

Cancer Stem Cells in Solid Tumors:  
Evidence for Their Existence and Some Implications for Therapy



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**Abstract:**

Cancer, the number one disease in the Netherlands, killed over 40.000 people in 2008 in the Netherlands. Years of intense studies on this subject shed new lights on the mechanism and the treatment of the disease. For years scientists believed that every single cell in the human body could mutate in a cancerous cell. Nowadays, this perspective has changed with the arrival of the Cancer Stem Cell (CSC) theory, which states that only tissue stem and progenitor cells can mutate into cancer cells. CSCs are at the base of growth and malignant properties of the tumor. There is still some controversy about the CSC and its existence. In this review the existence of the CSC will be confirmed and the CSC characteristics are linked to the tumor phenotype. CSCs are relatively resistant to anti cancer therapies because of their healthy stem cell origin. This review describes several pathways for drug resistance in CSCs. At the end, three tumors are discussed; glioblastoma multiforme, ovarian- and colon cancer. These tumors originate from CSCs and show therapy resistance, which both will be discussed.

## **Embryonic and Tissue Stem Cells**

The human body is, after fertilization of the ovum, formed from a single cell; the embryonic stem cell (ESC). This ESC gives rise to every single cell type in the body, from skin cells to the epithelial lining in the colon. To maintain tissues, cells must be continuously replaced and renewed. The maintenance of those old and damaged cells is regulated by stem cells.

The field of stem cell biology dates back to 1908, when the term was first introduced by Alexander Maximov. In 1964 the first stem cell like character cells are found in mouse bone marrow by Till JE, *et al.* In 1965 the first tissue stem cells were found in the brain (Altman J, Das GD. 1965), but these results were long ignored because of the contradicting results of this study. It was before thought that neurons were set for life and that no regeneration of neurons was possible. After the acceptance of these results and after several other studies, scientists knew that normal tissues also contained cells with a self-renewing and undifferentiated character. According to the definition of the stem cell provided by Loeffler and Roeder in 2002 stem cells must have seven properties which distinguish them from normal, differentiated tissue cells. Stem cells must for instance be undifferentiated cells capable of indefinite proliferation, they must give rise to nearly all different tissue cell types and they must be regenerative in tissues after injury (Loeffler M, Roeder I. 2002). In tissues stem cells account for only a small fraction of cells (~1%), but it is these cells which are crucial for normal tissue function (Boman, BM *et al.* 2008). T Reya *et al.* defined stem cells in a shorter version in 2001 “cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation”.

Stem cells reside in particular places of the tissue, called niches. The behavior of stem cells is not only regulated by its own genetics, but also the niches they reside in. For instance, if mouse fetal forebrain derived stem cells are transplanted into several regions of the brain, they give rise to differentiated cells that become correctly incorporated to the target tissue (Eriksson C, *et al.* 2003). Stem cells were thought to reside quiescent in their

niches and are responsible for normal tissue repair and regeneration, especially in tissues with a high turnover rate like the colon (reviewed by Haegerbarth A, Clevers H. 2009). Evidence suggests that signaling pathways activated in embryogenesis are also the key regulatory pathways in normal stem cell self-renewal. Those pathways include Sonic Hedgehog, p53, Notch, PTEN, BMI-1 and Wnt (reviewed by Boman BM, *et al.* 2008). p53 and PTEN are directly involved in regulating the cell cycle whereas Sonic Hedgehog, Notch, Wnt and BMI-1 are essential for stem cell function character. Those pathways will be mentioned tumor specific in later chapters.

A dividing stem cell usually has two products; the original self renewed stem cell and a differentiated progenitor cell, sometimes called Transit Amplifying (TA) cell. This progenitor cell usually has a high proliferation rate, however it has a limited number of divisions whereas the stem cell was thought to be relatively quiescent though not limited in its divisions. Also, the progenitor cell is destined to differentiate, in limited cell types compared to the stem cell. It always was thought that the TA cells make up for the rapid proliferation in tissues, but recently this hypothesis was adjusted which will be discussed later (Haegerbarth A, Clevers H. 2009). Stem cells divide much less than TA cells and therefore, there has been confusion between these cells. Identification, isolation and characterization of stem cells has long been a limitation to progress in stem cell biology. At first, stem cells could only be identified by their behavior in culture, e.g. their capacity for self-renewal and differentiation into multiple lineages. Studies have, since then, revealed stem cell markers which could be used in combination with techniques to separate stem cells from normal tissue cells.

