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Developing an Immunotherapy Strategy for the Effective Treatment of Oral, Head and Neck Squamous Cell Carcinoma

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The recent understanding of the role of tumor-infiltrating lymphocytes in different solid cancers suggests that only patients with evidence of an immune response will have durable responses to therapeutic intervention. Therefore, for more patients to achieve clinical benefit, it is necessary to find effective approaches to prime de novo antitumor immunity. This is particularly true for oral, head and neck squamous cell carcinomas (OHNSCCs), which are known for their immune-suppressive character and for which clinical outcome has not appreciably improved during the past 4 decades. The authors' group is exploring an immunotherapy strategy for the effective treatment of OHNSCC, which uses antibodies that modulate T-cell surface proteins with the use of next-generation vaccines and radiation therapy as an antigen source to prime T-cell response and induce immune-mediated rejection of tumors. The purpose of this review is to describe recent advances in immuno-oncology and the authors' developing therapeutic strategies.

The immune system consists of 2 components that offer protection from tumor cells (and viral and bacterial pathogens): innate and adaptive. The innate immune system provides the body's initial response to cancer by directing specialized preprogrammed leukocytes, such as natural killer (NK) cells and macrophages, toward tumor cells. In contrast, the adaptive immune system takes a longer time to initiate and is focused on specific antigenic targets in tumor cells. Adaptive immunity relies on lymphocytes called *T cells* and *B cells*. T cells recognize antigens associated with the tumor, secrete cytokines, and directly lyse or kill tumor cells in a granzyme- and perforin-dependent manner. B cells produce antibodies that can bind to the antigen on the surface of tumor cells and mediate cell lysis through NK cells and macrophages in a process termed *antibody dependent cellular cytotoxicity* (ADCC). These

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antibodies typically target proteins that are expressed on the surface of the tumor. Recently, ex vivo antibodies targeting proteins expressed on tumor cells and the immune cells of the tumor have been developed for the treatment of cancer and are part of an exciting new class of agents poised to have a profound effect on treatment outcome for patients with solid cancers. Therapeutic antibodies activate or block specific signaling pathways to modify the behavior of cancer cells (eg, epidermal growth factor receptors [EGFRs]) or T cells (eg, cytotoxic T-lymphocyte-associated protein-4 [CTLA-4]) and have recently formed the backbone of a type of cancer treatment called immunotherapy.

Tumor-Infiltrating Lymphocytes

It has long been observed that some OHNSCCs generate a robust lymphocytic inflammatory cell infiltrate within the tumor and others do not. Recent investigations into the microenvironment of several malignant tumor types have shown that the number, type, and location of the TILs could have prognostic importance.[1]

The realization that TILs can be predictive of biological behavior[2] has led to the development of a method of quantifying and qualifying the immune infiltrate called an *immunoscore*.[3-7] The potential of this new prognostic biomarker is based primarily on the density of cytotoxic (CD8) and memory (CD45R0) T cells (CD3 and CD45R0, CD3 and CD8, or CD8 and CD45R0) located at the tumor's center and at its invasive margin.[4-7]

The immunoscore has not been evaluated in OHNSCC, but there is evolving evidence that increased levels of TIL are associated with a good prognosis in human papilloma virus (HPV)-related oropharyngeal cancer,[8] and that this antitumor activity appears to be related to E6- and E7-specific T-cell response against viral epitopes.[9] Recently, Ward et al[10] found that TILs predict the outcome in HPV-positive oropharyngeal cancer. Furthermore, they could stratify patients with positivity for HPV into high-risk and low-risk groups based on TIL levels and developed a prognostic model for HPV-related tumors that incorporated TIL levels, heavy smoking, and T stage.

The authors' group is exploring the immunoscore concept further in the HPV-negative population, using digital imaging and objective assessment software combined with molecular sequencing of a panel of genes that regulate various immune cell types and populations, common tumor-associated antigens (TAAs), and other immune-related genes (Fig 1). Their goal is to develop a clinically relevant biomarker for OHNSCC based on expression profiles of T-cell surface proteins and genes that regulate immune cell function and to identify targetable molecules and pathways that can guide therapy. The authors and others hypothesize that the highly immune-suppressed microenvironment and pervasive systemic immune suppression that characterize OHNSCC could be reversed by a strategy that favors antitumor immunity.[11] To test this hypothesis, clinicians will need to better understand why OHNSCCs that are "immunoscore negative" lack immune cell infiltrate or manifest unfavorable gene expression profiles. Such information would provide a critical element in the design of clinical trials that would increase infiltration of anticancer immune cells and, the authors hypothesize, lead to cancer regression.

Tumor-Associated Antigens

Tumors express different proteins that are not usually expressed in healthy cells. Sometimes, owing to overexpression, gene mutation, or viral infection, T cells can recognize antigenic proteins in tumors. Mutated proteins associated with cancer can be a source of antigen, as can the products of nonmutated genes that are expressed by some cancers, such as cancer testis antigens. These TAAs also can be derived from the cancer's tissue of origin, such as melanosome-associated proteins, against which tolerance has not been developed. An example of antigenic protein that results from genetic mutation is the tumor-suppressor gene *TP53*.[12] In addition, oncoviruses, such as HPV and Epstein-Barr virus (EBV), are important antigen targets of E6 and E7 oncoproteins and EBV nuclear antigens, respectively.[13-15] The oncoproteins (E6 and E7), in particular, being nonself-antigens that are necessary for cancer development after HPV infection, are considered optimal targets of immunotherapy for HPV-related malignancy.

Immunotherapy

There are 2 types of immunotherapy approaches: passive and active. Passive immunotherapy involves the administration of therapeutic antibodies or patient-specific T cells (adoptive cell transfer) in an effort to boost immune-related tumor cell killing. Active immunotherapy is characterized by vaccine strategies, the administration of cytokines, or other immune therapies that activate the patient's existing immune cells to destroy cancer cells.

THERAPEUTIC VACCINES

There are 2 kinds of vaccines: prophylactic and therapeutic. Prophylactic vaccines have been used to prevent infectious diseases, such as polio, and in the prevention of cancers caused by viruses, such as liver cancer (hepatitis B virus) and cervical cancer (HPV). Unfortunately, the successful use of vaccines to treat established cancers has been elusive. Recently,