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REVIEW

Cancer stem cells in head and neck cancer

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Abstract: Cancer stem cells (CSCs), also called "cells that start the tumor," represent in themselves one of the most topical and controversial issues in the field of cancer research. Tumor stem cells are able to self-propagate in vitro (self-renewal), giving rise both to other tumor stem cells and most advanced cells in the line of differentiation (asymmetric division). A final characteristic is tumorigenicity, a fundamental property, which outlines the tumor stem cell as the only cell able to initiate the formation of a tumor when implanted in immune-deficient mice. The hypothesis of a hierarchical organization of tumor cells dates back more than 40 years, but only in 1997, thanks to the work of John Dick and Dominique Bonnet, was there the formal proof of such an organization in acute myeloid leukemia. Following this, many other research groups were able to isolate CSCs, by appropriate selection markers, in various malignancies, such as breast, brain, colon, pancreas, and liver cancers and in melanoma. To date, however, it is not possible to isolate stem cells from all types of neoplasia, particularly in solid tumors. From a therapeutic point of view, the concept of tumor stem cells implies a complete revision of conventional antineoplastic treatment. Conventional cytotoxic agents are designed to target actively proliferating cells. In the majority of cases, this is not sufficient to eliminate the CSCs, which thanks to their reduced proliferative activity and/or the presence of proteins capable of extruding chemotherapeutics from the cell are not targeted. Therefore, the theory of cancer stem cells can pose new paradigms in terms of cancer treatment. Potential approaches, even in the very early experimental stages, relate to the selective inhibition of pathways connected with self-renewal, or more specifically based on the presence of specific surface markers for selective cytotoxic agent vehicles. Finally, some research groups are trying to induce these cells to differentiate, thus making them easier to remove. For all these reasons, we have collected existing literature on head and neck cancer stem cells that correlate the biological characteristics of this subpopulation of cancer cells with the clinical behavior of tumors.

Keywords: head and neck cancer, cancer stem cells, tumor markers

Introduction

In the last 30 years, progress in the treatment of head and neck cancer has improved the quality of life of patients via the use of innovative surgical and endoscopic techniques that are aimed at the preservation of organ function, mainly in laryngeal tumors.[1–3] However, the survival of patients with advanced disease has not improved.[4,5] The main causes of death remain the recurrence of locoregional disease that is unresponsive to conventional treatments and distant metastases.[6–8] In addition, approximately 10% of patients in the early stage of disease have recurrence with unfavorable outcome.[9,10] Recently, the recurrence and lack of response to radiochemotherapy treatments of some tumors has been attributed to a small tumoral

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cell subpopulation termed cancer stem cells (CSCs). This cell subpopulation has been identified in several solid tumors, including head and neck cancer, and it shows certain characteristics that give it the ability to maintain the tumor population, metastasize, and be resistant to chemoradiotherapy. Thus, the CSC hypothesis was proposed as a hierarchical model of tumor origin.

History of the CSC theory

The idea that cancer can originate from a small population of cells with stem cell properties was proposed about 150 years ago by Francesco Durante in 1874. In "Nesso fisiopatologico tra la struttura dei nei materni e la genesi di alcuni tumori maligni [Nessus pathophysiological between the flaw structure of the mother and the genesis of some malignant tumors]," Durante explains why some aberrant epithelial or connective elements that remained inert for a long time take up highly tumultuous and abnormal activities.[11] His idea was that "aberrant embryonic stem cells remain in adult tissues and give rise to tumors." His theory was revived and popularized by the German pathologist Cohnheim, who lived during the same period (1839–1884).[12] The theory was revisited 90 years later by Till and McCulloch, and later by Pierce et al.[13,14]

However, Durante's scientific theory was redefined only in 2001 by Reya et al, as follows: "… a strict parallelism can be made between normal stem cells and cancer stem cells: tumors often originate from the transformation of normal stem cells, similar signals can adjust the self-regeneration in normal stem and in tumor cells, and tumor cells may include 'cancer stem cells,' rare cells with an indefinite regenerative potential that leads to tumor genesis."[15]

