CANCER GENOMICS & PROTEOMICS 7: 31-50 (2010)

Review

Genetic Basis and Gene Therapy Trials for Thyroid Cancer

HUSSAM AL-HUMADI, APOSTOLOS ZARROS, RAFAL AL-SAIGH and CHARIS LIAPI

Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, Athens, Greece

Abstract. *Gene therapy is regarded as one of the most promising novel therapeutic approaches for hopeless cases of thyroid cancer and those not responding to traditional treatment. In the last two decades, many studies have focused on the genetic factors behind the origin and the development of thyroid cancer, in order to investigate and shed more light on the molecular pathways implicated in different differentiated or undifferentiated types of thyroid tumors. We, herein, review the current data on the main genes that have been proven to (or thought to) be implicated in thyroid cancer etiology, and which are involved in several well-known signaling pathways (such as the mitogen-activated protein kinase and phosphatidylinositol-3-kinase/Akt pathways). Moreover, we review the results of the efforts made through multiple gene therapy trials, via several gene therapy approaches/strategies, on different thyroid carcinomas. Our review leads to the conclusion that future research efforts should seriously consider gene therapy for the treatment of thyroid cancer, and, thus, should: (a) shed more light on the molecular basis of thyroid cancer tumorigenesis, (b) focus on the development of novel gene therapy approaches that can achieve the required antitumoral efficacy with minimum normal tissue toxicity, as well as (c) perform more gene therapy clinical trials, in order to acquire more data on the efficacy of the examined approaches and to record the provoked adverse effects.*

The thyroid is a major human endocrine gland that controls heart rate, blood pressure, temperature and metabolism. Most thyroid tumors are benign, with primary malignant tumors accounting for fewer than 1% of all carcinomas (1). The incidence of thyroid cancer is increasing throughout the world and it is more frequent than ovarian, urinary bladder or pancreatic cancer. This fact could be due to the recorded enhancement in the detection of early thyroid cancer by the use of thyroid ultrasound, neck imaging and fine-needle aspiration biopsy; for the same reason, the 5-year relative survival rates for thyroid cancer have increased significantly from 93% in 1983-1985 to 97% in 1995-2001 (2, 3). In the United States of America (USA) alone in 2006, about 30,180 cases of thyroid cancer were diagnosed with an incidence of 25 per million per annum (4). Thyroid cancer can occur in any age group, with an incidence 3 times higher in women than in men (5), its aggressiveness increasing significantly in older patients. In spite of the progress in the treatment of thyroid malignant tumors by traditional methods (surgical and/or chemotherapy, radiotherapy), 10-20% of these patients die from advanced differentiated, poorly and undifferentiated thyroid tumors. Therefore, alternative therapies are of interest because of the possibility of developing selective targeting approaches, such as using therapeutic genes to target tumor cells by application of tissue-specific promoters such as thyroglobulin (TG), thyroid-stimulating hormone receptor (TSH-R), calcitonin (CT) and CT-related polypeptide alpha (CTRPα). These promoters have no or very limited expression elsewhere, thereby reducing extratumoral toxicity (*e.g.* in the liver or the lungs) even if the therapy leads to the destruction of all normal thyroid tissue (6).

Thyroid carcinomas arise from the two cell types present in the thyroid gland. The endodermally-derived follicular cells give rise to papillary, follicular, and probably anaplastic, carcinomas (PTC, FTC and ATC respectively), while the neuroendocrine-derived CT-producing C cells give rise to medullary thyroid carcinoma (MTC). PTC and FTC are well-differentiated thyroid carcinomas (WDTC), while ATC is characterized as undifferentiated. Moreover, poorly differentiated thyroid carcinoma is an intermediate class of malignant tumor between WDTC and ATC (7).

Correspondence to: Charis Liapi, MD, Ph.D., Associate Professor, Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, 75 Mikras Asias str, GR-11527, Athens, Greece. Tel: +30 2107462531, Fax: +30 2107462554, e-mail: cliapi@med.uoa.gr

Key Words: Thyroid cancer, genetic basis, gene therapy trials, mutations, antisense approach, combination approach, toxic gene therapy approach, review.

PTC is the most common form of thyroid cancer, accounting for about 75-80% of thyroid malignancies (1) with a 1% death rate and an approximately 5% recurrence rate (8). It represents the most common pediatric thyroid malignancy and most PTCs in adults occur between 20 and 50 years of age (3). The PTC patient has the highest 20-year survival (99%) in the low-risk group, whereas survival is only 43% in the high-risk group; distant metastases are uncommon but if present, they are lymphatic and the prognosis is poor.

FTC cases account for about 15% of all thyroid cancer cases. FTC is less common in younger adults and children than papillary cancer, while it is characterized by a 20-year survival of about 97% in the low-risk group and 49% in the high-risk group; it is more common among elderly patients. This type of thyroid cancer is more aggressive than PTC and tends to spread through the bloodstream to other parts of the body (vascular invasion is characteristic of follicular carcinoma), and therefore distant metastasis is more common (9, 10).

ATC is the least common (accounting for 5%-15% of primary malignant thyroid neoplasms) and the most deadly type of thyroid cancer. It is characterized by sudden, rapidly growing thyroid mass, mostly arising from a benign thyroid disease or a pre-existing differentiated thyroid carcinoma. It tends to occur most often among elderly women and in iodine-deficient areas, or in a pre-existing goiter, while it is also rapidly fatal with a mean survival of 6 months after diagnosis (11, 12). ATC is always refractory to conventional therapies including surgery, radioactive iodine and chemotherapy (13). Many tumors previously classified as ATC have now been recognized as lymphomas or poorly differentiated MTCs by means of immunohistochemistry (IHC) (14). Metastases to the neck lymph nodes are present in over 90% of cases at the time of diagnosis, while distant metastasis presents a very poor prognosis, even with aggressive therapy (11).

MTC is a neuroendocrine malignancy that secretes excessive amounts of CT, frequently metastasizes and has limited treatment. MTC accounts for 3 to 10% of all thyroid cancer cases, but bears significant mortality (15). Among MTCs, 25% are sporadic, whereas 75% are inherited as the predominant component of the multiple endocrine neoplasia type 2 (MEN2) syndromes (MEN2A, MEN2B and familial MTC) (16). MTC is less common and more aggressive than PTC or FTC, and tumor tends to spread to lymph nodes very early and to distant sites *via* the blood stream with excessive CT production (16). This cancer is treated with difficulty and has a much lower cure rate than do the 'well differentiated' thyroid carcinomas (PTC and FTC) (9, 10), but cure rates are higher than they are for ATC. The recorded overall 10-year survival is about 60-70% (16, 17).

The etiology of most thyroid carcinomas is not well understood, but as in every type of cancer, they range from environmental or therapeutic (radiation (18) iodine administration (19) and radioiodine exposure (20) *etc*., which have been shown to produce characteristic chromosomal translocations) to genetic factors. The genetic abnormalities are considered as bases for the development of thyroid cancer and have been extensively explored over the last two decades due to their importance as potential targets for novel therapeutic approaches. The reaction of the thyroid gland to these factors differs according to age, sex, immunological status *etc*., in addition to the effects of hormones, cytokines and growth controlling factors (18).

The aim of this review is to provide an up-to-date summary of the genetic background of the main types of thyroid cancer and to focus on the gene-targeting approaches that have been developed (in order to act as potential gene therapy approaches, in the future).

The Main Genes Implicated in Thyroid Cancer Development

The genetic abnormalities involved include rearrangements, point mutations or other gene disruptions, each of which plays a role in disease pathogenesis; most of them have been used as diagnostic tools and molecular targets through the difference in expression according to the type of tumor and its histological subtype. The main and most common genes that are found to be mutated and expressed, underexpressed or totally unexpressed in thyroid malignancies are described below (for a synopsis, see Table I).