### **Identification and Characterization of Stem Cells**

The discovery of membrane bound stem cell markers has given scientists a great benefit in stem cell research. Stem cell markers are not universal in every tissue type in the human body, for every tissue there is a different combination of markers. Stem cell markers are membrane bound molecules, those molecules could be receptors, antigens, adhesion molecules etc. The name Cluster of Differentiation (CD) is given to cell surface

markers, which include the stem cell markers. For instance, the surface marker prominin-1 (CD133) is seen on a wide collection of cells including several stem cells and progenitor cells (Wu Y, Wu PY. 2009). Stem cell markers are used to identify stem cells, by combining these markers with fluorescent activated cell sorting to separate cell populations based on their surface markers (reviewed by Boman BM, *et al.* 2008). In vitro, stem cells can be identified by sphere forming assays, only stem cells produce floating multicellular spheres in medium (Temple S, 2001).

Another technique for confirming the stem cell phenotype is by fluorescent activated cell sorting (FACS) analysis for fluorescence accumulation of Hoechst 33342. Hoechst 33342 is one of the substances pumped out of the cell by multidrug resistance (MDR) proteins. MDR proteins are ABC membrane transporters, which effectively pump drugs and other substances out of the cell. The cell population is analyzed with FACS, fluorescent activated cell sorting, a separation technique based on cell characteristics. Cells with a small collection of the dye in the cell are called the side population because during flow-cytometry analysis these cells are visualized as a negative stained “side population” to one side of the majority of cells on a density plot (reviewed by Dean, M *et al.* 2005). This side-population all have a high ABC MDR transporter expression, a trait shared by stem cells. Beside the MDR ABC transporters being expressed in CSCs, Breast cancer resistance protein (BCRP) has also been correlated with tumor resistance by expelling chemotherapeutics out of the cell in comparative manner as the ABC transporters (Eyler CE, Rich JN. 2008).

Since the identification of stem cell markers, not only a lot more is know about stem cells with respect to their behavior and characteristics, but also stem cell based therapies are starting to arise. The regenerative properties of stem cells could be used clinically in multiple cases. Recent studies showed that it is possible to create Induced Pluripotent Stem (iPS) cells from human fibroblasts by non-viral transfection of a single multi protein expressing factor (Kaji, *et al.*). Those iPs cells show expression of pluripotent cell markers thereby confirming the pluripotency of those cells. This study also suggests possibilities for the use of stem cells in several regenerative purposes. Although the use

of normal stem cells for regenerative therapeutic purposes is highly interesting, in this review the focus is on the recent concept of normal stem cells being at the basis of malignant transformation thereby forming cancer stem cells. Evidence for this cancer model is provided and implications for therapy are briefly discussed, including as examples CSCs identified in three different tumor types, ovarian, colon and brain tumors.

### **General Aspects of Cancer**

Since 2008 cancer is the number one disease in the Netherlands with over 40.000 deaths each year, followed by the 2006 leading disease; cardiovascular disease. Although the annual new cases of cancer rise every year, the relative death rate drops slightly due to improved screening techniques and timely diagnosis (Statline).

Cancer is a cellular dysfunctioning disease where the balance between newly formed and dead cells is disrupted locally or systemically by genetic defects. This disruption is caused by cells, which underwent DNA damage or changes of some kind which results in abnormal behavior (Hanahan D, Weinberg RA. 2000). This DNA damage is usually on sites where it affects DNA repair, cell cycle regulatory proteins, apoptosis, cell signaling and tissue architecture and it leads to uncontrolled cell proliferation where those cells behave antisocial in respect to their surroundings (Martin SJ, Green DR. 1995).

Cells must undergo a series of mutations in their DNA before they become cancerous, where one mutation is not enough to disturb the cells normal behavior, multiple mutations at precise sites are needed before a cancer cell is born. This is explained by the fact that a cell has many regulatory systems, if one is depleted, another may step in (Hanahan D, Weinberg RA. 2000). If several regulatory systems are damaged, the cell either can go in apoptosis or, when this mechanism is affected, it can develop into a cancer cell (Martin SJ, Green DR. 1995).

