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# Cancer Initiating Cells in Head and Neck Squamous Cell Carcinoma

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## 1. Introduction

The clonal genetic model of cancer defined it as a proliferative disease originating from mutated tumour cells. However, not all the cells in a tumour have the same characteristics and only particular cells have the capability of originating it. Cancer stem cells (CSCs) and cancer initiating cells are the main responsible of this property and are considered, due to their characteristics, the actual target for therapy. Moreover, recent evidences indicated that these cancer initiating cells are also responsible for the metastatic behaviour of tumours. Data obtained in human patients and in mouse models have revealed that CSCs are characterized, at the molecular level, by the expression of certain specific markers, a complex circuitry of transcriptional and epigenetic regulation, and, in solid tumours, by high predisposition to undergo the so called epithelial mesenchymal transition.
Head and neck squamous cell carcinoma is one of the most prevalent type of malignancy worldwide. The mortality due to HNSCC is mainly caused by local recurrence and local metastasis to cervical lymph node, and occasionally by distant organ metastasis. The primary tumours are characterized by cellular and functional heterogeneity. Numerous evidences have indicated the presence of cancer initiating cells or cancer stem cells in this type of cancer. Here the current evidences of such cell population in the context of putative markers, transcriptional and posttranscriptional regulation, in vivo genetically engineered mouse models and possible therapeutic approaches will be reviewed.

## 2. Relevance of head and neck cancers

The term Head and neck cancer comprises several epithelial-derived tumors arising in the oral cavity, pharynx, larynx and nasal cavity. The most common type of head and neck cancer, including oral cancer, is squamous-cell carcinoma (HNSCC). Head and neck cancers represent the sixth most common cancer worldwide, with roughly half million new cases each year, and its incidence is still increasing in several geographic areas and its trend is now affecting younger individuals (Leemans et al., 2011). Surgery and/or radiation therapy remain the gold standards for treatment of cancers of the lip and oral cavity. Recent evidence suggests that addition of chemotherapy may provide a survival advantage over radiation therapy alone in advanced stages of this disease (Bardet et al., 2011; Calais et al., 1999; Calais et al., 2004). Despite progress in surgery, radiation, and chemotherapy, the 5-year survival rate for oral cancer has not improved significantly over the past several

decades and remains at about 50–55% (Leemans et al., 2011). As for other types of cancer, the main risk factors are alcohol and/or tobacco use (up to 100 times higher for both) (Neville and Day, 2002); another important risk factor is HPV infection, which has been detected in around 20% of all cases and 50% of oropharynx cases (Leemans et al., 2011). An important aspect of these diseases is the significantly increased risk of developing subsequent second primary or recurrent tumor which shows similar genetic alterations as the original tumor (Braakhuis et al., 2006; Tabor et al., 2002; Tabor et al., 2001). This has led to the field cancerization concept (Slaughter et al., 1953), which reflects that premalignant clones are present at the surgical margins without macroscopic signs of disease that may allow the development of secondary tumors close to the areas where primary tumors arose (Bradley et al., 2007; Tabor et al., 2002; Tabor et al., 2001). The development of distant metastases, loco-regional recurrence or a second primary tumor cause in many circumstances the failure of the treatment, making essential an accurate analysis of the origin and spread of the disease.

As for other types of cancer, HNSCCs show marked heterogeneity affecting cellular morphology, proliferative and metastatic index, genetic lesions and therapeutic response. Such variability can occur between tumors arising in the same organ (intertumoral heterogeneity) identifying distinct tumor subtypes, which are characterized by their molecular profile, morphology and expression of specific markers. However, variation is also evident within individual tumors (intratumoral heterogeneity), as cells display different functional properties and express miscellaneous markers. Such heterogeneity within a single tumor is usually a reflection of the hierarchical organization within it. In addition, the intratumoral heterogeneity is an indirect indication of the existence of a subpopulation of self-renewing cells that can generate the full repertoire of tumor cells.

