring unlimited self-renewal potential. Stem cells seem to be the ideal candidates to support this model because of their long life span, which allows for the accumulation of multiple mutations events contrary to the differentiated one.

In hematological malignancies, tumor-initiating cells have been identify and strongly supports the stem cells model. In solid tumors, emergent observations argue for this theory but still require further investigation and validation.

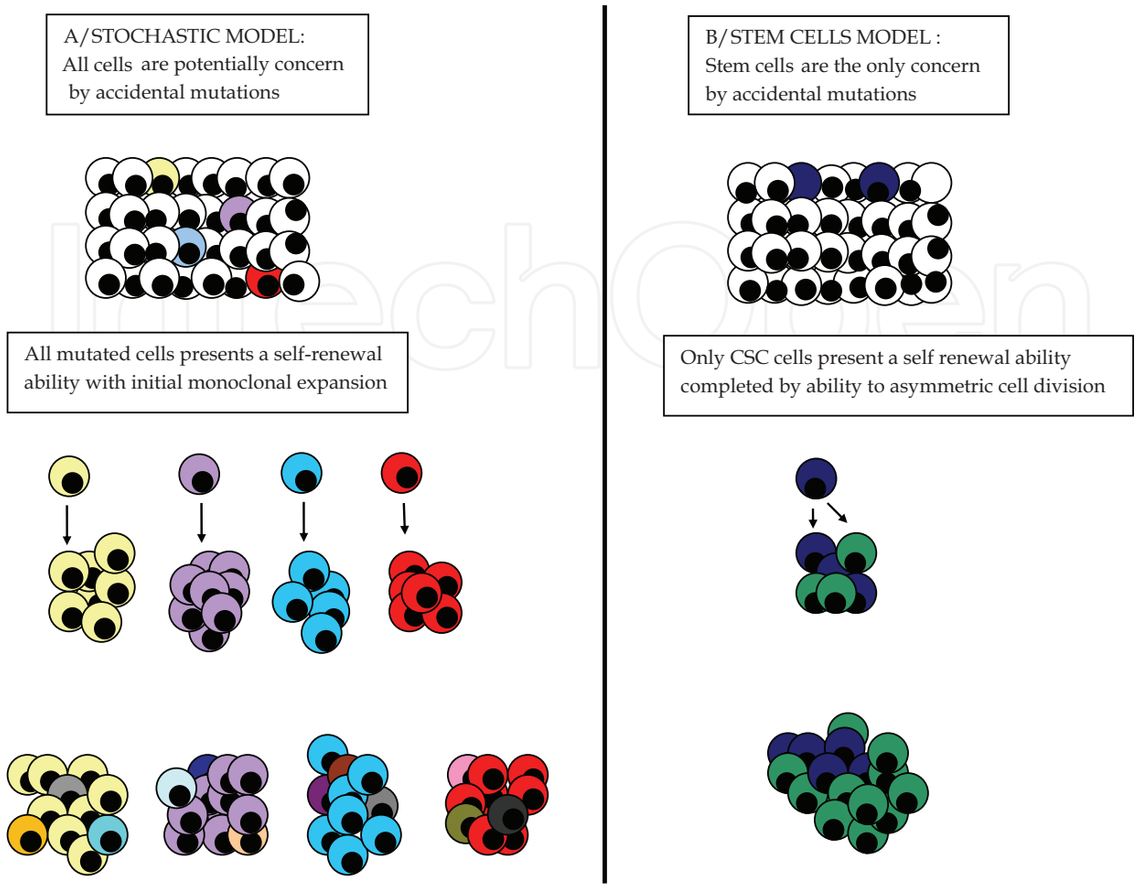

Fig. 1. Two models of tumor initiation

Using colon cancer as an example, this chapter will provide an overview of the complexity of CSC identification and characteristics. Based on the description of different implicated pathways, we'll try to tackle possible clinical applications.

2. Cancer stem cell: identification

As suggested above, CSC's can be differentiated from the bulk part of the tumor either by their specific surface markers or by the specific pathways involved. Considering the example of hematologic malignancy and the supposed origin of the CSC, the first markers used to identify this specific cells subset were common with the normal stem cells of the tissue of origin. The CD 133 antigen is for example commonly expressed both by malignant stem cells and the stem cells of the tissue of origin. On the contrary the CD20 antigen, highly express in colon tissue cells, is not express by the CSC.

2.1 Colon cancer stem cells: CD133

The CD133 antigen (Human prominin 1, PROML1) is a 5-transmembrane glycoprotein of 865 amino acids (120kDa) that is localized to membrane protrusions or microvilli in the colon. This antigen has been used as a marker to enrich for human hematopoietic stem cells and its expression has been correlated with CSC in solid tumors including prostatic cancer

(Richardson et al., 2004), kidney cancer (Florek et al., 2005), non-small cell lung carcinoma (Zhang et al., 2007), and ovarian cancer (Ferrandina et al., 2008).

Using 17 samples of human colonic cancer (6 primary, 10 liver metastases, 1 lung metastases), O'Brien *et al.* conducted serial xenograft implantations through the sub-renal capsule of diabetic (NOD)/severe-combined immunodeficient (SCID) mice (O'Brien et al., 2007). Only the CD133+ cells implanted for xenografts generated tumors. By immunohistochemistry (IHC) the CD133 expression ranged from 1.8 to 24% in the colon cancer samples and from 0.4 to 2.1% in the normal colon cells. The frequency of CSC in the CD133+ cells fraction was estimated to be 1 in 262 cells.

At the same time, Luccia Ricci-Vitani *et al.* reported their observation of both *in vitro* and *in vivo* culture of CD133+ cells sorted by flow cytometry shortly after tissue dissociation of colon cancer samples (Ricci-Vitiani et al., 2007). Again, only the CD133+ cells generated tumors in their xenograft models. The samples analyzed exhibited a very low frequency of CD133+ cells, with only 2.5 ± 1.4 % among a large excess of CD133- cells. In this study, the presence of CD133+ cells was barely detectable from normal colon tissues (Ricci-Vitiani et al., 2007). Several other reports confirmed the implication of CD133+ cells in tumor initiation (Vermeulen et al., 2008, Dalerba et al., 2007). Furthermore, the level of CD133 expression has been shown to be correlated between the primary tumor and corresponding metastasis in 94% of cases (15 patients out of 16) (Horst et al., 2009).

Thus, the definition and identification of colon CSC remains incomplete. As suggested by O'Brien *et al*, among these CD133+ cells only a few selected cells are expected to be "real CSC"(O'Brien et al., 2007). The heterogeneous cell population in colon cancer is partly highlighted by its multiplicity of the genetics combinations disorders found. Hence, it is likely that among CSC, several phenotypic profiles may exist, sharing some common markers and signaling pathways.

Several studies have investigated other potential CSC markers. It is important to note, that all the presented studies focused on colon CSC identification, actually isolated a "CSC-containing" subpopulation with different degree of sensitivity and specificity as there is probably no ideal single marker for CSCs in any tumor system.

2.2 Colon cancer stem cells: EpCAM, CD44 and CD166

The CD44 antigen is a cell surface glycoprotein expressed on lymphocyte, monocyte and granulocyte cells which has also been correlated to undifferentiated cells. Dalerba *et al.* explored therefore that alternative profile to identify CSC (Dalerba et al., 2007). They examined the expression profile of two markers, CD44 and EpCAM previously described as a key to identify CSC in breast cancer (Ponti et al., 2006).

EpCAM is a glycosylated 40 kDa type I transmembrane glycoprotein and functions as an intercellular adhesion molecule modulating cadherin-mediated adhesions and thereby adhesion strength. The physiologic expression of EpCAM in an adult's human tissue is restricted to the basolateral cell membrane of glandular, pseudo-stratified and transitional epithelia cells. Although the biological role of EpCAM is not fully understood, its overexpression has been observed in several cancers' types, including colon cancer (Stoecklein et al., 2006, Todaro et al., 2007, Munz et al., 2009, Gires et al., 2009). The study discriminated between two main expressions' profiles, $EpCAM^{high}$/CD44+ and $EpCAM^{low}$/CD44-, and measured their detectable in both colon cancer cells and normal epithelial colon cells. The frequency of the $EpCAM^{high}$/CD44+cells profile was higher in

some cancer cells population than in normal epithelial colon cells (mean frequency of 1.6% vs. 5.4%, respectively). The study went on to test the ability of these cell populations to form xenografts. While 10^4 EpCAM^{low}/CD44- cells graft failed to form a tumor *in vivo*, as few as 200 to 500 EpCAM^{high}/CD44+ cells consistently generated tumors.