A tumor can be restricted to a particular tissue, it has no intention of breaking this boundary and the cells cluster together, or it can be invasive; here the tumor tries (and succeeds) to cross this boundary and infiltrate other tissues in the body. An invasive tumor is called a cancer or malignant tumor, a tissue restricted tumor that does not grow

in a unlimited and metastatic manner is called a benign tumor (Barnhart BC, Simon MC. 2007).

The human body has some tumor suppressor mechanisms. For instance, to become a tumor cell, the cell needs multiple mutations in its DNA. The DNA of cells is precisely examined and repaired during each division and throughout the lifespan of the cell. When a mutation does occur and it results in genetic instability, the answer of the cell is nearly always apoptosis. Another cellular mechanism to control tumor cells is the expression of certain surface markers on cells. With the progression of the cancerous state of the cell, different surface markers are expressed. The markers are recognized by the immune system and these cells are lysed. Despite of these controlling mechanisms, some cells fail to activate the immune system or its DNA damage is not noticed, at this point a tumor cell is born (Hanahan D, Weinberg RA. 2000).

After a cell undergoes the mutations in its DNA required to become a cancer cell, it starts to divide. Cancer cells can divide indefinite because of mutations in their DNA responsible for the ageing of the cell, telomeres. Telomeres are at the ends of chromosomes and with every division, the telomere shortens. When the telomere is at its maximum of shortening, cell division is restricted (Hanahan D, Weinberg RA. 2000).

Another aspect of tumor cells is the insensitivity to foreign signals (Hanahan D, Weinberg RA. 2000). Every cell needs signals from other cells or itself to for instance, survival. Tumor cells grow insensitive for foreign halt signals and they sometimes secrete their own survival factors. A tumor therefore behaves antisocial in a tissue due to the lack of sensitivity to foreign signals and the high proliferation rate. The danger from tumors is that, because of this antisocial behavior, the expanding of the tumor is to the detriment of normal healthy tissue. Normal tissue functioning is inhibited because of the growing tumor, sometimes inhibiting healthy organs (Hanahan D, Weinberg RA. 2000).

A property of tumor cells is to invade and survive in other tissues. When this is locally, e.g. in one tissue or organ, the tumor can be relatively easily removed surgically, depending on the tissue type. A surgical treatment is considered an effective way of removing a tumor, with the least of all therapies chance of tumor recurrence. However,

when tumor cells spread throughout the body, the surgical approach for removing the tumor is somewhat more difficult. Tumor cells have the ability to metastasize, that is, to escape its tissue of origin and travel through the vascular or lymphatic tissue to another site in the body where new tumors are formed. This aspect is the least understood and the most feared because of the incapability of a surgical treatment of the tumor and the increasing number of tumors in the body (Hanahan D, Weinberg RA. 2000).

### **Different tumor models**

For years it was thought that any given cell in the body which accumulated the right set of mutations could give rise to tumors. The daughter cells are all equal to this one cell and all have the same characteristics with no hierarchy whatsoever between the cells. Also, tumors were seen as a bulk of all tumor cells, with blood vessels running through to provide the tumor of nutrients and the transport of waste products. Every cancer cell in the tumor was believed to metastasize, give rise to tumors elsewhere. This model is also referred to as the stochastic model (Pan Y, Huang X. 2008).

Recent studies shed a new light on cancer cell biology. It first was seen that tumors were no bulk of identical tumor cells, but merely an imitation of true tissue, with different cell types and hierarchy between the tumor cells. Non-stem tumor cells were seen to have limitations in their number of divisions in contrast to what was thought before. At the foundation of this new model is a new insight in cancer biology; the cancer stem cell (CSC) (Boman BM, Wicha MS. 2008).

### **Cancer Stem Cells**

This new model suggests that stem cells are responsible for tumor growth and maintenance. These stem cells are of course not the normal stem cells revised in the previous chapter, but newly emerged, mutated stem cell the CSCs. According to this model, it is likely that CSCs arise from healthy stem cells or progenitor/TA cells responsible for normal tissue functioning. Evidence for this hypothesis is provided in the

colon and hair follicle, where the same gene is expressed in healthy- and cancer stem cells (reviewed by Haegerbarth A, Clevers H. 2009). It is thought by some, however, that differentiated tissue cells with the right mutations could acquire the mechanisms to become a CSC, but this is not universally accepted. Other evidence for the healthy stem cell origin for CSCs, is the expression of the same surface markers as the healthy stem cells several of them are listed in table 1 (Pan Y, Huang X. 2008). Evidence showed poor patient prognosis with a high CSC percentage in a tumor (reviewed by Boman BM, Wicha MS. 2008).