The V-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene. The RAF proteins (ARAF, BRAF, and CRAF) are essential serine/threonine protein kinases; they play critical roles in cell proliferation, differentiation and apoptosis by signaling a complex kinase pathway that mediates cellular responses to growth signals [mitogen-activated protein kinase pathway (MAPK)] (21, 22). The BRAF protein is expressed at higher levels in hematopoietic cells, neurons, and testicles (23) and is the predominant isoform in thyroid follicular cells. Among the isoforms of RAF kinase, BRAF appears to be the most potent activator of the MAPK pathway and it is activated through multiple mechanisms (Figure 1). *BRAF* mutation is the most common genetic alteration in thyroid cancer, occurring in about 45% of sporadic PTCs, particularly in the relatively aggressive subtypes (24). A substantial body of data indicate that the *BRAF* mutational status is a significant predictor of poor clinical outcome, while a significant association of *BRAF* mutational status with extra-thyroidal invasion has been shown (25).

The RAS gene. The RAS oncogenes were the first to be associated with thyroid cancer. Members of the RAS family are signal-transducing proteins that share properties with the

Table I. *Malignant thyroid tumors and the main and most common genes that are usually found to be mutated and expressed, underexpressed or totally unexpressed in these tumors (for details, see text).*

Malignant thyroid tumor type	Associated genes*
Papillary thyroid carcinoma (PTC)	*BRAF*
	RAS
	RET
	NTRK1
	p53
	*APC***
	*PTEN***
Follicular thyroid carcinoma (FTC)	*RAS*
	PAX8-PPARγ1
	p53
	PTEN
	GRIM-1
	mtDNA
	*APC***
	*PTEN***
Anaplastic thyroid carcinoma (ATC)	*BRAF*
	RAS
	p53
	β-Catenin
	PTEN
Medullary thyroid carcinoma (MTC)	*RET*
	p53

*Full names of genes can be found within the text. **Familial syndrome-associated genes.

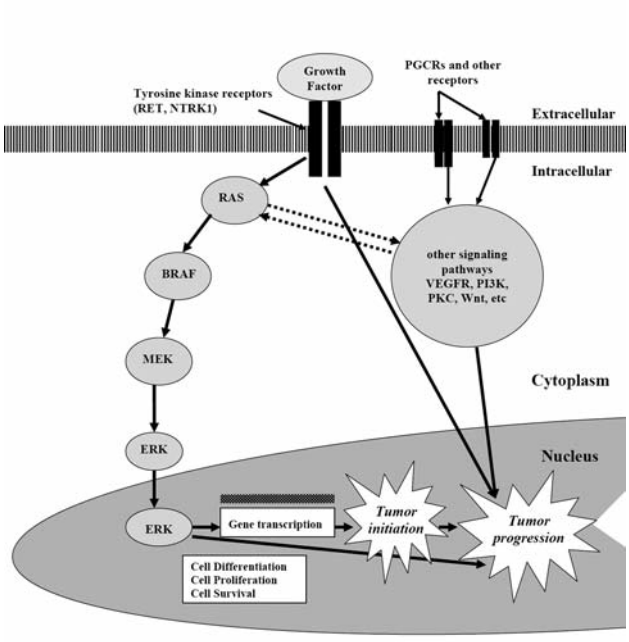

Figure 1. *Schematic representation of the main signalling pathways participating in cell differentiation, proliferation and survival of the thyroid.*

G-proteins. Point mutations in *H-RAS, K-RAS* and *N-RAS* have been described in many tumor types. The complex RAS-initiated MAPK-terminated cascade aberrant signaling is regarded as crucial for the development of thyroid cancer. Mutation of the phosphatidylinosibol-3-kinase catalytic alpha polypeptide (*PIK3CA*) gene [the product of which binds specifically with RAS and activates the phosphoinositide-3-kinase/Akt (PI3K/Akt) signaling pathway] also has an important role in thyroid tumorigenesis (26-28) (Figure 1).

The RET gene. There is substantial evidence for the involvement of the *RET* gene in thyroid cancer pathogenesis. Identified in 1985 (29), the *RET* gene is located on chromosome 10q11.2 and encodes a tyrosine receptor protein consisting of an extracellular domain with a ligand binding site, a transmembrane domain and an intracellular tyrosine kinase domain. The RET receptor is activated by interacting with a multicomponent complex that includes a soluble ligand family, the glial cell line-derived neurotrotrophic factors (GDNF), and also a family of cell surface bound co-receptors (30). Ligand binding results in receptor dimerization leading to autophosphorylation of the protein on tyrosine residues and initiation of the signaling cascade. RET plays a critical role in cell differentiation, proliferation and cell survival in nerve cells (Figure 1). Subsequent studies have demonstrated a novel form of RET (RET/PTC) resulting from different gene rearrangements. The most common rearranged forms of the *RET* gene are *RET/PTC1, RET/PTC2* and *RET/PTC3* and there are more than ten additional types of *RET* rearrangements, occurring primarily in radiation-induced tumors (31). Many of these mutations, particularly those leading to the activation of the MAPK pathway, are being actively explored as therapeutic targets for thyroid cancer.

The neurotrophic receptor-tyrosine kinase 1 (NTRK1) gene. The *NTRK1* gene is located on chromosome 1q22 and encodes the receptor for the nerve growth factor. Similar to *RET, NTRK1* undergoes oncogenic activation by chromosomal rearrangement (32) (Figure 1).

The paired box gene 8-peroxisome proliferator activated receptor gamma (PAX8-PPARγ) gene. Over the last few years, significant *PAX8-PPARγ* gene fusion has been identified in FTC cases in association with the presence of a recurrent translocation [t(2;3) (q13;p25)] (33). The consequence of this translocation is the fusion of the DNA binding domains of the thyroid transcription factor PAX8 to domains A-F of the *PPARγ1* (33, 34).

The p53 gene. The *p53* gene encodes a nuclear transcription factor that plays a key role in cell cycle regulation, DNA repair, and apoptosis and acts a tumor suppressor gene. Alterations of the *p53* gene are a peculiar feature in ATCs (35-38). Moreover, the accumulation of *p53* protein, frequently caused by *p53* mutations, characterizes tumoral areas with a less differentiated phenotype (38, 39). Conversely, *p53* gene mutations are rare in differentiated thyroid carcinomas (40). Accordingly, the introduction of mutated *p53* genes into thyroid cells blocks the expression of thyroid differentiation markers and suppresses the thyroid transcription factor PAX-8 (41). Interestingly, *p53* gene function is impaired in all thyroid carcinoma cell lines established from both differentiated and undifferentiated histotypes (42). All the above strongly suggest that *p53* inactivation is required for the establishment of human thyroid carcinoma cell lines.

The beta-catenin (β-catenin) encoding gene. The β-catenin protein is encoded by the *CTNNB1* gene (chromosome 3p22-21.3). This protein has a role in cell adhesion *via* its interaction with the cytoplasmic tail of cadherins (which are important transmembrane proteins) and in the Wnt signaling pathway (involving a complex network of proteins that play a crucial role in carcinogenesis) (43).

The adenomatous polyposis coli (APC) gene. The *APC* gene is a tumor suppressor gene that prevents the uncontrolled growth of cells that may result in cancerous tumors. The *APC* gene mutations seem to be associated with thyroid cancer development in a complex and mutated-codon-specific way (44).

The global receptor involved in myogenesis-1 (GRIM-1) gene. GRIM-1 is involved in the mitochondrial respiratory chain and in apoptosis (45).

