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Review Article

Head and Neck Cancer: Current Treatment Options and Associated Challenges

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Abstract: Head and neck squamous cell carcinoma (HNSCC) is one the most common malignancies in which significant advances in treatment modalities have yet to be translated into better clinical outcome except for cases associated with human papilloma virus (HPV) infection. New treatment strategies and new targets for treatment need to be discovered and explored. An area of much current interest is the existence and consequences of cancer stem cells (CSCs). This subpopulation of cells has been phenotypically and biologically identified in many human cancers including HNSCC. Their intrinsic properties suggest that they are resistant to conventional cancer treatments and their survival may explain the frequent local recurrences experienced by many tumors despite aggressive therapy. Targeted elimination of this subpopulation represents a potential breakthrough as an adjunct to current therapies. This review briefly describes the structure of the oral mucosa, epidemiology, major risk factors, common symptoms, and current treatment options and associated challenges of human HNSCC. It emphasizes the cancer stem cell (CSC) hypothesis and the importance of the cell surface antigen, CD44, as a biomarker of tumor aggressiveness in HNSCC. Finally, it discusses current treatment approaches and emerging nanoparticle-mediated therapeutic approaches to identify and eliminate this insidious cell population.
Keywords: Squamous Cell Carcinoma, Head and Neck Cancer, CD44, Cancer Stem Cells, Treatments, Nanoparticles.

Head and Neck Cancer

Head and neck cancers are a group of biologically similar cancers that originate from different regions of the upper aerodigestive tract including lip, oral cavity, nasal cavity, paranasal sinuses, pharynx, and larynx (Figure 1. Studies show that about 90% of all head and neck cancers are squamous cell carcinomas; a very common type of cancer that initiates from putative epithelial cells of these organs, also called squamous cells [1]. If a cancer is limited to the squamous layer of cells, it is called carcinoma in situ. If the cancer has grown beyond this cell layer and moved into the deeper tissue, then it is called invasive squamous cell carcinoma, and is categorized as local, regional, or distant based on the extent of spread.

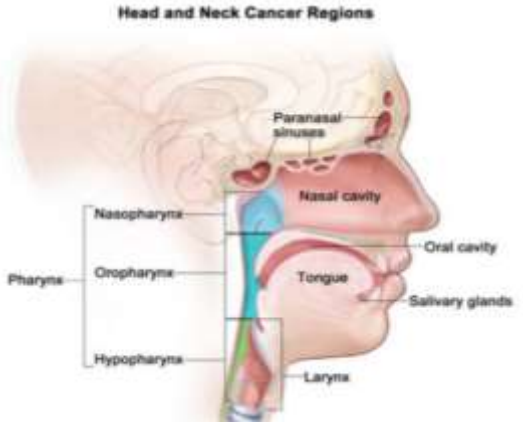

Fig-1: Figure shows the different regions of head and neck cancer (Source: http://www.cancer.gov/types/head-and-neck/head-neck-fact-sheet).

Structure and Function of the Oral Mucosa

The squamous epithelium is composed of keratinocytes that are organized into a multilayered, stratified structure. Epithelial tissues function together to protect the underlying tissue from physical, chemical, and microbial damage to maintain homeostasis [2]. The epithelium consists of a basal cell layer, a prickle or spinous cell layer, a granular cell layer and a keratinized or cornified cell layer [2, 3] (Figure 2). The basal keratinocytes are attached to the basement membrane by specialized structures called hemi-desmosomes which maintain the integrity of the entire epithelium [2-6].

