Cancer Stem Cells in Oral Carcinoma

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Core Message

A new paradigm has recently been developed concerning the etiology of oral cancer. It proposes that solely stem cells, a small proportion of cells, are able to be transformed into a tumor, maintain its growth, and contribute to metastatic spread. This new perspective on oral oncogenesis may have major repercussions for the treatment of oral squamous cell carcinomas. This chapter reviews the most important concepts related to stem cells and cancer stem cells, reporting information on their physiology, detection, and implications for tumor onset and therapeutic targeting.

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30.1 Introduction

In accordance with the clonal evolution model of oncogenesis, malignancy arises as the result of the cumulative addition of changes in genes or related with epigenetic aggression that can haphazardly disturb any cell in the oral epithelium, producing a progeny with growth gains that ultimately obtains the capacity for invasion. Nevertheless, there is growing evidence for the notion that not all cells in the epithelium have the ability to create a cancer and, on the contrary, only cells with prolonged survival, such as stem cells (SCs), can suffer the cumulative tumorigenic changes essential for oncogenesis. In this novel paradigm of oncogenesis, the malignant SCs, known as cancer stem cells (CSCs), would be responsible for the origin, continued growth, and distant spread of the cancer. So, the reduced survival for oral cancer might be due to the wrong choice of which cells to target during treatment because current oral cancer treatments usually target the total bulk of cancer cells. Cancer stem cells also appear to be resistant to radiotherapy and chemotherapy through protective mechanisms [1, 2]. This chapter offers a review of SCs and CSCs in relation to oral squamous cell carcinoma (OSCC), focusing on the anticancer defensive mechanisms of healthy SCs in relation to their proliferative patterns, as well as approaches for differentiating between healthy cells and cancer stem cells, and reviewing the current therapeutic strategies for targeting of CSCs.

Definition

Cancer stem cells are defined as a scant group of tumor cells that can self-renew and generate a phenocopy of the original tumor, being progenitors of tumor bulk cells and driving tumorigenesis.

30.2 What Are Stem Cells?

The central features that define SCs are their capacity to perpetuate themselves – self-renewal – and their ability to generate distinct cell types necessary for the formation of organs [3, 4]. SCs can be categorized as embryonic or adult [5–7]. A final category of SCs, known as CSCs, has now been recognized [4]. CSCs are capable of both self-renewal and of producing diverse cancer cell groups via abnormal differentiation.

30.2.1 The Physiological Pattern of SC Proliferation

SCs are a proportionally small subtype of all the cells in the epithelium of the oral mucosa and show reduced division activity compared to proliferating non-stem cells [8]. The normal way SCs reproduce is known as asymmetric division, a pattern that generates an amplifying transitory cell (ATC) and the persistence of the SC. ATCs proliferate intensively with 3–5 divisions and finally develop a population in terminal differentiation. This is a hierarchical model in which the renewal of the oral epithelium is consequent to the low proliferation of SCs and it is considered as a protective mechanism for these persistent cells (Fig. 30.1) [9–11].

Important

CSC paradigm proposes that only stem cells, a small cell population, are able to originate an oral tumor, maintain its growth, and contribute to metastatic spread.

30.3 Cancer Stem Cells

CSCs are responsible for tumor progression. A further key characteristic of CSCs is their resistance to the usual anticancer treatments. The notion of oral tumorigenesis based on the aberrant function of CSCs came from the diverse and heterogeneous appearance of oral cancers. Oral malignant cells are heterogeneous in pathological, molecular, and proliferative terms (Fig. 30.2) as well as having the ability to generate new malignancies [4, 12–18]. The CSC model is also supported by similarities between well-differentiated oral neoplasms and the healthy epithelium of origin (Fig. 30.3). So, a well-differentiated tumor could mimic both the structural and proliferative appearance of the oral epithelium. Well-differentiated cancer nests are characteristically structured in three compartments just as in the healthy epithelium. These are the basal CSC, ATC, and the inner differentiated cell layers. This replica of the proliferative hierarchy of healthy oral epithelium is more proof that epithelial replication is sustained by a sole kind of cancer cell, i.e., the CSC. Bonnet and Dick [19] were the first to suggest that a reduced subset of cancer cells is active in human acute myeloid leukemia (AML) in CD34+/CD38− cells. The authors reported that the AML is organized as a pyramid of cells that came from a primitive hematopoietic cell. The same was found in other malignancies like breast carcinoma [20] and HNSCC/OSCC where CD44+ cells were found to be capable of oral carcinogenesis [7].