## 3. Cancer initiating vs. cancer stem cells

For many researchers in basic and translational Oncology the concept "cell of origin" is mixed and used interchangeably with the cancer stem cell (CSC) notion. It is important to note these two concepts are not necessarily identical (Visvader, 2011). The cell of origin refers to the normal cell that acquires the first cancer-promoting mutation(s), whereas, the CSC is applied to the cells in the tumor that exclusively sustains the malignant growth. In other words, each concept refers to cancer-initiating cells and cancer-propagating cells, respectively, two cell populations that substantially differ in their phenotype. However, both cell types are also related because the nature of mutations acquired by the cell of origin may dictate whether a cancer follows a CSC archetype. The possible reasons for such confusing concepts come form the usual way in which CSCs are tested, by injecting or implanting into immunocompromissed mice, assuming the minimal number of cells required to develop a tumor. In this case both CSCs and initiating cells are the same, but this does not indicate that the original tumor initiating cell in the primary tumor was a CSC. Also the two current models of tumor evolution and intratumoral heterogeneity source can contribute to this confusing aspect: the "cancer stem cell" (CSC) and the "stochastic" or "clonal evolution" models. In the first one, the process of tumor growth follows a pattern highly similar to that of normal tissues relying on the presence of stem cells, and implicate that most tumor cells do not actually contribute to tumor perpetuation, and the heterogeneity is due to aberrant differentiation processes from these cancer stem cells. On the other hand, in the "clonal evolution model" the self-renewal and tumor maintenance are

mediated by the majority of tumor cells, and the differentiation, intraclonal genetic and epigenetic variation determine tumor heterogeneity. Whether which of these models is the correct one remains to be elucidated. However, the presence of multiple subclones in a single tumor and the identification of putative CSC in a very high proportion (20%) of the tumors strongly suggest that the real situation will be a combination of both. Accordingly, tumors that evolve through the CSC model, progress on acquiring additional mutations that resemble the clonal model. In this regard in, hematological malignancies it has been recently reported a tremendous genomic heterogeneity that evolve as clonal branches (Anderson et al., 2011; Notta et al., 2011)

## 4. Relevant signaling pathways in HNSCC

As in other solid tumors, the neoplastic process in HNSCC begins with the normal epithelium progressing through hyperplasia to dysplasia to carcinoma in situ and invasive carcinoma by a multistep process of genetic and epigenetic changes. The earliest manifestations of these lesions are leukoplakia or erythroplakia (white or red patches observed in the epithelium). The finding that only up to 20% of oral leukoplakias progress to malignancy (Braakhuis et al., 2004) indicates that it is possible to treat the disease at this premalignant stage.

As in other cancer types, HNSCC arises through the accumulation of genetic and epigenetic changes in genes to acquire a characteristic phenotype that includes limitless replicative potential, self-sufficiency in growth signals, insensitivity to anti-growth signals, ability to evade apoptosis, invasion and metastasis, and angiogenesis (Hanahan and Weinberg, 2000; Negrini et al., 2010). To acquire these properties HNSCC cells need to overcome multiple cellular brakes such as those imposed by cell cycle machinery and in particular the p53 and Rb-dependent pathways. Somatic mutations in TP53 are found in 60–80% of HNSCC cases (Smeets et al., 2011). Moreover, inactivation of p53, either by knock down, by expression of dominant-negative mutant p53R172H or by expression of the HPV16 oncoprotein E6, conferred extended lifespan on oral keratinocytes (Smeets et al., 2011). Similarly, the $p16^{INK4A}$–cyclin D–CDK4(6)–RB axis is inactive in most HNSCC. In these cases, the inactivation is attributable to mutation or methylation in combination with chromosome loss or, in most cases, by homozygous deletion of CDKN2A (coding for $p16^{INK4A}$) and amplification of Cyclin D1 locus (occurring in 80% of HNSCC cases). In addition, high-risk human papillomavirus (HPV) 16 and 18 infections are also frequent in certain HNSCC (Leemans et al., 2011). In these HPV-associated HNSCC, two oncogenes expressed by the HPVs, E7 and E6 target the p53 and the entire Rb family (pRb, p107 and p130) to degradation (zur Hausen, 2002).