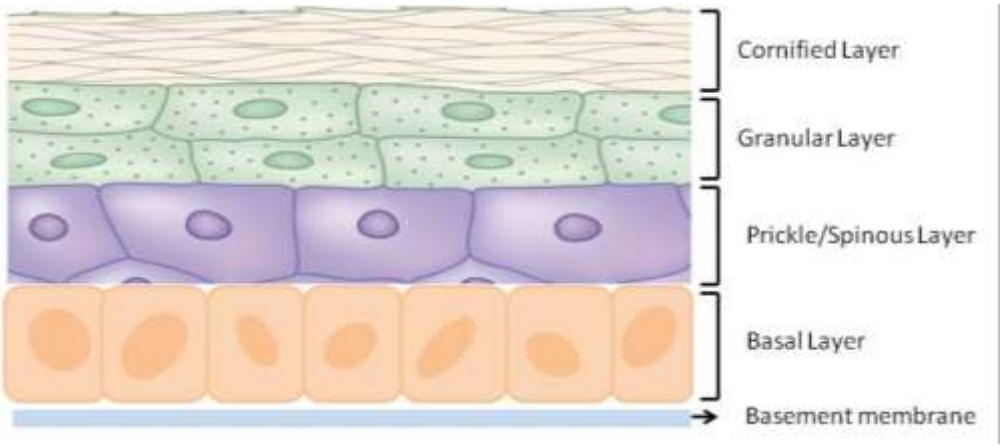

Fig- 2: Structure of the oral epithelium. Figure is adapted from Hsu *et al.;* 2014[116]

The basal cells undergo a well-defined differentiation program and produce new cells that preserve the thickness of the epithelium as cells are lost by desquamation at the surface. The basal cells comprise stem cells and transit-amplifying or progenitor cells, which are structured into units. The stem cell divides asymmetrically to produce another stem cell and a transit-amplifying cell [7, 8]. The transit-amplifying cell further undergoes several rounds of mitotic divisions; as a consequence, large numbers of daughter cells are produced that are required to colonize the epithelium [5, 8]. The stem cell divides infrequently, while the transit-amplifying cells divide more frequently due to their shorter cell cycles. The cell cycle kinetics maintains the oral epithelium in balance and supports epithelial regeneration following injury. The turnover time for oral epithelium is around 25 days and for gingiva 50 days [6, 9].

Following multiple rounds of cell division, the transit-amplifying cells commit to terminal differentiation. The cells loose the capacity to divide and start to accumulate cytoplasmic intermediate filaments called cytokeratins. Terminally differentiated squamous cells are ultimately lost by desquamation at the surface [5].

Epidemiology

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in humans, and every year 550,000 people are diagnosed with HNSCC worldwide [10]. Males are affected significantly more than females with a ratio ranging from 2:1 to 4:1. The incidence rate in males exceeds 20 per 100,000 in France, Hong Kong, the Indian subcontinent, central and Eastern Europe, Spain, Italy, Brazil, and among African Americans in the United States. Mouth and tongue cancers are more common in the Indian subcontinent, nasopharyngeal cancer is more common in Hong Kong, and pharyngeal and/or laryngeal cancers are more common in other populations [11, 12]. Head and neck cancer accounts for about 3% of all cancers in the United States. In 2015, 59,340 people (43,390 men and 15,950 women) were estimated to develop head and neck cancer, and 12,290 deaths (8,900 men and 3,390 women) were estimated to happen [13].

Risk Factors

There is a wide range of external factors that can cause head and neck cancer among various age groups such as excessive consumption of tobacco and alcohol, viral infection, and betel nut chewing [1, 14].

Excessive alcohol consumption and tobacco chewing or smoking habits are the major causes of cancers of the oral cavity, oropharynx, hypo pharynx, and larynx. About 70-80% of HNSCCs result from smoking and alcohol consumption. Cigarette smoking increases the risk 10 fold compared with non smokers, and heavy consumption of alcohol is an independent risk factor[1]. The combined effect of tobacco and alcohol is significantly greater than a multiplicative risk [14].

Over exposure to UV-light or some oncogenic chemicals can also result in head and neck cancer. For example, exposure to certain harmful products or chemicals such as wood or nickel dust or formaldehyde is a risk factor for cancers of the para nasal sinuses and nasal cavity.