One way for confirming CSC characteristics, is the transplantation of CSCs in immunodeficient mice. Via this technique it is possible to distinguish CSCs from other tumor cells due to the tumor forming abilities of the cells. Only CSCs can form tumors in these mice, TA and further differentiated tumor cells have a limit on their tumor generating properties caused by the absence of self renewal capacities of the cells. Multiple studies effectively reported these findings. One of these showed tumors were formed after transplanting as few as 100 CD133+ human brain tumor cells in immunodeficient mice, while CD133- cells did not initiate new tumors (Singh SK, *et al.* 2004). Just like healthy stem cells, CSCs in other tumors express stem cell markers, seen in table 1. These markers can be used to identify CSCs.

**Table 1.** Cell surface biomarkers associated with different cancer stem cells

Tumor type	Cell surface markers
Acute myeloid leukemia	CD34+CD38-
Breast cancer	CD44+CD24-ESA+
Brain cancer	CD133+
Colon cancer	CD133+
Head and neck cancer	CD44+
Prostate cancer	CD44+
Multiple myeloma	CD138-
Metastatic melanoma	CD20+
Colorectal cancer	EpCAM <sup>high</sup> CD44+CD166+
Pancreatic cancer	CD24+CD44+ESA+
Lung adenocarcinoma	Sca1+CD45-Pecam- CD34+
Bone sarcoma	Stro1+CD105+CD44+
Hepatocellular carcinoma	Very heterogeneous

Table 1: Stem cell markers in tumors (Pan Y, Huang X. 2008).

Gene profiling is the comparison of the global gene expression of CSCs with non-CSCs or healthy tissue. This was performed for breast cancer by Liu R, *et al* in 2007 and led to the identification of 186 genes differentially expressed by the CSC population. Comparison of those genes with previously reported gene signatures showed a significant CSC gene signature and both overall and metastasis free survival. These findings suggested a correlation between aggressiveness of a tumor and differentiation of the cells. Differentiation suggested to relate with patient prognosis (reviewed by Ailles LE, Weissman IL. 2007; Liu R, *et al.* 2007).

Another study identified the TGF- $\beta$  pathway to be associated with differentiation, where an inhibition of this pathway led to differentiation of CSCs (Shipitsin M, *et al.* 2007).

One group studied different colon cancer cells for metastatic properties and stemness factors. The metastatic colon cancer cell line, which expressed a majority of known CSC markers, closely resembling the patterns of expression in cells from several metastatic colon cancer patients, was selected as a reference material. Gene profiling revealed 4,351 differentially expressed genes with an overrepresentation of those responsible for apoptosis resistance, regulation of cell cycle, proliferation, stemness and developmental pathways (Botchkina IL, *et al.* 2009).

CSCs, like normal stem cells, reside in niches inside the tumor. This CSC niche is associated with the stromal components of the tumor, the site where fibroblasts and blood vessels are found (reviewed by Ailles LE, Weissman IL. 2007). In this niche, the factors essential for CSC survival and self renewal are secreted such as the TGF- $\beta$  mentioned earlier. The CSCs give rise to TA-like tumor cells with a high proliferation rate but without the potential to self renew.

These TA cells are the effective proliferative force of cancer. TA cells divide rapidly and CSCs were thought to remain relatively quiescent in a tumor (reviewed by Dean M, *et al.* 2005). Studies however provided evidence for colon stem cells and colon CSCs with a high proliferation rate, just slightly slower than their descending TA cells (reviewed by Haegebarth A, Clevers H. 2009). This might change the perspective of healthy and CSCs to be relatively quiescent in other tumors.

Metastasis mentioned earlier, is the process where tumor cells leave the tissue to invade other parts of the body. This process is seen to be induced by hypoxia and is driven by CSCs. CSCs respond very aggressive to hypoxia, as seen in figure 1 along with the activating pathways. Pronounced hypoxia correlated with poor patient prognosis (Barnhart BC, Simon MC. 2007). Another study suggested the CSC from the primary tumor as the metastasis developing cells in cancer (reviewed by Boman BM, Wicha MS. 2008). It has also been show that CSCs produce much higher levels of VEGF then non-CSCs, promoting angiogenesis in tumors (Eyler CE, Rich JN. 2008). CSCs for these reasons seem to be important targets for novel diagnostic and therapeutic strategies.