To specify the characterization of this EpCAM^{high}/CD44+ cell population, another set of surface markers was tested and included CD133, CD49f, ALDH and CD166 (Dalerba et al., 2007). Aldehyde dehydrogenase (ALDH) is an enzyme involved in intracellular retinoic acid production and has been linked to cellular differentiation during development, playing a role in stem cell self-protection (Croker et al., 2009). ALDH enzymatic activity was measured in the EpCAM^{high}/CD44+ and EpCAM^{low}/CD44- cells and found to be higher in the majority of the EpCAM^{high}/CD44+ cells. CD49f, also known as integrin alpha 6, functions in cell adhesion and cell-surface mediated signaling. CD49f expression was measured on the tumor cells and correlated with CD44 expression. The study found CD49f expression on tumor cells with higher levels of CD44.

In these experiments using EpCAM^{high}/CD44+ as CSC markers, the expression of CD133 in the selected cells appears heterogeneous. Some tumors displayed a homogeneous negative or positive expression, while others were composed of a mixture of positive and negative CD133 cells. When CD133 was expressed, the CD133+ population includes the CD44+ cells. The CD44+ antigen may be therefore more specific to identify the CSC than the CD133+, while the results do not always correlate between studies.

In Dalerba *et al.* experiments, the CD166 (cluster of differentiation 166), was found to be differentially expressed on colon cancer cells but all colon tumors contained a distinct fraction of EpCAM^{high}/CD44+/CD166+ cells. The study went on to compare the tumorigenic potential of the fraction of CD44+/CD166+ and CD44+/CD166- cells and found that in serial xenografts, only the CD44+/CD166+ cell population was tumorigenic.

Haraguchi *et al.* demonstrated that utilizing both CD133 and CD44 may enhance the selection of tumor initiation cells for colon cancer and by treated colon cancer cells with the differentiation's inducer, sodium butyrate (NaBT), there was a decrease in the expression of CD133 and CD44 (Haraguchi et al., 2008). In this study, the expression of CD44 and CD133 in clinical samples varied from 11.5% to 58.4% (mean 30%) and from 0.3 % to 82% (mean 35.5%) respectively, while the frequency of the CD133+/CD44+ cells population ranged from 0.2 to 15.73% (mean 7%). As expected, when injected in NOD/SCID mice, the CD44+ or CD133+ populations induced tumor formation, whereas the CD44- and CD133- did not. Interestingly, only the CD133+/CD44+ subset population was able to initiate tumors whereas CD133+/CD44- and CD133-/CD44- could not. The subset of CD133-/CD44+ population was too small to evaluate in a xenograft study.

Based on these observations, Du *et al.* proposed to discriminate the respective functional importance of CD44 and CD133 (Du et al., 2008). In contrast with previous reports, this study found that CD44+ cells displayed cluster growth and did not co-localize with CD133+ cells within colorectal cancer (CRC). As few as 100 CD44+ cells were able to initiate tumor formation in a xenograft model utilizing NUDE mice. Knockdown of CD44+ prevented clonal formation and inhibited tumorigenicity in a xenograft model, whereas knockdown of CD133+ did not.

2.3 Colon cancer stem cells: CD24 and other markers

Within spheroid cultures of primary cancer cells, Vermeulen *et al.* identified several heterogeneous subpopulations expressing stem cells markers such as CD133+, CD24+,

CD44+, CD166+, Lgr5 (Vermeulen et al., 2008). Using single-cell sorting of spheroid cells by flow cytometry, they established that only 1 out of 20 cells has the ability to induce monoclonal culture. Among these cells, those presenting a co-expression of CD133 and CD24 were identified as the clonogenic subset, whereas the co-expression of CD133 with CD44, CD166, Lgr5 or CD29 did not increase the selection.
Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) was initially identified as an orphan receptor and a Wnt target gene in colon cancer. On intestinal tissues, Lgr5 expression has been identified to be restricted to the bottom of the crypt and not the villi (Takahashi et al.). However, in premalignant lesions, Lgr5+ cells were not restricted to the crypt base, but were found also at the surface of the crypt (Becker et al., 2008). While its expression has not been precisely related to CSC, it has been recently shown to be overexpressed in ovary, liver cancers and colon cancer (Takahashi et al.).

Marker	Colon cancer	Other solid cancers	Function
CD133	(O'Brien et al., 2007), (Ricci-Vitiani et al., 2007), (Vermeulen et al., 2008, Dalerba et al., 2007), (Haraguchi et al., 2008), (Zhu et al., 2009)	Prostate (Richardson et al., 2004), Kidney (Florek et al., 2005) , Non-small lung (Zhang et al., 2007), Ovarien (Ferrandina et al., 2008), Hepatocarcinoma (Tomuleasa et al.)	transmembrane glycoprotein, self-renewal
CD44	(Dalerba et al., 2007), (Haraguchi et al., 2008), (Du et al., 2008)	Breast (Ponti et al., 2006), Head and neck (Joshua et al.), Glioblastoma (Anido et al.), Non-small cell lung (Leung et al.), Gallbladder (Shi et al.)	cell surface glycoprotein, cell adhesion, hyaluronic acid receptor
EpCAM	(Ponti et al., 2006)	Breast (Ponti et al., 2006), Esophagus (Stoecklein et al., 2006)	transmembrane glycoprotein, intercellular adhesion molecule
CD24	(Vermeulen et al., 2008)	Pancreatic (Rasheed et al.), Breast (Huang et al.), Gastric (Takaishi et al., 2009)	cell surface glycoprotein Cell adhesion
CD166	(Ponti et al., 2006)	Prostate (Rowehl et al., 2008)	cell surface glycoprotein Cell adhesion
ALDH	(Huang et al., 2009)	Lung (Sullivan et al.), Sarcoma (Awad et al.)	Enzyme, cellular differentiation
Lgr5	(Takeda et al., Takahashi et al.)	Colon, Rectum,	G protein-coupled receptor, Wnt targeted gene

Abbreviations: EpCAM: Epithelial cell adhesion molecule; ALDH: Aldehyde dehydrogenase; Lgr5: Leucine-rich repeat-containing G-protein coupled receptor 5

Table 1. CSC markers explored according to the cancer type

Using the same process, cells expressing ALDH1 were demonstrated to be highly tumorigenic compared to the ALDH1- ones. Among them further isolation of cancer cells using a second marker like CD133 or CD44 modestly increased the enrichment in tumor-initiating cells (Huang et al., 2009).

3. Cancer stem cell, definition

The CSC are defined by their ability of self-renewal, asymmetric cell division and differentiation. In colon cancer, the evidence of existing CSC was first reported in 2007 by two groups independently (Ricci-Vitiani et al., 2007, O'Brien et al., 2007). Using a xenograft model of colon cancer cells into nude mice, these studies demonstrated that only a small subset of tumor cells was able to generate a tumor. Based on previously known stem cells markers, they demonstrated that only the tumor cell fraction harboring the CD133 or CD44 marker was tumorigenic.

3.1 Tumor initiation (in vivo)

All tumors are comprised of a heterogeneous population of cancer cells. In xenograft experiments, it has been demonstrated that only a minority of the cells from heterogeneous cell population retain the ability to generate a tumor.

Since the early 1900's, xenografts of colon cancer cells into irradiated hamsters were successfully performed (Murphy, 1914). The implantation of human colonic cancer cells into diabetic NOD/ SCID mice to induce tumor formation was later routinely used to investigate cancer metabolism. Yet, limiting dilutions experiments demonstrated that this tumor initiation was dependent on the amount of injected cells (O'Brien et al., 2007, Ricci-Vitiani et al., 2007). These studies demonstrate that if $1x10^5$ cells or more were injected, tumor formation occurred systematically but below this, the efficiency of these grafts decreased. To clarify this decrease in engrafting efficiency, cancer cells were separated in several fractions before being injected into NOD/SCID mice at different dilutions of cells. The cells were fractioned utilizing the CD133 stem cell marker into CD133+ and CD133- cells. After injecting CD133- cells, less than 2% of the mice transplanted with the higher cell dose generated a tumor. In contrast, tumors were generated systematically after injection of 1×10^3 or more CD133+ cells. Moreover, only the CD133+ cells were able to initiate tumor growth in secondary and tertiary mice, providing the first evidence of the existence of a small sub-population of cancer initiating cells (O'Brien et al., 2007, Ricci-Vitiani et al., 2007, Ieta et al., 2008, Varnat et al., 2009). By extension, this suggests that only this small fraction of cancer cells may be responsible for the metastasis development, while it has never been demonstrated.

3.2 Self-renewal (in vitro)

Cancer cells are defined by their ability to divide and renew endlessly. This observation has been confirmed by the *in vitro* experiments, yet this tumoral property is limited to a specific subset of cells among a tumor.