30.3.1 The CSC Hypothesis of Oral Carcinogenesis

Two different models attempt to explain the growth and heterogeneity of malignant tissues: the stochastic or clonal evo-

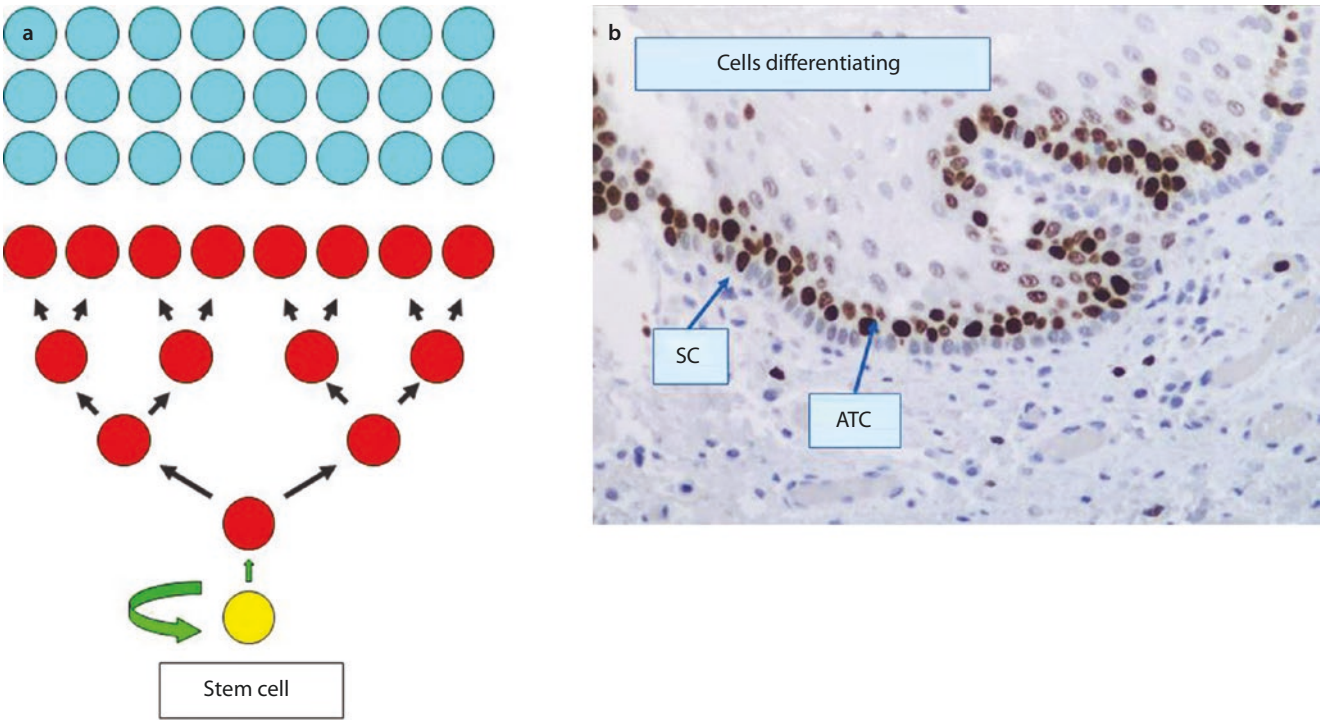

Fig. 30.1 **a** Schematic representation of the physiological asymmetric proliferation pattern of normal oral epithelial stem cells (SCs). At each division, a SC persists in the basal layer and gives rise to an amplifying transitory cell (ATC). ATCs in parabasal layers are able to proliferate rapidly for two or three cycles. The ATC population then loses its proliferative capacity and starts a process of terminal differentiation followed by desquamation. **b** The asymmetric proliferation pattern can be observed in this ki-67 staining of oral epithelium, showing scant proliferating basal cells, presumably SCs, numerous proliferating parabasal cells, ATCs, and quiescent superficial cells in terminal differentiation phase

lution model and the CSC model. In the first model, malignant transformation arises from a haphazard mutation that may occur in any cell leading to clonal development of mutated progeny with increased proliferation, genomic instability, and the gradual saturation of the tissue with more aberrant groups of daughter cells [1, 21, 22]. In the CSC model, carcinogenesis only arises from CSCs [19, 20, 23–33].