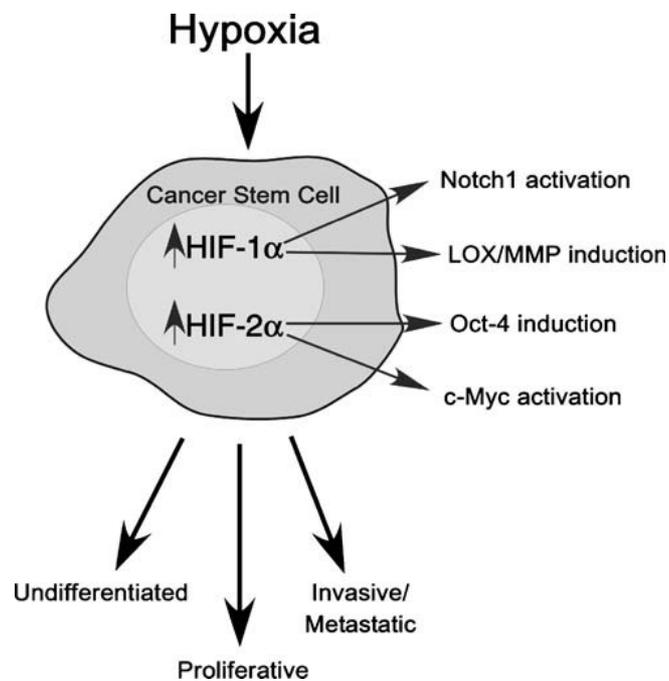


Figure 1. CSC response to hypoxia (Barnhart BC, Simon MC. 2007)

These data suggest that the CSC is self supporting in its needs and therefore mimics the normal, healthy stem cell.

## CSC and Drug Resistance

CSCs show an increase in drug resistance compared with non-CSCs. This relative resistance could be an explanation for tumor relapse after treatment and the occurrence of resistant tumors. Recurrent tumors are in most cases resistant to multiple drugs (reviewed by Dean M, *et al.* 2005). It was thought that CSCs were quiescent and that therefore therapies had no effect on these cells. Anti cancer therapies target fast proliferative cells, and it was thought that the TA cells would account for those cells. New insights in CSC mechanisms have, as mentioned before, changed the view on the role of the CSC in a tumor (reviewed by Haegerbarth A, Clevers H. 2009). This shows that the drug resistance by CSCs is not due to its activity, there must be other causes.

Normal stem cells fulfill an important role in the maintenance and regeneration of tissues. If CSCs arise from normal tissue stem cells, CSCs share those mechanisms. It is known that CSCs have a high expression of ABC transporters, for instance the ABCC1 gene, an important MDR gene (reviewed by Dean M, *et al.* 2005). Normal stem cells have dominant DNA repair mechanisms and have resistance to apoptosis not seen in differentiated cells. CSCs have shown to be resistant to radiation in vivo and in vitro, due to DNA damage checkpoint activation and DNA repair capacity not seen in differentiated cancer cells. Due to genetic instability of cancer cells, the mutation rate is greater than in healthy cells. These mutations can cause drug resistance for instance by overexpressing MDR proteins, drug inactivation or elimination. After treatments, a fast recovery of the tumor can sometimes be seen probably because of CSC behaviour. CSCs mimic the role of healthy stem cells in tissue recovery, causing fast re-growth of the tumor (reviewed by Boman BM, Wicha MS. 2008).

Several molecular mechanisms have been implicated in resistance for therapy, unfortunately not one can be linked to overall drug resistance by CSCs. Recently the Wnt/ $\beta$ -catenin pathway was implicated with radiation resistance in tumors. Wnt and  $\beta$ -catenin induced hyperplasia's showed a higher proportion side population. Stem cell antigen cells showed a higher levels of  $\beta$ -catenin (Eyler CE, Rich JN. 2008). The Wnt/ $\beta$ -catenin pathway has been shown to induce genomic instability in colon cancer