Studies have shown that colonic tumoral cells can be cultivated in serum-free medium containing epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2). In this medium, the culturing of four weeks of CD133+ cells resulted in colon spheres formed by aggregates of exponentially growing undifferentiated cells whereas CD133- cells invariably died. This CD133+ tumor spheres maintained their tumorigenic potential when injected into

SCID mice and generated rapidly growing tumors. Tumor xenografts derived from colon sphere can be their self-maintained in culture for at least twelve months (Ricci-Vitiani et al., 2007, Todaro et al., 2007).
By contrast, in serum-containing medium without EGF and FGF-2, CD133- cells demonstrated growth for 2 weeks before declining in number. In this medium, it was observed that CD133+ cells gradually migrated and began to differentiate.
In these experiments, the CD133- population cells exhibited only limited self-renewal ability, resulting in cell death when cultured *in vitro*. Yet this population of cells is still classified as tumoral due to their displaying features of gastro-intestinal cancer like carcinoembryonic antigen (CEA) expression, and adenomatous polyposis coli (APC) or p53 mutation. These observations clearly demonstrated *in vitro* the differential property of tumoral cells to survive in culture conditions.

3.3 Differentiation ability

3.3.1 *In vitro*

The differentiation's ability of the CSC accounts for the tumor heterogeneity. In serum-containing medium without EGF and FGF, after one day of culture, CD133+ cells gradually migrate and differentiate into large and adherent cells. During their differentiation, these cells acquired CK20 expression and a similar morphology to the original tumor. In the meantime, they lost their ability to transfer tumor in SCID mice (Ricci-Vitiani et al., 2007).
This differentiation is not correlated to accidental mutations as the experiments with CD133 or CD44 cells consistently reproduced the same behavior *in vitro*. This asymmetric division is more likely the result of highly specific cells controls supporting the stem cells model compared to the stochastic model.

3.3.2 *In vivo*

The relative proportion of "CSC-cells" profile varied among xenograft lines but is conserved within each line of successive *in vivo* transplantation in immunodeficient mice. CD133+ tumor cells isolated from xenograft of colon cancer samples demonstrated a similar ability to generate tumors that contained the same range of CD133+ and CD133- cells compared to the original tumor (O'Brien et al., 2007, Todaro et al., 2007, Ricci-Vitiani et al., 2007, Vermeulen et al., 2008). Similarly, tumor generated from EpCAM ^{high}/CD44+ cells (other putative CSC markers) reproduced the same phenotypic heterogeneity as their parents and contained both and in same proportion EpCAM^{high}/CD44+ and EpCAM^{low}/CD44- populations (Vermeulen et al., 2008).
During the differentiation process, the cells gradually lose their stem cell markers. These markers allow us to precisely discriminate the subset of CSC among a large excess of other cells, including bulk tumor part cells and circulating cells. It is critical to note that this differentiation process always reproduces the heterogeneity of the parental tumor. A tumor must be therefore considered as a complex hierarchically organized tissue involving specific pathways and regulatory mechanism.

3.4 Treatment resistance

Because the CSC are the only tumor initiating cells, it is suggested that the cancer treatment will not be successful unless this population of cells can be completely eradicated. Yet, there is growing evidence that CSCs are naturally resistant to both radiation, and the majority of chemotherapies (Pajonk et al.).

The CSC intrinsic radio-resistance, has been demonstrated in different solids tumors such as breast cancer (Phillips et al., 2006), medulloblastoma (Blazek et al., 2007), or glioma (Chang et al., 2009) with an enrichment of the CSC fraction post tumor treatment. In colon cancer, the tumor exposition to chemotherapy and radiotherapy has been shown to increases the CD133+ cells fraction proportionally to the time and intensity of the application (Saigusa et al.).
The relative chemoresistance of the CSC compared to the stemness cells is now becoming a part of the CSC definition (Song et al., 2009). The CSC chemoresistance resulted from the conjunction of two phenomenon. First, several pathways are differentially expressed by the CSC compared to the bulk counterpart. Some of these have been implicated in chemo-resistance and will be further described. Second, in analogy with the adult stem cells, the CSC are postulated to remain quiescent in their niche and therefore responsible for late recurrent disease. As most of the chemotherapeutic agents act through disruption of the mitotic phase of cancer cell-cycle, quiescent CSC are relatively protected against these cytotoxic agents. This hypothesis has been confirmed by several experiments. In xenograft model, the CSC from colon cancer labeled with dye injected to nude mice demonstrated to remain in a state of quiescence. This quiescence was shown to be reversible when mice were exposed to chemotherapy (paclitaxel) (Kusumbe and Bapat, 2009).
Consequently to this chemo-resistance, it has been shown that tumor submitted to chemotherapy demonstrated enrichment in the stem cell fraction. In hepatocarcinoma, CSC were injected into nude mice treated with different doses of chemotherapy (cyclophosphamide). Cells sorted from these generated tumors presented in vitro a self-renewal potential increasing with the increasing dose of chemotherapy. Similarly, in a secondary xenograft, the ability of these enriched cells to produce xenograft in mice was also dependent on the chemotherapy dose (Tan et al., 2009). In breast cancer, doxorubicin-selected cells demonstrated a higher tumorigenic potential in Matrigel when compared to the parental cells (Calcagno et al.).

4. CSC pathway and possible applications

Considering the CSC theory, effective anticancer drugs should target not only the tumor bulk but also specifically the tumor initiating cells. As CSC are considered to be drug-resistant compared to their bulk counterpart due to their elevated expression of the family of ATP-binding cassette (ABC) transporters and their low proliferation rate. This drug resistance is thought to be responsible for tumor recurrences, as these cells persist after treatment and compose the "minimal residual disease". Characterizations of specific signaling pathway exclusively used by CSC, and not by the tumor bulks cells or by the normal colon cells or the normal colon stem cells is indeed a new challenging area for research. First it enriches the CSC definition by physiological understanding, and second it allows the development of new specific CSC targeted therapy. Similarly to normal stem cells, CSC's rely on pathways that govern development, cell-renewal and apoptosis. It is excluded to realize a thorough review of the explored pathway as the variety of explored pathways is important and as most of these publications consisted in initial observations which need to be further examinated. This chapter will thus focus on several of the major implicated pathways in CSC pathology.

4.1 Wnt/β-catenin signaling pathway

The Wnt signaling pathway plays a critical role in cellular processes including proliferation, differentiation, mobility, survival and apoptosis (Takahashi-Yanaga and Kahn). The

relevance of Wnt signaling is underlined by the frequency of its aberrant activation in a large diversity of malignancy. The regulation of this pathway is provided by the cytoplasmic concentration of β-catenin. In the cytoplasm, β-catenin concentration is maintained at a low level by the adenomatous polyposis coli (APC) complex. This complex of axin, APC and glycogen synthase kinase-3β (GSK- 3β) phosphorylates β-catenin which results in its degradation mediated by the proteasome. Wnt binds to a transmembrane receptor complex comprised of Frizzled (Fz)/ low-density lipoprotein receptor-related protein (LRP) and disheveled (Dvl) leads to its phosphorylation thereby inhibiting GSK-3 β (so the APC complex) and unphosphorylated β-catenin then localizes to the nucleus. In the nucleus, β-catenin functions in conjunction with a transcriptional complex composed of TCF (T-cell transcription factor), LEF (lymphoid enhancer-binding factor), and co activators including CBP, cAMP, and p300 to activate the expression of "Wnt-targeted genes". E-cadherin functions as a sequestering protein of β-catenin on the cell membrane (Wang et al., 2004). In spheroidal culture, CSC's (defined as CD133+/CD166+cells) showed heterogeneity in the Wnt signaling network accompanied by heterogeneity in β-catenine localization, although these cells all carried an APC mutation (Vermeulen et al.). On microarray analysis of these CSC CD133+/CD166+, two capital fractions were described. The TOP-GFP^{high} cells fraction (reporter that provides the evidence of Wnt signaling activation) demonstrated up-regulation of the expression of stem-cell-associated genes like LGR5, and revealed a higher clonogenic potential *in vitro*. *In vivo*, this fraction demonstrated the ability to induce tumors in immunodeficient mice. In contrast, the TOP-GFP^{low} cells fraction expressed epithelial differentiation associated gene like mucin 2 (MUC2), cytokeratin 20 (CK20), keratin 20 (KRT20) and fatty acid binding protein 2 (FABP2) (Vermeulen et al., Sikandar et al.).

As in spheroidal culture, each cell lines remains independent and therefore it can be concluded that the regulation of the Wnt pathway is insured at least in part by the cells intrinsic features.