In 1997, Bonnet and Dijk were the first to isolate "cancer stem cells" in samples of acute myeloid leukemia. In their work, they speculated that myeloid leukemia originated by mutation of a normal stem cell according to a "hierarchical model."[16] Regarding solid tumors, in 2003 Al-Hajj et al first identified and isolated a population of cancer stem cells from breast cancer, showing that only a subset of them, which exhibited expression of the surface markers $CD44^{+}/CD24^{-/low}$, had tumorigenic capacity.[17]

Later, populations of cancer stem cells were identified and isolated in other solid tumors, such as brain, prostate, colorectal, pancreatic, and lung cancers.[18–22] In head and neck tumors, Prince et al first identified and isolated a cellular subpopulation expressing the surface marker CD44 that exhibited stem-like characteristics and was capable of reproducing when a tumor was implanted in immunosuppressed mice.[23]

Characteristics of CSCs

The basic characteristics that distinguish CSCs are: (1) promotion of tumorigenesis when they are transplanted into immunosuppressed mice; (2) possession of specific cell-surface markers that are not expressed by noncancer stem cells; (3) tumors that arise from CSCs include both tumorigenic and nontumorigenic cells (heterogeneity); and (4) capacity for self-renewal in seriated transplants over several generations.[19,20,24–27] These characteristics are derived from the intrinsic properties of CSCs, which reside in their ability to duplicate, differentiate, and control homeostasis.

Origin of CSCs

Two main hypotheses exist regarding the origin of CSCs: (1) origin from a somatic tissue cell that undergoes genetic mutations, becomes cancerous, and acquires stem characteristics; and (2) derivation from embryonic stem or adult cells as a result of genetic mutations. The first theory does not exclude the second, because the mode of onset may depend on the location of the origin of the tumor.

In squamous cell carcinoma of the oral cavity; for example, the most accepted theory is that the CSCs are derived from the processing of a somatic stem cell. This idea springs from the observation that the time of renewal of epithelial cells of the oral mucosa is about 14–24 days (which is insufficient to accumulate the genetic mutations required for processing). The only accredited hypothesis is that CSCs residing for a long time in the oral mucosa can accumulate sufficient mutations to produce carcinoma of the oral cavity over time.[28]

These new insights have led to a new theory to explain the onset of solid tumors that suggests a hierarchical model, as opposed to the known stochastic model.

The stochastic model

In 1976, Nowel proposed the stochastic model. According to this theory, tumors originate from a single cell, and tumor progression is derived from a more aggressive subpopulation selected within an original clone over time.[29] The concept of multistep progression foresaw the stochastic accumulation of numerous genetic mutations underlying the process of neoplastic transformation of solid tumors; it also justified the transition from precancerous to invasive carcinoma as a consequence of the progressive accumulation of genetic mutations, which ultimately determines the origin of a predominant clone and results in a selective advantage over other changed cell populations.[7,30–32]

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The hierarchical theory

The hierarchical theory hypothesizes that the tumor originates from embryonic stem cells or somatic cells (present in all tissues) undergoing mutations. These changed stem cells give rise to stem cells that are further altered. Unlike the previous theory, in the hierarchical model, during cell division, one of the two daughter cells retains the ability to replicate, whereas the other loses this capacity and differentiates. Differentiated CSCs represent the majority of the tumor; further mutations that alter the characteristics of the parent cells may intervene during the process of CSC duplication, giving rise to cells that are functionally different. Unlike the stochastic model, the hierarchical model considers that tumorigenicity resides in a small subpopulation of cells composing the tumor that retain the capacity of stemness (Figure 1).

Therefore, a tumor can be compared to an aberrant organ that is maintained in a manner similar to that of normal tissues. This body contains a small proportion of CSCs that feed tumor growth, give it the ability to resist radio- and chemotherapy, and promote local or distant metastasis. The remaining cellular components of the tumor represent the tumor mass formed by aberrantly differentiated cells that have lost the ability to replicate.[23]

During tumor progression, the CSC population can perform several tasks. Thus, the following CSC subpopulations can be distinguished: stationary CSCs, which remain incorporated in the epithelia, are not able to spread, are responsible for resistance to chemo- and radiotherapy, and serve to increase tumor volume; and movable CSCs, which are capable of migrating, are localized at the host–tumor interface, and are responsible for the ability to metastasize locoregionally and/or remotely. These specificities of CSCs give rise to two phenomena: niches and the epithelial–mesenchymal transition (EMT) process.