(Hadjihannas MV, *et al.* 2006). Chk1/2, cell cycle arrest modulators are modulated by Wnt/ $\beta$ -catenin pathway (Eyler CE, Rich JN. 2008). Therefore the Wnt/ $\beta$ -catenin pathway was suggested to promote genomic instability while the tumor cell survived after radiation, promoting genetic changes in the tumor. In fact, loss of the tumor suppressor phosphatase and tensin homolog (PTEN), which has reduced activity in any tumors as a result of silencing or mutation and which functions to oppose epithelial growth factor receptor (EGFR)-mediated signaling through the Akt kinase, has been shown in mouse embryonic stem cells to prevent cell cycle arrest in response to radiation by restricting Chk1 to the cytoplasm, ultimately leading to genetic instability (Eyler CE, Rich JN. 2008). Notch1 and Notch2 may act as tumor suppressors and regulate stem-cell maintenance, proliferation, and apoptosis. BCC is the most common form of skin cancer and is strongly correlated with Notch deficiency and Notch 1/2 downregulation as well as the increase in Sonic hedgehog (SHH) signal transfer and activation of the Wnt pathway in which result in the malignant transformation (Hombach–Klonisch S, *et al.* 2008).

In the following part three examples of CSC in solid tumors are described in some more detail with respect to identification and characterization.

### **CSCs in Ovarian Cancer**

Epithelial Ovarian Cancer (EOC), the fifth leading cancer deaths among women, originates from the surface epithelium from the ovary and consists of a multiple subtypes within one tumor. This cancer has a low survival rate (45% after five years) and a high recurrence of the disease despite of advances in surgery and chemotherapy. Most patients develop a drug resistant form of this disease after treatment (reviewed by Pan Y, Huang X. 2008). Growing evidence suggests that tissue stem cells contribute to the normal repair and regeneration of the ovarian epithelial lining, and therefore suggests a role for CSC in EOC.

It has been showed that SP cells from EOC formed measurable tumors, whereas non-SP did not. Chemotherapeutic agent doxorubicin inhibited non-SP cells significantly more than SP cells (Szotek PP, *et al.* 2006). From these findings they concluded the CSC stem cell character for the SP. Another study analyzed the two subtypes of CD133, CD133-1 and CD133-2 in EOC, CD133 being a known stem cell marker in several tissues. Ovarian tumors showed a higher percentage of CD133+ cells than normal tissue or benign tumors. The CD133+ cells showed a higher proliferative potential compared to the CD133- cells (Ferrandina G, *et al.* 2009). Analyzing the markers on CSC from EOC, Szotek *et al.* saw a variable expression on CSCs and could not find a specific EOC stem cell marker (Szotek PP, *et al.* 2006).

These results suggested the existence of epithelial ovarian cancer stem cells and demonstrated their proliferative properties. Research thus far has failed to identify a specific marker for EOC stem cells, but these cells can be sorted with Hoechst accumulation in cell sorting technique as well as CD133+ analysis. Identification of EOC, selected the surface marker CD44 as CSC markers in this tumor. CSC from EOC had an increase in Myeloid Differentiation Factor 88 (MyD88), responsible for downstream activation of the NFκB signalling pathway, leading to tumor initiation and progression (Ayesha B, *et al.* 2009).

## CSCs in Colon Cancer

The human intestinal lining has a high turnover rate of 5 days in mice and 6 days in humans. This process is driven by stem cells in the crypts of the colon previously identified as crypt base columnar cells. It was long known that stem cells resided near the crypts in a stem cell zone but recently a stem cell marker has been found. The Wnt pathway has been shown to control proliferation in the intestinal lining and therefore the target genes (~80) of this pathway were tested. Inadequate stimulation of Wnt on the target genes together with other mutations results in colon cancer (Pinto D, Clevers H. 2005; Garcia M, *et al.* 2009). Wnt has also been linked to the self renewal of stem cells and CSCs (Reya T, Clevers H. 2005). Several genes were found to be expressed in the crypts but one in particular showed a restricted expression in the crypts bottom. This gene was Leucine-rich repeat G protein-coupled receptor 5 (LGR5/GPR49) or short Lgr5 gene. A combination of Lgr5+ cells with the gene adenomatous polyposis coli (Apc), a tumor suppressor gene deleted leads to transformation of the Lgr5+ cells. This transformation gives rise to microadenomas within days eventually growing into a tumor. If the Apc gene is knocked out in TA Lgr5- cells, a transformation is seen but the adenome does not grow into a tumor. These results give further evidence for the CSC theory and its mechanism in colon cancer. Lgr5 is also seen in a restricted area in the eye, brain, mammary gland, reproductive organs and stomach. This suggests a universal role for Lgr5 as a stem cell gene, however this needs further analysis (Haegebarth A, Clevers H. 2009). It is seen that in colon cancer, the upregulation of MDR genes is responsible for the drug resistance to some chemotherapeutics (Toffoli G, *et al.* 1994). A difference in colon cancer stem cells behavior is seen opposing normal stem cells or stem cells in other cancers. The Lgr5+ cancer stem cell in colon cancer is not quiescent but divides relatively often, about once every day. The TA cells in this cancer divide more often, about once every 12-16 hours (Barker N, *et al.* 2009).