Among the different signaling pathways acting in HNSCC, EGFR seems to be crucial. Upon binding to its ligands, this receptor triggers activating signals predominantly through the Ras–MAPK and PI3K–PTEN–AKT pathways, and may also concur to activate Stat3-dependent transcription. EGFR is overexpressed in many cases of HNSCC (Grandis and Tweardy, 1993; Hama et al., 2009; Ozanne et al., 1986). This results in the clinical use of EGFR-specific antibodies in combination with radiotherapy that showed increased efficacy to treat HNSCC patients (Bonner et al., 2006). As mentioned, EGFR activation proceeds though the PI3K–PTEN–AKT signaling pathway. This is also of extreme relevance in HNSCC. Both, activating mutations in PIK3CA as well as inactivating mutations of PTEN, have been found in HNSCC. In addition, PIK3CA gains are very frequent in this type of

tumors. All these alterations lead to AKT activation. Although the possible presence of activating mutations in Akt genes (AKT1, 2 and 3) and gene amplifications remains to be confirmed, overall the wide majority of HNSCC display active Akt signaling (Segrelles et al., 2006). This induces not only resistance to apoptosis, but also facilitates an angiogenic switch (Segrelles et al., 2004) and cell proliferation (Paramio et al., 1999). This raised the possibility of using anti Akt therapies in HNSCC (Moral and Paramio, 2008).

## 5. Identification of putative CSC in HNSCC

Based on cell surface markers, dye efflux, in vivo tumorigenicity in immunocompromised mice, slow-cell cycle progression, upregulation of stemness-related genes, resistance to therapy and clonogenic proliferation assays, it has been possible to demonstrate the presence of tumor-initiating cells exhibiting stem cell properties in tumors of breast, colon, brain and melanoma. All these approaches have been also used to identify the putative ideal cancer stem cell marker, which should impart all the acquired hallmarks of self-sufficiency in growth signals, anchorage-independent growth, apoptotic/drug resistance, invasiveness, metastatic potential and impinge a high self-renewal capacity. However, it is worth noting that certain controversies in the experimental systems and the assays followed in these characterizations precluded a broad applicability for the cancer stem cell theory in all solid tumors. Nonetheless, several cell markers have been used to identify and characterize the putative CSCs in HNSCC.

### 5.1 CD44

CD44 is a broadly distributed polymorphic cell surface glycoprotein that mediates important processes such as wound healing and tumor metastasis (Naor et al., 2008; Orian-Rousseau, 2010). Based on its involvement in breast cancer stem cells, several studies have analyzed the relevance of CD44 in HNSCCs. In primary HNSCC CD44 expression is confined within the basal epithelial cells and is co-expressed with the stem cell-related gene BMI-1. The CD44+ cells usually found to be less than 10%, could self-renew indefinitely and produce CD44− progeny, and could generate the original tumor heterogeneity after serial transplants in NOD/SCID mice (Prince and Ailles, 2008; Prince et al., 2007). Importantly, the binding of CD44 to extracellular molecules can drive the activation of PI3K/Akt (Ghatak et al., 2005; Gilg et al., 2008; Misra et al., 2005) and MAP kinase and Ras signaling pathways (Cheng et al., 2006). However, the CD44+ fraction is probably not a pure population of CSCs and, unlike breast CSC, the use of CD44 and CD24 does not appear to further enrich the CSC population (Prince and Ailles, 2008; Prince et al., 2007). It is worth mentioning that CD44 is actually a family of several proteins that differ in the extracellular domain by insertion of variable regions through alternative splicing (Ponta et al., 2003). In particular two isoforms CD44s and CD44 v6 are widely expressed in oral epithelia (Mack and Gires, 2008). However, these proteins were abundantly expressed in carcinomas and also in normal tissue (Mack and Gires, 2008) thus indicating that the value of CD44 as a marker for a small CSC population in HNSCC needs to be reconsidered.

### 5.2 CD133

This is a 5-transmembrane glycoprotein, also known as Prominin-1 identified in a subpopulation of human hematopoietic cells, and has been used for the identification and isolation of putative CSCs stem cells from different human tumors such as brain, prostate,

colon, liver, pancreas and hematological tumors (Mizrak et al., 2008). Of note, CD133 expression doe not solely identify these CSC; for instance, in a large series of prostate tumors, CD133 is expressed in combination with CD44+ and $\alpha 2\beta 1^{hi}$ in approximately 0.1% of cells, irrespective of their grade or metastatic state. These cells were capable of self-renewal, proliferation, and multi-lineage differentiation in vitro to recapitulate the original tumour phenotype, consistent with CSC properties (Collins et al., 2005). CD133 expression has been found in a small fraction (1-2%) of HNSCC tumors and cell lines (Zhang et al., 2010). Interestingly this subpopulation of CD133+ cells possess distinct properties characteristic of CSCs, including higher potential for clonogenicity, increased expression of specific stem cell markers, invasion and in vivo tumorigenicity as well as increased chemoresistance as compared with their CD133− counterparts (Zhang et al., 2010). The possibility that CD133 is a bona fide CSC for HNSCC however remains to be elucidated in wide number of tumor samples.

