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Jacobs Journal of Cancer Science and Research

Review article

Immunotherapeutic Approach to Oropharyngeal Cancer

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Received: 04-22-2016

Accepted: 05-23-2016

Published: 05-27-2016

Abstract

Oropharyngeal cancer accounts for more than 13000 new cases in the US, which is more common in older men, and male:female ratio is 2.7:1. Immunotherapy is an alternative to chemotherapy and radiation therapy to treat cancer. It is utilized to stimulate one's own immune system to fight the tumor. Giving someone synthetic immune system proteins to fight cancer can also be done in immunotherapy. In this paper, we will discuss the pathophysiology of Oropharyngeal Cancer itself and as part of the squamous cell cancer of head and neck, the active molecules involved in immunotherapy, as well as potential ways to fight these cancers using immunotherapy.

Keywords: Oropharyngeal Cancer; HPV; Head and Neck Cancer; Immunotherapy; Epidermal Growth Factor Receptor; Kinase Inhibitor; Monoclonal Antibody; Cytokine; Vaccine; T Cell

Abbreviations

HNSCC: Head and Neck Squamous Cell Carcinoma;

HPV: Human Papilloma Virus;

LOH: Loss of Heterozygosity;

Rb: Retinoblastoma;

EGFR: Epidermal Growth Factor Receptor;

mTOR: mechanistic Target of Rapamycin;

DBD: DNA Binding Domain;

RTK: Receptor Tyrosin Kinase;

CTL: Cytotoxic T Lymphocyte;

TA: Trojan Antigen;

APC: Antigen Presenting Cells;

TGN: Trans Golgi Network

Introduction

Oropharyngeal squamous cell carcinoma refers to cancer of the tonsil, base and posterior one third of the tongue, soft palate, and posterior and lateral pharyngeal walls. Squamous cell carcinoma comprises over 95% of oropharyngeal cancers. In the US in 2015, there were an expected > 13,000 new cases of oropharyngeal cancer. Although the incidence of oropharyngeal cancer is increasing, its cure rates are also improving. Like most head and neck cancers, oropharyngeal cancer is more common among older men with a mean age of 63. The male:female ratio is 2.7:1. However, recently, oropharyngeal cancer patients have become younger and more commonly female as HPV infection has emerged as an etiology [1].

Etiology/Predisposing factors:

The cause of Oropharyngeal cancer may vary from region to region. The risk of developing oropharyngeal cancer is 16 times higher in HPV-positive patients. In Europe and North America, HPV infection accounts for about 70 to 80% of oropharyngeal cancers. Nonetheless, tobacco and alcohol remain important risk factors for oropharyngeal cancer. Patients who smoke more than 1.5 packs/day have about a 3-fold increased risk of cancer, and patients who drink 4 or more drinks/day have about a 7-fold increased risk. People who both drink and smoke heavily have 30 times the risk of developing oropharyngeal cancer [1,2].

• **Smoking:** Smoking tobacco is one of the most important risk factors for HNSCC.

• **Chewing Tobacco:** Chewing tobacco is the main reason for oral and oropharyngeal SCC in India and various parts of China, Taiwan, and South-East Asia. It is particularly harmful when ingested in betel quid, comprised of areca nut [3].

• **Alcohol:** Alcohol is another high risk factor for HNSCC. Avoiding alcohol intake could decline the development of HNSCCs up to 90%, especially hypopharyngeal and laryngeal tumors [3].

• **HPV Infection:** Current studies state that the HPV infection (HPV-16 and -18) is liable for HNSCCs and is presently the leading cause of oropharyngeal SCC (particularly squamous cell carcinoma (SCC) of the tonsils and the base of the tongue) [4].

Sign/Symptoms

The possible signs of Oropharyngial cancer may include: a persistent sore throat, pain or difficulty with swallowing"Odynophagia", unexplained weight loss, voice changes, tinnitus, a lump in the back of the throat or mouth, a lump in the mouth, dull pain behind the sternum and cough [5,6].