30.3.2 Source of CSCs

If SCs are the origin of CSCs, they need to have potent preventive mechanisms to decrease the chance of malignant transformation. The central way is probably through a physiological asymmetric proliferation of SCs, which generate ATCs whose function is to renew the epithelium and, ultimately, desquamate. This way the oral epithelium avoids cumulative genetic damage before the first carcinogenic event. This physiological pattern of division implies furthermore a small ratio of SC proliferation, decreasing the hazard of oncogenic changes at each division. The selective DNA segregation in the normal proliferation of the SC, giving the new DNA strand to ATCs, allows the epithelium to eliminate cells that receive the modified or mutated DNA. This mechanism also appears, for example, in the SCs of the bowel or breast [34, 35]. Lastly, the DNA of healthy SCs should be extremely stable, preserving their DNA restoration mechanisms [34].

In addition to the abovementioned source of CSCs, there should be other sources of CSCs. For example, a CSC could appear as the result of the intimate union between a HSC and a mature epithelial cell (Fig. 30.4a) [36], which may lead to a cell with genomic instability and at risk of having had summative carcinogenic events. Another source of CSCs could be from the union between a HSC and a mutated epithelial cell, developing into a premalignant cell with SC characteristics and able to acquire new carcinogenic events [37, 38]. A CSC could also derive from the abnormal evolution of a differentiated cell (Fig. 30.4b) through the effect of carcinogenic damage and then this mature cell would regain its capacity for self-renewal losing the ability for terminal differentiation [39]. Further carcinogenic assaults may lead to malignant transformation [40]. At the cellular level a process of reprogramming is needed to acquire SC capabilities [41]. There are five important transcription factors involved in different stages of the reprogramming related with oncogenesis: c-Myc [42, 43], OCT-4, Sox-2, Klf-4, and 4YTF [41].

Researchers have demonstrated epithelial-mesenchymal transition (EMT) – the cellular process relating to the acquisition of mesenchymal attributes in epithelial cells – as a mechanism of achieving a CSC-like state that allows for the invasiveness of such a cell. The Snail, Twist 1, and ZEB 1 transcription factors are involved in the acquisition of an EMT phenotype [44–47] which mainly downregulate E-cadherin expression [48].