In addition, the diet and lifestyle of an individual can also play an important role. For example, disproportionate consumption of processed food and red meat are associated with an increased rate of head and neck cancer. The betel nut chewing habit in Southeast Asia is another major cause of oral cancer.

Until relatively recently, only a few strains of viruses such as Epstein-Barr virus (EBV) infection has been associated with head and neck cancer, in particular, nasopharyngeal cancer [15] and salivary gland cancer [16].

More recently, human papilloma viruses (HPV) infection has been recognized as a significant, emerging cause of developing oropharyngeal cancer [17, 18]. In recent studies, the HPV-associated development of oropharyngeal cancer is increasing at a faster rate than any other cause of oropharyngeal cancers [19]. Interestingly, the overall incidence of HNSCC has dropped while the incidence of HPV-induced cancer has increased in the past 30 years, especially tonsil and tongue based cancers [19, 20]. HPV- positive malignancies represent 5-20% of all HNSCC and 40-90% of oropharyngeal cancers at variable rates depending on different factors [20-22]. Among all HPVs, HPV-16 is the most common high risk HPV genotype detected in oropharyngeal squamous cell carcinoma, and the only absolute carcinogenic genotype for the head and neck cancer [23, 24]. HPV-induced cancers are more common in younger male patients (below 50 years), non-smokers, non-drinkers or mild to moderate drinkers, with higher socioeconomic status and better performance status than patients with HPV-unrelated cases [25, 26]. HPV positive carcinomas have better treatment response and modality-independent survival benefit compared to the HPV-unrelated carcinomas [23].

Further, multiple genetic factors and pathways may also contribute to an increase in risk of head and neck cancer, and these factors may interact with other known risk factors [27]. For example, metabolic polymorphisms influence the exposure to the carcinogens in tobacco smoke, DNA repair gene polymorphisms, as well as variations in other pathways contributing to carcinogenesis.

Symptoms

The majority of symptoms of head and neck cancers start with a minor swollen lymph node next to the neck, mild pain, infection, sore throat and/or a horse sounding voice. For example, the symptoms of cancer originating in the oral cavity starts with a white or red patch on the gums, the tongue, or the lining of the mouth, and a swelling of the jaw that causes uncomfortable and unusual bleeding or pain in the mouth. In case of cancer originating from paranasal sinuses and the nasal cavity, sinuses are blocked and do not get cleared. Chronic sinus infections are treatment resistant and cause bleeding through the nose, frequent headaches, swelling or other trouble with the eyes, pain in the upper teeth/problems with dentures, etc. Difficulty in breathing or speaking, pain when swallowing, persistent pain in the neck or the throat, frequent headaches, pain or ringing in the ears or trouble in hearing are common for pharynx and larynx cancers. These symptoms become worse as they grow, and if they are not treated on time could result in death.

Treatments and Challenges

The major treatments of HNSCC are surgery, radiation therapy, chemotherapy, hormone therapy, immune therapy, and targeted therapy etc. or a combination of these treatments. The treatment strategy for an individual patient depends on a number of factors, comprising the exact location of the tumor, the stage of the cancer, and the person's age and general health. However, these possible multi-modality treatments have not improved the 5-year survival rates of locally advanced head and neck cancers (~50%) in the past few decades [28, 29].

Currently, surgery, radiation, and chemotherapy are the primary treatment modalities. HNSCC is categorized into three stages: early-stage (stage I/II), loco regionally advanced stage (stage III/IV) and recurrent/metastatic [30]. Early stage HNSCC is treated relatively well with a single-modality treatment such as either with surgery or radiation alone, and each of the treatments have similar survival rates ranging 60-90% based on tumor site and extension of the disease [31]. However, patients with advanced HNSCC (stage III and IV) have poor outcomes. The regimens of advanced HNSCC comprises of multi-modality treatments with surgery, radiation, and chemotherapy. Cisplatin-based chemoradiation is the standard treatment regimen for loco regionally advanced disease, and palliative platinum-based chemotherapy in conjunction with best supportive care for recurrent/metastatic HNSCC disease [30]. However, these therapies are often destructive [32] and introduces substantial side effects.

