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Mini-Review

Markers of cancer stem cells in head and neck squamous cell carcinoma (HNSCC)

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ABSTRACT

This review is aimed at exploring the literature on the clinical manifestation and role of cancer stem cells, specific markers which provide improved and accurate detection of cancer stem cells, the influence of chemotherapy and radiotherapy on cancer stem cells that develop in head and neck squamous cell carcinoma and its resistance to these therapies. Cancer stem cells are a subpopulation of tumor cells that possess the ability for self-renewal, self-reestablishment, and often exhibit resistance to existing treatment methods. Disruption of the communication between cancer stem cells and endothelial cells offers treatment advantages, specifically in treatment methods that eradicate the vessel system around the tumor to disrupt the tumor niche. Due to their great similarity with normal stem cells, it is very necessary to establish effective and accurate identification methods for cancer stem cells in order to support prognosis and treatment methods. The molecular markers such as CD44, ALDH and CD133 have been recognized as effective molecular markers for head and neck squamous cell carcinoma. These molecular markers provide improved and accurate detection and identification methods for the characterization of cancer stem cells and provide advantages in designing target therapies.

KEYWORDS: stem cell, HNSCC, CD44, CD133, ALDH.

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) usually arises from the mucosal membrane of the respiratory and digestive tract, including the paranasal sinuses and nasal cavity, the pharynx region and hypopharynx, trachea, larynx, and the oral cavity [1-6]. HNSCC is considered to be the 6th most common cancer in the world. According to estimates of the American Cancer Society, 58,000 individuals developed head and neck cancers by the end of the year 2014 [1-6]. The severity is higher in industrialized countries due to increased carcinogen levels in the environment, along with increase in smoking, substance abuse and human papilloma virus infection [7]. The prognosis and diagnosis rates of HNSCC are very low due to its identification at very late stages of the disorder, due to its fast spread to local lymph nodes and high rates of metastasis to different parts of the body [6].

Some of the cancers in this group are outlined below.

1.1. Oral cavity cancer

The primary sites of cancer include lips, a major front section of tongue, the inside section of the cheek, gums, the floor of the mouth, the undersection of the tongue, the area of the gums and the bony palate. The common symptoms include the appearance of any sore or ulcer in the oral cavity that does not heal, appearance of lumps in the oral cavity, difficulty in swallowing and chewing, bleeding and appearance of red or white patches in the mouth [8-9].

Usual diagnostic procedures that are usually employed are ultrasound, MRI, and X-ray of the neck section, and fine needle aspiration if the lymph nodes in the neck area appear swollen or abnormal. Most of the methods employed are greatly dependent on the grade and stage of cancer [10]. Diagnosis in early stages can result in usage of less invasive methods of treatment which reduce the negative impact on the patient. Surgery is often the main treatment for oral cancer; during surgery the lymph nodes are also removed from the neck region to minimize the possibility of metastasis and recurrence of cancer in the neck region. Usually, tumors on the lip are treated with a surgical treatment called Mohs micrographic surgery (MMS); then the tumor is removed in thin layers until no cancerous cells are identified, which ensures that all the tumor cells have been surgically removed with minimal damage to rest of the healthy tissues. Radiation therapy in oral cavity cancer involves brachytherapy or internal radiation therapy, where the radioactive material is sealed in a pellet or capsule and implanted for a limited period, and external therapy, where the section is exposed to radiation. Treatment with cytotoxic drugs is hoped to restrict metastasis.

1.2. Nasopharyngeal cancer

The nasopharynx is a section of the throat located at the back of the nose. Types of nasopharyngeal cancer include basaloid squamous cell carcinoma, squamous cell carcinoma, and non-keratinising carcinoma; one of the prevalent risk factors is the Epstein-Barr virus which causes glandular fever. The usual symptoms include blocked nose, earache, leakage of fluid from ear and nose, headache and impairment in hearing; treatment usually involves radiation therapy along with chemotherapy. The two organizing frameworks utilized are the TNM framework and the number framework. In TNM system, T represents the size, location and extent of the tumor, N represents the tumor cells in the lymphnodes, and M represents the metastasis. The number system represents the extent of cancer in the body [10].

1.3. Oropharyngeal cancer

The oropharynx lies behind the oral cavity or mouth. The most common type of oropharyngeal cancer is squamous cell cancer; other infrequent types include melanoma, salivary organ cancer, lymphoma, sarcoma and small cell cancer. Alcohol and tobacco consumption are the risk factors. The causative factor for oropharyngeal cancer may be human papilloma virus (HPV) infection, where the virus can stay in a dormant state for a long period (latency) before transforming healthy cells into cancer cells [11]. Symptoms include lumps or swellings in the neck, that are not painful, trouble in eating, change in voice and significant weight loss [12].