As expected, the TOP-GFP^{high} fraction cells, when cultivated in serum-containing medium progressively acquire differentiation marks and lost CSC markers. Interestingly, when co-cultured with myofibroblast cell lines (MCFM), their morphological and molecular differentiation was prevented and their clonogenicity highly improved (50 fold). A cytokine antibody array revealed that the hepatocyte growth factor (HGF) was one of the most abundant factors present in MFCM. This HGF modulated nuclear β-catenin activity though c-MET. Exposure of TOP-GFP^{low} cells to MCFM induced their re-expression of CSC markers and restored their clonogenic potential. Regarding these results, CSC are not only independent cells clones driving the tumor growth but their activity is highly related to their microenvironment.

This cooperation between the CSC and the corresponding stromal cells is crucial and established a link between the CSC and previous reports concerning the tumor progression. These results has been confirmed in several solid tumors models including the pancreatic cancer (Moriyama et al.).

Another study, focused on the respective correlation between CD133+ cells and Wnt pathway. Mice model were generated with a knock down for one or two of the CD133 alleles were created (Zhu et al., 2009). Surprisingly, mice completely knockout for CD133 were viable and demonstrated normal development. The tissue expression of CD133 in heterozygote embryos (CD133+/CD133-) was initially found to be restricted to the central nervous system, kidney, intestine and skeletal system. As the mouse developed, the CD133 expression expanded to other organs either by differentiated or undifferentiated cells. In the small bowel, the CD133+ expression was relatively restricted in the crypt base and

overlapped with that of LGR5. The activation of the endogenous Wnt pathway in heterozygous CD133+/CD133- mice resulted initially in a complete disruption of the crypt architecture related to a major proliferation of CD133+ cells at the base of the crypt. Lineage-tracing demonstrated that the entire intestine mucosa was replaced by the progeny of these cells resulting in high-grade focal neoplasic formation.

Wnt pathways play an important role in cells maintenance of pluripotency, however it is also involved in differentiation of embryonic cells. Recently it has been demonstrated by Kahn *et al.* that in the Wnt pathway, the co-activateurs CBP and p300 are the mediator of this balance pluripotency/differentiation (Ma et al., 2005). Indeed, CBP/β-catenin-mediated transcription is involved in undifferentiated stem cells maintenance while CBP/β-catenin-mediated transcription is involved in cells differentiation. They demonstrated *in vitro* that combined treatment with imatinib mesylate (IM) and ICG-001, which is specific inhibitor of binding to the N-terminus of CBP, significantly inhibited colony formation of chronic myeloid leukemia progenitors isolated from two patients resistant to IM (Kim et al.). The Wnt pathway is one of the most important pathways being evaluated in stem cell research and is therefore a target for new cancer therapy development.

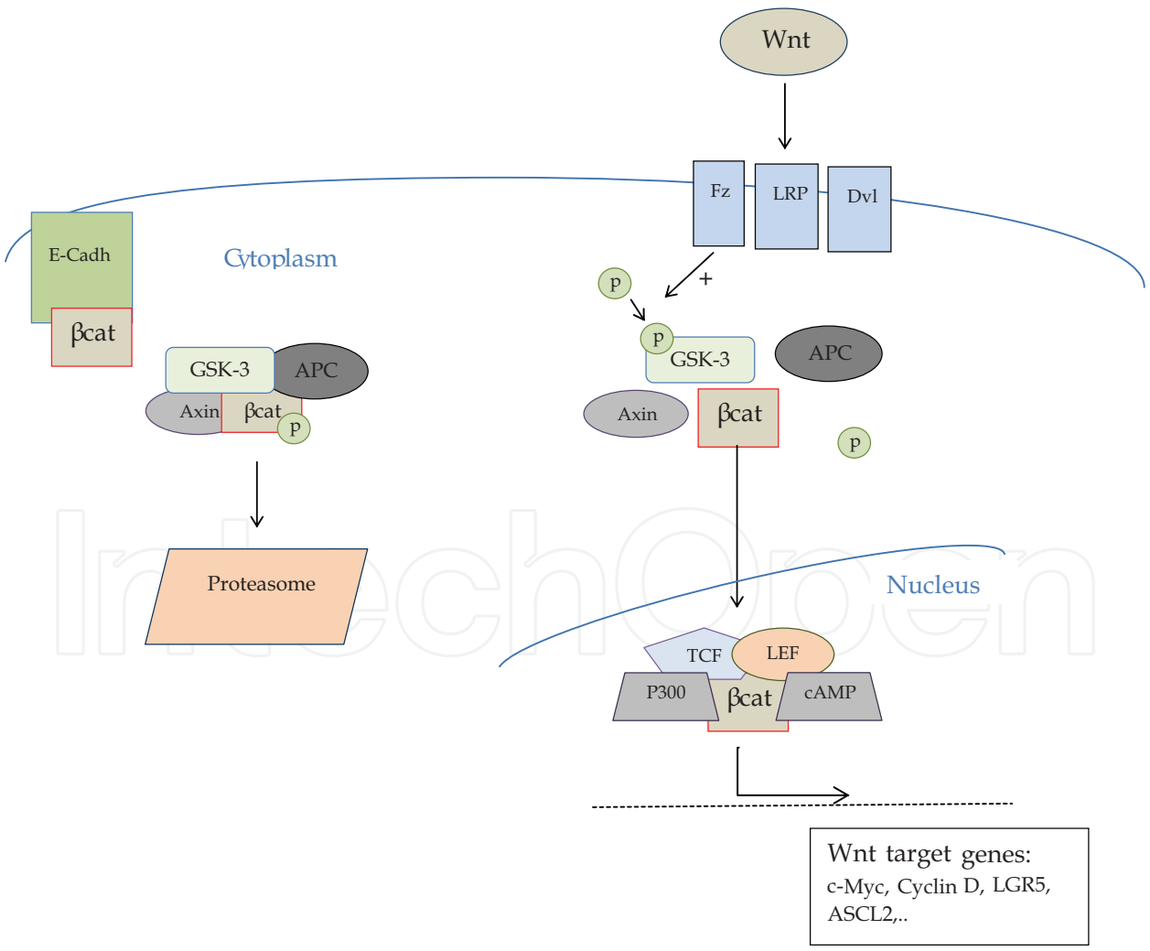

Fig. 2. Wnt/βcatenin signaling pathway

4.2 NOTCH signaling pathway

In mouse models, it has been shown that NOTCH signaling plays an important role in the intestinal tumor initiation. In colon CSC, the NOTCH signaling components are highly expressed compared to the usual colonic cell lines. This expression plays a critical role in CSC self-renewal. Knockdown of the NOTCH pathway in APC mutant cells resulted in cells differentiation into post-mitotic goblet cells. In plate culture, colon CSC treated with NOTCH inhibitors could no longer form adenocarcinoma glands but only disorganized cells cluster without self-renewal capacity (Sikandar et al.).