### 5.3 Emmprin

CD147, also known as EMMPRIN (extracellular matrix metalloproteinase-inducer) TCSF (tumor cell-derived collagenase stimulatory factor) and Basigin, is a transmembrane glycoprotein that is upregulated during the early stage of premalignant lesions and remains stable during invasive and metastatic progression (Vigneswaran et al., 2006). EMMPRIN/ CD147 is a major candidate mediating anchorage-independent growth, angiogenesis, drug resistance, hypoxic survival and invasion, all of which are essential molecular events of carcinogenesis (Vigneswaran et al., 2006). Moreover, its ability to stimulate the production of hyaluronan by tumor cells, which in turn promotes cell survival and anchorage-independent growth via activation of Akt, Erk and FAK (Marieb et al., 2004), and the fact that it is a CD44 binding patner may indicate that the combination of CD147/CD44 positive cells may help to define a potential subpopulation of CSCs in HNSCC (Richard and Pillai, 2010).

### 5.4 The side population (SP)

The existence of a cell population that exclude the dye Hoechst 33342 was described in bone marrow stem cells (Goodell et al., 1997). Subsequently SP cells have also been found in a number of other stem cell populations, including putative CSCs for neuroblastoma, glioblastoma, breast, cancer and ovarian cancer. Using the same approach Harper et al., (Harper et al., 2007) identified a small cell population in HNSCC cell lines. More recently Zhang et al., (Zhang et al., 2009) characterized this SP in cell lines and clinical samples of HNSCC; they observed that SP+ cells give rise to SP+ and SP-, whereas SP- only produced SP- cells. They studied their clonogenic and tumorigenic properties and suggested that these cells posses tumor stem cell phenotypes. Moreover, they also observed that these cells expressed higher amounts of Bmi1, NSpc1, CD44 and Oct4 together with several members of the ABC tansporter family (ABCG2 and ABCB1), thus further reinforcing their suggestion. However, although SP+ were clearly more tumorigenic than SP- cells, $10^4$ cells are required, a number enough high to exclude that a small subpopulation is the actual responsible for tumorigenesis potential.

### 5.5 ALDH1

The aldehyde dehydrogenase (ALDH) families of enzymes are cytosolic isoenzymes that are responsible for oxidizing intracellular aldehydes and contributing to the oxidation of retinol

to retinoic acid in early stem cell differentiation (Yoshida, 1992). ALDH1 is expressed at early stages during HNSCC progression and studies have shown that ALDH1 is a CSCs marker and that its presence strongly correlates with tumor malignancy as well as self-renewal properties of stem cells in different tumors, including breast cancer, hepatoma, colon cancer, and lung cancer. Accordingly, ALDH1 cells were isolated from HNSCC patients (Chen et al., 2009). These cells showed the ability to form tumorspheres, displayed increased migratory properties and demonstrated higher abilities to induce tumor growth (Chen et al., 2009). In addition, the expression of ALDH1 correlates with stage and grade of tumors and inversely related to poor outcome in HNSCC patients, probably due to an increased predisposition to undergo epthelial-mesenchymal transition and increased Snail1 expression (Chen et al., 2009). Of note, these capacities were more evident in CD44+CD24-ALDH1+ cells, suggesting that this CD44+CD24-ALDH1+ cell population is likely a CSC in human HNSCC.