The main drawbacks of traditional chemotherapy are severe off-target side effects and unwanted toxicity due to the systemic distribution of the chemotherapeutic drugs [33]. Another major challenge is the development of multidrug resistance (MDR) by

the tumor cells; which ultimately makes chemotherapy less effective [34-36]. Therefore, to improve the treatment outcomes in HNSCC, there is an urgent need to establish other targeted therapies in combination with radiation and/or chemo to improve conventional radiation and chemo therapies.

Targeting EGFR in HNSCC

The main goal of the molecular targeted therapy is to eliminate tumor cells by targeting specific protein or signal transduction pathways upregulated in the tumor or specifically targeting the tumor microenvironment or vasculature without affecting normal cells. Targeting the epidermal growth factor receptor (EGFR) pathway has been widely studied in HNSCC, and antibody therapies against EGFR have been extensively investigated for therapeutic efficacy [37-40].

EGFR-targeted therapies have been shown to inhibit cellular proliferation, survival, invasion and angiogenesis as well as acting synergistically with chemoradiation therapies [38]. One of the most common EGFR inhibitors is cetuximab (Erbitux, IMC-C225), an IgG1 mAbs used for targeting EGFR in HNSCC malignancies. Cetuximab blocks downstream EGFR signaling pathways essential for tumor survival [37], stimulates antibody-dependent cellular cytotoxicity (ADCC) by recruiting activated immune cells into tumors to enhance tumor cell killing [41-43], and enhances radiosensitivity in HNSCCs [44, 45]. In HNSCC, cetuximab is approved for using in combination with radiotherapy in the treatment of patients with unresectable, loco regionally advanced HNSCC [46], as a monotherapy in Europe and the United states in platinum-refractory recurrent disease [47], and as a first-line therapy in recurrent/metastatic disease in the United States in combination with carboplatin or Cisplatin and 5-fluorouracil (5-FU) [47-49].

For the treatment of HNSCC with curative intent, cetuximab in combination with radiation therapy definitely has benefit compared with radiation therapy alone [46]. However, it is not clear whether cetuximab is superior to the cisplatin-based chemoradiation, which remains the standard care for HNSCC chemotherapy [50]. Further, besides cetuximab, no targeted treatment approaches are currently approved that have significant response rates [31, 49] in HNSCC. The treatment resistance can be attributed to the persistence of aggressive tumor associated HNSCC CSCs dependent on EGFR expression [51]. Studies suggest that EGFR plays critical roles in the survival, maintenance and function of CSCs, and blocking the EGFR-mediated CSC pathways might be an effective treatment for HNSCC [51- 52].

Cancer Stem Cell Hypothesis

As described earlier, the oral mucosa is an organized structure that contains a population of stem cells within its basal layer that are responsible for the continual regeneration of the mucosa in response to cell loss by desquamation of the surface. Similarly, the heterogeneous nature of HNSCC has been demonstrated by histological, phenotypical and karyotypical analyses [53, 54]. This heterogeneity was initially attributed to the model of clonal expansion, in which various clones are frequently generated from a single mutated cell that underwent various sequential genetic and/or epigenetic mutations in response to certain carcinogens during cancer development [55]. This model was able to explain the pathophysiologic basis for development of preneoplastic lesions (dysplasia) and their subsequent, gradual progression to neoplasm. According to this model, neoplastic disease is considered a proliferative disease, and all neoplastic cells are identical and possess the same potential of proliferation and carcinogenesis. This model had a great influence on the development of current anti-cancer therapies. However, it was unable to explain fundamental facts regarding the phenotypic heterogenicity of neoplastic cells, their tumorigenic potential, and their ability to metastasize by characterizing only a small proportion of tumor cells [56, 57]. Thus, a new theory of cancer development related to a cancer stem cell (CSC) model was established [58] .