1.4. Paranasal sinus cancer

Paranasal sinuses are a group of four paired air-filled spaces that surround the nasal cavity. They affect the tone and sound of the voice. The risk factors include formaldehyde, chromium, nickel, mineral oils, wood dust, use of tobacco, chemical fumes and HPV infection. Symptoms include excessive pain in the sinus section with repetitive nosebleeds, difficulty in inhalation and exhalation through nose, headache, changes in the tone of voice, numbness in nose section, lips or cheeks and blocked nasal passages. Interventions include endoscopic surgeries in which the tumor is removed through nose or mouth, transoral surgery through incision inside the mouth and open surgery through cuts made on the neck and face [10-12].

2. Head and neck squamous cell carcinoma

HNSCC affects the areas such as oral cavity, the oropharynx, the paranasal sinuses and nasal cavity, the upper section of throat close to nasal section, the larynx, and the hypopharynx. Squamous cell carcinoma usually causes dysplastic lesions in the squamous epithelial lining of the mucous membranes of the GI tract and airway passages [4, 11]. Some of the symptoms are ulcers and painful sores in the throat and mouth, continuous bleeding from the mouth and nose, sinus blockage, ear infection, sore throat, difficulty in inhalation and exhalation, change in voice and painful feeling during gulping and swallowing [13-14]. A number of genetic mutations in individuals with HNSCC have been identified, although the presence of these mutations is yet to be clearly linked to the development and progression of cancer. These genes include NOTCH1, TP53, and CDKN2A,

which play a role in cell cycle regulation and act as tumor suppressor genes [10, 13, 14]. HNSCC occurs due to somatic mutations influenced by environmental or genetic factors, and is not hereditary [15].

3. Cancer stem cells

The role of cancer stem cells as initiators of metastasis with capabilities of self-reestablishment and self-renewal provides strong evidence for establishment of cancer. These self-proliferating stem cells are responsible for the development of tumor in head and neck squamous cells [16-17]. Following are the three primary characteristics of cancer stem cells: 1) the cancer stem cell must show strong tumor initiation, 2) and when the cancer stem cell is recovered from the cancerous tissue and implanted it must exhibit self-restoration and reactivation after sequential or primary and tertiary transplantations, 3) and the cancer stem cells must exhibit an innate capacity for differentiation, permitting the establishment of heterogeneous genealogies of the offspring derived from the stem cells [10, 14, 18]. To summarize, these transplanted cancer stem cells undergo further mutations and genetic alterations resulting in dedifferentiation and aquisition of the characteristics of cancer stem cells [10, 18]. Cancer stem cells have been extracted from hematopoietic cancers and many other tumors which include brain, breast, prostate, colon, lung, liver, head, and neck cancers, and skin melanoma [14], and the characterization of cancer stem cells in different tumors has clearly indicated that these cells are unique for different tissues [13].

4. CD44

CD44 has been distinguished as an effective biomarker in colon, breast, central nervous system, pancreas, head and neck and prostate tumors. The cell surface glycoprotein CD44, which has been utilized in the research of cancer cancer stem cells and metastasis progression, is required for cell adhesion and mobility [3]. CD44 is associated with hyaluronic acid (HA), heparin sulfate, and chondroitin sulfate, which helps in communication with growth factors, metalloproteinases, as well as in collagen disruption and invasion [1-2].

New tumors from very limited number of CD44+ developed whereas CD44− cells were unable to establish or further the progress of any tumor. Similarly a number of studies demonstrated that the population of cells containing CD44+ markers, obtained from both cells lines and tissues, displays a higher potential for expansion, differentiation, and metastasis, an aggressive and invasive nature as well as resistance to different treatment methods [2, 18]. CD44 isoforms were also connected with tumor stage, treatment inefficacy, metastasis, and decreased survival, showing that CD44 is a potential marker for head and neck cancer squamous cell carcinoma and a possible target for effective treatment methods.

5. ALDH

Aldehyde dehydrogenase (ALDH), a cytosolic isoenzyme family member is expressed in high quantities in stem cells. These enzymes function to incorporate retinoic acid that is critical for development and differentiation in stem cells and for the catalysis of intracellular aldehyde. Similar to CD44, ALDH expression has been observed in stem cells in HNSCC. ALDH1+ cells extracted from tissues of head and neck squamous cell carcinoma cell lines have exhibited self-reestablishment capabilities, formation of tumor, increased invasive abilities and sphere formation (*in vitro* 3D culturing technique) [19-20]. ALDH1 has a positive relationship with the staging and development of head and neck squamous cell carcinoma and a negative connection to patient survival. In the animal models, ALDH1+ cells could also establish tumors by showing better capacity of invasion, metastasis, spheroid formation, and increased resistance to treatments. There is a significant correlation between the CD44 and ALDH cells with 75% of ALDH1+ cells also having high levels of CD44 and 25% of CD44 cells showing elevated activity of ALDH indicating that ALDH1 may be a more effective and explicit marker of cancer stem cells in head and neck squamous cell carcinoma [1-2, 18].

