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Review article

Immunotherapeutic Approach to Laryngeal Cancer

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Abstract

Laryngeal cancer is a rare cancer, accounts for 12,630 new cases in 2014 out of a total number of 1,665,540 new cancer cases in the United States. It is mostly Squamous in origin as the rest of head and neck tumors. 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 discuss the pathophysiology of Laryngeal 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: Laryngeal Cancer; Head and Neck Cancer; Immunotherapy; Epidermal Growth Factor Receptor; Kinase Inhibitor, Monoclonal Antibody; Cytokine; Vaccine; T Cell; HPV; P53

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

Laryngeal cancer is a type of head and neck cancer that arise from squamous cells, the thin, flat cells lining the inside of the larynx. [1]Smoking is the most important risk factor for laryngeal cancer. Death from laryngeal cancer is 20 times more likely for heaviest smokers than for nonsmokers.[2] Heavy chronic consumption of alcohol, is also significant risk factor. When combined with smoking, these two factors appear to have a synergistic effect. People with a history of head and neck cancer especially head and neck squamous cell cancer (HNSCC), are known to be at higher risk (about 25%) of developing a second cancer of the larynx, or lung cancer, additionally the infection with HPV is considered as an independent risk factor sd the HPV DNA has been detected in benign (papillomatosis), indolent (verrucous carcinoma), and malignant (squamous cell carcinoma) lesions of the larynx with approximately 25% of laryngeal squamous cell carcinomas harbor HPV infections on meta-analysis [3] There is evidence that alterations in the p53 gene are important for tumour progression as has been shown in malignancies with distinct sequential morphological features such as those which occur in the development of cancer in the oral cavity [4].

In laryngeal carcinogenesis dysplasia usually precedes the development of in situ and invasive SCC. Several studies indicate significant overexpression of stable p53 protein in invasive SCC of the larynx [5] and this phenomenon has been associated with heavy smoking [6,7] closely resembling that observed in lung carcinomas [8].

It seems that the mutated p53 gene plays a significant role in the early stages of carcinogenesis and may determine progression of the disease during the late stages of tumour development [7].The most common symptoms of laryngeal cancer are : hoarseness of voice , lump in the neck , stridor , sore throat , persistent cough, bad breath and pain when swallowing (odynophagia) [9]

Epidemiology

Laryngeal cancer is relatively rare, with an estimated incidence of 12,630 new cases in 2014 out of a total number of 1,665,540 new cancer cases in the United States [10]. Five year survival rates in the United States are 60%. The larynx is divided into three anatomical regions: the glottis, the supraglottis and the subglottis. Almost all laryngeal malignancies occur in the glottis (2/3) and supraglottis (1/3), with subglottic tumors accounting for only 2 %[11].

Pathophysiology and Molecular Basis of Laryngeal Cancer:

Larynx as it's 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.[12-14]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 pre-invasive lesions (Figure1). [13-14]The most common genetic alteration, the loss of chromosomal region 9p21, is found in 70-80% cases of HNSCC.[13,15-16] The CDKN2A gene locus, found inchromosome 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.[17]

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 [18]

The loss of chromosome region 3p is another genetic modification, which occurs in the HNSCC. [17,19-21] The specificlocusresponsible for the tumor suppressor phenotype of 3p remainsuncharacterized,but investigators have recognized at least four different regions of allelic loss.[17, 20,21,22]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. [16,23]

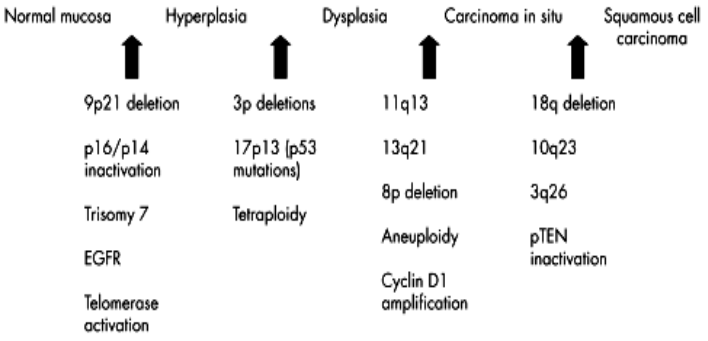

Figure 1. HNSCC carcinogenesis hypothetical model [13]

Loss of heterozygosity (LOH) of 17p and the point mutations of the p53 are seen in about 50% of the cases of HNSCC.[12] Around 64% of the p53 mutation take place in G nucleotides in HNSCC, consistent with the exposure to the carcinogens such as tobacco. [24]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.[25-27]CyclinD1 induces the phosphorylation of retinoblastoma (Rb), thereforeenabling 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. [28]

The gene expression microarrays suggest that a large number of transcriptional alterations take place during the transition from normal mucosa to premalignant lesions, insteadof 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. [29]

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.[30] 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. [31,32]

PI3K/AKT/mTOR: The PI3K activation starts with the phosphorylation of PIP2 (phosphatidylinositols) and successive AKT activation, one of the maineffectors ofthe PI3K signaling pathway.[33]The AKT also activates mTOR,which is responsible for combining various cellular signals such asstress, cellular energy stores, and nutrient levels. The PI3K is also indirectly activated through EGFR when itheterodimerizes with ERBB3. [34]