Pathophysiology and Molecular Basis of Orophrynx Cancer

As the Oropharynx is a part of the head and neck shares a common pathophysiology as they are mostly of squamous cell of origin, the Head and Neck Squamous Cell Cancer HNSCC arises from a common premalignant progenitor followed by the outgrowth of clonal populations. This is associated with cumulative genetic alterations and phenotypic progression to the invasive malignancy [7-9]. These genetic modifications inactivate tumor suppressor genes and activate proto-oncogenes through gene amplification, point mutations, deletions, and promoter methylation (Table-1). The various microsatellite marker analyses have permitted the description of a genetic progression model for HNSCC. This is based on the regularity of these genetic alterations in the different invasive tumors and preinvasive lesions (Figure 1) [8,9]. The most common genetic alteration, the loss of chromosomal region 9p21, is found in 70-80% cases of HNSCC [8,10,11]. The CDKN2A gene locus, found in chromosome 9p21, encodes two different types of transcripts. They are p14ARF and p16. These are liable for regulating the G1 phase of the cell cycle and also for the degradation of MDM2 of p53. The p16 is frequently inactivated by the homozygous deletion, promoter methylation, or, less commonly, through point mutations [12].

Loss of Heterozygosity (LOH)	Percentage
LOH 9p	**70–80%**
LOH 3p	60–70%
LOH 17p	50–70%
LOH 11q	30%
LOH 13q	30%
Inactivation of p16ink4A (homozygous deletion, promoter methylation, point mutation)	80%
Inactivation of *FHIT* and *RASSF1A* p53 mutation	50–80%
Cyclin D1 amplification	30%

Table 1. Common molecular abnormalities in HNSCC [13]

The loss of chromosome region 3p is another genetic modification, which occurs in the HNSCC [12,14-16]. The specific locus responsible for the tumor suppressor phenotype of 3p remains uncharacterized, but investigators have recognized at least four different regions of allelic loss [12,15-17]. These regions include 3p14, 3p21, 3p22, 3p24, and 3p26. Among these,

3p14 contains the delicate histidine triad gene or FIHT, which is a putative tumor suppressor gene. Researcher has found that it is inactivated by exonic deletions in different type of tumors and in a small percentage of HNSCC [11,18].

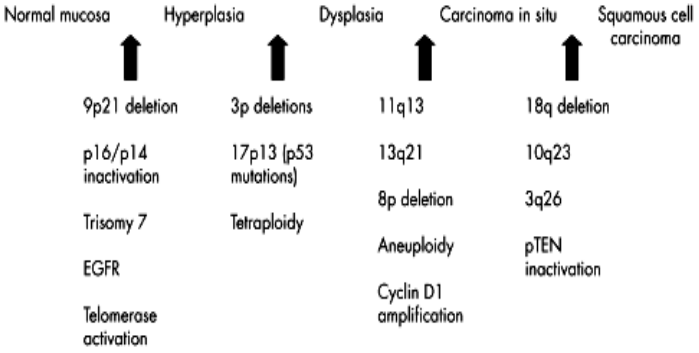

Figure 1. HNSCC carcinogenesis hypothetical model [18]

Loss of heterozygosity (LOH) of 17p and the point mutations of the p53 are seen in about 50% of the cases of HNSCC [7]. Around 64% of the p53 mutation take place in G nucleotides in HNSCC, consistent with the exposure to the carcinogens such as tobacco. [19]The overexpression of cyclin D1 and the amplification of 11q13 is found in the 30-60% of the cases of HNSCC. They have also been related with an improved rate of lymph node metastases and overall poor prognosis [20-22]. CyclinD1 induces the phosphorylation of retinoblastoma (Rb), therefore enabling the progression from G1 to S phase of the cell cycle. It is also found that the phosphorylation of Rb and the progression in the cell cycle from G1 to S phase is increased due to both inactivation of p16 and amplification of cyclin D1 [23].

The gene expression microarrays suggest that a large number of transcriptional alterations take place during the transition from normal mucosa to premalignant lesions, instead of the alteration from premalignant lesion to invasive carcinoma. A study conducted by PK et al. compared normal mucosa with premalignant lesions. The latter expressed 108 upregulated genes and 226 downregulated genes. On the other hand, invasive carcinomas had 5 upregulated genes and 13 down regulated genes when compared with other premalignant lesions [24].