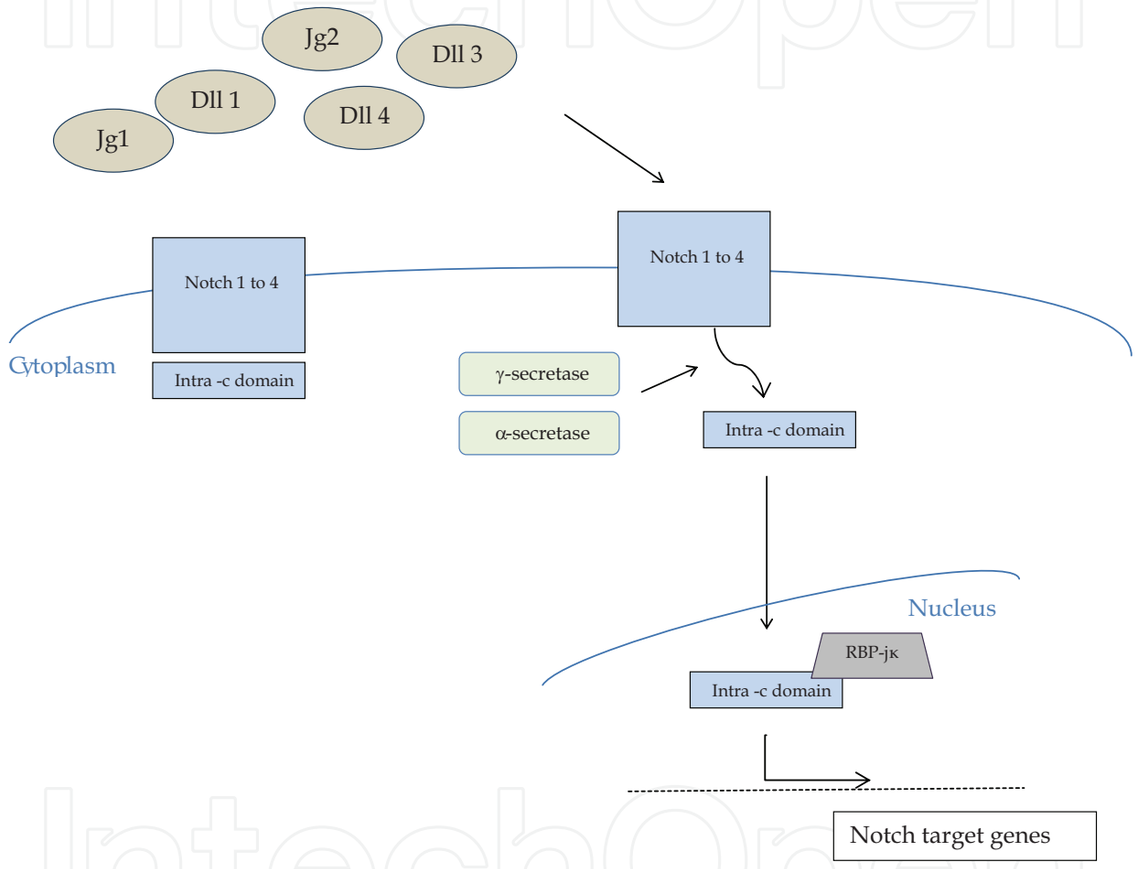

Fig. 3. Notch signaling pathway

4.3 Akt and MAPK signaling pathway

An activation of the epidermal growth factor receptor (EGFR) turns on at least five different signaling pathways: the mitogen-activated protein kinase (MAPK), phospholipase C, phosphatidylinositol 3-kinase (PI3K)/AKT, signal transducer and activator of transcription (STAT), and SRC/FAK pathways. These pathways form an intersecting biochemical network that, when mutated, drives cell growth in a manner unrestricted by environmental cues (Laurent-Puig et al., 2009).

To assess the critical role of these pathways in CSC, a cDNA GeneChip analysis was performed in cells marked CD133+ or CD133- extracted from samples of metastatic colon cancer (Wang et al., 2006). In total, 321 genes were up-regulated and 65 down-regulated in

CD133+ cells compared with the CD133- cells. The gene expression (examined by real-time PCR) confirmed that the changes preferentially concerned PI3K/AKT, NOTCH, Janus kinase/signal transducer and activator of transcription STAT, MAPK and transforming growth factor (TGF)-β pathways. More precisely, AKT was significantly activated and Erk1/2 up-regulated in CD133+cells. When cultured in soft-agarose in the presence of the AKT inhibitor II (SH-5), AKT inhibitor IV or MAPK inhibitor (U0126), there was a decrease in the ability of the CD133+ cells to form colonies formation by 3 to 11 fold. In gene knockdown experiment, cells transduced with AKT and Erk shRNA also demonstrated a reduction in their ability to form colonies.

4.3.1 AKT

Previous studies have suggested the implication of the AKT pathway in colorectal CSC. Thymosin-β-4 (Tβ4) is an ubiquitous G-actin sequestering molecule and has been shown to be involved in a great number of cellular functions such as adhesion, differentiation, migration, angiogenesis, apoptosis and metastasis. Aberrant expression of Tβ4 has been reported in CRC and associated with inducing tumoral progression therefore, Ricci -Vitani *et al.* focused on the Tβ4 expression as a potential critical factor in CSC metabolism (Ricci-Vitiani et al.). Real-time PCR confirmed by Northern and Western blot analysis showed an over-expression of Tβ4 in CSC (CD133+/CD44+) compared to normal epithelial colon cells. Transduced knockdown cells for Tβ4 resulted *in vitro* in their reduced tumor growth ability (50% lower) and their impairment of migration capacity. These knockdown cells showed a decrease of ALDH1 and LGR5 expression (mainly expressed by colon stem cells) whereas differentiation genes like CK20 and trefoil factor 1 were up-regulated. In a xenograft model, tumor growth ability was decreased. Further analysis reveals that Tβ4 over-expression was responsible for increasing integrin-linked kinase and decreasing PTEN expression resulting in AKT pathway activation.

In other experiment, PI3K inhibition of CD133+/CD24+ CSC cells resulted in their "enterocyte-like" differentiation (Vermeulen et al., 2008).

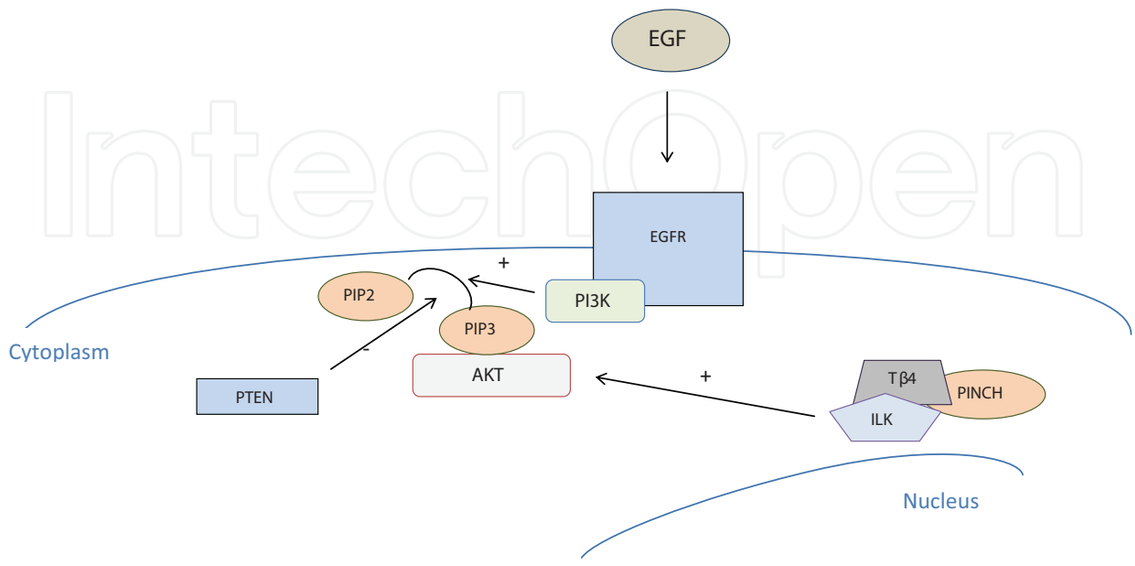

Fig. 4. AKT signaling pathway

4.3.2 TLR and MAPK

Toll-like receptors (TLR) are a family of transmembrane receptors that contribute to activate the MAPK pathway via the adaptator molecule myeloid differentiation primary-response protein 88 (My88). In their study, Grimm *et al.* gave evidence of a correlation between TLR expression and tumor progression (Grimm et al.). Tumoral colon tissues demonstrated a higher expression of TLR 7, 8, 9 or 10 when compared to normal colon tissue. The intensity of this expression correlated with tumor stage and was higher for the late stage tumor (in the UICC classification). The TLR 7 and 8 were shown to be co-expressed with CD133+ in tumor cells.

4.4 CSC and Interleukin-4

Confirming the CSC drug resistance, Todaro *et al* (Todaro et al., 2007) analyzed the cell viability of primary colon cancer cells following exposure to oxaliplatin and/or 5-fluorouracil (5-FU). *In vitro*, the CD133-cells fraction showed a dose-dependent high sensitivity to these drugs whereas CD133+ cells were largely resistant, even at higher dose. Among the different pathways implicated in drug resistance the interleukin-4 (Il 4) mediated signaling has been shown to strongly modulate the death receptors and chemotherapy-induced apoptosis. In this study, colon cancer samples showed a high level expression of IL4 compared to normal colon specimens. More precisely, the CD133+ cells fraction was shown to express both IL4 and IL-4Receptorα. A significantly increase in overall death was observed *in vitro* when CD133+ cells treated with either oxaliplatin and or 5-FU alone or in combination with an IL4-neutrilizing antibody ($p<0.001$). Anti-IL4 treatment resulted in a decrease in the protein expression of anti-apoptotic molecules, cFLIP, Bcl-xl, and PED. *In vivo*, nude mice were engrafted with CD133+ cells and treated by an intraperitonal injection with IL-4DM (IL-4 Rα antagonist) followed (24 hours later) by chemotherapy (oxaliplatin or/and 5-FU). The co-treatment resulted in a marked synergistic effect on the tumor growth compare with single agent chemotherapy.