Niches

One of the main factors contributing to the maintenance of stem cells is a microenvironment called a niche.[33] Stem cells are stabilized in niches that are specific anatomical locations and help maintain an environment that supports the growth of stem cells.[34] The maintenance of the microenvironment is mediated by factors that are secreted from stem cells and from the extracellular matrix.[35] The niche protects CSCs via differentiation and apoptosis and maintains self-regeneration via cell–cell and cell–matrix interactions. Niches also play a fundamental role in resistance to chemo- and radiotherapy and contribute to the genetic instability of CSCs (Figure 2).

Epithelial–mesenchymal transition

The EMT process is a fundamental stage of embryogenesis.[36,37] During EMT, epithelial cells break cell–cell and cell–matrix connections and migrate elsewhere.[38] The aberrant activation of this physiological process is involved in various pathological conditions, such as fibrosis, inflammation, and cancer. During tumor progression, some CSCs undergo EMT and acquire the ability to infiltrate surrounding tissues and metastasize (Figure 3).[39]

It has been shown that non-EMT cells are unable to metastasize without the action of EMT cells, suggesting that the latter are required for invasiveness and metastasis. EMT occurs when the cells are dissociated from each other, lose the expression of epithelial markers and earn the expression of mesenchymal markers, and change their polarization and cytoskeletal structure to establish new cell–matrix interactions.[40] Once an epithelial cell assumes a mesenchymal appearance and reaches its destination, it can undergo the reverse process of mesenchymal–epithelial transition.[38]

The identification of biomarkers of stem cells with EMT characteristics may facilitate the development of chemotherapeutic agents targeting EMT CSCs. In head and neck tumors, the overexpression of tyrosine kinase receptor B corresponds to an altered expression of the molecular mediators of EMT, including the downregulation of E-cadherin and the upregulation of Twist (a transcription factor that regulates differentiation, adhesion, and proliferation).[41]

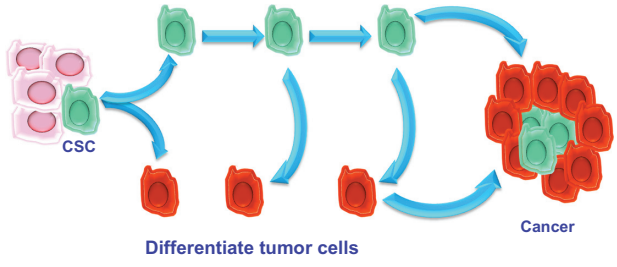

Figure 1 The hierarchical model.
Abbreviation: CSC, cancer stem cell.

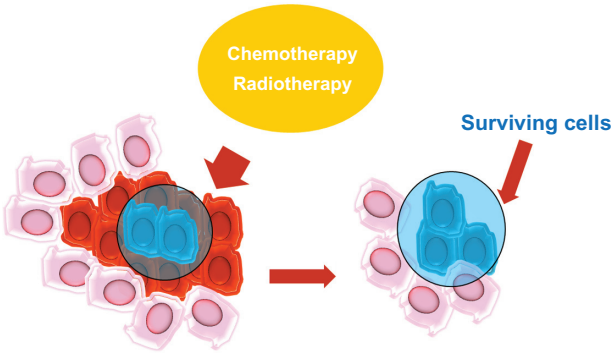

Figure 2 The niches.

