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Immunotherapeutic Modalities in the Treatment of Head & Neck Squamous Cell Carcinoma

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Abstract:

Attempts of establishing vaccines against cancers are in process of becoming authentic in future due to its successful clinical trials. But, there are problems in assessing the prognosis of the diseases due to the weakness and specificity of tumor associated antigens in stimulating an effective immune response. A surplus combination of immunotherapeutic cancer strategies and appropriate checkpoint inhibitors becomes a promising area for successful cancer disease control. This article summarizes the understanding of tumor antigens (specific or associated) in head and neck squamous cell carcinoma, which allow some space with a positive and hopeful view of the future.

Keywords: Antigens; Cancer; Immune response; Tumor; Vaccines.

Introduction

The human immune system is a multifaceted system comprising of tissues, organs, and motivated cells that act individually or in a group to defend the body. Cancer, by definition, is a heterogeneous disease of the genes. A gene is a functional unit of DNA (Deoxyribonucleic acid), which is the chief molecule of the cell. Genes make "proteins," which allow all the processes that permit us for healthy living. An alteration (mutation) to the DNA molecule can disrupt the genes and produce faulty proteins. This causes the cell to proliferate abnormally and lose its restraints on growth and results in cancer formation[1].

Cancer is the leading cause of death and a major health problem worldwide requiring new treatment modalities and new strategies to combat the disease. Among all the treatment modalities like chemotherapy, radiotherapy and adaptive immunotherapy and vaccine based immune response against the tumor is thought to be the only way to prevent the cancer for lifetime. The immunotherapies have the potential to destroy tumor cells with significantly better specificity and safety by the development of humoral and/or cellular immune response against the malignant tissue. Advancement in the area that have the potential to divulge more immunogenic proteins to target site, leads to stronger and more stable responses to treatment. Tumor immunotherapy generally consists of administration of cells/antibodies ex vivo (Passive) and (Active) with administration of vaccine, against the Tumour Specific Antigen (TSA) and Tumor Associated Antigens (TAA) [2 – 7].

Immunology of the cancers particularly Head and Neck cancers are closely associated with host immune system, hence the reversal of immunosuppression is the ultimate therapeutic goal in treating these types of tumors. A plethora of immunotherapeutic approaches have been investigated in an attempt to enhance the immune reactivity towards cancers. These includes

1. Cell mediated Immunotherapy
2. Targeted Immunotherapy
3. Antibody Immunotherapy
4. Cancer Vaccines

Cell Mediated Immunotherapy

This therapy aims to stimulate the patient's own immune system to plinth an antitumor response by stimulating cytotoxic T cells to kill the cancer cells. Systemic Cell mediated immunotherapies are nonspecific because it attempts to replace the entire immune system by mounting either a systemic and/or loco regional antitumor response [8]. The antitumor effect is elicited loco regionally and/or systemically by delivering proinflammatory cytokines under cytokine treatment approaches. Various cytokines are being explored for the treatment of head and neck cancers. Some of them are discussed below:

1. Interleukin (IL) – 2 – It is a major proinflammatory cytokines produced by T cells. The use of IL – 2 as cytokine immune therapy has shown increased cytokine levels and Natural Killer (NK) cells within the tumor along with the increased activity of tumor infiltrating lymphocytes. It has been suggested that the therapy causes tumor shrinkage, tumor specific delayed type of hypersensitivity and long lasting immune memory. Mode of delivering IL – 2 includes intralesional/intratumoral injections and synthetic gene delivery system. Study by De Stefani et al.[9] showed that perilymphatic administration of IL – 2 significantly increased overall and disease free survival of the patients with HNSCC. The high doses of IL – 2 can be toxic and can induce Treg (regulatory T- cells) expansion which suppresses the surrounding lymphocytes.
2. Interleukin (IL) – 12 – IL -12 promotes T cell growth and affects both the innate and adaptive immune response. When administered intratumorally into Head and Neck Squamous cell carcinoma (HNSCC) patients, Van Herpen et al. [10] observed it increases the level of B cells within the tumor, stimulates B cell proliferation, increases B cell expression of IFN - $_{\gamma}$ and skews the plasma antibody profile towards the Th1 phenotype. It regulates cellular immunity by the production and activation of cytolytic T cells and NK cells and also induces the production of cytokines.
3. Granulocyte – Macrophage – Colony Stimulating Factor (GM-CSF) –It is expressed in most of the tumor types with an evidence of antitumor activity. Giersten et al. [11] showed in his study that, synthetic mutant raspeptides with GM-CSF as an adjuvant are highly effective at generating high-level ras peptide-specific T-cell responses. The adjuvant effect of the cytokine GM-CSF is related to the maturation and activation of DCs, which after antigen uptake will move to an adjacent lymph node and activate effector T cells[11 – 14]. Further experimental studies are required to expand the treating strategies for the patients with HNSCC.
4. IFN (Interferon) - $_{\gamma}$ - The effectiveness of IFN - $_{\gamma}$ treatment demonstrated varying levels of immunological and clinical responses by Richtsmeier WJ et al. [15] in 1990. In his 22 days of treatment of 4 stable HNSCC, a case of carcinoma in situ in the piriform sinus showed healing and the other 3 showed tumor shrinkage by 40%, 40% & 18%. IFN - $_{\gamma}$ has not been studied well in HNSCC, hence needs attention and more clinical trials.
5. IFN – α – Interferon – α has been combined with other drugs as an adjuvant for the treatment of head and neck cancers. Urba SG et al. [16] in 1993, used combined therapy of INF – α2a and IL – 2 in their patients and observed partial response in 18% of patients, instead treatment was associated with substantial toxicity. Bazarbashi S et al. [17] adopted combination of IFN– α, cisplatin and 5-fluorouracil in the treatment of esophageal cancer which resulted in a 61% response with significant side effects.
6. IRX (Murine *Iroquois* gene family) – 2 - A promising mix of cytokines in cell mediated immunotherapy employs a multifaceted approach towards stimulating immune response. IRX – 2 is a natural cell derived cytokines mixture including IL - 1β, IL – 2, IL – 6, IL – 8, IFN- $_{\gamma}$, TNF– α and GM – CSF. IRX – 2 is produced by stimulating peripheral blood mononuclear cells (PBMCs) from healthy donors and is sterile, serum free and endotoxin-free. IRX – 2 treatments resulted in an increased expression of co-stimulatory markers as well as markers for maturation and migration. The ability of IRX-2 to activate both DCs (dendritic cells) and T and even B and NK cells makes it especially attractive since it is this combination of immune cell subsets that coordinate the immune response against tumor antigen expressing cells. Berinstein et al.[18] demonstrated increased number of tumor infiltrating lymphocytes in patients following IRX – 2 treatments. Also, correlated high lymphocyte immune infilterate with decrease tumor size and improved 5-year survival. IRX -2 accelerates antitumor effects of immune cells, such as 'up regulation of key signaling molecules', expression on dendritic cells so as to increase their functions [8, 18].

Targeted Immunotherapy

The amalgamation of the methodology has enabled us to identify different kinds of tumor associated antigens as therapeutic target agents. The therapeutic efficacies of the tumor associated antigens are under investigation for the following targets. First, towards tumor – specific mutated proteins, which are unique to the tumor and may contribute to the malignant phenotype? Second, tumor specific antigens or germ cell antigens or cancer tests antigens which remain quiescent in normal tissue but can be reactivated in some malignant tumors. Third, is towards over expression of the antigens in tumors [8].

Tumor associated antigens such as EGFR (Epidermal growth Factor receptor), HPV (Human Papilloma Virus) or Hsp65 (Heat Shock Proteins 65) have been clinically trialed in HNSCC but the results were not satisfactory. The major drawbacks which were reported was profound immune suppression, the mechanism for which are multifocal. In HNSCC, several immune inhibitory mediators are released which inhibits the T cell functions. Also, functionally impaired and defective maturation of dendritic cells are observed in HNSCC patients because of the mediators released by the tumor cells causes shift in the dendritic cell signaling production [19].

The EGFR family encompasses four receptor proteins, namely ErbB-1/EGFR-1 to -4 (also called HER 1-4) that are expressed on cell surface and exhibit tyrosine kinase activities. Overexpression and/or gene amplification of EGFR confer malignancy to diverse tissues. Active mutants of EGFR are found in different cancers, and are often associated with poor prognosis. Triggering of EGFR induces antibody dependent cell – mediated cytotoxicity via the recruitment of NK (Natural Killer) cells and macrophages [20, 21].

