Review Article

HEAD AND NECK CARCINOMA STEM CELLS. DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC TARGETS

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Abstract. *Head and neck carcinoma arise from the mucosal lining of the upper aerodigestive tract, affecting more than half a million people worldwide each year. Although cancer tissue has many heterogeneous cells with different phenotypes, only a subset of these cancer cells proliferate extensively and have the potential to give rise to all other tumor cells. They are named carcinoma stem cells (CSC). CSCs can persist in tumors and cause relapse and metastasis by producing a new tumor. Head and neck carcinoma stem cells (HNCSC) share a common CD44+ phenotype. What drives a normal stem cell into a malignant stem cell is insufficiently understood. Many regulating pathways were analyzed, such as:Receptor Tyrosine Kinases, Sonic Hedgehog, Notch, Wnt, and Bmi1. Also, the miRNA ratio or epigenetic alteration pattern is potential subject of further studies. This would enable better therapy and survival rate in HNSCC.*

Key words: *head and neck cancer, cancer stem cell, epigenetics, prognostic factor, therapy target.*

Introduction

Head and neck squamous cell carcinomas (HNSCC) represent biologically similar cancers originating from the mucosal surface of the upper aerodigestive tract, such as lip, oral cavity, nasal cavity, paranasal sinuses, pharynx, and larynx. Major risk factors are smoking and alcohol abuse. More than half a million people worldwide each year are affected [1].

Treatment of HNSCC includes surgery, radiotherapy, chemotherapy, and targeted agents. Advances in treatment strategies have improved quality of life and survival rates. A better understanding of the biology of HNSCC is needed for more effective therapies and better results. The introduction of the term "stem cell model of cancer" frorm recent studies should enable a better understanding of tumor pathogenesis [2].

Until recently, cancer was considered as a group of heterogeneous cells with different phenotypes, and the general possibility of extensive proliferation. The new model of cancer proposes that only a subset of these cancer cells can proliferate extensively. These cells are named cancer stem cells (CSC). Other cancer cells, known as "non - stem" cells, have only a limited proliferative potential. CSCs may form new tumors on transplantation, and may even persist in tumors and cause relapse and metastasis by giving rise to a new tumor [3].

The analogies between CSCs and "normal" stem cells were confirmed. Extensive proliferative potential and the ability to give rise to new (normal or abnormal) tissues are characteristic of both types of cells [3]. Both tumors and normal tissues have heterogeneous combinations of cells, different phenotypic characteristics, and different proliferative potentials. Most tumors have a clonal origin, so phenotypically diverse progeny is formed. Self-renewal and differentiation of normal stem cells are characteristics of CSCs [3].

Current therapy of HNSCC is aimed to decrease the size of the tumor, which is effective for a limited amount of time. However, CSCs are less sensitive to the current chemotherapy agents, and they remain virtually untouched. So, further research is directed to targeting mainly the CSC population within the tumor mass, which prevents tumor growth and eventually cures the patient.

Review of Literature

Evidence for HNCSCs

Extensive proliferation of a small subset of tumor cells was confirmed in multiple myeloma and leukemia. Mouse myeloma cells from mouse ascites formed colonies in only 1 in 10,000 up to 1 in 100 cancer cells [4]. Also, only a small proportion of human AML stem cells can transfer AML from human patients to immunocompromised nonobese diabetic or severe combined immunodeficient (NOD/SCID) mice [5].

Identification and confirmation of proliferation and differentiation abilities of CSC were recently investigated [6]. Implantation of specimens obtained from patients undergoing surgical resection under the skin of NOD/SCID mice produced mouse xenograft models of HNSCC. Also, CD44^{+} cells could form tumors, contrary to CD44^{-} cells. Only a small number of CD44^{+}Lin^{-} cells from a patient's tumor produced new tumors that were phenotypically diverse for CD44 expression. Additionally, CD44^{+} expression was confirmed in the basal layer and not in the well- differentiated cells in moderately to well- differentiated HNSCC. These tumors had cytologi-

cal and architectural features similar to normal squamous epithelium, with differentiation from a basal layer toward an apical layer, and the formation of keratin.

HNCSC Markers

Antigenic markers helped to isolate CSCs in various solid tumors, as demonstrated in various studies. For example, Human Brain Tumor Stem Cells have been confirmed using expression of the cell surface marker CD133 [8], while Prostate Cancer Stem Cells have a CD44+/A2B1^{hi}/CD133+ phenotype [9], and Pancreatic Stem Cells a CD44+CD24+ESA+ phenotype [10]. Cells with a specific cell-surface antigen profile (CD44-positive and CD24-negative) from patients with advanced metastatic breast cancer could become tumor xenografts [11]. Also, the experiments with immunodeficient mice and transplantation of cells under the skin provided an environment similar to that in HNSCC.