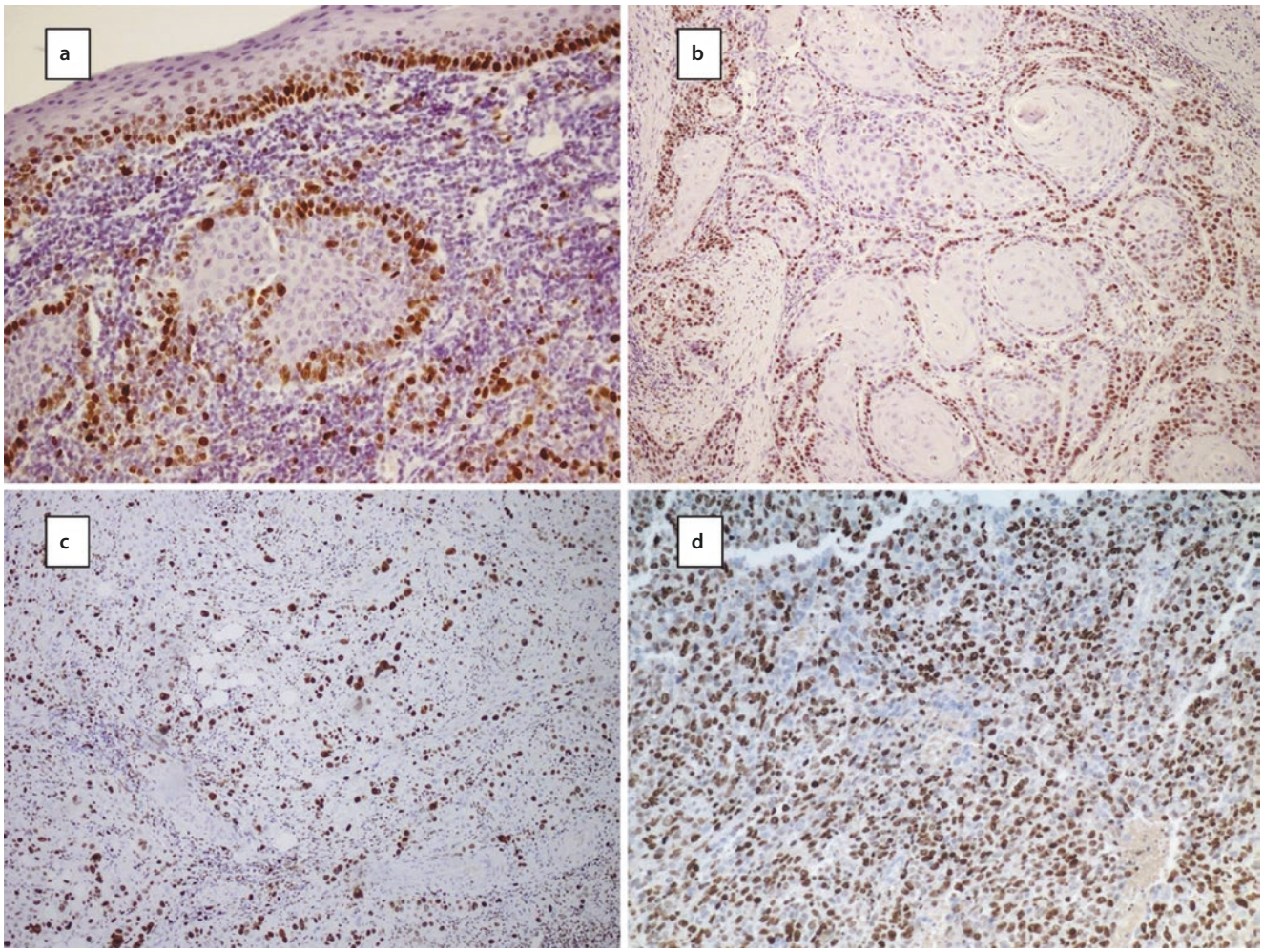

Fig. 30.2 Expression of proliferation marker ki-67 in OSCC and in adjacent non-tumor tissue. Distinct proliferative patterns can be observed in different tumors, with ki-67 expression in peripheral layers of well-differentiated tumor nests **a**, **b** or anarchic expression **c**, **d**

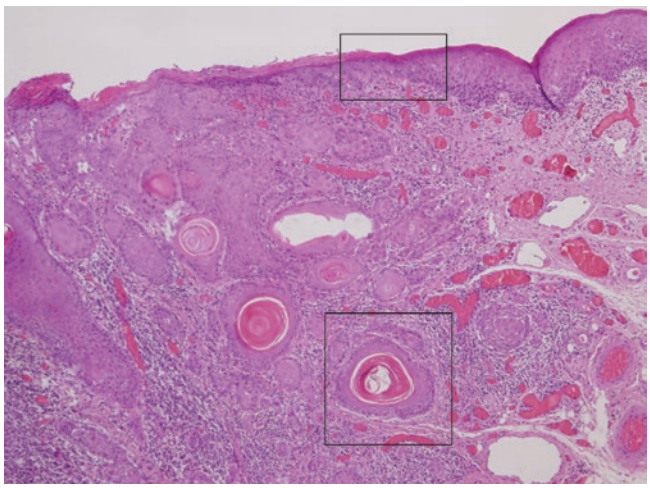

Fig. 30.3 Well-differentiated nests are usually organized in three compartments as in normal epithelium: basal CSC compartment, ATC compartment, and innermost differentiated cell compartment. This reproduction suggests that tumor growth is maintained by a single type of tumor cell, i.e., the CSC

Recently published research derived from the study of proliferative patterns of the oral epithelium [49] has suggested a different source for CSCs/premalignant SCs [50, 51]. The similarities in the structural organization of the basal cell layer when comparing healthy oral epithelium, precancerous epithelium, and well-differentiated oral cancer nests reflect a change from the physiological process of asymmetric division to a symmetrical proliferative pattern. In this symmetrical pattern the CSCs do not produce an ATC and a CSC but rather give rise to two CSCs located in the basal layer. This is seen in both premalignant epithelium and in the peripheral layer of well-differentiated nests. Proliferation of premalignant SCs would overcome the ability of the basal space to contain them, generating progressive parabasal layers encroached by premalignant SCs (Fig. 30.3).