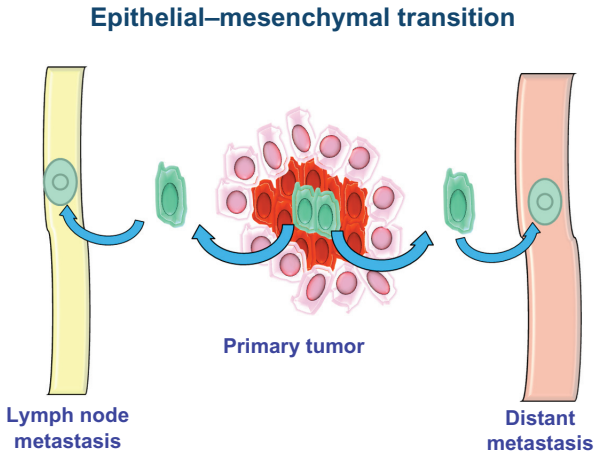

Figure 3 Epithelial–mesenchymal transition.

Chen et al have shown that aldehyde dehydrogenase 1$^+$ (ALDH1$^+$) CSCs exhibit upregulated levels of Snail (a transcriptional repressor of genes involved in EMT via E-cadherin) and Twist and show a significant increase in the expression of mesenchymal markers (characteristics of myofibroblasts).[42]

Methods for CSC identification and isolation in solid tumors

The methods used for the identification and isolation of tumor stem cell populations apply the same techniques used to identify normal stem cells from their differentiated progeny. Cancer stem cells can be identified via surface markers, determination of ALDH activity, ability to efflux vital dyes, and ability to form tumor spheres in vitro.

The subpopulation identified and isolated using these methods is then subjected to tests that prove their tumorigenic ability: quantitative assays of xenografts (which test tumorigenicity) and methods to assess self-renewal in vivo (which test self-renewal capacity).

Surface antigens

The identification and isolation of CSCs using surface markers is most commonly used for implantation into nonobese diabetic/severe combined immunodeficient mice to grow xenografts. The surface antigens used are the same that are used to identify normal stem cells. The surface antigens involved in solid tumors are CD133, CD44, and CD24.

CD133 is a transmembrane pentaspan glycoprotein localized on the protrusions of the cell membrane; its presence has been reported in various solid tumors, such as brain, prostate, colorectal, and lung cancers.[43–46] In head and neck tumors, CD133 has only been studied in cell lines. Cells with high expression of CD133 exhibit high clonogenicity, the ability to form spheres, and tumor and tumorigenic capacity in xenograft models compared with cells with low CD133.[47–49] However, no studies have used CD133$^+$ cells derived from primary head and neck cancer for quantitative assays of xenotransplantation.

CD44 is a surface glycoprotein that is involved in cell migration and adhesion. It is a known receptor of hyaluronic acid and interacts with other "ligands," such as matrix metalloproteases.[50,51] Prince et al first demonstrated that CD44 expression could be used to isolate a subpopulation with increased tumorigenicity in head and neck tumors. Those authors were able to demonstrate that a small proportion of CD44^{high} cells (<10%) that form a tumor can regenerate the tumor when transplanted in the side of immunosuppressed mice, whereas higher concentrations of CD44^{low} tumor cells are not able to reform a tumor.[23] However, the limitation of this study was that in two-thirds of the samples used, it was necessary to pass the cells initially in immunocompromised mice to have a sufficient number of tumor cells to isolate CSCs. This could have altered the expression patterns of native CSCs.

CD24 is a mucin adhesion molecule expressed by pre-B lymphocytes and neutrophils. Functionally, CD24 promotes metastasis, as it has been identified as a ligand of P-selectin, an adhesion receptor found on activated endothelial cells and platelets. Lim and Oh showed that the cytoplasmic expression of CD24 was associated with adenocarcinoma of the colon, stomach, bladder, and ovaries, whereas there is no evidence of this activity in head and neck cancer.[52]

ALDH activity

ALDH is an intracellular enzyme that is present normally in the liver. Its best-known functions are the retinol conversion to retinoic acid and the oxidation of toxic aldehyde metabolites, such as those formed during the alcohol metabolism and certain chemotherapeutic drugs (eg, cyclophosphamide and cisplatin).[53–55]

Clay et al have shown that a small percentage of ALDH^{high} tumor cells can produce new head and neck tumors when transplanted into immunosuppressed mice. The majority of isolated cells with ALDH^{high} also exhibited high expression of CD44.[56]

Side populations

Another strategy used to identify highly tumorigenic cellular subpopulations is based on the ability of these cells to efflux a fluorescent dye that binds to DNA. The cell populations isolated using this method are called side populations.

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Using this technique, side populations have been identified in normal tissues and in solid tumors.[57]

The dye used to isolate side populations is Hoechst 33342. Cells that are able to expel the dye, similar to certain chemotherapeutic drugs, express a group of transmembrane transporters, such as multidrug resistance transporter 1. They are involved in resistance to chemotherapy because of their ability to efflux the drug from the cell and prevent the action of the chemotherapeutic agent.[58] Cells of head and neck carcinoma were isolated using this method and exhibited increased clonogenicity and tumorigenicity in xenotransplantation.[59–60]

Formation of tumor spheres

CSCs grown in culture conditions without serum retain an undifferentiated state. The addition of growth factors guides them toward proliferation and formation of cell aggregates that are termed tumor spheres.

In oral cancer, tumor spheres derived from cell lines and from primary tumors show a high tumorigenic capacity in orthotropic xenografts. Okamoto et al reported that CSCs isolated from cell lines from carcinoma of the oral cavity were highly capable of forming spheres and expressed high levels of CD44.[61] Chiou et al studied two cell lines and primary tumors of the oral cavity and showed that the isolated CSCs had a high capacity to form tumor spheres and expressed high levels of CD133.[62] However, in a study on 43 primary tumors of the head and neck, Lim et al reported that only 6% (3/43) of the primary tumors formed spheres.[63]

Tumor progression and metastasis

The tumor stem cell subpopulations identified and isolated using the techniques described above have a demonstrated ability to regenerate a new tumor if replanted in vivo. The next phase of the study of these cells is the determination of the phenotypic characteristics of the isolated populations to understand the mechanisms that underlie the different behaviors of cancer stem cells in terms of ability to metastasize to regional lymph nodes, distant metastasis, and resistance to chemo- and radiotherapy treatments, which are factors that strongly influence survival in patients with head and neck tumors.

Clinical studies

Existing studies on the clinical significance and applicability of the CSC theory are mainly based on the clinical significance of the tumor expression of stem cell surface markers and on the determination of ALDH expression.

Regarding head and neck tumors, numerous papers have addressed the expression of CD44 and its isoforms. These studies started in the 1990s, before Prince et al demonstrated that the expression of CD44 in head and neck tumors was associated with a tumor subpopulation with stem characteristics. This is because of the known CD44 property as a glycoprotein involved in mechanisms of adhesion and cell migration; hence the hypothesis that the altered expression of this marker could be related to tumor invasiveness. Subsequently, the expression of CD44 in head and neck tumors was linked to subpopulations with characteristics of CSCs.[64]

Studies focused on case studies in different ear, nose, and throat (ENT) locations are clearly not comparable and are contrasting. Some authors argue that the expression of CD44 and its variants shows no significant differences in terms of intensity and percentage of positive cells between normal epithelia and invasive carcinoma, thus CD44 is not a marker of invasiveness, and even reduced CD44 expression appears to correlate with high invasiveness.[65–68]

In contrast, later papers showed that high CD44 expression seems to correlate with a greater ability for lymph-node metastasis, higher recurrence, resistance to radiotherapy, and poor prognosis.[69–71] In 2009, Wang et al discovered a high correlation between the expression of the CD44v3 isoform and lymph-node metastases and between the expression of the CD44v10 isoform and distant metastases and failure of radiotherapy.[70]

Papers addressing single sites also exhibit discrepancies; however, data obtained on the basis of the different biological behavior of head and neck tumors depending on the site of origin are clearer. Studies on head and neck tumors are limited by the fact that most of them group tumors from various ENT sites with different biological characteristics.

The evidence on squamous cell carcinomas of the oral cavity seems to indicate that low expression of CD44 correlates with a greater capacity of metastasis and recurrence, with negative or no significance on prognosis.[72–78]

Because studies related to the oropharynx are few, the discrepancy in the results reported becomes even more evident. Bloor et al and Carinci et al found no evidence of association between CD44 expression and prognostic significance, whereas Lindquist et al and Kokko et al found a correlation between high expression of CD44 and poor prognosis.[77–80] However, it must be noted that the number of patients evaluated in the latter two works is greater than that of previous studies.