The purified CD44 positive cells could differentiate into cells similar to those found in the bulk tumor population [6]. Aldehyde Dehydrogenase (ALDH) expression as a potential functional marker for stem cells and CSCs was confirmed. The majority of HNSCC cells had low ALDH activity [12]. A majority of highly tumorigenic ALDH^{high} cells overlapped with the CD44+ population of cells, and only a small number of CD44+ cells expressed a high ALDH activity. It has been suggested that CD44+ cells contain a mixture of CSCs and non CSCs because some 5000 cells are needed to produce a tumor. So, probably the best way to identify CSCs in HNSCC is a CD44+/ALDH^{high} phenotype.

Side Population (SP) cell sorting represents another common method of identifying CSC. SP cells efflux Hoechst dye 33342 and contains differentiation and proliferation abilities of CSCs [13]. SP cells in a human laryngeal cancer cell line are consistent with cancer stem or stem-like cells [14]. Also, SP cells had higher proliferation rates, than those of non-SP cells. However, proliferating SP cells give rise to both SP and non-SP cells. High proliferation rates, differentiation capacity, serum-free growth, and sphere formation in vitro imply that SP cells have CSC characteristics [14].

Genetic and Epigenetic Alterations in HNSCSs

Cancer is a consequence of genetic and epigenetic alterations of normal tissue. Also, normal stem cells renew under the control of several signaling pathways. Mutation could lead to the unlimited and uncontrolled proliferative potential of cells. Wnt, Sonic Hedgehog, Notch, PTEN, Bmi1, and EGFR are identified pathways and genes for the proliferation of HNSCC.

Increased production of growth factors, overexpression of growth factor receptors on the cell membrane, and mutations in the receptor can produce abnormal cell signaling via the Receptor Tyrosine Kinases (RTK) causing proliferation, block of apoptosis, angiogenesis, and metastasis [15, 16].

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane receptor consisting of a family of 4 members: EGFR, HER2, HER3, and HER4. EGFR leads to intracellular phosphorylation and exposure of the catalytic cleft, activating different signaling pathways. EGFR is up-regulated in more than 90% of HNSCCs [17]. It is postulated that over-expression rather than mutation promotes HNSCC [19–21].

The Hedgehog signaling pathway is essential for the regulation of proliferation and differentiation of various types of stem cells during embryogenesis. There is an association between Sonic Hedgehog (SHH) and carcinogenesis [22, 23]. SHH overexpression was found in different malignant tumors, such as small cell lung carcinoma [24], medulloblastoma [25], and basal cell carcinoma [26]. Overexpression of SHH signaling was found in HNSCC after concurrent chemo radiation, just before the rise in tumor proliferation rates [27]. This further supports CSC model, with the remaining chemo radio resistant portion after the bulk of the tumor mass has been destroyed.

The Notch signaling pathway has four Notch receptors (Notch1–Notch4) and five structurally similar Notch ligands (Delta-like1, Delta-like3, Delta-like4, Jagged1, and Jagged2). Activation of the Notch pathway results in self-renewal of stem cells or differentiation along a particular lineage [28, 29].

The canonical Wnt signaling is initiated by engaging of Wnt ligand with its Frizzled receptor along with the LDL receptor family member, Lrp 5/6, causing the accumulation of β-catenin activating target genes. A mutation of the Wnt pathway is considered a cause of colorectal cancer, leukemia, and HNSCC [30, 31].

BMI1 or B-cell-specific Moloney murine leukemia virus insertion site 1 controls the cell cycle and self-renewal of tissue stem cells [32]. BMI1 influences the proliferation of many normal human stem cells, but is over-expressed in different malignancies, including HNSCC [33].

These pathways are related to each other. For example, secreted Shh influences the cell fate switch executed by Notch [34]. Hedgehog and Notch regulate normal development with a feedback loop, with deregulation leading to cancer. It is not clear whether the Hedgehog pathway and the Notch pathway can regulate stem cell self-renewal through downstream targets other than Bmi-1. This model could help understand regulation normal and malignant stem cell self-renewal [35].

Cancerogenesis is nowadays not exclusively connected to the alterations in pathways and genetic material. Indeed, many recent papers indicate that mechanisms other than changes in the DNA sequence can lead to malignant processes. Epigenetic alterations include DNA methylation, histone modifications, and nucleosome positioning. Global hypomethylation, specific CpG hypermethylation, overall miRNA downregulation, a global reduction of monoacetylated H4K16, global loss of active mark H3K4me3, silencing of tumor suppressors BRG1 and BRM by hypermethylation is the most fre-

quent types of epigenetic alterations [36]. In HNSCC it includes promoter hypermethylation of genes p16^{INK4a}, DAPK and MGMT [37].

Diagnostic and Prognostic Potential of HNCSCs

The improvement of the survival rate of HNSCC is slower than for other common carcinomas. Also, many patients with HNSCC present with locally advanced, stage III or IV disease that requires different therapy modality is mandatory for improvement of the results.