The phosphatase and tensin homolog (PTEN) gene. The *PTEN* gene, which acts as a tumor suppressor gene, encodes a protein that helps in the regulation of cell division and plays a role in intracellular signaling. Mutations of this gene contribute to the development of a multitude of cancer types (46). It should be noted that PTEN acts in opposition to PIK3, therefore it is considered as a regulator for cell cycling, translation, and apoptosis (47).

The mesenchymal epithelial transition factor (MET) gene. The *MET* gene encodes for a tyrosine kinase membrane receptor, which functions, primarily, as a receptor for hepatocyte growth factor/scatter factor (HGF/SF). Overexpression of *MET* has been reported in multiple human PTC cases.

The C-erbβ-2 gene. The protooncogene erb beta (*erbβ*) encodes two functional thyroid hormone receptors (β-1 and β-2). The *C-erbβ-2* gene is overexpressed or underexpressed depending on the thyroid tumor type (48).

Epigenetic alteration. This is a widely described molecular mechanism involved in thyroid tumorigenesis. The aberrant methylation of tumor suppressor genes is an example of epigenetic alteration (49).

Gene Expression in Differentiated Thyroid Cancers (PTC and FTC)

Familial genetic predisposition for differentiated thyroid cancer such as an association of PTC and colorectal disease as well as FTC and breast disease have been described in at least two hereditary cancer syndromes, familial adenomatous polyposis (FAP) (50) and a hereditary hamartoma syndrome (51). The genes for the two syndromes are characterized by *APC* (52, 53) and *PTEN* gene mutations (54), respectively.

In PTC, *BRAF* mutations have been identified in approximately 40-70% of the tumors (55, 56), and can occur early (57). The $BRAF^{T1799A}$ mutation is the most common genetic change in PTC (24). *RET/PTC* mutations are also regarded as one of the most common mutations in this type of cancer, while at least 15 types of *RET/PTC* rearrangements have been described (48, 58). The frequency of *RET/PTC* rearrangements in the adult population in the USA is approximately 30-40%. In recent studies, *RET* rearrangements have emerged as the second most common genetic abnormality found in PTCs. *RET/PTC1* and *RET/PTC3* account for 60-70% and 20-30% of cases, respectively, while *RET/PTC2* has been implicated in approximately 10% of cases. *RET* rearrangements are particularly common in tumors in pediatric patients (50-60%) and in patients exposed to accidental/therapeutic radiation during childhood (60-70%) (59); thus ionizing radiation considered as the major risk factor for development of PTC, can directly induce *RET* recombination events. Several studies have emphasized the fact that RET rearrangements are associated with PTCs lacking evidence of progression to poorly differentiated or anaplastic carcinomas (60, 61). $BRAF^{T1799A}$ (V600E) mutations are unique to PTC, not being found in any other form of well-differentiated follicular neoplasm or MTC (24). Practically, in PTCs, there seems to be no overlap between *RET/PTC, BRAF* or *RAS* mutations, which altogether were found to be present in 66% of cases (62). *NTRK1* rearrangements are considerably less frequently found in PTCs than are *RET* rearrangements (63). The prevalence of *NTRK1* rearrangements is approximately 3% in post-Chernobyl PTCs (64). *RAS* point mutations are uncommon in PTCs of conventional type, with an overall frequency of less than 10% (65, 66). Differentiated thyroid carcinomas (PTCs and FTCs) both seem to be largely unaffected by *p53* gene mutations (36). Studies of transgenic

models have demonstrated that lack of functional *p53* in *RET/PTC* mice promotes anaplasia and invasiveness of thyroid carcinomas (67). Gimm *et al*. (46), showed a 20-30% frequency of hemizygous deletions of the *PTEN* gene in PTCs.

In FTC, rearrangements of *PAX8-PPARγ1* have been reported in about 53% of cases (33, 34, 68). In a comparative study of *RAS* mutations and *PPARγ* rearrangements, 49% of conventional FTC had *RAS* mutations, 35% had *PAX8-PPARγ* rearrangements, only 3% had both abnormalities (57), while 12% were negative for both. *p53* mutations account for less than 10% (36). *PTEN* mutation is presented in about 20-30% of FTCs (46). Deletions and point mutations of mitochondrial DNA (mtDNA) are common in both neoplastic and normal oncocytic follicular cells, but the role of these abnormalities in the development of oncocytic tumors is unknown (69). Mutations of the *GRIM-1* gene have been implicated in the pathogenesis of oncocytic thyroid tumors (45).

Gene Expression in Poorly Differentiated and Undifferentiated Thyroid Cancer

The genes for p53 and β-catenin have been implicated in the progression of these tumors. Inactivating mutations of *p53* occur in approximately 25% of poorly differentiated carcinomas and 60% of undifferentiated carcinomas (36, 38, 70). β-Catenin has a role in cell adhesion *via* its interaction with the cytoplasmic tail of cadherins and in the Wnt signaling pathway. Garcia-Rostan *et al*. (43), have demonstrated that *CTNNB1* mutations and nuclear (rather than plasma membrane) localization of β-catenin are restricted to poorly differentiated or undifferentiated carcinomas. These findings highlight the important role of *CTNNB1* mutations in the progression of thyroid cancer. *BRAF* and *RAS* mutations appear to confer a predisposition to the development of both poorly differentiated and undifferentiated carcinomas (25, 57).

Several studies have emphasized the fact that *RET* rearrangements are associated with PTCs that lack evidence of progression to poorly differentiated or ATCs (60, 61), while 60% of undifferentiated carcinomas had hemizygous *PTEN* deletions (46).

Gene Expression in MTC

RET point mutations are crucial for the development of MTC. Activating germline point mutations of *RET* are present in more than 95% of patients with MEN2A, MEN2B and familial MTC (71, 72). In sporadic MTC, somatic mutations are present in up to 70% of cases (73), while a statically significant correlation between the presence of somatic mutation and more advanced pathological TNM states has been recently observed (74).

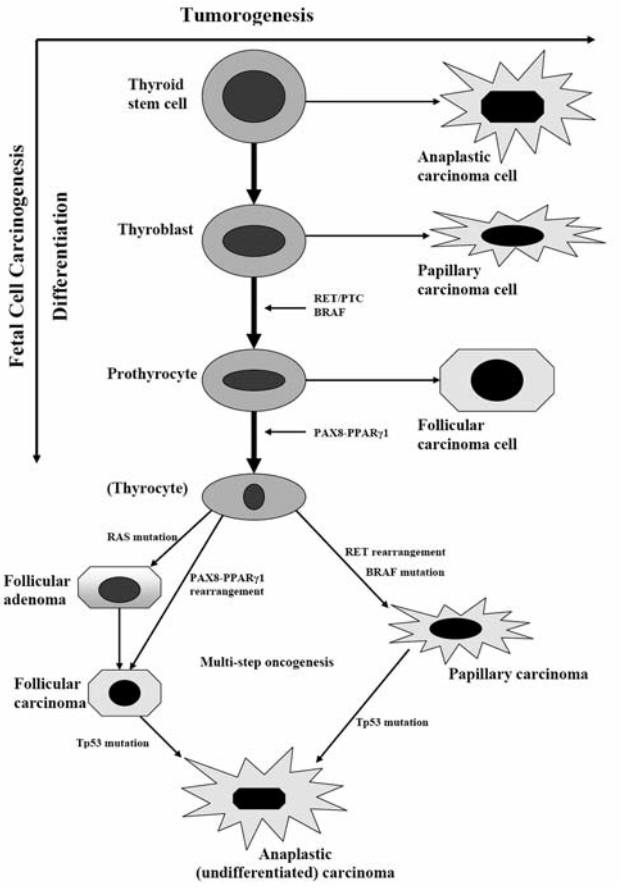

Figure 2. *Schematic representation of the two hypotheses that have been developed in order to explain the cascade of the events that take place from the initiation until the development of a thyroid tumor.*

Tumorigenecity Hypotheses

In order to explain the cascade of events that take place from the initiation until the development of a thyroid tumor, taking into consideration all the precipitating factors and their interaction with genetic susceptibility, two hypotheses have been developed.