4.5 CSC and Taxoid-T-1214

To assess the efficiency of a new-generation taxols, Botchkina et al designed an experimental protocol to discriminate the respective effects of Taxoid (SB-T-1214) both on CSC and on the tumor bulk (Botchkina et al.). CSC were isolated form three tumor CRC cells lines (DLD-1, HCT116 and HT29) according to their CD133high/CD44high phenotypic expression. The cytotoxic effect of SB-T-1214 on CSC was studied in two different setting which promoted the stemness phenotype. First, the CSC's were seeded as an adherent monolayer to type I collagen in the serum-free medium for 2 days and then incubated with increasing doses of SB-T-1214 for 48 hours (100nM to 1µM). The vast majority of cells underwent apoptosis (89-96%). The 4-11% of survival cells displayed multiple abnormality including enlarged size, multiple nuclei and severe vacuolization.

Secondly, the experiments were performed in spheroidal cultures. Again, administration of of increasing does SB-T-1214 induced apoptosis in greater than 90% of the CRC cells. The few surviving cells lost their ability to form secondary spheroids colonies.

4.6 Aurora Kinase-A

Aurora-A (STK15/BTAK), is a member of a serine/threonine kinase family, and is involved in mitosis entry, control of centrosome maturation, and segregation during mitosis. Aurora-

A is a key regulator of the p53 pathway, and it's over expression abrogates the wild-type function of p53 such as growth regulation and apoptosis and further confers resistance to chemotherapeutic agents. Cammereri et al. explored this pathway regulation in CSC isolated from colon cancer specimen (defined CD133+CD29+CK20- cells phenotype) (Cammareri et al.). RNA analysis showed that Aurora-A, barely was undetectable in normal colon control cells whereas it was clearly expressed in tumoral cells (CD133+ and CD133-). Immunoblot analysis revealed a higher expression of Aurora-A in tumor specimen compared with normal tissue. Moreover, although in primary tumor cells immunoreactivity was cytoplasmic-located, the Aurora expression in CSC was also nuclear-located. The rare CD133+ cells presents in the normal colon population were mostly negative for Aurora-A expression. Eight of the 15 Aurora-A over expressing CSC cells lines were p53 wild type. Further investigation revealed that knockdown of Aurora-A in CSC resulted in significant growth inhibition, inhibition of migration in vitro, and in a limitation in their tumorigenic capacity on xenograft models with an increase in their susceptibility to chemotherapy induced death.

5. Limits

The experiments discussed above provide evidence that within a tumor, the high cell diversity is not result of a heterogeneous accumulation of diver mutations, but it is more probable that the diversity is the result of a complex regulation program where only a subset of cells are responsible for tumor initiation and development. However, the isolation, characterization and the driving pathways of CSC's are poorly understood and need to be further investigated.

5.1 Stem cells and cancer stem cells

The origin of CSC's cells is thought to be from tissue stem cells. Thus it has been demonstrated that under specific conditions, it is possible to reprogram cells to have a stem-like phenotype. Yu et al. demonstrated that the induction of expression of several gene expression (like Oct4, sox2, nanog, and LIN28 (Yu et al., 2007) or Oct3/4, sox2, Klf4, and c-Myc) in human dermal fibroblasts can convert them into pluripotent cells with a phenotype virtually indistinguishable from embryonic stem cells. In another report, Takahashi et al. (Takahashi et al.) showed that expression of c-Myc can achieve the same result. The evidence that the proto-oncogene c-Myc may be part of the reprogramming of genes supports the hypothesis that under some conditions, it may be possible to reprogram a cell to have a stem-like phenotype.

5.2 Stem cells identification

As an example, while CD133 is supposedly one of the most efficient markers to identify CSC, its biological function on the cell remains unknown. In CRC cell lines (Caco-2 and LoVo) the knock-down for CD133 by siRNA resulted in a significant decrease in both the level of CD133 mRNA and protein expression without any evidence of impairing the cells in vitro rate of proliferation, migration or invasion.

While widely utilized as a CSC marker, CD133 may not be the best marker due its expression as not being restricted to stem cells. Analysis of CD133 knockout mice revealed that this antigen is expressed in epithelial differentiated tissues of several adults' organs including: parietal layer of the Bowman capsule, epithelium of proximal tubules, bile ducts,

and pancreatic duct (Shmelkov et al., 2008). On the colon of mice, CD133 was mostly expressed on the surface and the center of the intestinal crypt, which is typically composed of differentiated columnar absorptive cells.

It has been shown that some tumors do not exhibit CD133+ cells (Dalerba et al., 2007, Ricci-Vitiani et al., 2007, Ieta et al., 2008). In those cases, some of the CD133- cells have been reported as initiating cells with various and contradictory phenotypic profiles such as CD133-/CD44+/CD24- (Shmelkov et al., 2008) or CD133-/CD44-/CDX-2+/CK20+/CK7- (Navarro-Alvarez et al.).

In addition to the characterization of a unique CSC profile, it is of critical importance to be able to discriminate the best sets of markers needed identify the CSC. Consequently to the genetic diversity of colon cancers, the CSC may also be varied among divers tumors but sharing similar phenotypic and function specificity.

Different pathways implicated in CSC functions have been investigated. The hyper-activation of pathways implicated in tumor proliferation and self-renewal has led to the development of several specifically targeted treatments to down regulate these pathways and improve the chemo sensitivity. Until now, these pathways are not specifically used by CSC and the efficiency and side effects when distributed throughout the whole body remains unknown.

6. Conclusion

In conclusion, research has demonstrated that with the heterogeneity of cells within a tumor, only a portion of these cells are tumorigenic. The characterization of these cells needs to be further investigated. The understanding of the implicated pathways will be critical for the development of new targeted therapies that are able to selectively treat the CSC population thereby to reduce a tumor's ability to recur.

7. References

Anido, J., Saez-Borderias, A., Gonzalez-Junca, A., Rodon, L., Folch, G., Carmona, M.A., Prieto-Sanchez, R.M., Barba, I., Martinez-Saez, E., Prudkin, L., Cuartas, I., Raventos, C., Martinez-Ricarte, F., Poca, M.A., Garcia-Dorado, D., Lahn, M.M., Yingling, J.M., Rodon, J., Sahuquillo, J., Baselga, J. & Seoane, J. TGF-beta Receptor Inhibitors Target the CD44(high)/Id1(high) Glioma-Initiating Cell Population in Human Glioblastoma. *Cancer Cell,* Vol. 18, No. 6, pp. 655-68.

Awad, O., Yustein, J.T., Shah, P., Gul, N., Katuri, V., O'Neill, A., Kong, Y., Brown, M.L., Toretsky, J.A. & Loeb, D.M. High ALDH activity identifies chemotherapy-resistant Ewing's sarcoma stem cells that retain sensitivity to EWS-FLI1 inhibition. *PLoS One,* Vol. 5, No. 11, pp. e13943.

Becker, L., Huang, Q. & Mashimo, H. (2008). Immunostaining of Lgr5, an intestinal stem cell marker, in normal and premalignant human gastrointestinal tissue. *ScientificWorldJournal,* Vol. 8, pp. 1168-76.

Blazek, E.R., Foutch, J.L. & Maki, G. (2007). Daoy medulloblastoma cells that express CD133 are radioresistant relative to CD133- cells, and the CD133+ sector is enlarged by hypoxia. *Int J Radiat Oncol Biol Phys,* Vol. 67, No. 1, pp. 1-5.

Botchkina, G.I., Zuniga, E.S., Das, M., Wang, Y., Wang, H., Zhu, S., Savitt, A.G., Rowehl, R.A., Leyfman, Y., Ju, J., Shroyer, K. & Ojima, I. New-generation taxoid SB-T-1214

inhibits stem cell-related gene expression in 3D cancer spheroids induced by purified colon tumor-initiating cells. *Mol Cancer,* Vol. 9, pp. 192.

Calcagno, A.M., Salcido, C.D., Gillet, J.P., Wu, C.P., Fostel, J.M., Mumau, M.D., Gottesman, M.M., Varticovski, L. & Ambudkar, S.V. Prolonged drug selection of breast cancer cells and enrichment of cancer stem cell characteristics. *J Natl Cancer Inst,* Vol. 102, No. 21, pp. 1637-52.

Cammareri, P., Scopelliti, A., Todaro, M., Eterno, V., Francescangeli, F., Moyer, M.P., Agrusa, A., Dieli, F., Zeuner, A. & Stassi, G. Aurora-a is essential for the tumorigenic capacity and chemoresistance of colorectal cancer stem cells. *Cancer Res,* Vol. 70, No. 11, pp. 4655-65.