Epidermal growth factor receptor (EGFR): EGFR is activated through its ligand binding. The EGFR heterodimerizes or homodimerizes with different types of receptors in the ErbB family. This dimerization leads to tyrosine kinase activity followed by auto-phosphorylation in its cytoplasmic tail [25]. This function initiates various downstream signaling cascades as well as JAK/STAT, RAS/RAF/MAPK, and PI3K/AKT/mTOR pathways, which are significant regulators of metastasis, invasion, proliferation, and angiogenesis [26,27].

PI3K/AKT/mTOR: The PI3K activation starts with the phosphorylation of PIP2 (phosphatidylinositols) and successive AKT activation, one of the main effectors of the PI3K signaling pathway [28]. The AKT also activates mTOR, which is responsible for combining various cellular signals such as stress, cellular energy stores, and nutrient levels. The PI3K is also indirectly activated through EGFR when it heterodimerizes with ERBB3 [29].

Pathophysiology based on HPV: The following pathways are involved in the HPV associated head and neck squamous cell cancer:

TP53: TP53 is made up of 393 amino acids. It carries 4 domains as well as a highly preserved DNA binding domain (DBD). Inactivation of p53 may be through the deletion of CDKN2A (negatively regulates MDM2). It may also happen through amplification or over-expression of MDM2 (a negative regulator of p53). [30] The p53 inactivation also arises in HPV-positive tumors. The p53-mediated apoptosis does not come out to be the leading form of cell death in the epithelial tumors. [31]

E6 and E7 are the two oncogenes of HPV, which inactivate Rb and p53, respectively. E6 decreases the activity of p53 through binding with E6-AP (UBE3A). It also targets p53 for degradation and ubiquitination [32].

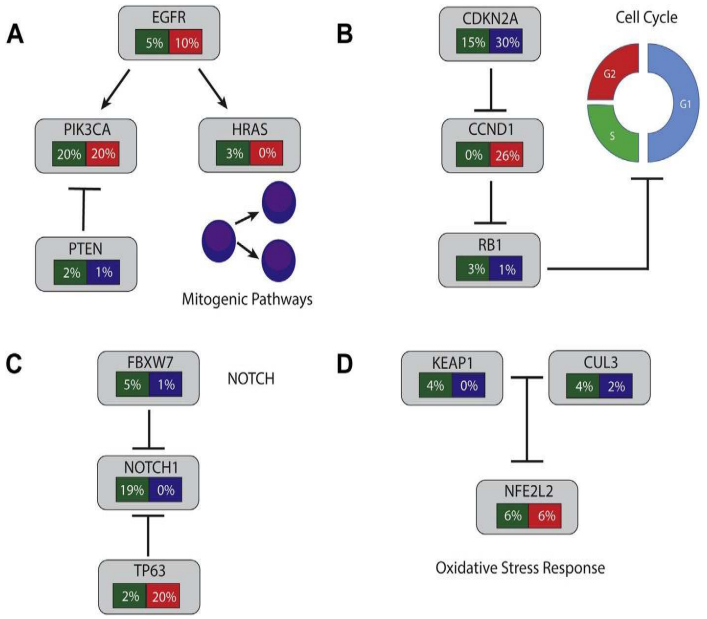

Figure 2. (A) Mitogenic Pathway Alterations. (B) Cell Cycle Alterations. (C) NOTCH Signaling. (D) Oxidative Stress Response. Green: Frequency of mutations. Red: frequency of amplification. Blue: Frequency of deletion [33]

Immunotherapy for Oropharyngeal cancer:

A. Kinase Inhibitor Drugs:

a. Non-FDA approved kinase inhibitor drugs: There are some drugs that are not currently approved by FDA for oropharyngeal cancer. However, many drugs are under clinical trials in phase I, II, and III as in Table 10 below.

1. Erlotinib: A quinazoline derivative with antineoplastic properties. Competing with adenosine triphosphate, erlotinib reversibly binds to the intracellular catalytic domain of epidermal growth factor receptor (EGFR) tyrosine kinase, thereby reversibly inhibiting EGFR phosphorylation and blocking the signal transduction events and tumorigenic effects associated with EGFR activation.