HPV associated cancers often express targets such as oncogenic E6 and E7 proteins, exogenous protein, which can be targeted through vaccination. Schoenfeld [22] suggest that immune reaction directed against these antigens is expected to spare the normal host tissue and hence, it is easier to overcome immune tolerance and generate antitumor immune response. Another virus associated with tumor genesis is EBV (Epstein Barr Virus), an attractive site for immunotherapy. Reactivation of EBV enhances the immune recognition.

Immune inhibitory cells areTreg cells, immature CD34+ progenitor cells and tumor derived VEGF (Vascular Endothelial Growth Factor). Treg cells level is correlated with CD8+ cells, as the ratio of these are associated with the increase/decreased survival. Treg cells hydrolyse ATP (Adenosine Triphosphate) to greater level which increases the levels of adenosine-mediated suppression of T cells. The homeostasis of Treg cells depends on the inhibitory protein CTLA-4 (Cytotoxic T-lymphocyte associated antigen – 4). CTLA-4 functions as a natural break to prevent autoimmunity by down regulating the T cell activation and proliferation. It is presumed that the blockage of CTLA-4 may improve the immune suppression in HNSCC [23 – 25].

Immature CD34+ progenitor cell levels were shown to be increased in the peripheral blood of HNSCC. These mediate their immune suppression behavior by the production of (Transforming Growth Factor) TGF – β. CD34+ cells can be mobilized by the tumor cells to differentiate into granulocytes/monocytes, endothelial cells and/or dendritic cells. These CD34+ cells are expected to be the precursor of immune inhibitory cells in various stages of differentiation. Myeloid - derived suppressor cells (MDSC) are the differentiating product, found in various tumors, and it suppresses immune activity via reactive oxygen species, which can be blocked by blocking NADPH (Nicotinamide adenine dinucleotide phosphate) oxidase.

Immune inhibitory endothelial cells causes immune suppression, induced by tumor – derived VEGF, through the production of prostaglandins which blocks production of INF - γ, T – cell proliferation, perforin and granzyme B. All these will inhibit T - cell helper and cytotoxic effect [26 – 28].

Antibody Immunotherapy

To increase the antitumor effect, treatment following production of monoclonal antibodies towards the specific target and tumor cell apoptosis with better tolerance was the fascinating field of research. Studies have shown an absence of lymphocytic infiltration as a result of antibody treatment. EGFR is often expressed in most of the HNSCC and its overexpression is correlated with the poor clinical outcome. Anti – EGFR antibodies clinical trials in HNSCC patients showed down regulation of EGFR expression. Anti – EGFR antibodies blocks EGFR phosphorylation and downstream signaling along with complement mediated tumor lysis. This cytolytic action can be enhanced by combining antibodies directing against different non overlapping epitopes [20, 21].

The antitumor effect can also be brought about by targeting the vascular system of the tumor. VEGF binds to the receptors present on the vascular endothelial cells thus inducing angiogenesis. VEGF has three membrane receptors (VEGFR 1 – 3) expressed on the endothelial cells. The VEGF & VEGFR interaction provides the key roles in vasculogenesis and angiogenesis. Hence, the inhibition of angiogenesis and decrease micro vascular permeability can be achieved by blocking the binding/interaction of VEGF to VEGF receptor. This has been suggested to increase the effectiveness of chemotherapeutics [29, 30].

Carcinoembryonic antigen (CEA) is an oncofetal glycoprotein and a tumor associated antigen observed on the surface of most of the HNSCC. CEA is observed in high levels in colon epithelial cells during the embryonic development but it significantly lowers in the colon tissue of adults. CEA levels can be elevated during the inflammation, benign and malignant tumors. In humans CEA genes encodes messenger RNA and is uniquely linked via lipid into the membrane through the glycosylphosphatidylinositol moiety, but its exact function in tumor cells is not clear. Hence it serves as recognition markers [31].

CEA is used as immunotherapeutic agents to the site of an established tumor. It is suggested that when anti-CEA antibodies binds to the surface of a tumor cell it activates complement cascade/antibody directed cellular cytotoxicity, which can result in the destruction of an antibody marked cells. But as CEA is found in a heterogeneous pattern within a tumor mass, it makes it very difficult to eradicate all the cells.

CEA also produces anti-tumor response by mediating CD4+ and CD8+ T cells by shedding the CEA proteins by the tumor cells. These proteins are taken by the dendritic cells or antigen presenting cells and processed into smaller peptide fragments and presented to CD8+ and CD4+ T cells via major histocompatibility complex (MHC) I & II. CEA is recognized by T cell receptors (TCRs) which leads to release of cytokines (IL – 2) by CD4+ cells and mediates CD8+ cells either by perforins or by receptors that are involved in apoptosis of cells [32, 33].

Cancer Vaccines

These are medicines that are considered as biological response modifiers. There are two broad types of cancer vaccines:

- **Preventive (or prophylactic) vaccines**, which focuses on cancer immune prevention.
- **Treatment (or therapeutic) vaccines**, which focuses on cancer immunotherapy

Prophylactic vaccines are far away from its application in human subjects and only limited as research mode in animal models. On the other hand, therapeutic vaccines have been recently used in research on transgenic mice, prone to cancer development and tried to prove that it can lead to complete tumor prevention and normal life restoration. The two most common types of therapeutic cancer vaccines are peptide-protein based and dendritic cell based cancer vaccines [35].

Peptide – Protein Based Cancer Vaccines

Protein or peptide had been used to build immunity against the cancer employing broad range of proteins such as heat shock proteins (HSP), Agonist Peptides, Anti-idiotype antibodies. It has been suggested by Rosenberg in 2008thatvaccines based on proteins have stronger response on the generation of CD4+ lymphocytes, and less effective on production of Cytotoxic T lymphocytes [36 – 38].

Weak immunogenicity of single protein, Tumors evading antigen recognition through mutation loss and HLA restriction of proteins, for vaccine development can be overcome by use of long peptides and using dendritic cells loaded with protein as adjuvant to boost immunity. For the therapeutic effect, an adjuvant is combined with one or more peptide-proteins, which are commonly, expressed in head and neck squamous cell carcinoma (HNSCC) cancers such as p53, MAGE, HPV. These adjuvants are compounds that serve to improve the extent, scope, superiority and durability of specific immune responses to antigens, but have minimal toxicity or long-term immune effects on their own. These vaccines anticipate to stimulate T-lymphocyte responses to tumor-specific antigenic peptides presented on the surface of tumor cells through MHC I molecules. Most of the tumor antigens originate in tumor cell cytosol or cellular organelles where they complex with the surface MHC I molecules. These synthetic peptide vaccines may also enclose some distinct complexes or supramolecular structures like polymer particles, liposomes, micelles;etc. can specifically or nonspecifically trigger our immune system immune to synthetic fragments and thereby initiating the immune response [39 – 41].

The vaccines based upon several peptides sequences which have been tried for cancer immunotherapy in phase I/II/III clinical trials are Heregulin (HER)-2/neu peptide, Carcinoembryonic antigen (CEA), Mucin-1 (MUC-1) peptide, Prostate-specific membrane antigen (PSMA), ALVAC-CEA (Canarypox viruses) vaccine, Rasoncoprotein, Melanoma antigens IFN, peptide-granulocyte-macrophage colony-stimulating factor, GP2 vaccine, AE37 vaccines, E75 vaccine, GPC3-derived peptide vaccine, GV-1001 Dendritic Cells pulsed with four AFP peptides modified DNA aptamer TLS11aGC, anti-OX40 monoclonal antibody with two folate binding protein peptide, Vaccines (E39 and J65), Bombesin/Gastrinreleasing peptide.

Peptide vaccines have several advantages; they are easily produced, inexpensive, safe, synthesized in big quantities and represent a standardized, well defined antigen, allowing post vaccination immune response monitoring.2,23 Their main disadvantage is MHC I allotype restriction that makes them useful only in the patients matching MHC I allotype [42, 43].

Dendritic Cell - based Cancer Vaccines

In 1991, Ralph Steinman identified the dendritic cell (DC) as the key player involved in controlling the immune response. DCs consititute only 0.5% of blood leukocytes. Like other cell types within the immune system, they arise from a common CD34+ progenitor in the bone marrow whose expansion and differentiation is influenced by a variety of cytokine growth factors including stem cell factor, fetal liver tyrosine kinase-3 (Flt-3) ligand, IL-3 (Interleukin), granulocyte/macrophage colony stimulating factor (GMCSF), TNF-α (tumor necrosis factor) and TGF-β (Transforming growth factor)[44 – 46].

The family of human DC displays considerable heterogeneity and plasticity at the level of phenotype and function. The majority of experimental systems clearly demonstrate that tumor immunity is largely provided by CD4+ T lymphocytes, CD8+ T or NK cells [47 – 53].