Among the few clinical trials available for squamous cell carcinomas of the tongue, Fonseca et al reported a relationship

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between high CD44 expression and lymph-node metastasis; in contrast, Mostaan et al, Rodrigo et al, and Masuda et al described a correlation between low CD44 expression and propensity to metastasis and poor prognosis.[81–84]

Studies of squamous cell carcinomas of the hypopharynx and larynx are more numerous, which is probably related to the greater representation of this cancer among head and neck tumors. The high CD44 expression seems to correlate with a poor prognosis more prominently in laryngeal tumors than in the other ENT sites because of the higher metastatic capacity (locoregional and distant) and resistance to chemoradiation of cells with high CD44 expression.[77,85–91] Moreover, it seems increasingly clear that differences in the propensity to locoregional or distant metastasis and radiotherapy resistance appears to depend on the overexpression of specific variants of CD44.[90–94]

These results refer to studies performed in the mid-2000s, whereas the previous and even fewer studies reported data correlating a low CD44 expression with poor prognosis and a high risk of recurrence and metastasis.[95] These differences in reported data may depend on many factors, in addition to the site of origin: differences in the characteristics of the patients selected for these studies (age and tumor, node and metastasis system), different treatments, and different methods used.

Recently, the expression of CD44 was studied in saliva samples from patients with head and neck cancer. Emerging data suggest that CD44 may be a useful diagnostic marker.[96–99]

Only one study is available on the clinical significance of CD133 expression, the work of Lu et al,[91] in which the authors detected a correlation between the expression of CD44 and CD133 and lymph node metastases in supraglottic tumors; however, those authors believed that the expression of CD133 was not specific to CSCs because of a high percentage of positive cells (70%–85%) in each slide examined compared with the 5%–10% of cells that were positive for CD44.

Studies of ALDH

Studies on the clinical significance of ALDH expression in head and neck tumors are still very few, which may explain their strongly contrasting results. According to the study of Koukourakis et al, high ALDH expression corresponds to a favorable prognosis, whereas according to Xu et al, it corresponds to a poor prognosis.[71,100] However, this variation in the clinical significance of ALDH expression may depend on the expression of the B-cell-specific Moloney murine leukemia virus insertion site 1 (BMI1) protein. BMI1 controls the cell cycle and the regeneration of stem cells. The downregulation of p16-mediated BMI1 expression promotes the progression of the cell cycle. According to these studies, the suppression of BMI1 in $ALDH^+$ tumors appears to increase the response to radiation, whereas BMI1 overexpression in ALDH-positive tumors increases node metastasis.[101,102]

These data seem to agree with the results described in our recent report based on patients with laryngeal cancer, in whom high BMI expression correlated with metastatic capacity and BMI^{low} expression in association with absence of p16 expression seemed to select a subset of patients at high risk for lymph-node metastasis.[103,104]

Conclusion

Clinical studies on CSC characterization are still few and conflicting. Thus, the data derived from them are not sufficiently reliable for clinical application. To establish the clinical significance of the expression of stem cell markers, larger studies are necessary that involve each headquarter of ENT sites; further studies should verify the functional role of these markers via the analysis of their functionality.

CSC-specific markers may be used to identify specific subpopulations that are resistant to therapy and require more aggressive treatment strategies. In addition, a greater understanding of the microenvironmental factors that support niches and the knowledge of the intercellular mediators that underlie the EMT process may lead to the identification of new potential therapeutic targets.

Future fields of application:

- acknowledge time tumors that damage lymph-node metastases
- recognize tumors that exhibit distant metastasis
- identify mechanisms of radioresistance
- identify new molecules that reach niches.

The many questions that remain unanswered should stimulate further research to confirm or refute the data obtained to date. Both assumptions represent an achievement of research.

Disclosure

The authors report no conflicts of interest in this work.

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