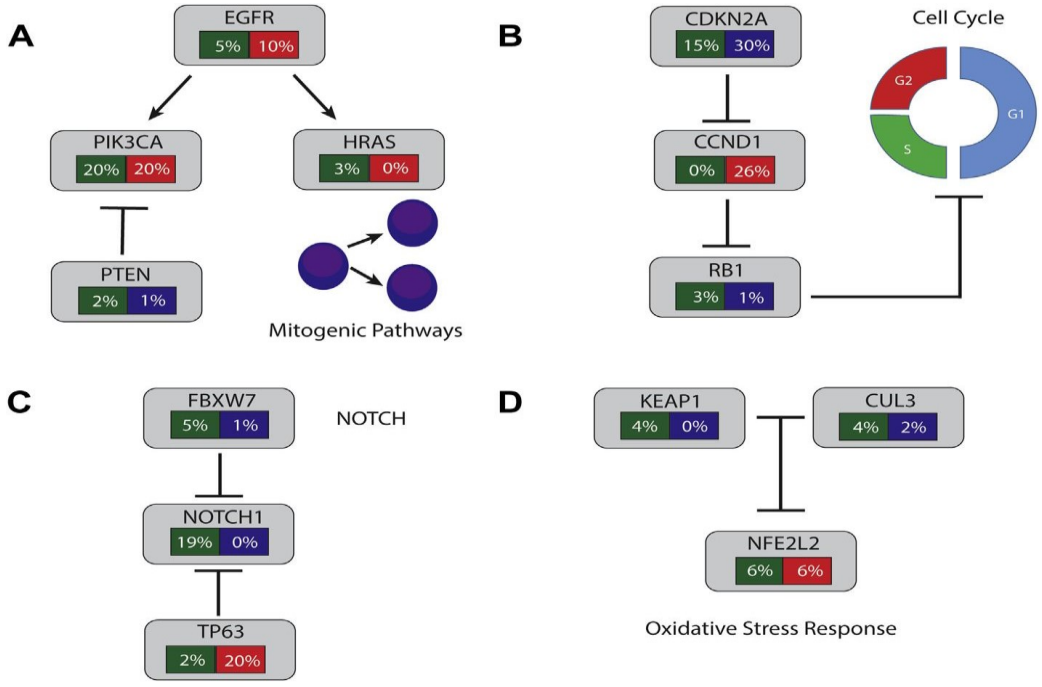

Figure 2. (A) Mitogenic Pathway Alterations. (B) Cell Cycle Alterations. (C) NOTCH Signaling. (D) Oxidative Stress Response. Green: Frequency of mutations. Red: frequency of amplification. Blue: Frequency of deletion [38]

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). [35] The p53 inactivation also arises in HPV-positive tumors. Thep53-mediated apoptosis does not come out to be the leading form of cell death in the epithelial tumors. [36]

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. [37]

Targeted therapy for Laryngeal cancer:

A. Kinase Inhibitor Drugs:

There are no drugs that are currently approved by FDA for laryngeal cancer. However, few drugs are under clinical trials in phases I, II, and III as in Table 2 below.

1. Erlotinib: An aquinazoline derivative withantineoplastic properties. Competing with adenosine triphosphate, erlotinib reversibly binds to the intracellular catalytic domain of epidermal growth factor receptor (EGFR) tyrosine kinase. This reversibly inhibits EGFR phosphorylation and blocks 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. This includes 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.

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[39-40]

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.

B. Vaccine Therapy:

There are no vaccines that are currently approved by FDA for laryngeal cancer. However, only vaccine that is under clinical trials in phases I-III is listed in Table 2 below.

1. MAGE-A3 HPV-16 vaccine: A multi-epitope "Trojan antigen" (TA) construct vaccine with immune stimulatory and antitumor activities that consists 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). 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 endopeptidasefurin 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 class 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
MAGE-A3 HPV-16 vaccine	NCT00704041	Phase I	Randomized, Open Label, Safety/Efficacy Study,	Cancer cells

Table 3. Non-FDA approved vaccines [41]

C. IDO Inhibitors:

Indoleamine-pyrrole 2,3-dioxygenase (IDO or INDO EC 1.13.11.52) is a heme-containing enzyme that in humans is encoded by the *IDO1* gene. This enzyme catalyzes the degradation of the essential amino acid L-tryptophan to N-formylkynurenine using the superoxide anion as an oxygen donor [42].

It has been shown that IDO permits tumor cells to escape the immune system by depletion of L-Trp in the microenvironment of cells. A wide range of human cancers such as prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, lung, etc. overexpress human IDO (hIDO). The details of the study are listed in table 3 below:

D. PI3k Inhibitors:

A phosphoinositide 3-kinase inhibitor (PI3K inhibitor) is a class of medical drug that functions by inhibiting one or more of the phosphoinositide 3-kinase enzymes, which are part of the PI3K/AKT/mTOR pathway. Within this pathway there are many components, inhibition of which may result in tumor

Drug	Clinical trial identifier number	Phase	Study design	Target
MK-3475	NCT02178722	Phase I/II	A Phase 1/2 Study Exploring the Safety, Tolerability, and Efficacy of MK-3475 in Combination With INCB024360 in Subjects With Selected Cancers (INCB 24360-202 / MK-3475-037 / KEYNOTE-037)	Cancer cells.

Table 4. IDO inhibitors [43]

suppression. [44]. The details of the study are listed in table 4 below:

Drug	Clinical trial identifier number	Phase	Study design	Target
Buparlisib	NCT01816984	PhaseI/II	Buparlisib and Cetuximab in Treating Patients with Recurrent or Metastatic Head and Neck Cancer	Cancer cells.

Table 5. PI3K Inhibitors [45]

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 target therapy treatment has not been realized and/or utilized in laryngeal cancer. Proper pre-clinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

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