The first hypothesis, known as the 'multi-step carcinogenesis hypothesis' was put forward in the 1980s. According to this hypothesis, cancer cells, including those of the thyroid, are considered to be derived from well-differentiated normal cells (such as thyrocytes) *via* multiple incidents of damage to their genome. Oncogenes or tumor suppressor genes, which accelerate proliferation or foster malignant phenotypes (such as the ability to invade the surrounding tissue or metastasize to distant organs) are mainly altered (75) (Figure 2). For example, under this hypothesis, PTCs are derived from some unknown precursor cells generated by normal thyrocytes by rearrangements of

the *RET* gene (*RET/PTC*) or by *BRAF* mutations (76, 77), while FTCs are generated from thyrocytes *via* follicular adenomas by *RAS* mutations or the *PAX8-PPARγ1* rearrangement (33, 34, 78). Poorly differentiated ATCs are generated by both FTCs and PTCs through genomic changes, such as *p53* mutations (75, 79).

In 2000, a novel hypothesis of thyroid carcinogenesis was proposed, namely the 'fetal cell carcinogenesis', according to which cancer cells are derived from the remnants of fetal thyroid cells instead of thyrocytes. A considerable number of researchers have concluded that cancer cells derive from immature progenitor or stem cells, but not from well-differentiated cells (80, 81).

Takano (81) has explained well the clinical and biological features of this hypothesis and the recent molecular evidence that supports his novel hypothesis for thyroid carcinoma, according to which cancer cells derive from the remnants of three types of fetal thyroid cells, instead of normal thyroid follicular cells (Figure 2). This hypothesis suggests that thyroid cancer cells are generated from fetal cells by proliferation without differentiation, and that oncogenes play an oncogenic role by preventing fetal cells from differentiation. Therefore, any defect or inhibition of differentiation may lead to cancer cell formation. ATC, PTC and FTC derive from thyroid stem cells, thyroblasts and prothyrocytes, respectively. *BRAF* mutations and rearrangements of the *RET* (*RET/PTC*) prevent the differentiation of thyroblasts into prothyrocytes, resulting in the generation of PTCs, while the *PAX8-PPARγ1* rearrangement prevents differentiation of prothyrocytes into thyrocytes, resulting in the generation of FTCs.

Different clinical and molecular features support one or the other hypothesis, but the exact mechanism of thyroid carcinoma development remains unclear. Therefore, in the etiology of thyroid carcinoma, it remains controversial as to whether thyroid tumors of different histologies share a common origin and whether they follow an adenoma-carcinoma progression.

Gene Therapy Approaches in Thyroid Cancer

The American Association of Clinical Endocrinologists (AACE) in collaboration with the American Association of Endocrine Surgeons (AAES) emphasized the Guidelines for the Management of Thyroid Carcinoma and the importance and prevalence of thyroid cancer as a "forgotten cancer" (1). Thyroid cancer occurs as frequently as other well-publicized types of cancer, and despite multimodality treatment for thyroid cancer (including surgical resection, radioiodine therapy, TSH-suppressive thyroxin treatment, and chemotherapy/radiotherapy), survival rates remained not improved over the last decade. Therefore, development and evaluation of novel treatment strategies, including gene therapy, are very important. Gene therapy has been regarded as a promising future therapeutic approach for any thyroid cancer, especially for types that do not respond to traditional treatment aiming to restrict the therapy to target tissues and to decrease extratumoral toxicity, by maintaining a high therapeutic index.

Gene Therapeutic Strategies for Obtaining Efficient Targeting for Cancer

Because of the non-selectivity of the available gene delivery systems, which renders cancer gene therapy strategies potentially toxic to normal cell populations, selectivity of gene therapy for tumor cells has been achieved either by direct intratumoral administration of recombinant vectors or by integration of tissue promoters and/or enhancers into gene delivery vectors that are activated in transformed, but not normal cells. However, cell-specific promoters, such as the CT or TG promoters, maintain a highly-regulated expression but are often weaker than nonspecific promoters, such as the commonly employed cytomegalovirus (CMV) promoter. In addition, these promoters can be modified to achieve more potent tissue specificity such as *CALCA* (the gene encoding both CT and CTRPα) that has been modified to produce a promoter (TSE2.CP1) (82). Targeting viruses specifically to tumors would be very desirable and has been partially accomplished in some systems by changing viral tropism or employing an agent bound to the virus in order to direct it to a specific receptor on the tumor cell surface. For example, an antibody can be bound to the viral rod protein, and this antibody can be coupled with a tumor-targeting molecule (*e.g.* a growth factor or a second antibody) (83). A new strategy has been developed functioning through the attachment of target ligands to adenovirus (Ad) proteins or receptors by chemical or genetic modification of Ad particles. A recent example involves the identification of human MTC-specific peptide ligands (one of which has the potential to be covalently linked to Ad particles while maintaining effective cellular internalization properties) (84, 85). This strategy allows Ads to be retargeted to MTC cells with high transduction efficiency and no effect on the liver. Other interventions to achieve efficient targeting depend on the selection of viruses or on the use of inducible promoters and the use of the Cre-loxP system. The latter is a recently developed approach to target gene transcription in tumor cells and enhance the function of the thymidine kinase/ganciclovir (tk/GCV) system and the expression of tk under control of TG promoter to target expression (86). In general there are two main strategies for achieving efficient gene therapy treatment: (a) maintenance of tissue specificity, and (b) efficient transduction of target cells.

By considering the above two strategies, gene therapy can be effective and safe, but for each approach there is an

Table II. *Different gene therapy approaches related to thyroid carcinoma.*

1.	Introduction of a (non-normally present) gene in order to confer a new function to cells or tissues.
2.	Replacement of a mutation-induced deactivated gene by reintroduction of a normal form of that gene into tumor cells.
3.	Production of a mutated protein (by a mutated gene) that blocks the function of an oncogenic protein.
4.	Reintroduction or amplification of a specific gene expression.
5.	Antisense approach.
6.	Use of ribozymes.
7.	Use of siRNA.
8.	Use of replication-competent viral vectors (replication-selective, viral-mediated oncolysis).
9.	Combinatorial approach.
10.	*NIS*-introducing or toxic gene therapy approach.

siRNA: Small interfering RNA; *NIS*: sodium iodide symporter gene.

evaluation between the degree of effectiveness and undesirable toxic effects that may occur due to non-selectivity. Further investigation is required in order to obtain a more efficient method with better results but less adverse effects. These approaches are summarized below (see also Table II).

