21. GENE THERAPY FOR THYROID CANCER

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INTRODUCTION

The original concept of gene therapy is to treat and cure diseases caused by a known monogenic defect by introducing and expressing a normal copy of the mutated or deleted gene into the host cells. In this regard, gene therapy for cancer should be aimed at correcting gene alternations in cancer cells, that is, replacement of tumor suppressor genes and inactivation of oncogenes. However, cancer gene therapy has evolved in somewhat different directions. These include (i) transfer of suicide genes that convert inactive prodrugs into cytotoxic compounds, (ii) transfer of genes coding immunostimulators such as cytokines and chemokines to enhance anti-tumor immunity, (iii) transfer of genes coding anti-angiogenic factors to inhibit angiogenesis in solid tumors, (iv) transfer of drug resistant genes into normal hematopoietic stem cells to render them resistant to high-dose myelosuppressive chemotherapeutic agents. These strategies do not constitute "gene-replacement therapy" as defined above, and might instead be called "DNA therapeutics" for instance (1). Cancer gene therapy can be defined simply as "the transfer of nucleic acids into cancer or normal cells to eliminate or reduce tumor burden".

Genes can be introduced into target cells *ex vivo* and placed back into the host or directly into target cells *(in vivo).* Viral or non-viral vectors are used to facilitate the transfer of genes into target cells. This chapter discusses recent advances in gene therapy of the thyroid cancer field. Attention is focused on the therapeutic genes used.

STRATEGIES USED FOR THYROID CANCER GENE THERAPY

Silencing of oncogenes

According to the original concept of gene therapy, that is, "gene-replacement therapy", we may speculate that correction of a mutated or an aberrantly overexpressed oncogene might reverse malignant phenotype. On the other hand, some may contend that since cancers generally arises as the culmination of a multiple process that involves a variety of somatic gene alternations (see Chapter 1 for more detail), it is impossible to correct all the genetic abnormalities, as neither to restore normal gene function in every cancer cells with currently available vectors.

Several mutations or overexpression of oncogenes have been identified in thyroid cancers. The former includes RAS mutations and RET gene rearrangements in follicular and papillary carcinomas, respectively (2), and the latter overexpression of c-myc and high mobility group I (Y) protein [HMG I (Y)] in some thyroid cancers with highly malignant phenotypes (3, 4). Theoretically, suppression of gene expression can possibly be achieved with antisense, ribozyme, intracellular single-chain antibodies or RNA interference. For instance, suppression by antisense method of expression of c-myc and HMG I (Y) protein is reported to induce growth inhibition and cell death, respectively, in thyroid cancer cell lines with overexpression of a respective gene (3, 4).

Replacement of tumor suppressor gene

Among numerous mutations in different tumor suppressor genes so far identified in distinct types of cancers, the gene for tumor suppressor p53 (5) is well known to be frequently mutated in anaplastic, not well-differentiated, thyroid carcinoma (6–8). These mutations are closely associated with de-differentiation of thyroid cancer, and therefore thought to be the late event in thyroid carcinogenesis.

One can expect that introduction of wild type (wt)-p53 gene into thyroid cancer cells defective in normal p53 might reverse malignant phenotype or induce re-differentiation. Indeed it has been reported that reintroduction of wt-p53 by stable transfection into p53-defective follicular cell-derived thyroid cancer cell lines and a medullary thyroid cancer (MTC) cell line led to cell cycle arrest and growth inhibition (presumably the cells expressing p53 at relatively low levels survived) (9–16). Re-expression of wt-p53 is accompanied by chemosensitization, radiosensitization and re-appearance of the differentiated markers such as TPO, TSHR and PAX8 (9–11,14,15). Besides, of interest, despite *in vitro* cell growth inhibitory, not cell-killing, effect of wt-p53 in an anaplastic thyroid cancer cell line FRO, FRO cells stably expressing wt-p53 exhibits poor tumorigenicity in nude mice (16). Thus, tumors can not grow more than a few mm in a diameter. Tumors are found to be in an angiogenesis-restricted dormant state, that is, growth of FRO cells is counterbalanced with apoptotic cell death induced by anti-angiogenic effect of wt-p53. Wt-p53 appears to exert more complex anti-cancer actions than expected from *in vitro* data.

In contrast, however, high level expression of wt-p53 achieved with recombinant adenovirus clearly induces apoptotic cell death *in vitro* (17, 18). Furthermore, in *in vivo* experiments in nude mice, intratumoral injection of adenovirus expressing wt-p53