30.4 Identification of Stem Cells and CSCs

The lack of specific markers for recognizing SCs restricts our understanding of their role in the genesis of cancer. The only

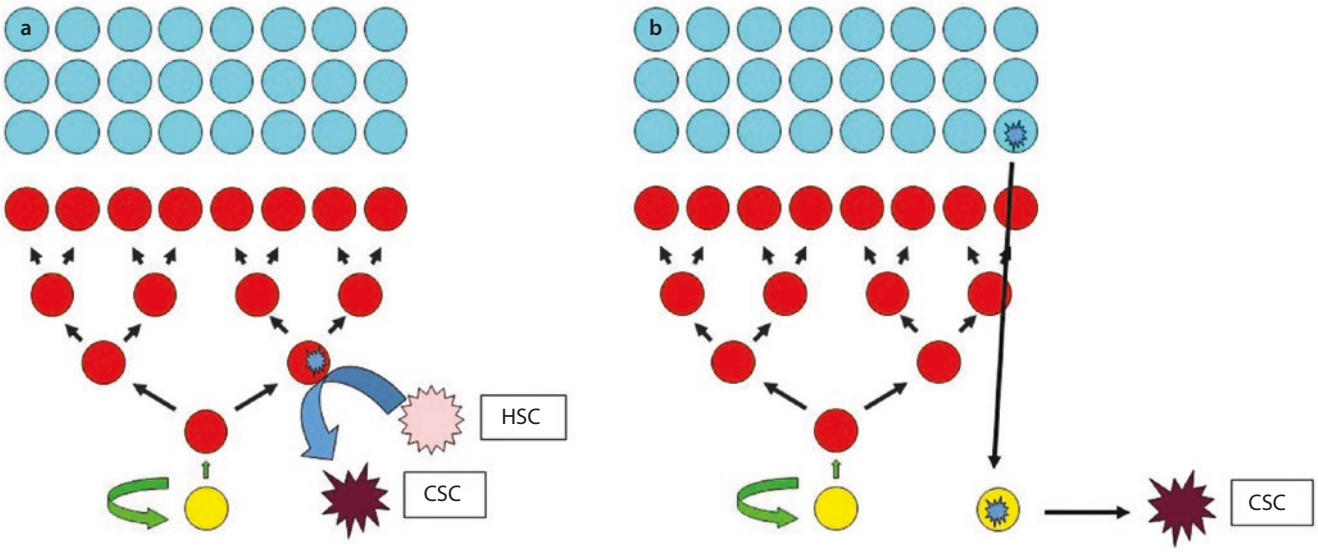

Fig. 30.4 **a** CSC could originate from the fusion of an HSC with a differentiated epithelial cell, which would acquire the self-renewal property of the HSC; this process could create genomic instability and promote the accumulation of new oncogenic events. Another possibility is the fusion of an HSC with a mutated epithelial somatic cell, which gives rise to a mutant cell with SC features that could then accumulate further oncogenic events. **b** A CSC might also result from the dedifferentiation of a mature cell. As a result of oncogenic events in mature epithelial cells, they can retrieve their ability to self-renew and lose their terminal differentiation capacity. These cells may acquire additional mutations, leading them to transform. Differentiated cells must be reprogrammed to acquire SC features

recognized approaches have been the study of their proliferative behavior in vitro and the recognition of long-surviving cells in tissues [11].

In vitro clonal trials have revealed that epithelial cells in low-density cultures produce diverse kinds of colonies in relation to the characteristics of their predecessors, essentially holoclones [52], paraclones, and meroclones. Cells producing holoclones are categorized as SCs, while cells that generate paraclones are ATCs. This clonal assay offers a strong technique for SCs and CSCs recognizing and typifying their reaction to drug treatments [11]. Flow cytometry using surface markers (β-1 integrin, α-6 integrin, CD71, E-cadherin, β-catenin, CD44), expressed in holoclones, is another method for identifying CSCs [53]. The best technique for recognizing CSCs in culture is to use flow cytometry to detect cells with the capacity to eliminate the DNA dye Hoechst 33342 [54], selecting cells able to maintain the dye and a characteristic minor non-dyed residents cells (side population, SP), that express SC markers. The capability of the SP to expel Hoechst 33342 dye is conferred by the action of the large family of ABC cell transporters. Human ABCG2, a member of the ABC gene family, is recognized as a CSC marker [55]. In head and neck carcinomas, SP is extremely carcinogenic [54, 56] and expresses SC markers such as ABCG2 [56].