Cancer stem cells are defined as a small fraction (<10%) of tumor cells that display properties of self-renewal, high tumorigenicity and drug resistance, resulting in cancer metastasis and recurrence [59, 60]. CSCs most likely derive either from normal stem cells, in which mutations accumulate resulting in genetic instability, or from mutated progenitor stem cells [7]. According to the CSC model, heterogeneity and hierarchy among all of the cells exists as a consequence of asymmetric division of CSCs within the tumor mass, and all other cells comprising the tumor bulk are the result of differentiated CSCs [61]. As previously mentioned CSCs have ability to self-renew and form pools of precursors like normal stem cells. However, CSCs demonstrate deregulated self-renewal/ differentiation processes and generate daughter cells that arrest at various stages of differentiation [7].

Under the normal circumstances, as a result of asymmetric division of stem cells, two groups of cells may coexist within the same niche (microenvironment); one being dedicated to restoring a stem cell reserve and the other being dedicated to the differentiation (Figure 3A). In carcinogenesis, when a CSC divides, it generates a CSC and a transformed “progenitor-like” cell that has limited self-renewal ability but is highly proliferative, similar to a transit amplifying population in normal tissues. These progenitors give rise to more or

less partially differentiated bulk tumor cells through a combination of proliferation and abortive differentiation (Figure 3B).

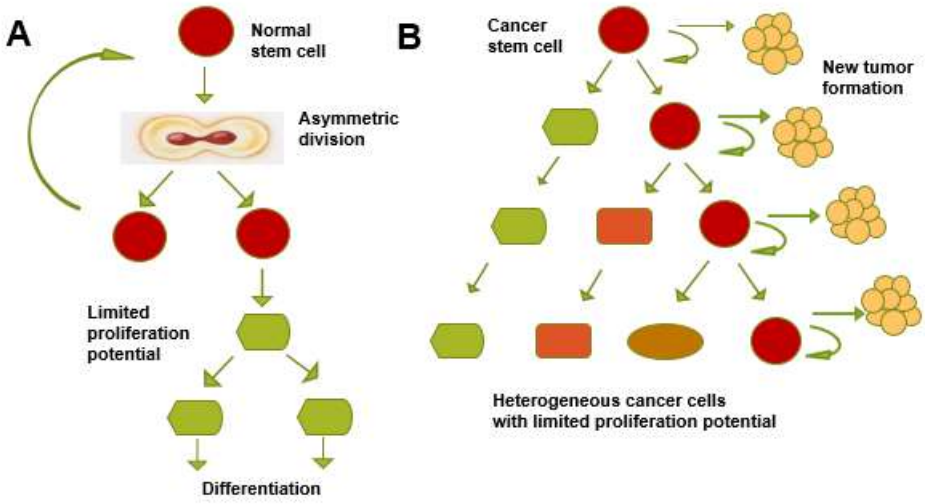

Fig- 3: (A) A stems cell division in steady state. (B) A cancer stems cell division in tumorigenesis.

Currently there is much interest in the existence and activity of CSCs in HNSCC as a potential reason why conventional therapies fail to eradicate tumors [7] . CSCs possess intrinsic survival mechanisms that may protect them from radiotherapy and chemotherapy [62]. As CSCs constitute a small percentage of cancerous tumor cells, identifying genetic/ epigenetic changes as well as expression of, among other things, transcription factors remains extremely difficult [63].

CD44 as a Cancer Stem Cell Marker

CSCs can be identified by using surface markers, determining ALDH1A1 enzyme activity or their ability to efflux vital dyes (side population – SP) and form tumor spheres in vitro [64]. The development of new strategies is currently hampered by the lack of reliable markers to identify CSCs. However, there are several surface markers including CD34, CD133, CD24, CD29, CD31, and CD44 which are usually either singly or in combination used for identifying CSCs [64, 65].