## **CSCs In Glioblastoma Multiforme**

Glioblastoma multiforme (GBM) is the most lethal type of brain tumor and aggressively infiltrative (Oka N, *et al.* 2009), with a high resistance to ionizing radiation (IR) and chemotherapy (Mukherjee B, *et al.* 2009). Despite of advances made in therapies, the survival rate remains low. IR remains the most effective therapy after surgery, though these tumors invariably recur after radiation (Eyler CE, Rich JN. 2008). Although GBM cells show similar histological phenotypes, differences in cellular biology and clinical prognosis suggest that GBM might harbor different subtypes descending from cells of different origin and/or at different points of differentiation (Tchoghandjian A, *et al.* 2009). In GBM, cells are found with stem cell-like capacities which share features with the neural stem cell (NSC) and express the surface marker GT3, a ganglioside expressed on the surface of cells which conduct several functions, and some express CD133. It is shown that GT3+ cells are the CSCs in GBM, where CD133+ does not affect the stem cell like properties of these cells (Tchoghandjian A, *et al.* 2009).

The resistance by this tumor to therapies has been linked to a mutation in a key glioma-specific mutation, epidermal growth factor receptor variant III (EGFRvIII). This regulates double strand breaks repair via hyperactivation of protein kinase catalytic subunit (DNA-PKcs) (Mukherjee B, *et al.* 2009). GBM stem cells have an increased activation of DNA damage checkpoint thereby making it resistant to IR. Just as in the other discussed tumor types, MDR genes seem to be responsible for chemotherapy resistance in GBM CSCs (Jin F, *et al.* 2008).

## **Conclusion**

Stem cells are, compared with differentiated cells, equipped with a more dominant cell cycle control system, DNA repair mechanisms and are not limited in their divisions. This review summarizes available evidence for the existence of cancer stem cells (CSCs) in solid tumors. The stochastic and the new CSC tumor model are discussed to create an objective view on the different models. The discussed evidence confirms the existence of CSCs in solid tumors and the malignant phenotype of tumors is linked to CSC biology. Identification of CSCs is described, as well as some of the known cell surface markers for CSCs. CSCs show an increase in drug resistance which can be linked to their healthy stem cell origin. CSC mimics the role of stem cells in injured tissues which can lead to fast recurrence after therapy. Three tumors are described; glioblastoma multiforme, ovarian- and colon cancer, according to their CSC presence, CSC surface markers and possible drug resistance pathways. The expression of MDR genes is seen to be responsible for chemotherapy resistance by CSCs in the discussed tumor types. The surface markers differ between these types, but overall CD133 is seen on a great part of the CSC population throughout the body though this surface marker cannot be linked solely to stem cells. Finally, it can be concluded that CSCs are, because of their stem cell origin, the source for malignancy by tumors and that because of this origin, they are a relatively difficult target for therapy.

## **Discussion: CSC or Stochastic model and CSC proliferation**

The field of the CSC hypothesis is relatively young and with every new hypothesis, there is some disagreement. This new hypothesis seems very promising, but is it reality? In 2006, the American Association of Cancer Research (AACR) convened a workshop to discuss the rapidly emerging CSC model. Stem- and non-cell experts were present to talk about this new hypothesis. While the people who stood behind the new model were discussing the definition of the CSC, others were debating the existence and importance of CSCs in tumors (Clarke MF, *et al.* 2006). Most disputes were actually based on misunderstandings between scientists about CSCs and its definition linked to the normal stem cell biology. The outcome of this workshop yielded a definition for the CSC and almost all controversions about CSCs were rejected (Jordan GT, 2009). A newly arisen dispute, caused by evidence in colon cancer, is whether or not (cancer) stem cells remain quiescent in their niche. It is seen that healthy and CSCs divide rapidly in crypts of the colon, this behaviour has not yet seen before. According to this behaviour, new studies can clarify healthy and CSC proliferation behaviour in different tissues.

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