Unfortunately, the processes described are incompatible for routine use and don't allow for the topographic location of SCs in normal or malignant tissue to evaluate their replicative activity or the relationships with their daughter cells. The following markers display some potential for identifying SCs: *β-1 integrin*, a protein probably needed for preserving epithelial cells in an immature state [57–59]. *Oct3/4* [60, 61], *Sox* [62], and *Nanog* [63, 64] *are considered to be transcription factors* and are critical in preserving self-renewal in embryonic and adult SCs [65–67]. *Oct3/4* is known as one of the preeminent markers of stemness activity [68, 69]. These transcription factors can be found in head and neck cancer tissue [70]. Studies on *CD 133* [71] in oral cancer have reported that a small rate of cancer cells (1–3%) are positive for this marker, although they are intensively clonogenic, and carcinogenic, with chemotherapy resistance [71]. *CD44* [72, 73] *anchors* MMP-9, indispensable for metalloproteinase action, which may promote cancer invasiveness. The first CSC marker applied in breast cancer was CD44, and Prince et al. [32] described that a subset of CSC-enriched CD44+ cells in head and neck cancer might be successively passaged in vivo, replicating the cancer of origin. These cells had elevated Bmi-1 expression levels as well as self-renewal and differentiation properties. Nevertheless, queries have been raised about the significance of CD44 as a CSC marker in oral cancer (Fig. 30.5), since it is expressed by a considerably higher amount of cancer and normal cells in oral epithelium [74–77] versus the amount of CSCs considered existing in oral tissues. Contradictory findings have also been published on the value of CD44 as a marker of OSCC progression and prognosis, with some authors associating the elevation of CD44 with greater cancer aggressiveness [78–81] and others associating its reduction or loss with a poor prognosis [74, 77, 82–85]. Some studies in head and neck cancer have presented cells expressing ALDH CSCs, particularly in view of their increased carcinogenic capacity [77, 86, 87]. *E-cadherin* fixes to actin in the cell skeleton via essential relations with caten-

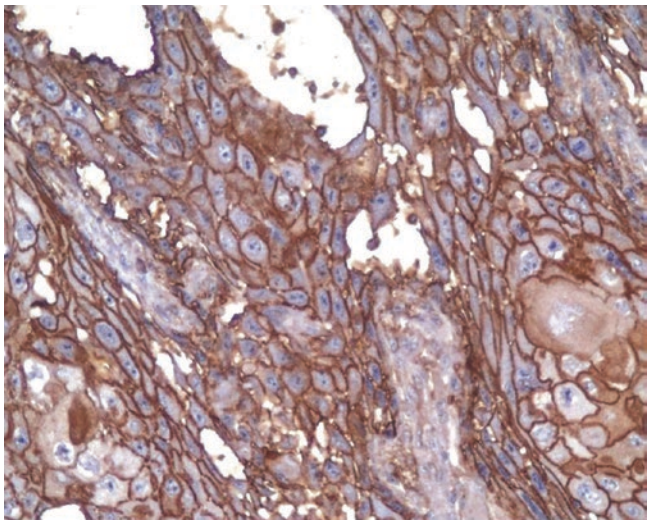

Fig. 30.5 CD44 expression in a high percentage of tumor cells from a patient with OSCC. A large number of OSCCs show extensive expression of CD44, calling into question the usefulness of this adhesion molecule as a reliable marker of CSCs, theoretically a very small cell population

ins [88]. E-cadherin is a suppressor of invasiveness [89], and it is a prognostic factor for a worse evolution when it is down-expressed [90]. The loss of E-cadherin is a critical step for epithelial-mesenchymal transition and confers SC-like characteristics to cancer cells [91].

30.5 Cancer Stem Cell Niche

A specific environment has been shown to control normal stem cells and CSCs [92]. This CSC environment facilitates the division of CSCs, allowing them to give rise to progenitor daughter cells while self-renewing and preserving CSCs in a primitive developmental state. The cells of the microenvironment (stromal and vascular) have the ability to stimulate signaling that might support the survival of cancer stem cells. The niche protects cells from genotoxic damage and promotes their radioresistance. Myofibroblastic cells expressing HGF (hepatocyte growth factor) activate the Wnt cascade of signaling, influencing the dedifferentiation of malignant cells to return to cancer stem cell behaviors. Consequently, it appears that the microenvironment influences the dynamism of the malignant tissue [93, 94]. The vascular endothelium of head and neck cancers has been revealed to be significant in the advancement of these tumors. Both interleukin-6 and EGF are produced by cells of the endothelium and it is known to promote stemness characteristics in malignant cells of the surrounding perivascular niche, favoring motility and evading anoikis [95, 96].