One such surface marker is CD44, a transmembrane glycoprotein that is involved in cell migration and adhesion, and is considered a potent marker for the identification of CSCs in different carcinomas of squamous cells including HNSCC [66]. Emerging studies show that CD44 is an important biomarker of a cellular subpopulation of CSCs that are capable of self-renewal and have the capacity for initiation, progression, invasion, metastasis, tumor recurrence, and resistance to chemo and radiotherapy [67]. In addition, CD44 serves as a receptor for hyaluronan (HA) and certain matrix metalloproteinases (MMPs) [68, 69]. HA is a member of the glycosaminoglycan family and is composed of tandem disaccharide repeats of b-1, 4-D-glucuronic acid-b-1,3-D-N-acetylglucosamine [70, 71]. As an important component of the extracellular matrix, HA has been proved to be directly involved in many cellular processes for example cell adhesion, cell migration, innate immunity, and wound healing [72]. In HNSCC the association of HA and CD44 activates epidermal growth factor receptor pathways (EGFR) which eventually lead into tumor cell growth, tumor cell migration and chemotherapy resistance resulting in head and neck cancer [73]. An accumulation of evidence indicates that HA-CD44 interaction in the extracellular domain promotes multiple signaling pathways which play a crucial role in tumor cell progression in a variety of solid tumor malignancies [66, 74].

The existence of the CSCs subpopulation in vivo was confirmed for the first time by a seminal study performed by Bonnet and Dick using samples of acute myeloid leukemia (AML) in 1997 [75]. CSCs have also been identified in many solid tumors. For example, Al-Hajj *et al.;* [76] experimentally verified the presence of CSCs in breast cancer samples in 2003. They showed that transplanted stem cells were able to restore a phenotypically heterogeneous structure of a tumor. Further, Singh *et al.;* [77] identified CSCs in brain tumors, Collins *et al.;* [78] in prostate tumors, Dalerba *et al.;* [79] in colorectal cancers, Li *et al.;*. [80] In pancreatic tumors, and Ho *et al.;* [81] in lung tumors.

In 2007, Prince *et al.;* [62] first characterized the existence of CSCs in HNSCC based on the expression of CD44 surface marker by flow cytometry. According to them, a cellular subpopulation in head and

neck tumors expressing the surface marker CD44 with stem-like characteristics was capable of reproducing the original tumor when implanted into immune-compromised mice. Following this study, Harper et al. studied the expression of CD44, CD29, and CD133 as apparent markers of CSCs in cell lines derived from head and neck tumors; they reported that the highest expression of CD44 correlated with increased clonogenicity [82].

Cancer Stem Cells and Therapeutic Implications

As previously described the existence and activity of CSCs in HNSCC is a potential reason for the failure of the conventional therapies to eradicate tumors [7] since CSCs possess intrinsic survival mechanisms that may protect them from radiotherapy and chemotherapy [62]. Therefore, targeting CSCs in HNSCC may support an effective battle against this malignant disease. Due to the disparities between the presence of a single surface CSC marker, prognosis, and tumor invasiveness [83], therapy targeting CSCs is not significantly used in clinical trials. Targeted elimination of CSCs directly or through a CSC niche such as targeting antiangiogenic agents are potential treatment strategies, which are currently under development.

In HNSCC, a splice-variant of CD44 isoform 6 (CD44v6) was targeted by mAbs and their immunoconjugates prior to the recognition of CD44 as a CSC marker, since CD44v6 expression in HNSCC is correlated with tumor invasion, lymph node metastasis, and shorter survival [84]. Verel *et al.;* [85] developed Bivatuzumab mAb as a stand-alone antibody against CD44v6, which was further developed as radiolabelled conjugates with Technicium-99, and Rhenium-186 [86, 87], as well as with mertansine, an microtubule inhibitor [88, 89]. The radio-labelled bivatuzumab antibodies showed moderate responses in radio-immunotherapy trials. However, bivatuzumab mertansine, conjugated to CD44v6, tested in two parallel metastasis HNSCC patients of phase I trials, were discontinued due to the severe toxicity of epidermal necrolysis [88, 89].