### 5.6 The niche

Both stem cell maintenance and tumor growth are supported by the stroma. This is not a casual event, as in both cases the intricate interrelationship appears to be essential. It is worth mentioning that such stroma is composed, in the case of tumors, by a plethora of different cells such as fibroblasts and different inflammatory cells. Although there is almost no data regarding the niche of oral stem cells, there is mounting evidence that stem cells in other stratified epithelia, such epidermis, are supported at least in part by extensive cross talk between these cells and their environment (Blanpain and Fuchs, 2009; Fuchs, 2009; Moore and Lemischka, 2006). Whether there is a unique microenvironment surrounding these progenitors remains to be determined (Moore and Lemischka, 2006). Interestingly, cancer stem cells derived from epidermal tumours may exist independently of the classic skin stem cell niche, yet also have stem cell properties, including multi-lineage differentiation (Ambler and Maatta, 2009). A relative quiescent state is an important feature distinguishing adult stem cells. Such quiescence is, in part, directed by external cues expressed in the presumptive microenvironment, the niche, although the cellular and molecular nature of the niche remains obscure in most SC systems. In turn, this may lead to specific restrictive proliferation in the stem cell population (Lopez et al., 2009; Lorz et al., 2010). Similarly, tumor maintenanace and progression is modulated in part by the extracellular matrix and the cells components of the stroma such as fibroblasts (Bhowmick and Moses, 2005; Bhowmick et al., 2004b; Kalluri and Zeisberg, 2006), and/or inflammatory cells (Andreu et al., 2010; Coussens et al., 1999; Coussens et al., 2000; Kerkela and Saarialho-Kere, 2003; Spadaro and Forni, 2004; van Kempen et al., 2003). Recently, it has been suggested that these cells may affect the tumor growth by specific signaling pathways such as TGFβ (Bhowmick et al., 2004a; Cheng et al., 2005b), HGF/cMet axis (Daly et al., 2008; Grugan et al., 2010; Knowles et al., 2009), SDF-1 (Daly et al., 2008; Kojima et al., 2010; Orimo et al., 2005), and that the induction of senescence in the stromal cells (Krtolica et al., 2001; Liu and Hornsby, 2007; Parrinello et al., 2005; Yang et al., 2006) or the specific inactivation of certain pathways such LKB1 (Katajisto et al., 2008) or PTEN (Trimboli et al., 2009) in these cells may effectively influence tumor progression. It becomes obvious that such crosstalk may also influence CSCs. However, with the clear exception of colon cancer (Borovski et al., 2011; de Sousa et al., 2011; Vermeulen et al., 2010), the possible effect that the niche can exert on this cell population remains unknown.

## 6. Genomics of HNSCC CSC

### 6.1 Gene expression studies

The sequentiation of the human genome and the development of high throughput technologies, in particular gene-expression profiling, have provided the opportunity to describe biological features, including pathologies, in a quantitative manner. Accordingly, there is a astonishing number of genomic studies in cancer, and also the number in HNSCC is huge (Braakhuis et al., 2010; Hunter et al., 2005; Molinolo et al., 2009). Subsequently, genomics studies of CSCs purified on the basis of cell surface marker have been characterized for various tumor types including leukemia, glioblastoma, sarcoma, breast, colorectal, lung, pancreatic and prostate cancer. In an early study using quantitative RT-PCR analysis performed in CD44+ cells from HNSCC demonstrated an increased expression of the stem cell regulator Bmi1 (Prince et al., 2007). Later on, using microarray studies on CD44+CD24-ALDH1+ vs CD44+CD24-ALDH1- permitted the identification of 1082 genes differentially expressed (Chen et al., 2009). Remarkably among the upregulated genes there are stemness genes (OCT4, NANOG, SOX2, KLF4, BMI-1, and NESTIN), Cell cycle regulators (CCNA2, GTSE1, MAD2L1, MCM7, RAD21), transcriptional modulators (FOSL1, HMGB1, MBNL1, POU5F1, TPX2), modulators of epithelial mesenchymal transition (DKK1, SNAI1, SNAI2, TWIST1) and other signaling proteins belonging to mTOR, Cytokine and ABC transporter families or pathways (Chen et al., 2009). Importantly the upregulated genes included CD147 (Chen et al., 2009). It is therefore conceivable that this genomic study may have identified a CD147+CD44+CD24-ALDH1+ population with enriched CSC properties.

### 6.2 MicroRNA studies

miRNAs are a class of endogenous non-coding RNAs that function as important regulatory molecules by negatively regulating gene and protein expression. They have been implicated to control a variety of cellular, physiological, and developmental processes and the aberrant expression of miRNAs is connected to human diseases such as cancer acting as a tumor suppressors or oncogenes (Brown and Naldini, 2009; Croce, 2008; Croce, 2009; Esteller, 2008; Garzon et al., 2009; Garzon et al., 2010; Inui et al., 2010; Ryan et al., 2010). In addition, miRNAs are important factors in stem cell function and the expression levels of certain miRNAs in stem cells are different from other normal tissues, thus implying that they may have a unique role in stem cell regulation (Alvarez-Garcia and Miska, 2005; Cheng et al., 2005a; Croce and Calin, 2005; Gangaraju and Lin, 2009; Hammond and Sharpless, 2008; Sartipy et al., 2009; Wang et al., 2009; Zhang et al., 2006). The obvious parallelism indicates that miRNAs may exert specific roles in self-renewal, proliferation, and differentiation of cancer cells including CSCs. In agreement, recent studies have shown involvement of several miRNAs in the regulation of CSCs. For example, the miR-200 family inhibits CSC functions in breast and pancreatic cancer. Functional inhibitory roles have been suggested for miR 125b, miR 183 and miR-34, whereas miR 30 and miR-17-19b may play essential roles in maintaining the stem-like features of cancer cells (Croce and Calin, 2005; Inui et al., 2010; Wang et al., 2009). Since there are a relatively large number of studies describing the altered expression of miRNAs in HNSCC (Liu et al., 2009; Tran et al., 2010), it is likely that in the near future the link between miRNAs and CSCs in HNSCC will find further experimental support.