MicroRNAs (mina) are small, non-coding RNAs that can regulate gene expression and seem very important for the prognosis of HNSCC. They downregulate many of their target transcripts and the amount of protein encoded by these transcripts [38]. Few cancer-specific miRNA fingerprints have been identified in different types of cancer.

An abnormal expression of miRNA was confirmed in cancer cells, and also in premalignant stages. The examples are the reduced expression of miR-143 and miR-145 in colon adenomas [39] and reduced expression of miR-16-1 and miR-15s in pituitary adenomas [40]. Also, miR-221, highly overexpressed in, is also overexpressed in papillary thyroid tumors and in normal thyroid tissue adjacent to tumors, but not in normal thyroid tissue [41]. Clinical implications of such overexpression are significant for disease control. Indeed, the difference of miRNA expression in HNSCC tumor tissue compared with normal head and neck epithelia was found. Also, miRNA-21, miRNA-18a, miRNA-221 and miRNA-375 were differentially expressed in HNSCC. The ratio of miRNA-221:miRNA-375 had the strongest predictive ability to distinguish tumor from normal tissue with both high sensitivity (0, 92) and specificity (0, 93). This expression ratio can be applied to determine the potential of malignant alteration in precancerous lesions, or for screening for HNSCC in saliva or mouthwash [42].

A potential marker for prognosis and also a predictor of treatment response could be the different level of DNA methylation. DNA hypermethylation can be documented in the saliva of patients with HNSCC. Studies confirmed aberrant promoter DNA hypermethylation (p16, MGMT, and DAPK genes) in 56% of head and neck primary tumors, contrary to the control group. So, it seems possible to determine abnormal promoter hypermethylation in saliva DNA in HNSCC with earlier detection and better results of treatment of cancer [43].

Therapeutic targets of HNCSCs

The cancer stem cell theory implies that the failure in the treatment of HNSCC by chemoradiotherapy is a consequence of remaining small groups of cancer cells with stem potential, present after the removal of the bulk of the cancer tissue.

In the future more effective therapy modalities have to be found and implemented into clinical praxis; therapy that target cells with high differential and proliferative potential. Another area of interest is the microenvironment of CSCs. Their surrounding niches, extracellular matrix, and soluble factors are critical for the maintenance of cell steaminess. Also, CSC were found in the proximity of blood vessels [44], as well as in perinecrotic hypoxic microenvironment [45]. This further implies that the therapeutic target could be directed not only to cells, but also to the perivascular hypoxic environment, or the CSC niche.

CSCs resistance to chemo and radiotherapy is of utmost importance. It was confirmed that after irradiation or chemotherapy CSCs showed an up-regulation of specific "markers". The quiescence of CSCs, high expression of ABC drug pumps, enhanced resistance to oxidative DNA damage, and other factors could lead to the negative response to this treatment [46, 47].

The epithelial-mesenchymal transformation (EMT) could also cause cancer progression. As a consequence, epithelial cells` acquire traits typical for mesenchymal cells; cell-cell junctions dissociate and gain the ability to migrate. This process is essential for the invasion, progression, and metastasis of HNSCC. The interruption of EMT is a potential aim of treatment [48].

Elimination of CSCs through the targeting of specific markers is another important therapeutic modality. Possible targets in studies were: Wnt and Frizzled receptors, Sonic Hedgehog and Notch signaling. Also, the effects of antibodies to the extracellular domains of Wnt-1 and Wnt-10b were studied in HNSCC showed tumor growth, apoptosis, or elimination of tumor cells by complement, or antibody dependent cellular toxicity [49]. Increased chemo radioresistance of CSCs may be solved by targeting SHH pathway. One option is the administration of blocking antibody against SHH or PTCH-1, while application of SMOH inhibitors (cyclopamine), and the knockdown of GLI-1/GLI-2 with specific small interference RNA [50–52].

Further studies are mandatory to document the need to investigate the effectiveness of targeted therapy and clinical efficiency. The applicability of epigenetic therapy to solid tumors is yet to be confirmed [53].

Conclusion

The cancer stem cell model has proposed a new perspective in the process of cancerogenesis in HNSCC. It states that only some tumor cells have the ability to differentiate and proliferate extensively. Such cells can be confirmed the expression of surface molecules, such as CD44 in HNSCC, and by filtration of CSCs is Side Population cell sorting and ALDH activity. The transformation of a normal stem cell into malignant stem cell is a poorly understood mechanism. The potential regulating pathways include Receptor Tyrosine Kinases, Sonic Hedgehog, Notch, Wnt, and Bmi1. Epigenetic alterations, such as promoter hypermethylation of genes p16^{INK4a}, DAPK, and MGMT, are also an important factor. Better knowledge of these facts offers clinical implementation, and potential improval of the survival rate of HNSCC.

The microRNA expression ratio miRNA-221:miRNA-375, or the promoter hypermethylation pattern of p16, MGMT, and DAPK genes could enable the early detec-tion from saliva or mouthwash samples. Potential thera-peutic targets and blockage of a specific signaling path-way demand further investigation.

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