Tumor environments could likewise offer a state of hypoxia that promotes quiescence in cancer stem cells and favors, during radiotherapy, resistance to oxidative stress [97].

30.6 Radioresistance

SCs have a natural advantage in resisting DNA damage produced during radiotherapy [98]. Cancer stem cells, in a similar way to healthy SCs, are likewise resistant to radiation. They can avoid death from radiation through a variety of mechanisms, such as effective means for DNA repair, elevated free radical scavenging, and the stimulation of signaling pathways, among others [99–101].

DNA repair subsequent to radiotherapy can be measured through histone phosphorylation. In this sense, γH2AX may be considered as a measure of radiotherapy toxicity [102]. A study by Zhang et al. [103] establish that after 48 hours of irradiation, cancer stem cells presented significantly fewer γH2AX foci in comparison to other kinds of cells, thus indicating a prominent ability for DNA repair in the clonogenic population compared to other cancer cells. Moreover, stimulation of kinases Chk1 and Chk2, which stop the advance of the cell cycle, allows for additional effective DNA repair in CSCs and helps them to avoid cellular damage [14, 99, 104, 105]. This capacity was show to be reversed in experimental trials when precise inhibitors of kinases were tested in order to block this regulatory function of the cell cycle [106].

Autophagy is a cellular mechanism whereby healthy cells exposed to stress might catabolize their contents and use the substrates for biosynthesis or energy. This procedure could also be used by cells to remove toxins and pathogens [107]. Lomonaco et al. [108] established that radiation could induce the autophagy mechanism to a greater degree in $CD133^+$ cells compared to CD133- cells, indicating that the use of autophagy could confer radioresistance and be used for cellular protection and reparation.

CSC radioresistance could produce radiotherapy failure. Although the cancer bulk can be diminished after radiation therapy, residual cancer stem cells might persist through their capacity to resist the effects of radiotherapy [92].

30.7 Therapeutic Targeting of CSCs: A New Approach to Cancer Treatment

According to the CSC hypothesis, the only effective way to treat cancer is the eradication of CSCs. This has striking implications for the therapeutic approach to cancer. It appears that the recurrence of OSCC cannot be prevented by reducing tumor volume alone. The sensitivity of HNSCC/OSCC cells to cisplatin was increased by CD44 knockdown, and the efficacy of radiotherapy in nude mice transplanted with ALDH1+ CSCs was enhanced by the knockdown of Bmi-1, a stem-cell-related gene [109]. Chen et al. [110] studied the role of SNAI1 in cancer cell growth and described its metastatic potential in different malignancies [111]. They found [110] that ALDH1 expression was reduced, CSC-like characteristic were inhibited, and carcinogenesis in CD44+/ALDH1+ cells was decreased by the endogenous co-

expression of ALDH1+ and Snail. Snail is a big family of zinc-finger transcription factors that participate in embryonic EMT control. It is important for maintaining CSC properties via EMT regulation and might be useful in the treatment of HNSCC/OSCC. Increased research on resistance mechanisms in HNSCC/OSCC CSCs is warranted to develop therapeutic approaches that might prevent metastasis development and recurrence.

30.7.1 Targeting Stem Cell Niches

The CSC microenvironment may play an important role in the radioresistance of CSCs. Targeting of perivascular CSC niches in HNSCC is supported by reports that the exact ablation of tumor-associated endothelial cells with an inducible Caspase-9 reduced the fraction of cancer stem cells in HNSCCs in an SCID mouse model of human tumor angiogenesis [87]. Therefore, antiangiogenic drugs such as bevacizumab may be useful in the treatment of head and neck and oral cancers by reducing the proportion of HNSCC/OSCC CSCs (see ► Chapter 27). It is possible that the dependence of cancer stem cells and vessel endothelia can be used to decrease the risk of head and neck and oral cancer reappearance and metastasis [112–121].