Chang, C.J., Hsu, C.C., Yung, M.C., Chen, K.Y., Tzao, C., Wu, W.F., Chou, H.Y., Lee, Y.Y., Lu, K.H., Chiou, S.H. & Ma, H.I. (2009). Enhanced radiosensitivity and radiation-induced apoptosis in glioma CD133-positive cells by knockdown of SirT1 expression. *Biochem Biophys Res Commun,* Vol. 380, No. 2, pp. 236-42.

Croker, A.K., Goodale, D., Chu, J., Postenka, C., Hedley, B.D., Hess, D.A. & Allan, A.L. (2009). High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability. *J Cell Mol Med,* Vol. 13, No. 8B, pp. 2236-52.

Dalerba, P., Dylla, S.J., Park, I.K., Liu, R., Wang, X., Cho, R.W., Hoey, T., Gurney, A., Huang, E.H., Simeone, D.M., Shelton, A.A., Parmiani, G., Castelli, C. & Clarke, M.F. (2007). Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A,* Vol. 104, No. 24, pp. 10158-63.

Du, L., Wang, H., He, L., Zhang, J., Ni, B., Wang, X., Jin, H., Cahuzac, N., Mehrpour, M., Lu, Y. & Chen, Q. (2008). CD44 is of functional importance for colorectal cancer stem cells. *Clin Cancer Res,* Vol. 14, No. 21, pp. 6751-60.

Ferrandina, G., Bonanno, G., Pierelli, L., Perillo, A., Procoli, A., Mariotti, A., Corallo, M., Martinelli, E., Rutella, S., Paglia, A., Zannoni, G., Mancuso, S. & Scambia, G. (2008). Expression of CD133-1 and CD133-2 in ovarian cancer. *Int J Gynecol Cancer,* Vol. 18, No. 3, pp. 506-14.

Florek, M., Haase, M., Marzesco, A.M., Freund, D., Ehninger, G., Huttner, W.B. & Corbeil, D. (2005). Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. *Cell Tissue Res,* Vol. 319, No. 1, pp. 15-26.

Gires, O., Klein, C.A. & Baeuerle, P.A. (2009). On the abundance of EpCAM on cancer stem cells. *Nat Rev Cancer,* Vol. 9, No. 2, pp. 143; author reply 143.

Grimm, M., Kim, M., Rosenwald, A., Heemann, U., Germer, C.T., Waaga-Gasser, A.M. & Gasser, M. Toll-like receptor (TLR) 7 and TLR8 expression on CD133+ cells in colorectal cancer points to a specific role for inflammation-induced TLRs in tumourigenesis and tumour progression. *Eur J Cancer,* Vol. 46, No. 15, pp. 2849-57.

Haraguchi, N., Ohkuma, M., Sakashita, H., Matsuzaki, S., Tanaka, F., Mimori, K., Kamohara, Y., Inoue, H. & Mori, M. (2008). CD133+CD44+ population efficiently enriches colon cancer initiating cells. *Ann Surg Oncol,* Vol. 15, No. 10, pp. 2927-33.

Horst, D., Scheel, S.K., Liebmann, S., Neumann, J., Maatz, S., Kirchner, T. & Jung, A. (2009). The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer. *J Pathol,* Vol. 219, No. 4, pp. 427-34.

Huang, E.H., Hynes, M.J., Zhang, T., Ginestier, C., Dontu, G., Appelman, H., Fields, J.Z., Wicha, M.S. & Boman, B.M. (2009). Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res,* Vol. 69, No. 8, pp. 3382-9.

Huang, M., Li, Y., Zhang, H. & Nan, F. Breast cancer stromal fibroblasts promote the generation of CD44+CD24- cells through SDF-1/CXCR4 interaction. *J Exp Clin Cancer Res,* Vol. 29, pp. 80.

Ieta, K., Tanaka, F., Haraguchi, N., Kita, Y., Sakashita, H., Mimori, K., Matsumoto, T., Inoue, H., Kuwano, H. & Mori, M. (2008). Biological and genetic characteristics of tumor-initiating cells in colon cancer. *Ann Surg Oncol,* Vol. 15, No. 2, pp. 638-48.

Joshua, B., Kaplan, M.J., Doweck, I., Pai, R., Weissman, I.L., Prince, M.E. & Ailles, L.E. Frequency of cells expressing CD44, a Head and Neck cancer stem cell marker: Correlation with tumor aggressiveness. *Head Neck*.

Kim, Y.M., Ma, H., Oehler, V.G., Gang, E.J., Nguyen, C., Masiello, D., Liu, H., Zhao, Y., Radich, J. & Kahn, M. The gamma catenin/CBP complex maintains survivin transcription in beta-catenin deficient/depleted cancer cells. *Curr Cancer Drug Targets,* Vol. 11, No. 2, pp. 213-25.

Kusumbe, A.P. & Bapat, S.A. (2009). Cancer stem cells and aneuploid populations within developing tumors are the major determinants of tumor dormancy. *Cancer Res,* Vol. 69, No. 24, pp. 9245-53.

Laurent-Puig, P., Cayre, A., Manceau, G., Buc, E., Bachet, J.B., Lecomte, T., Rougier, P., Lievre, A., Landi, B., Boige, V., Ducreux, M., Ychou, M., Bibeau, F., Bouche, O., Reid, J., Stone, S. & Penault-Llorca, F. (2009). Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol,* Vol. 27, No. 35, pp. 5924-30.

Leung, E.L., Fiscus, R.R., Tung, J.W., Tin, V.P., Cheng, L.C., Sihoe, A.D., Fink, L.M., Ma, Y. & Wong, M.P. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. *PLoS One,* Vol. 5, No. 11, pp. e14062.

Ma, H., Nguyen, C., Lee, K.S. & Kahn, M. (2005). Differential roles for the coactivators CBP and p300 on TCF/beta-catenin-mediated survivin gene expression. *Oncogene,* Vol. 24, No. 22, pp. 3619-31.

Moriyama, T., Ohuchida, K., Mizumoto, K., Cui, L., Ikenaga, N., Sato, N. & Tanaka, M. Enhanced cell migration and invasion of CD133+ pancreatic cancer cells cocultured with pancreatic stromal cells. *Cancer,* Vol. 116, No. 14, pp. 3357-68.

Munz, M., Baeuerle, P.A. & Gires, O. (2009). The emerging role of EpCAM in cancer and stem cell signaling. *Cancer Res,* Vol. 69, No. 14, pp. 5627-9.

Murphy (1914). Heteroplastic tissue grafting effected though roentgen-ray lymphoid destruction. *J. Am. Med. Assoc.,* Vol. 62, pp. 1459.

Navarro-Alvarez, N., Kondo, E., Kawamoto, H., Hassan, W., Yuasa, T., Kubota, Y., Seita, M., Nakahara, H., Hayashi, T., Nishikawa, Y., Hassan, R.A., Javed, S.M., Noguchi, H., Matsumoto, S., Nakaji, S., Tanaka, N., Kobayashi, N. & Soto-Gutierrez, A. Isolation and propagation of a human CD133(-) colon tumor-derived cell line with tumorigenic and angiogenic properties. *Cell Transplant,* Vol. 19, No. 6, pp. 865-77.

O'Brien, C.A., Pollett, A., Gallinger, S. & Dick, J.E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature,* Vol. 445, No. 7123, pp. 106-10.

Pajonk, F., Vlashi, E. & McBride, W.H. Radiation resistance of cancer stem cells: the 4 R's of radiobiology revisited. *Stem Cells,* Vol. 28, No. 4, pp. 639-48.

Phillips, T.M., McBride, W.H. & Pajonk, F. (2006). The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst,* Vol. 98, No. 24, pp. 1777-85.

Ponti, D., Zaffaroni, N., Capelli, C. & Daidone, M.G. (2006). Breast cancer stem cells: an overview. *Eur J Cancer,* Vol. 42, No. 9, pp. 1219-24.

Rasheed, Z., Wang, Q. & Matsui, W. Isolation of stem cells from human pancreatic cancer xenografts. *J Vis Exp*, No. 43.

Ricci-Vitiani, L., Lombardi, D.G., Pilozzi, E., Biffoni, M., Todaro, M., Peschle, C. & De Maria, R. (2007). Identification and expansion of human colon-cancer-initiating cells. *Nature,* Vol. 445, No. 7123, pp. 111-5.

Ricci-Vitiani, L., Mollinari, C., di Martino, S., Biffoni, M., Pilozzi, E., Pagliuca, A., de Stefano, M.C., Circo, R., Merlo, D., De Maria, R. & Garaci, E. Thymosin beta4 targeting impairs tumorigenic activity of colon cancer stem cells. *FASEB J,* Vol. 24, No. 11, pp. 4291-301.