Introduction of a (Not Normally Present) Gene in Order to Confer a New Function to Cells or Tissues

Suicidal gene induction as a 'cytoreductive gene therapy' approach [such as prodrug approach, sometimes known as gene-directed enzyme prodrug therapy (GDEPT)] utilizes an agent which can be directly injected into the tumor to allow the anticancer prodrug to be converted to its cytotoxic form (Table III). This approach depends on the use of specific promoters for tumor cells or residual normal cells as in the case when tumor cells express herpes simplex virus thymidine kinase (*HSV-tk*, suicidal gene). In this case, the administration of GCV (prodrug), which is phosphorylated by *HSV-tk* and competes with deoxyguanosine triphosphate in DNA polymerization, results in the arrest of DNA synthesis and cell death (87). Braiden *et al*. (88), by transducing three thyroid cell lines, namely Fischer rat thyroid cell line-5 cells (FRTL-5), FRO cells (human ATC cell line) and FRTC cell line (a malignant cell line developed from FRTL-5 cells) with a retrovirus (Re) expressing HSV-tk under control of the TG promoter, achieved cell death in all cell lines except for the FRO cells, which do not express TG. It should be noted that this vector has to be given by direct injection into the tumor. In another study, an Ad with TG promoter driving expression of tk (Ad-TG-tk) was used to transduce FRTL-5 cells, and when GCV was added, more than 90% of the cells were killed; a minimal effect was observed in the other cell lines studied (HEPG2, COS-1, rat and human MTC, HeLa, GH3, P98G, and CA77). Moreover, when an Ad using nonspecific CMV promoter to express tk (Ad-CMV-tk) was given intravenously (*i.v.*) to rats treated with GCV, severe liver damage was induced. In contrast, when the same amount of Ad expressing tk under control of the TG promoter was given (*i.v.*), neither significant changes of serum transaminase levels and histologic liver abnormalities were observed, nor adverse effects (89). For enhancing tk expression in TG-expressing cells, Nagayama *et al*. (86), combined the function of the tk/GCV system with the Cre-loxP system. As a result, the cytotoxic effect was 5 to 10-fold higher than that with TG promoter alone.

These studies demonstrated the ability of the TG promoter to transcriptionally target therapeutic genes to thyroid carcinoma cells, despite the lower efficacy compared to constitutive viral promoters such as the CMV early promoter. On the other hand, the Re vector was expected to have effects distant from the injection site (bystander effects) which have an important role in antitumor therapy (86-88), while the Ad vector in rat MTC (rMTC) cells through the HSV-tk/GCV system was limited to a low bystander effect *in vitro*. The latter correlated well with the limited antitumor efficacy *in vivo*. Intratumoral application of an Ad carrying the *HSV-tk* gene under the control of the CMV promoter followed by GCV treatment resulted in the destruction or stabilization of smaller tumors without a therapeutic effect in larger tumors (90). The latter confirms that a significant bystander effect is an important advantage for effective suicide gene therapy.

Many scientists took advantage of this point using a virus expressing a cytokine gene (as a suicidal gene) for increasing antitumor immunity and making its effect persist for months or longer. Interferon-γ (*IFNG*), interleukin-2 (*IL2*), tumor necrosis factor-alpha (*TNFA*), *IL12*, granulocyte-macrophage colony-stimulating factor (*CSF2*), and lymphotactin (*XCL1*) are among the genes employed. This type of suicidal gene approach is enrolled within what the scientists called 'immunomodulatory gene therapy', which depends upon the principle of many types of cancer expressing tumor-associated antigens that can be recognized by the immune system (released from tumor cells either physiologically or after cytotoxic therapy). Mobilization of the immune system, by delivery of genes that enhance the immunogenicity of the tumors and the responsiveness of the immune system, is associated with a number of advantages including inherent specificity of the immune system (decreasing normal tissue toxicity), a systemic immunogenic effect, signal amplification, and permanent antitumor immunity because of inherent memory of the immune system. Local expression of certain cytokines is able to elicit an immune response against the tumor by stimulating surrounding immunocompetent cells, targeting cytotoxic T-cells and natural killer cells, thereby

Table III. *Suicidal gene approaches associated anticancer prodrug with or without immunomodulatory cytokines gene insertion in different gene therapeutic trials with different vectors and cell lines (for details and the meaning of the abbreviations used, see text).*

Study/Reference	System	Cell line type	Effects	Side-effects
Nagayama *et al*. (86)	Re-TG-tk / Cre-loxP	FRTL-5, FRTC, FRO	Enhances the expression of TK in TG-expressing tissues	---
Nishihara *et al*. (87)	Re-CMV-tk	FRO, WRO	Cytotoxic Bystander effect Radiosensitization	Extrathyroidal tissue toxicity
Braiden *et al*. (88)	Re-TG-tk	FRTL-5, FRTC, FRO	Cytotoxic only to TG-expressing tissues	Not effective in the anaplastic form
Zang *et al*. (89)	Ad-TG-tk, Ad-CMV-tk	FRTL-5, HEPG2, COS-1, MTC, HeLa, GH3, P98G, CA77	Cytotoxic only to TG-expressing tissues	Not effective in the anaplastic form Severe liver damage
Zhang and DeGroot (90)	Ad-CMV-tk	Rat MTC	Bystander effect *in vitro* Limited antitumor effect *in vivo*	No effect on large tumors
Zang *et al*. (91, 92)	Ad-CMV-mIL-2	Mouse MTC	Destruction of smaller tumors and stabilization of larger tumors Development of long-term antitumor immunity	---
Lausson *et al*. (93)	---	Rat MTC	Tumor progressively rejected	---
Zhang and DeGroot (94, 96)	Ad-CMV-mIL-12	Rat MTC	Development of long-term antitumor immunity	---
Yamazaki *et al*. (95)	Ad-CALC1-mIL-12	Rat MTC	Development of long-term antitumor immunity	---
Soler *et al*. (97)	Re-CMV-tk-IL-2	MTC	Development of long-term antitumor immunity (superior to that of each system alone)	---
Zhang and DeGroot (98)	Ad-CMV-tk-IL-12	Rat MTC	Enhanced antitumor activity (superior to that of each system alone)	---
Yamazaki *et al*. (99)	Ad-TCP-tk+ Ad-TCP-mIL-12	Rat MTC	Enhanced antitumor activity (superior to that of each system alone)	Minimal toxicity
Barzon *et al*. (100, 101)	Re-TG-tk-IL-2	Human differentiated thyroid cancer cells and ATC	Bystander effects both *in vitro* and *in vivo* Increased anticancer effects	Effective only in the differentiated cell line
Barzon *et al*. (102)	Re-tk-IL-2 (intra-tumor)	Human ATC	Activation of a local and systemic immune-inflammatory response Radiological examination demonstrated tumor necrosis	Transduction efficiency low with mild side-effects

inducing rejection of tumor cells. Zhang *et al*. (91, 92), used a replication-defective Ad harboring the *IL2* gene for treatment of MTCs in mice and rats that resulted in tumor regression of smaller tumors and tumor stabilization of larger tumors, with low *in vivo* toxicity after systemic application of the Ad. The antitumor effect was shown to be dependent upon cytotoxic T lymphocyte activity against the tumor, which also prevented tumor growth after reinjection of tumor cells (indicating the development of long-term antitumor immunity); similar results have been obtained by Lausson *et al*. (93), supporting the possibility of a future vaccine production for those at risk of developing cancer. IL-12 is one of the antitumor cytokines used in different studies for the treatment of follicular cell-derived and MTC, with a significant therapeutic effect and long-term antitumor immunity (94-96). In order to express both these genes in one vector, various vectors have been developed (Re or Ad vectors) expressing both *HSV-tk* and *IL2* or *IL12* (human or murine), and these have been shown to have systemic and long-term antitumor immunity superior than that of each gene system alone (97-99). Similar results regarding the feasibility and efficiency of a combined immunomodulating and suicide gene therapy approach for thyroid carcinomas was obtained by Barzon *et al*. (100), who used a Re vector in differentiated and ATC cells with bystander effects both *in vitro* and *in vivo*. For the achievement of larger selectivity, a transcriptionally targeted Re vector was generated, replacing the viral enhancer with the enhancer sequence of the human TG gene, which allowed selective transgene expression and cell killing in differentiated thyroid tumor cells, but not in ATC cells or non-thyroid cells (101).