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It appears reasonable to consider that novel approaches to oral cancer should be targeted against CSCs, requiring the development of strategies to identify and elucidate the molecular pathways that maintain the SC state.

30.7.2 EMT and Targeting Pathways

EMT permits a polarized epithelial cell to take on a mesenchymal appearance and increased capacity for motility and invasion. EMT due to crosstalk between OSCC cells and other cells in the cancer environment can increase the motility of tumor cells and give them SC characteristics. The post-EMT invasive phenotype allows cells to penetrate the lymphatic and/or angiogenic vasculature, and a possible therapeutic strategy in OSCC may be to block EMT by inhibiting the crosstalk between tumor and stromal cells. The targeting of signaling pathways involved in EMT development may be useful to treat HNSCC/OSCC, and clinical trials are under way on the efficacy of Wnt/beta-catenin pathway inhibitors against various cancers [122–124]. Numerous molecules that target the Wnt pathway are under study, and ongoing phase 1 trials are focused against Wnt/receptor interactions and cytosolic and nuclear signaling [125, 126]. The JAK/STAT pathway is also under investigation in HNSCC, with some promising results. The combination of radiotherapy with a STAT3 inhibitor suppressed tumorigenesis and improved survival in CD44 + ALDH1+ HNSCC transplanted immunodeficient mice [79]. Drugs have been developed against other pathways involved in CSC development, including Notch or Hedgehog, but problems have arisen regarding the preservation of normal stem cells from their effects. In nasopharyngeal carcinomas, E-cadherin repressor ZEB2 targets the beta-catenin signaling pathway by using miR200a and induces stemlike characteristics, i.e., a CD133+ side population, the formation of spheres with increased Oct4 and ALDH expression, and carcinogenicity in vivo [127]. Finally, TrκB is a 145-KDa receptor tyrosine kinase implicated in EMT and the invasiveness of HNSCC cells, and its regulation was found to inhibit tumor growth [128].

Warning

There are no procedures that are suitable for routine application that permit SCs to be topographically localized in healthy or tumor tissue for assessment of their proliferative activity or spatial relationships with their progeny.

30.7.3 CSCs and Immunotherapy

CSC-targeted therapies in HNSCC/OSCC have also been directed against immune escape mechanisms of CSCs. Their antigen presentation machinery can be defective through the infraregulation of human leukocyte antigen (HLA) surface expression [114], and CSCs in a heterogeneous cancer might produce therapeutic failure and disease progression by escaping immunotherapy. Recently, a CD8-defined T-cell epitope of ALDH1, which is known to be a source of antigens, elicited a humoral immune response in head and neck cancer and was considered as a possible target [129]. ALDH1A1 peptide was found by Visus et al. to be an HLA-A2-restricted and naturally presented CD8+ T-cell-defined cancer antigen [129], and ALDH1 peptide-specific CD8+ T cells recognized HLA-A2+ HNSCC cell lines expressing ALDH1 but not a human fibroblast cell line. Liao et al. also reported that CSCs with the ALDH1 phenotype can be recognized and differentiated from non-CSC cells by the host immune system [115].

The development of antitumor vaccines is another promising approach to the targeting of CSCs, and significant progress has been achieved by targeting against the antigen ALDH1A1. For instance, ALDH1A1-specific cytotoxic T lymphocytes proved capable in vivo of destroying ALDH cells present in HLA-A2+ head and neck cancer cell lines and of exerting antitumor action in adoptive immunotherapy [116]. Duarte et al. [118] reported that an ALDH1-targeted vaccine markedly decreased tumor onset and volume in a rat colon carcinoma syngeneic model and that half of the vaccinated animals were resistant to cancer progress, with a 99.5% decrease in cancer bulk versus controls. These studies not only amplify our knowledge of the immune biology of CSCs but also demonstrate that vaccination targeting CSCs can eradicate H&N cancer stem cells, decrease cancer volume, and avoid cancer reappearance.

30.8 Conclusions

A wide field of future research has been opened up by the novel model of carcinogenesis centered exclusively on CSC action. There is a particular need to develop precise markers to recognize these cells in routine laboratory diagnosis. This would give additional accurate information as to the kinds of cells that produce a cancer, their tissue spreading abilities, the relations with their clonal populations, and the repercussions of their replicative behavior for the prognosis of cancer patients.

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Readings Especially Recommended

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