Richardson, G.D., Robson, C.N., Lang, S.H., Neal, D.E., Maitland, N.J. & Collins, A.T. (2004). CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci,* Vol. 117, No. Pt 16, pp. 3539-45.

Rowehl, R.A., Crawford, H., Dufour, A., Ju, J. & Botchkina, G.I. (2008). Genomic analysis of prostate cancer stem cells isolated from a highly metastatic cell line. *Cancer Genomics Proteomics,* Vol. 5, No. 6, pp. 301-10.

Saigusa, S., Tanaka, K., Toiyama, Y., Yokoe, T., Okugawa, Y., Kawamoto, A., Yasuda, H., Morimoto, Y., Fujikawa, H., Inoue, Y., Miki, C. & Kusunoki, M. Immunohistochemical features of CD133 expression: association with resistance to chemoradiotherapy in rectal cancer. *Oncol Rep,* Vol. 24, No. 2, pp. 345-50.

Shi, C., Tian, R., Wang, M., Wang, X., Jiang, J., Zhang, Z., Li, X., He, Z., Gong, W. & Qin, R. CD44+ CD133+ population exhibits cancer stem cell-like characteristics in human gallbladder carcinoma. *Cancer Biol Ther,* Vol. 10, No. 11, pp. 1182-90.

Shmelkov, S.V., Butler, J.M., Hooper, A.T., Hormigo, A., Kushner, J., Milde, T., St Clair, R., Baljevic, M., White, I., Jin, D.K., Chadburn, A., Murphy, A.J., Valenzuela, D.M., Gale, N.W., Thurston, G., Yancopoulos, G.D., D'Angelica, M., Kemeny, N., Lyden, D. & Rafii, S. (2008). CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest,* Vol. 118, No. 6, pp. 2111-20.

Sikandar, S.S., Pate, K.T., Anderson, S., Dizon, D., Edwards, R.A., Waterman, M.L. & Lipkin, S.M. NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. *Cancer Res,* Vol. 70, No. 4, pp. 1469-78.

Song, B., Wang, Y., Xi, Y., Kudo, K., Bruheim, S., Botchkina, G.I., Gavin, E., Wan, Y., Formentini, A., Kornmann, M., Fodstad, O. & Ju, J. (2009). Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. *Oncogene,* Vol. 28, No. 46, pp. 4065-74.

Stoecklein, N.H., Siegmund, A., Scheunemann, P., Luebke, A.M., Erbersdobler, A., Verde, P.E., Eisenberger, C.F., Peiper, M., Rehders, A., Esch, J.S., Knoefel, W.T. & Hosch, S.B. (2006). Ep-CAM expression in squamous cell carcinoma of the esophagus: a potential therapeutic target and prognostic marker. *BMC Cancer,* Vol. 6, pp. 165.

Sullivan, J.P., Spinola, M., Dodge, M., Raso, M.G., Behrens, C., Gao, B., Schuster, K., Shao, C., Larsen, J.E., Sullivan, L.A., Honorio, S., Xie, Y., Scaglioni, P.P., DiMaio, J.M., Gazdar, A.F., Shay, J.W., Wistuba, II & Minna, J.D. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. *Cancer Res,* Vol. 70, No. 23, pp. 9937-48.

Takahashi-Yanaga, F. & Kahn, M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? *Clin Cancer Res,* Vol. 16, No. 12, pp. 3153-62.

Takahashi, H., Ishii, H., Nishida, N., Takemasa, I., Mizushima, T., Ikeda, M., Yokobori, T., Mimori, K., Yamamoto, H., Sekimoto, M., Doki, Y. & Mori, M. Significance of Lgr5(+ve) Cancer Stem Cells in the Colon and Rectum. *Ann Surg Oncol.*

Takaishi, S., Okumura, T., Tu, S., Wang, S.S., Shibata, W., Vigneshwaran, R., Gordon, S.A., Shimada, Y. & Wang, T.C. (2009). Identification of gastric cancer stem cells using the cell surface marker CD44. *Stem Cells,* Vol. 27, No. 5, pp. 1006-20.

Takeda, K., Kinoshita, I., Shimizu, Y., Matsuno, Y., Shichinohe, T. & Dosaka-Akita, H. Expression of LGR5, an intestinal stem cell marker, during each stage of colorectal tumorigenesis. *Anticancer Res,* Vol. 31, No. 1, pp. 263-70.

Tan, S., Chen, J.S., Sun, L.J. & Yao, H.R. (2009). Selective enrichment of hepatocellular cancer stem cells by chemotherapy. *J Int Med Res,* Vol. 37, No. 4, pp. 1046-56.

Todaro, M., Alea, M.P., Di Stefano, A.B., Cammareri, P., Vermeulen, L., Iovino, F., Tripodo, C., Russo, A., Gulotta, G., Medema, J.P. & Stassi, G. (2007). Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell,* Vol. 1, No. 4, pp. 389-402.

Tomuleasa, C., Soritau, O., Rus-Ciuca, D., Pop, T., Todea, D., Mosteanu, O., Pintea, B., Foris, V., Susman, S., Kacso, G. & Irimie, A. Isolation and characterization of hepatic cancer cells with stem-like properties from hepatocellular carcinoma. *J Gastrointestin Liver Dis,* Vol. 19, No. 1, pp. 61-7.

Varnat, F., Duquet, A., Malerba, M., Zbinden, M., Mas, C., Gervaz, P. & Ruiz i Altaba, A. (2009). Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *EMBO Mol Med,* Vol. 1, No. 6-7, pp. 338-51.

Vermeulen, L., De Sousa, E.M.F., van der Heijden, M., Cameron, K., de Jong, J.H., Borovski, T., Tuynman, J.B., Todaro, M., Merz, C., Rodermond, H., Sprick, M.R., Kemper, K., Richel, D.J., Stassi, G. & Medema, J.P. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol,* Vol. 12, No. 5, pp. 468-76.

Vermeulen, L., Todaro, M., de Sousa Mello, F., Sprick, M.R., Kemper, K., Perez Alea, M., Richel, D.J., Stassi, G. & Medema, J.P. (2008). Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci U S A,* Vol. 105, No. 36, pp. 13427-32.

Wang, J.C. & Dick, J.E. (2005). Cancer stem cells: lessons from leukemia. *Trends Cell Biol,* Vol. 15, No. 9, pp. 494-501.

Wang, J.Y., Wu, C.H., Lu, C.Y., Hsieh, J.S., Wu, D.C., Huang, S.Y. & Lin, S.R. (2006). Molecular detection of circulating tumor cells in the peripheral blood of patients with colorectal cancer using RT-PCR: significance of the prediction of postoperative metastasis. *World J Surg,* Vol. 30, No. 6, pp. 1007-13.

Wang, W.S., Chen, P.M., Hsiao, H.L., Wang, H.S., Liang, W.Y. & Su, Y. (2004). Overexpression of the thymosin beta-4 gene is associated with increased invasion

of SW480 colon carcinoma cells and the distant metastasis of human colorectal carcinoma. *Oncogene,* Vol. 23, No. 39, pp. 6666-71.

Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, II & Thomson, J.A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science,* Vol. 318, No. 5858, pp. 1917-20.

Zhang, H.Z., Wei, Y.P., Wang, M., Wu, C., Yang, Y.Q., Chen, J. & Cao, Y.K. (2007). [Association of CD133 and endothelin-converting enzyme expressions with prognosis of non-small cell lung carcinoma]. *Nan Fang Yi Ke Da Xue Xue Bao,* Vol. 27, No. 5, pp. 696-9.

Zhu, L., Gibson, P., Currle, D.S., Tong, Y., Richardson, R.J., Bayazitov, I.T., Poppleton, H., Zakharenko, S., Ellison, D.W. & Gilbertson, R.J. (2009). Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature,* Vol. 457, No. 7229, pp. 603-7.

Cancer Stem Cells - The Cutting Edge

Edited by Prof. Stanley Shostak

ISBN 978-953-307-580-8
Hard cover, 606 pages
Publisher InTech
Published online 01, August, 2011
Published in print edition August, 2011

Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Benhaim Leonor, Labonte Melissa and Lenz Heinz-Joseph (2011). What do We Know About Cancer Stem Cells? Utilizing Colon Cancer as an Example, Cancer Stem Cells - The Cutting Edge, Prof. Stanley Shostak (Ed.), ISBN: 978-953-307-580-8, InTech, Available from: http://www.intechopen.com/books/cancer-stem-cells-the-cutting-edge/what-do-we-know-about-cancer-stem-cells-utilizing-colon-cancer-as-an-example

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen