1. INTRODUCTION

1.1. Genetics of Cancer

It is generally recognized that the unregulated growth of cancer cells results from sequential acquisition of mutations in genes that control growth and/or differentiation of cells or are involved in protection of the genome. Cancer develops when the accumulation of these alterations allows for a growth advantage over normal surrounding cells (1). The pathogenesis of cancer can be described as follows: Oncogenes are altered normal genes (called proto-oncogenes) that mediate normal cell growth and differentiation. Gain-of-function (dominant) mutations affect these genes to induce the neoplastic phenotype. Tumor suppressor genes are genes that normally inhibit cellular function. Loss-of-function (recessive) mutations alter their inhibitory properties, leading to unimpeded proliferation. Gene therapy aims to change these genetic alterations so that cancer cell growth can be suppressed. After a gene is transferred into a cell, mRNA is transcribed, and then its protein product is translated.

Alterations of the tumor suppressor genes p53 and p16 have been implicated in the development of head and neck squamous cell cancer (HNSCC) (2,3). The p53 gene is mutated in approx 33 to 45% of HNSCC (2–4). The p53 protein acts by inhibiting the cell cycle, promoting apoptosis, and regulating transcription (5). The p53 protein upregulates the cell cycle inhibitor p21, which further acts on cyclin-dependent kinases (cdks) to cause cell cycle arrest. The p53 gene also causes apoptosis in cells that have undergone severe DNA damage from radiation or chemotherapy exposure and inhibits DNA repair and synthesis. Furthermore, p53 regulates transcription by stimulating transactivator proteins (5). The suppressive effects of p53 genes lead one to believe that clinical behavior can be correlated to alterations in the gene. A handful of protooncogenes have been implicated in the development of HNSCC, e.g., Her2/neu and cyclin D1. Her2/neu, a transmembrane tyrosine kinase receptor that functions to promote cellular proliferation, is overexpressed in approx 40 to 50% of HNSCC (6,7). Cyclin D1 (a promoter of cdk 4/6 and the cell cycle) is overexpressed in 12 to 54% of HNSCC (8), because of the amplification of chromosomal area 11q13 (9,10).

1.2. Tumor Immunology

The body’s immune system has a surveillance function that seeks out and destroys tumor cells. A “hierarchy of immunosuppression” exists in patients with HNSCC (11) that enables tumor cells to avoid detection. Immune reactivity is maximally suppressed in tumor infiltrat-
ing lymphocytes (TILs), followed by lymph node lymphocytes (LNLs) and peripheral blood lymphocytes (PBLs) (11). Immune cells, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, attack cancer cells. Cytokines, such as interleukins and interferons, activate the immune system. Tumor-specific antigens are expressed on tumor cells and help the host recognize and mount a specific immune response against these cells.

Induction of a T-cell immune response by antigen-presenting cells (APCs) occurs in three distinct stages. Initially, a nonspecific adhesion occurs between an APC and a T cell, followed by antigen-major histocompatibility complex (MHC) of the APC crosslinkage with the T-cell receptor (TcR). The final step occurs when a second or costimulatory signal is delivered by the APC to the T cell, enhancing response. Presently, the best characterized second signal occurs when the B7.1 or B7.2 ligand of the APC binds to the CD28 receptor on the T cell, resulting in enhanced cellular activation (12). The goal of genetic immunotherapy is to promote this T-cell response against cancer cells.

2. APPROACHES TO GENE THERAPY FOR CANCER

The approaches to gene therapy are as follows: (1) corrective gene therapy, (2) cytotoxic therapy, (3) immunotherapy, and (4) combination adjuvant therapy (Table 1).

2.1. Corrective Gene Therapy

Gene therapy can be used to correct any molecular aberrations in the control mechanism of cell proliferation. For example, replacement of a mutated tumor suppressor gene with a copy of the wild-type gene results in appropriate cell death. Alternatively, an oncogene can be inhibited either by transfecting the antisense cDNA so it binds to the mRNA of the oncogene or by adding a gene that regulates and inhibits the transcription of an oncogene.

2.2. Cytotoxic Therapy

Gene therapy can be used to augment cytotoxic therapy by either a drug sensitization or a resistance approach. In the drug sensitization approach, a gene is transfected to convert a prodrug into its active metabolite. This allows for drug conversion and a high level of active drug only in the tumor bed. One example is the herpes simplex virus thymidine kinase (TK) gene, which converts ganciclovir into its cytotoxic triphosphate. Another way to augment cytotoxic effects of chemotherapy is to use a drug resistance approach. A drug-resistant gene, such as multiple drug resistance (MDR1) gene, is added into cells that are sensitive to chemotherapy, such as hematopoietic stem cells, so they can resist the toxicity of chemotherapy. Therefore, higher doses of chemotherapy can be used since the most sensitive cells are now resistant to these levels of chemotherapy.

2.3. Immunotherapy

Immunotherapy can help decrease the immune suppression described above by “revving up” the immune system’s tumor killing capabilities. Cytokine gene transfer is a method used to stimulate the immune system. Cytokine gene transfer is performed in vivo whereby tumor cells or immune cells, such as TILs and CTLs, are transfected in the body, upregulating the immune and anti-tumor response. Ex vivo cytokine gene transfer is performed after fibroblasts, immune cells (such as TILs, CTLs, or APCs) or irradiated cancer cells are removed from the body, and then these cells are placed back into the body to obtain high levels of a cytokine with a resulting immunological effect. Irradiated tumor cells are used not only to produce high levels of cytokine but also to provide tumor antigens for immune cells.