2. Afatinib: An orally bioavailable, antineoplastic, anilino-quinazoline derivative and inhibitor of the receptor tyrosine kinase (RTK) epidermal growth factor receptor (ErbB; EGFR) family. Upon administration, afatinib selectively and irreversibly binds to and inhibits the epidermal growth factor receptors 1 (ErbB1; EGFR), 2 (ErbB2; HER2), and 4 (ErbB4; HER4), and certain EGFR mutants, including those caused by EGFR exon 19 deletion mutations or exon 21 (L858R) mutations. This may result in the inhibition of tumor growth and angiogenesis in tumor cells overexpressing these RTKs. Additionally, afatinib inhibits the EGFR T790M gatekeeper mutation, which is resistant to treatment with first-generation EGFR inhibitors. EGFR, HER2 and HER4 are RTKs that belong to the EGFR superfamily; they play major roles in both tumor cell proliferation and tumor vascularization and are overexpressed in many cancer cell types.

Drug	Clinical trial identifier number	Phase	Study design	Target
Erlotinib	NCT01316757	Phase II	Open label, Efficacy Study	EGFR
Afatinib	NCT01824823	Phase II	Randomized, Open Label, Efficacy Study	EGFR1,2,3,4

Table 2. Non-FDA approved kinase inhibitor drugs [34, 35]

B. Vaccine Therapy:

a. Non-FDA approved vaccines: There are some vaccines that are not currently approved by FDA for oropharyngeal cancer. However, many vaccines are under clinical trials in phase I, II, and III as in the Table 11 below.

1. Mutant p53 peptide pulsed dendritic cell vaccine: A cancer vaccine consisting of autologous dendritic cells, which have been pulsed with a mutant p53 peptide. Vaccination with mutant p53, peptide-pulsed dendritic cells may stimulate the host immune system to mount a cytotoxic T lymphocyte (CTL) response against tumor cells expressing mutant p53, resulting in tumor cell lysis. Many tumor cells overexpress mutant p53 proteins, resulting in the loss of apoptosis regulation and abnormal cell proliferation.

2. MAGE-A3 HPV-16 vaccine: A multi-epitope "Trojan antigen" ("TA") construct vaccine consisting of human melanoma antigen A3 (MAGE-A3) and human papillomavirus (HPV) 16 peptide epitopes linked by the furin-sensitive linker peptide RVKR (arginine-serine-lysine-arginine) with immuno-stimulatory and antitumor activities. The TA construct enters the cytoplasm of antigen-presenting cells (APC) and is processed by the endoplasmic reticulum (ER) and the trans-Golgi network (TGN), where the endopeptidase-furin releases the epitopes from the RVKR linker peptide and, together with various exopeptidases, generates MHC class I-binding peptides. Expressed on the cell surfaces of APC, these MHC-I binding peptides stimulate a cytotoxic T lymphocyte (CTL) response against tumor cells that display the same peptide epitopes on their cell surfaces.

Drug	Clinical trial identifier number	Phase	Study design	Target
Mutant p53 peptide pulsed dendritic cell vaccine	NCT00404339	Phase I	Randomized	CTL
MAGE-A3 HPV-16 vaccine	NCT00704041	Phase I	Randomized, Open Label, Safety/Efficacy Study,	Cancer cells

Table 3. Non-FDA approved vaccines [36, 37]

C. Miscellaneous Drugs:

1. EGFR Antisense DNA: A synthetic sequence of DNA constructed in the antisense orientation to a sequence of DNA in epidermal growth factor receptor (EGFR), a member of the erbB gene family. EGFR antisense DNA suppresses the expression of EGFR by tumor cells, thereby inhibiting tumor cell proliferation and decreasing tumor growth. This agent also appears to reduce the invasiveness of certain breast cancer cells. Members of the erbB gene family are over-expressed in many cancers and play roles in carcinogenesis and the regulation of cell proliferation.

2. BKM120 (Buparlisib): Buparlisib specifically inhibits class-I PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway in an ATP-competitive manner. It is an orally bioavailable specific oral inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases with potential antineoplastic activity. This inhibits the production of the secondary messenger phosphatidylinositol-3,4,5-trisphosphate and activation of the PI3K signaling pathway. This may

result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a

Conclusion

The recent activities have increased our understanding of the tumor microenvironment, various immunotherapeutic modalities and combination therapies (like chemotherapy with immunotherapy). Additionally, the effects of such modalities in combination with chemotherapy in cancer patients are still in the exploratory phase. The complete perspective of immunotherapy treatment has not been realized and/or utilized. Proper pre-clinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

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