## 7. Mouse models of HNSCC

The mouse models have proven indispensable in addressing the cellular origin of cancers. Two primary approaches have been used to tackle this question in HNSCC. One is based on the induction of squamous malignancies by the application of carcinogens to the skin, the so called two stage carcinogenesis protocol. The second is a more refined system using transgenic or conditionally targeted gene technologies to explore the effects of oncogenes and tumour suppressors in different cellular contexts.

### 7.1 The two-stage model of mouse skin chemical carcinogenesis

This system has proved of use in the understanding of the development of squamous tumors from a molecular point of view (DiGiovanni, 1992; Slaga et al., 1995; Yuspa, 1998). In this model, tumors progress through three sequential steps termed initiation, progression and conversion. Initiation is an irreversible and inheritable change that does not lead to phenotypic alterations; this can be achieve through the use of dimethylbenzanthracene (DMBA), which frequently induces mutations in the Ha-Ras oncogene (Quintanilla et al., 1986; Zhang et al., 1998). Promotion refers to the selection and expansion of the initiated population, giving rise to papillomas; this step is typically induced by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Some of these papillomas proceed through the conversion phase, forming malignant squamous cell carcinomas (DiGiovanni, 1992; Slaga et al., 1995; Yuspa, 1998). Interestingly, this model displays some parallelisms with certain human tumors, including head and neck cancer (Amornphimoltham et al., 2008; Amornphimoltham et al., 2004; Segrelles et al., 2006). Such parallelism stands for more than a similar pathology or histology. In this system there are two main players that are also essential actors in the HNSCC scenario: Akt (Segrelles et al., 2006; Segrelles et al., 2002 Molinolo et al., 2009) and Stat3 (Kim et al., 2007; Sano et al., 2008). In agreement, deregulated Akt activity in transgenic mice leads to heightened sensitivity to this experimental carcinogenesis protocol (Segrelles et al., 2007). Accordingly tumors generated through injection of Akt-transfected, papilloma derived PB keratinocytes display many molecular alterations similar to those found in human HNSCC (Segrelles et al., 2006). The two stage chemical carcinogenesis model has allowed the study of the possible role of adult stem cells in cancer development (Blanpain and Fuchs, 2006; Blanpain and Fuchs, 2009; Owens and Watt, 2003), thus allowing to important aspects of CSC. However, there might be intrinsic differences between the epidermal stem cells and oral stem cells. Of note these studies have allowed to propose mTOR as a suitable molecule for the treatment of HNSCC targeting possibly also CSCs (Amornphimoltham et al., 2008; Amornphimoltham et al., 2005; Castilho et al., 2009; Molinolo et al., 2007).

### 7.2 Genetically modified mice

The evolution of gene manipulation techniques in mouse has allow to express or ablate virtually any gene, including oncogenes and tumour suppressors, in a target tissue. The technical refinement also permits to generate such modification only in a small subsets of cells in a tissue in a time controlled manner, thus recapitulating what really happens in human sporadic tumors (Jonkers and Berns, 2002; Meuwissen et al., 2001). A number of mouse models have tried to recapitulate human HNSCC. The first report of a genetically engineered mouse model was the targeted expression of cyclin D1 transgene to the oral-esophageal epithelium (L2-cyclinD1 transgenic mice) (Nakagawa et al., 1997). These mice