Table IV. *Gene therapy trials for reintroduction of the wild-type p53 gene (alone or with chemotherapy) in different neoplastic cell lines (for details and the meaning of the abbreviations used, see text).*

Study/Reference	Cell line type	Results
Nagayama *et al*. (107)	FRO	Cell growth inhibition *in vitro,* tumorigenecity inhibition *in vivo*.
Moretti *et al*. (108)	ARO	Inhibition of cell proliferation, restoration of a more differentiated phenotype, as well as TG, thyroid peroxidase and TSH-receptor expression increase.
Fagin *et al*. (109)	NPA, ARO, FRO, WRO	Re-expression of thyroid peroxidase, significant cell differentiation.
Imanishi *et al*. (110)	FRO, WRO	Increased transcriptional activity of *p53* (by 10-100 times) with introduction of histone deacetylase inhibitor, enhanced apoptotic killing by *p53* transfer.
Moretti *et al*. (111)	FRO, WRO	FRO: no effects in term of proliferation and activity. WRO: strong growth inhibition.
Narimatsu *et al*. (112)	FRO	Radiosensitivity enhancement, cell-growth inhibition.
Nagayama *et al*. (113)	ARO, FRO, NPA, WRO, FRTC	Dose-dependent killing, chemosensitivity enhancement.
Blagosklonny *et al*. (114)	BHT-101, SW-1736, KAT-4	Chemosensitivity enhancement.
Kim *et al*. (115)	NPA	Cell-growth inhibition, chemosensitivity enhancement.

The first report of gene therapy for ATC on humans was by Barzon *et al*. (102), where two patients with end-stage ATC were treated by direct intratumor injection of Re vector producer cells followed by GCV. The Re vector carried human *IL2* gene and *tk* gene of herpes simplex virus type 1. Treatment was safe and associated with only mild adverse effects, while it increased the production of T helper type 1 cytokine expression in peripheral blood mononuclear cells with local tumor necrosis.

An alternative GDEPT approach is based on the *Escherichia coli* enzyme purine nucleoside phosphorylase (PNP), which converts a nontoxic prodrug, fludarabine phosphate, to 2-fluoroadenine. The active metabolite is an adenosinetriphosphate (ATP) analog that inhibits ATP-dependent reactions; it kills both proliferating and non-proliferating cells by inhibition of protein and RNA synthesis. The PNP-fludarabine system also induces a very efficient local bystander effect which is under control of the modified *CALCA* (TSE2.CP1) promoter in Ad (103).

Replacement of a Mutation-induced Deactivated Gene by Reintroduction of a Normal Form of that Gene into Tumor Cells

p53 is a transcription factor mediating critical cellular responses, including cell cycle arrest and apoptosis, after exposure to DNA-damaging stimuli (104-106). Mutations of the thyroid *p53* tumor suppressor gene are found in most poorly differentiated tumors (which have lost expression of normal p53) as part of the process of becoming unregulated carcinomas, while reintroduction of the wild-type *p53* (wt-*p53*) has been used in a variety of experimental thyroid cancer models (Table IV). The expression of wt-*p53* in a *p53*-null thyroid carcinoma cell line (FRO) resulted in reduced cell growth *in vitro* and in inhibition of tumorigenesis *in vivo* in nude mice (107). As a result of the p53-induced antiangiogenic effect, due to down-regulation of vascular endothelial growth factor (VEGF) and to up-regulation of thrombospondin (106), not only are p53-transduced cells killed but so are the surrounding non-transduced cells by the transduction of their neighbors, leading to a reduction in the level of transduction efficiency required for successful gene therapy. In ATC cell lines, *p53* reintroductions resulted in inhibition of cell proliferation and restored a more differentiated phenotype retaining the ability to respond to TSH with an increased expression of thyroid-specific genes such as *TG*, thyroperoxidase and *TSHR* (108, 109). In order to enhance the apoptotic killing by *p53* gene transfer in ATC cell lines (FRO and WRO), Imanishi *et al*. (110), used the histone deacetylase inhibitor (HDAC-I) depsipeptide, suggesting that this combinatorial treatment approach might also be useful in the treatment of undifferentiated thyroid carcinomas. This treatment also takes advantage of the fact that a replicating Ad (or a non-replicative form) is used in order to infect the tumor cells, while the expression of the p53 protein leads to apoptosis of the tumor cells. Apparently, the gene expression in normal cells, which already have an intact and expressed p53, causes less, or in some cases no, harm (111, 112). Nagayama *et al*. (113), re-expressed *p53* in four human ATC cell lines harboring *p53* mutations (ARO, FRO, NPA, WRO) and in normal human thyroid follicular cells *in vitro* and *in vivo* by the use a replication-defective Ad. The evaluation of the therapeutic efficacy of *p53* expression resulted in a dose-dependent cell killing in both normal and carcinoma cells, but normal thyroid cells were relatively resistant to p53-mediated cell death despite their higher Ad infectivity. The mechanism of cell killing was shown to be apoptosis.

In addition, wt-*p53* expression sensitized some of the cell lines to the chemotherapy and this approach clearly offers

potential for therapy, especially when there is difficulty in transducing all cells by direct tumor injection (86). *In vitro*, Blagosklonny *et al*. (114), found that there is an increased chemosensitivity of ATC to doxorubicin. *In vivo* experiments using FRO and NPA cell xenografts in nude mice showed inhibition of tumor growth following direct injection of the Ad expressing wt-*p53*. This effect was augmented by combination with doxorubicin, resulting in tumor regression (113), similar results were obtained by Kim *et al*. (115), when a retroviral p53 gene transfer into *p53* mutant PTC cells (NPA) resulted in a dose-dependent inhibition of tumor cell growth and enhanced chemosensitivity to adriamycin (doxorubicin) *in vitro* and *in vivo*.

Production of a Mutated Protein (by a Mutated Gene) that Blocks the Function of an Oncogenic Protein

The human *RET* proto-oncogene encodes for a transmembrane receptor that consists of three functional domains: the extracellular ligand binding domain, the transmembrane segment and the intracellular domain formed by a tyrosine kinase. Mutations of the *RET* gene result in constitutive activation of RET tyrosine kinase with aberrant downstream signaling and initiation of tumor formation in many malignant tumors including thyroid malignancies. Drosten *et al*. (116, 117), demonstrated that more than 95% of MTCs harbor dominant activating mutations in the *RET* proto-oncogene, which play a central role in the development of tumor. Ad vectors expressing dominant-negative *RET* mutants were used in human MTC cells under the control of a *CALCA* gene-related promoter. Dominant-negative *RET* mutant protein has amino acid changes in the extracellular domains and because of these changes, the glycosylation process is disturbed, resulting in hampered protein transport to the cell surface. The dominant-negative mutant protein dimerizes with oncogenic RET protein in the endoplasmic reticulum (ER), thereby preventing expression of both dominant-negative and oncogenic RET protein on the cell surface. A pronounced shift of endogenous oncogenic RET protein localization from the cell surface to the ER was demonstrated, resulting in strong inhibition of cell viability caused by induction of apoptosis *in vitro*. Furthermore, *ex vivo* infection of thyroid tumor cells with this C-cell-targeted dominant-negative *RET* mutant Ad resulted in suppression of subcutaneous tumor growth in nude mice, whereas direct injection of this vector into pre-established, subcutaneous, xenograft thyroid tissue tumors retarded tumor growth in only a limited number of mice. In contrast to *p53* restoration, this approach requires high levels of *ex vivo* transduction efficiency, because it does not affect surrounding cells, thereby limiting its therapeutic efficacy *in vivo*.