6. CD133

CD133 has been noted as a potential cancer stem cell marker in prostate, skin, lung, colorectal, and liver cancers. CD133+ cells extracted from head

and neck squamous cell carcinoma cell lines showed increased expansion of different clones or clonogenicity. Various studies have proposed that CD133+ cells extracted from head and neck squamous cell carcinoma cell lines show self-reestablishment, potential for development of tumor sphere, expanded clonogenicity, metastasis, and tumorigenicity and indicated that the level of CD133 marker in cancer stem cells is directly linked with stages of cancer, as stage III and IV tumors showed more elevated amounts of CD133 than earlier stages such as I and II stages [2]. Flow cytometry has been used to identify the level of expression of CD33. Cancer stem cells can also be extracted by means of the anchorage-free culture test, and cells must have the ability to establish and progress towards tumor growth [20-21]. To assess this characterization animal transplantation models have been used [22].

7. Clinical implication of cancer stem cells in head and neck squamous cell carcinoma

Studies were carried out to understand the pathological pathway of cancer stem cells and the studies identified a target gene Bmi-1, which is expressed in high quantities in cancer stem cells [23-24]. Other molecules identified in some of these studies include PTEN, Wnt, Hedgehog, and Notch and were investigated through microarrays, for their therapeutic capabilities. These studies provide an understanding of how cancer stem cells are regulated differentially as compared to normal cells or tissues. One of the studies highlighted the importance of micro RNA such as MicroRNA-200c in the control and maintenance of the tumorigenicity in stem cells [18]. Cancer stem cells (CSCs) depend on their associations with the perivascular niche for survival and reestablishment [6]. Disrupting the niche or microenvironment of cancer stem cells results in the loss of their basic capacity to survive and greatly compromises their ability for self-reestablishment [1-2]. Anti-angiogenic therapy may impact by modifying the microenvironment and reducing the viability of exceedingly vascular tumorigenic cancer stem cells in HNSCC.

Tumor cells might undergo phenotypic change to escape from the changing tumor niche or microenvironment created by drug therapy and this process is known as evasive resistance [25]. CD44+ is a known marker of localized tumor that relapses even after radiotherapy is carried out in laryngeal cancer [26]. On the basis of literature reviews, it can be established that the high expression rate of CD44+ and ALDH+ is directly correlated with progressive neoplastic illness. A collection of CD133+ cells in oral squamous cell carcinoma is identified by their more prominent proliferative ability, intrusiveness and tumorigenesis in correlation with CD133-cells and it has also been identified that these cells are resistant to standard chemotherapy methods and techniques. ALDH1+ cells from head and neck squamous cell carcinoma have higher potential for tumor development and are more resistant to radiotherapy than normal cells [27]. In mice, cancer cells expressing high levels of ALDH marker have higher potential for tumor development, whereas cells expressing low ALDH marker resulted in minimal tumors [2]. In head and neck squamous cell carcinoma, where the first and central strategy for treatment includes surgical evacuation of the tumor, cancer stem cells may have already metastasized at the time of surgical intervention. These cells have an innate resistant mechanism against these therapeutic methods rendering these methods ineffective [1]. These cancer stem cells play a role in the recurrence and relapse of tumor in head and neck cancer.

It is critical to understand the niche or micro-environmental elements of the tumor [16]. Disruption of the association and communication between cancer stem cells and endothelial cells in head and neck cancer can provide treatment advantages, specifically in anti-angiogenic treatment methods, that disturb the perivascular microenvironment or niche of the tumor and intercede in the communication between cancer stem cells and endothelial cells [28]. Tumors with cancer cells may be regarded as a functional organ where altered and mutated cells communicate with stromal cells around the tumor region [20]; however, not all tumor cells exhibit similar properties and characteristics [29-30]. In one of the studies carried out by Civenni *et al.* [31], CD271+ cells extracted from melanomas produced a larger number of tumors as compared to CD271-cells, providing evidence for the

cancer stem cell theory. Similarly an experimental study by Taghizadeh *et al*., [32] also showed that CXCR6+ cells establish aggressive tumors as compared to CXCR6-cells in melanoma cells. The concept of tumorigenic cells was investigated in leukemia, where low quantities of leukemic cells established secondary tumors in recipient mice [30, 33].

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CONFLICT OF INTEREST STATEMENT

Neither author reports any conflicts of interest.

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