exhibited dysplasia in the tongue, esophagus, and forestomach by 16 months of age but did not develop tumors (Nakagawa et al., 1997) unless they were crossbred with p53 heterozygous mice, which resulted in invasive oroesophageal cancer development in L2-cyclinD1/p53+/- mice (Opitz et al., 2002). More recently, using inducible systems, it has been reported that the activation of an oncogenic K-rasG12D allele in the oral cavity of the mouse induces oral tumor formation in mice, but these lesions were classified as benign squamous papillomas that progress to squamous malignancy upon activation a point mutated p53 gene (Caulin et al., 2007). In contrast, transgenic mice expressing the K-ras G12D oncogene under the control of tet-regulated responsive elements in the basal layer of stratified epithelia developed multiple lesions ranging from hyperplasias, papillomas, and dysplasias to metastatic carcinomas in the skin and oral mucosa. (Vitale-Cross et al., 2004). Finally, the TGFβRII deletion in combination with activation of either K-ras or H-ras in mouse head-and-neck epithelia caused HNSCC (Lu et al., 2006), in contrast with the targeted ablation of this gene in all stratified epithelia, which results in anal and genital SCCs (Guasch et al., 2007), indicating that progression to cancers occurs rapidly when the TGFβRII null epithelial tissues are exposed to activated oncogenes and/or loss of additional tumor suppressors. Nonetheless, although in many circumstances the transgene expression or gene ablation affect the stem cell compartment no careful analysis of this cell population or the possible involvement of CSCs have been addressed. Similar to the above mentioned models a transgenic mouse model expressing a constitutive active Akt1 kinase has been generated. These mice develop spontaneous tumors in various organs and display increased sensitivity to two stage skin carcinogenesis protocols (Segrelles et al., 2007). Remarkably, these mice also display altered development of several ectodermal organs and disturbed homeostasis of the epidermal stem cells (Segrelles et al., 2008). In oral tissues, the lesions that these mice develop rarely progress to overt tumors due to induction of premature senescence (Moral et al., 2009a). Remarkably genomic analyses in these tissues and in primary cells have been used to validate the upregulation of the Kruppel-like factor 4 (Klf4/Gklf/Ezf) as a potential biomarker for HNSCC (Moral et al., 2009b), in agreement with the elevated Klf4 levels found during the early stages of oral squamous-cell carcinomas development (Foster et al., 1999) and the induction of squamous epithelial dysplasia by the ectopic Klf4 expression in transgenic mice (Foster et al., 2005). Importantly, the ablation of the tumor suppressor Trp53 in the same cells that express the active Akt kinase (or the ablation of PTEN and p53 genes in these cells) allowed progression of the tumors to squamous cell carcinomas that also showed locoregional and distant metastasis, which can be followed by imaging in vivo, and display alterations in most of the relevant signaling pathways found in human HNSCC (Moral et al., 2009a). Interestingly, both in the tumors in vivo and in cell lines derived form these mice, the expression of putative CSC markers (CD44, CD133) was noticed along with other stem cell markers (CD34, K15 and ΔNp63) (Moral et al., 2009a), thus suggesting that this model can be highly valuable for the experimental analysis and characterization of CSCs in HNSCC.

## 8. Conclusion

Overall, although the way leading to the identification of the potential CSCs in HNSCC is still long, the achieved findings so far are very promising. Identification of the cell of origin in these tumors may allow to the genetic analyses of the lesions involved in tumor initiation and progression, thus becoming a unique platform for the identification of early disease

biomarkers. In addition, it may have important implications for new preventive therapeutic approaches to suppress or reverse the initial phase of disease. On the other hand, the identification of CSCs will have a tremendous relevance in the therapy of these tumors. In this regard, the available mouse models, and cell lines derived from them, will turn into essential tools in uncovering the cellular origins of cancer and the impact of specific mutations on tumorigenesis. However, a major disadvantage coming form the inherent different systems, human and mouse, makes essential exhaustive validation. Since this is particularly achievable using functional and comparative genomics, this potential problem can also become a benefit as may allow to the identification of genetic or epigenetic changes common in both systems that may permit the identification of suitable targets of potential therapeutic benefit.

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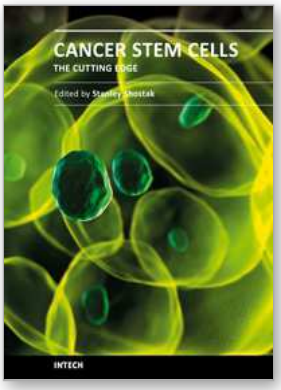


**Cancer Stem Cells - The Cutting Edge**

Edited by Prof. Stanley Shostak

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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.

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