Clinical trials of DC vaccination have been made possible by the development of methods for obtaining large numbers of human DCs. Three general approaches have been exploited for use in clinical trials that includes 1) Direct isolation from blood or in vivo by growth factors 2) From CD14+ monocytes and 3) From CD34+ hematopoietic progenitors.

Various ranges of different antigenic preparations for charging DCs are available which can be either known tumor associated antigens or approaches when antigens are unknown. Known tumor associated antigens includes a) Synthetic or eluted peptides b) Recombinant or purified protein c) Non-peptide antigens d) Transfection with cDNA or RNA e) Recombinant viruses. When antigens are unknown then the approaches includes a) Differentiation of DCs from malignant cells b)Tumor-DC fusions c)DC-derived exosomes d)Tumor RNA e) Apoptotic or necrotic tumor cells f)Tumor lysates.

The use of whole protein antigens, DNA, RNA or recombinant viruses encoding the antigen of choice allows human leukocyte antigen (HLA) molecules to select the appropriate peptide epitope to form peptide-MHC complex on the cell surface. Although, this approach does not require analysis of MHC molecules, butthe awareness of the fact that spectrum of epitopes seen by effector T cells might be restricted, as certain peptides are not presented by DC due to incomplete processing at the level of the proteasome. Newer approaches to use the entire antigenic content of a tumor cell for the vaccination, to present it as tumor antigens is possible to the immune system, which minimizes the occurrence of immune escape variants. This could be achieved by either pulsing DCs with whole tumor cell lysate, tumor derived RNA, DNA or fusion of tumor cells and DCs.But the limitation of this approach is the availability of tissue serving as a source of tumor lysate or tumor derived RNA. Another major disadvantage in using whole tumor in the form of lysates, RNA or DC tumor fusions, is that, the effector cells functions in vitro and in vivo is difficult to achieve and monitor [54 – 58].

Delivery or administration of the DC based vaccines in an effective way to the cancer patients is also an important aspect. Clinically, subcutaneous, intradermal, intravenous and intranodal approaches to deliver DCs have been evaluated. The intravenous route of administration resulted in the accumulation of DCs to lung, liver, spleen and bone marrow, but not the lymph nodes or in the tumor site. The intranodal approach bypassed the migration to lymphoid tissue and simply relied on to express effective T cell stimulatory capacity. In contrast, studies using intradermal injection of monocyte derived DCs have demonstrated direct migration of DCs to the draining lymph nodes. However, immature DCs were used in these particular studies, and only 1% of DCs migrated to the regional lymph node and the majority remained at the injection site [59 – 61].

Most of the published clinical studies have shown that generation of antigen-loaded DCs ex vivo on a clinical scale is possible and DCs vaccination is safe and well tolerated. But DCs carries a risk of inducing autoimmune responses against self-antigens, precisely if the target antigen is also expressed by normal cells. As the cancer patients are known to exhibit defect in DC-based functions, it becomes vital when autologous DCs are prepared as vaccine. This might include defect in antigen presentation, and DC maturation. Also, tumor cells can secrete various mediators including IL-6, IL-10, and VEGF that have recently been found to inhibit DC differentiation and/or maturation. Hence, an in vitro treatment of DCs must be enhanced to resolve this issue [62 – 65].

Future Prospective

Cancer vaccine development is challenging due to lack of Tumor Specific antigen and weak strength of Tumor Associated antigen to stimulate strong immunogenic response. The research in the future needs to stress on the following – 1) Recombinant Vector based vaccine to direct strong immunogenic response against weakly immunogenic Tumor Associated Antigens 2) Mechanism of activation of Antigen Specific cytotoxic T lymphocytes, Decreased TREG numbers functionality and antigen cascade mechanism during immunological reactions 3) Computational and mathematical modeling to analyze the efficacy of cancer vaccines for anti-cancer approach 4) T cell epitope prediction and binding to find potential antigenic determinant for binding of MHC molecules using the in sillico approach.

Conclusion

Cancer cells are too heterogeneous in nature, the antigens they express and their susceptibility to immune-mediated killing, hence many immunotherapeutic agents are introduced to work in different ways and to shift the balance towards antitumor activity. Immunotherapeutic agents are actively tested in patients with head & neck squamous cell carcinoma, but still it remains questionable whether an immune reaction alone will be able to eradicate large tumor masses in advanced-stage of diseases. Further research to describe the finest approach towards vaccination and intervention at initial stages of tumors will further progress the effectiveness of these immunotherapeutic drugs and/or vaccines.

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