Reintroduction or Amplification of a Specific Gene Expression

Most poorly differentiated and ATCs have lost the ability to express the *TG* gene, which is accompanied by loss of transcription factors [thyroid transcription factor-1 (*TTF1*), *TTF2* or *PAX8*] interacting with the TG promoter. Non-TG-expressing ARO and WRO thyroid cell lines (which had low intrinsic TG) transfected with the *TTF-1* and *PAX-8* genes, resulted in increased function of the TG promoter (118). In addition, co-transduction of *TTF1* with the TG promoter by Ad vectors reactivated the TG promoter in dedifferentiated thyroid cells but exhibited only little effect in non-thyroid cells (119). Another study depending on this approach has been conducted by Chung *et al*. (120), who provided an explanation for the significantly decreased levels of growth arrest and DNA damage 45 gamma (*GADD45G*) RNA in ATC cells, compared to normal primary cultured thyrocytes and by Ad-mediated re-expression of GADD45γ in ATC cells (ARO, FRO, NPA). It should be noted that GADD45 family proteins have been implicated in a variety of growth-regulatory mechanisms, including DNA replication and repair, G_2/M checkpoint control, and apoptosis. The above modifications resulted in a significant inhibition of the proliferation due to apoptosis. In addition to this, the expression of tyrosine phosphatase eta (PTPη) gene, which encodes a receptor-type tyrosine phosphatase protein with tumor-suppressor activity, had been used in several studies (121-123), resulting in cell growth inhibition by interfering with RET autophosphorylation. The latter reduces its kinase activity and leads to the inhibition of downstream Ras-ERK (Ras GTPase-extracellular signal-regulated kinase), and AKT-PI3K signaling pathways (Figure 1), which, in turn, reduces oncogenic activity.

Antisense Approach

The antisense approach aims to turn off a mutated gene in a cell by targeting the mRNA transcripts copied from the gene in order to disrupt the transcription of the faulty gene. High mobility group I (HMGI) proteins are overexpressed in several human malignant tumors and by using an adenovirus carrying the *HMGI(Y)* gene in an antisense orientation (Ad-Yas) has led to the suppression of *HMGI(Y)* protein synthesis and prevention of thyroid cell transformation. Such virus-induced programmed cell death was successful in two human ATC cell lines (ARO and FB-1) and in a variety of other tumor cell lines, but not in normal thyroid cells (124). Another study by Hassan *et al*. (125), applied this approach by transfecting FTC cell line (FTC-133) cells with oligodeoxyribonucleotide phosphorothioates (ODNs) (mutant *Tp53* knockout) and finally managed to impair VEGF secretion in the undifferentiated FTC-133 cell line.

Use of Ribozymes

Another approach having the same outcome as that with antisense uses ribozymes. The ability of ribozymes to recognize and cut specific RNA molecules makes them exciting candidates for human therapy. Among the characteristic examples of this method is a synthetic ribozyme that destroys the mRNA encoding a receptor of VEGF and also the ribozyme designed by Parthasarathy *et al*. (126), to cleave the sequence that results from the mutation of codon 634 from TGC to TAC in the *RET* proto-oncogene. Cleavage specificity was demonstrated by the observation that transformed NIH3T3 cells that expressed the active ribozyme (*RETC634*) no longer formed colonies in soft agar, unlike NIH3T3 cells that expressed wt-*RET* codon 634.

Use of Small Interfering RNA (siRNA)

This approach utilizes siRNA (similarly to the antisense approach) in order to prevent the production of the faulty protein. Overexpression of antiapoptopic genes, such as *BCL2* (B-cell chronic lymphocytic leukemia/lymphoma 2) and *BIRC5* encoding for survivin protein, was found in MTC tissues and MTC cell line. Targeting the *BIRC5* gene with siRNAs reduced the expression of survivin protein, which led to an increase in apoptosis concomitant with suppression of *in vitro* MTC growth (127).

Antisense, ribozymes and siRNA approaches were introduced without bystander activity in non-targeted tissue, and therefore require high vector transduction efficiency to increase the efficiency of transduction.

Use of Replication-competent Viral Vectors (Replication-selective, Viral-mediated Oncolysis)

The development of an Ad system that can replicate exclusively in wt-*p53*-deficient cells by using a double infection approach has been achieved in order to infect the cells with two recombinant viruses. The recombinant virus contains an expression unit of the synthetic p53-responsive promoter and the Cre recombinase gene, and the other Ad contains two expression units: the first consists of the *E1A* gene flanked by a pair of loxP sites downstream of the constitutive CAG promoter, and the second consists of the *E1B19K* gene under the control of the CMV promoter. Co-infection of these two Ads into *p53*-expressing cells leads to expression of Cre recombinase gene, which then excises the *E1A* gene that is flanked by a pair of loxP sites, thereby stopping virus replication. However, in cells without *p53* expression, Cre recombinase is not expressed, the *E1A* gene is not excised, and virus replication takes place, thereby causing cell lysis (128).

Another anticancer therapeutic approach is through achieving selective replication oncolytic viruses (virotherapy), which represents a novel targeted form by using the *dl*1520 (ONYX-015) virus was genetically engineered for replication-selectivity for treatment in cancer patients (129-130). Oncolytic viral therapy offers several advantages over conventional anticancer drugs, viruses can be rapidly modified by recombinant DNA technology, allowing for the rational creation of 'designer viruses'. In addition to the ability of these viruses to self-replicate within the cancer cell and their unique pharmacokinetic properties that are distinct from conventional therapeutics, Ads and other viruses have been engineered for selective replication within neoplastic cells. The most common goal is the deletion of the viral gene whose product is necessary for replication in normal cells but expendable in cancer cells. dl1520 was expected to replicate selectively in a high percentage of human cancer types (where the p53 pathway is non-functional in about 50% of human neoplasia). Recently, it has been shown that *E1B55K* mediates late-viral RNA transport, and *dl*1520 tumor-selective replication is determined by a unique property of tumor cells to efficiently export late viral RNA in the absence of *E1B55K*. Thus, loss of E1B-55K-mediated late viral RNA export, rather than p53 degradation, is the major determinant of *dl*1520 selective replication in neoplastic cells (131). The use of *dl*1520 oncolytic virus as a monotherapy has demonstrated limitations in efficacy because of the multifunctional nature of many viral proteins; the gene deletion approach for viral selective replication frequently results in reduced replication and therapeutic potency. Moreover, the complexities of tumor biology and the heterogeneity of human tissues necessitate further investigation for the design of strategies in order to increase the tumor-eradicating potential of such a method (132). It is almost impossible to deliver the virus to all the tumoral cells; therefore, the uninfected tumoral cells will continue to grow and complete tumor regression will only be obtained by the association of ONYX-015 with chemotherapeutic drugs (133).

Recently, a sizable fraction of undifferentiated or poorly differentiated thyroid tumors were shown to contain mutations in β-catenin. A conditionally replicative Ad (named HILMI) has been developed, which replicates specifically in cells with an active Wnt/β-catenin pathway. As a result, several thyroid cancer cell lines derived from undifferentiated or anaplastic tissues and possessing an active Wnt/β-catenin pathway are susceptible to cell killing by HILMI. Furthermore, viral replication in ATC cells as xenograft tumors in nude mice was observed, and prolonged survival of mice with ATC tumors was observed following administration of the HILMI therapeutic vector (134).

ONYX-411 is a new virus that replicates selectively and induces cell death in eight human ATC cell lines. The cytopathic effect of the virus is specific to cells with retinoblastoma dysfunction, which appears to be frequent in

ATC with high expression of the coxsackievirus and adenovirus receptor (CAR) in all ATC cell lines, demonstrating the potentially universal application of this oncolytic viral therapy to ATC, it was shown to suppress the growth of xenograft tumors in nude mice (135).