Emerging Nanoparticle Research

Currently, the use of nanoparticles in cancer treatment has opened up some exciting possibilities, including the possibility of destroying malignant tumors with minimal damage to healthy surrounding tissues and organs, as well as the detection and elimination of cancer cells before they form tumors. Most of the techniques for improving cancer diagnosis and treatment are at the developmental stage, while a few of them have reached the pre-clinical or clinical trial stage cancers [90-92]. Nanoparticles have been actively developed for tumor imaging in vivo, biomolecular profiling of cancer biomarkers, targeted drug delivery, and magnetic hyperthermia [93]. Their nanometric dimensions and large surface to volume ratio renders them suitable for attaching multiple copies of a variety of ligands. In addition, nanoparticles unique magnetic, optic, or fluorescent properties make them suitable for biological imaging [94-97].

Although nanoparticles can be nonspecifically taken up by macrophages, their surface modification with specific ligands to actively target tumor cell specific receptors can potentially enhance the efficiency and selectivity of the delivery [94], [98].For targeting CD44 in cancers, investigators have used HA-mediated nanocarriers coupled to anticancer drugs such as epirubicin[99], doxorubicin (DOX) [100], paclitaxel (PTX) [101], and mitomycin C (MMC) [99], as well as siRNA, that can deliver these agents to CD44 over expressing cells [102, 103]. The nanoparticles used in these studies have been diverse and include quantum dots [104], carbon nanotubes [105] and nanodots[103, 106], graphene [107], gold nanoparticles [108], iron oxide nanoparticles [109], and silica nanoparticles.

In HNSCC, nanoparticles have been increasingly used for ablative or thermotherapy for treating cancers, offering patients an alternative, minimally invasive, and inexpensive treatment compared to other standard treatments [90, 110]. One promising thermotherapy strategy is targeted magnetic hyperthermia. Magnetic hyperthermia can be achieved by delivering magnetic nanoparticles to the cancer cells/tissue and creating local heat in the presence of an externally applied alternating magnetic field (AMF), which results in thermo-ablation of the cancer cells/tissue [111].

In contrast to other nanoparticles, super paramagnetic iron oxide nanoparticles (SPIONs) have been increasingly investigated for the treatment of HNSCC [90-93] via magnetic hyperthermia as their inducible magnetic properties facilitate them to be aligned in a defined location in the presence of an externally applied alternating current (AC) magnetic field[111]. This property of inducible magnetism of SPIONs renders them suitable for many biological applications in tumor biology, ranging from diagnostics (MRI) to therapeutics (magnetic hyperthermia), and magnetically assisted transfection of cells [112-115]. Modifying SPIONs by conjugating targeting ligands such as hyaluronan has the potential to specifically deliver hyperthermia to the CSC population.

In summary, the advances in the pre-clinical/clinical stage suggest that the use of nanoparticles in biomedical application is currently a rapidly developing area of nanotechnology that nurtures new possibilities in the diagnosis and treatment of human HNSCC cancers.

Summary

HNSCC is a disease with modest prognosis and treatment outcome due to the limited success of conventional radio- and chemotherapy. There is compelling evidence that tumor associated CSCs plays a crucial role in treatment failure in HNSCC and developing optimism that targeting CSCs regulated signaling pathways may improve treatment efficacy. CD44 has been identified and an important biomarker for prognosis, a putative stem cell-associated gene and a potential therapeutic target. Nanoparticle-mediated therapeutic approaches represent a promising approach to supplement conventional therapy.

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