Combinatorial Approach

The *dl*1520 virus has been used in conjunction with chemotherapeutic agents in clinical trials with evidence for potential synergistic antitumor activity. Synergistic cell killing effects of *dl*1520 with doxorubicin or paclitaxel were observed in ATC cell lines (113, 114). Enhanced sensitivity to chemotherapeutic regimens in ONYX-015-infected cells may depend on the *E1A* gene expression. In fact, *E1A* expression activates the cell cycle and increases cellular sensitivity to p53-independent apoptosis (136, 137). Therefore, the apoptotic effects of anticancer agents can be enhanced by the *E1A* Ad gene product (138). Recently, findings by Kim *et al*. (139) indicate that doxorubicin enhances transgene expression under the control of the CMV promoter and that doxorubicin might be used as an adjuvant to radioiodine therapy by sodium iodide symporter (*NIS*) gene transfer in ATC.

Increased efficacy of the *dl*1520 virus has been repeatedly shown following ionizing radiation. Portella *et al*. (140), showed that ATC ONYX-015-treated cells were very sensitive to radiation-induced apoptosis. Moreover, lovastatin in combination with *dl*1520 virus has also been used to increase oncolytic activity, since lovastatin is a drug that increases Ad replication and enhances the effects of oncolytic Ad *in vitro* and *in vivo* against ATC cells (141). Lovastatin (which is a 3-hydroxy-methylglutaryl-CoA reductase inhibitor), a drug used for the treatment of hypercholesterolemia, has also been reported to exert growth inhibitory activity *in vitro* and *in vivo*. Farnesyl- (FPP) and geranyl-geranyl-pyrophosphate (GGPP), intermediates in the cholesterol synthetic pathway, are needed for isoprenylation, a crucial step for membrane attachment of cellular proteins such as Ras, Rho, Cdc42 and Rac, and thus, by inhibiting protein isoprenylation, lovastatin induces a reduction of cell proliferation and apoptosis (142, 143). Moreover, it has been demonstrated that low doses of lovastatin induce apoptosis of ATC cells (144, 145). In addition, it was observed that lovastatin significantly enhanced the antineoplastic activity of *dl*1520 against ATC cells and its replication.

Another oncolytic herpes simplex virus, G207, presents a promising activity against human ATC, when combined with paclitaxel. A significant enhancement of paclitaxel effect (increase in antitumoral activity, including microtubule acetylation, mitotic block, and apoptosis) in addition to direct oncolytic effects of G207 was shown (146).

NIS-Introducing or Toxic Gene Therapy Approach

Normally, iodide is accumulated in the thyroid gland in concentrations that reach 20 to 40-fold over the plasma levels (147) and this accumulation is under the control of NIS which is a transmembrane glycoprotein present in the basolateral pole of the thyroid follicular cells that were firstly cloned in rats (148) and then in humans (149). The entire process is under the control of TSH. It should be noted that a major limitation for the treatment of some thyroid carcinomas with radioactive iodide (150) has been attributed to the progressive reduction or loss in the ability of these carcinomas to take up radioactive iodine due to iodine metabolism abnormalities (such as defects in iodide uptake and/or organification) (151-153). Induction of hypothyroidism in order to elevate endogenous TSH or the administration of human recombinant TSH is used by physicians in such cases to enhance iodide uptake, proving that cancer cells still maintain a certain degree of differentiation and hormonal responsiveness. However, when thyroid cancer undergoes total loss of differentiation, no iodide uptake is observed, and the prognosis is clearly worse. Overall, low or an absence of *NIS* expression was thought to occur under these circumstances (153-155). Surprisingly, after the cloning of *NIS*, in some studies that were based on IHC and reverse-transcriptase PCR (RT-PCR), it was revealed that up to 70-80% of thyroid tumors expressed or even overexpressed NIS, and this expression was mainly cytoplasmatic and not targeted to the basolateral membrane (156-158). These observations pointed out that targeting of, and retention in, the plasma membrane are essential for *NIS* to be fully functional, explaining why iodide uptake is diminished in thyroid cancer.

The principle of introducing *NIS* gene for developing a novel cytoreductive or toxic gene therapy strategy in the treatment of thyroidal and extrathyroidal malignancies was based on the transfer of *NIS* gene followed by radioiodine therapy (159), either by transfection of *NIS* cDNA (148, 149, 160) or with Re or Ad vectors (161, 162). Iodide was, finally, uptaken by the transduced cells and by coupling with ^{131}I administration as a treatment or with a recombinant Ad (*Ad-NIS* radioiodine administration), a procedure that appears to be a very promising strategy for treating tumors of various origins (163). Application of the *NIS* gene extends the diagnostic and therapeutic use of ^{131}I in the management of differentiated thyroid cancer to the treatment of non-thyroidal cancer and dedifferentiated ATC and MTC. In addition, *NIS* gene transfection is associated with a bystander effect, because not only *NIS*-transduced cancer cells, but also surrounding non-transduced cells are destroyed by the crossfire effect of the β-emitter ^{131}I (164). Early studies in malignantly transformed rat thyroid cells (FRTL-Tc) without iodide transport activity showed that transfection with rat *NIS* cDNA using electroporation is able to restore

radioiodine accumulation both *in vitro* and *in vivo* (165). More recently, stable transfection of a NIS-defective follicular thyroid carcinoma cell line (FTC-133) with the *NIS* gene was able to re-establish iodide accumulation activity both *in vitro* and *in vivo* (166). Petrich *et al*. (167), transiently transfected a variety of PTC, FTC and ATC cell lines (B-CPAP, K1, WRO, 8505C, FTC-133) with the *NIS* gene, thereby inducing perchlorate-sensitive accumulation of ^{125}I. These studies show that NIS gene delivery into thyroid cancer cells is capable of restoring ^{131}I accumulation and might therefore represent an effective therapy for dedifferentiated and anaplastic thyroid tumors that lack iodide-accumulating capacity. Lee *et al*. (168), used a recombinant Ad to transduce a panel of human thyroid carcinoma cell lines (ARO, FRO, NPA) with the *hNIS* gene. They demonstrated significant iodide accumulation associated with rapid iodide efflux *in vitro* that may limit the therapeutic potential of *hNIS*/radioiodide-based treatment, but more recent trials still confirmed the potential of the *hNIS*-mediated ^{131}I gene therapy for ATC (169).

There is a significant correlation between low *NIS* expression and more aggressive tumors (170), and BRAF-positive PTC correlated with high recurrent rate and impairment of NIS targeting to the membrane as assessed by IHC (171). In addition to the role of other important gene mutations such as those of *RAS* family genes and *RET* rearrangements, all of them contribute to the partial or complete loss of differentiation of thyroid cancers.

Conclusion

More understanding of the molecular pathways and tumorigenesity is remaining the only key for developing new gene therapy approaches to achieve the efficient and successful antitumoral efficacy with minimum normal tissue toxicity by taking into consideration the subsequent trials in each approach alone and then interpretation of all findings and modifications. The selection of the most promising approach must be based on the balance between therapeutic efficacy and unwanted effects in accordingly designed clinical trials.

Acknowledgements

The Authors wish to acknowledge their appreciation to Mr Vasileios Stolakis (B.Sc.) for his help in reference gathering and manuscript preparation.

References

1 Thyroid Carcinoma Task Force: AACE/AAES medical/surgical guidelines for clinical practice: management of thyroid carcinoma. American Association of Clinical Endocrinologists. American College of Endocrinology. Endocr Pract *7*: 202-220, 2001.

2 Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ: Cancer statistics, 2007. CA Cancer J Clin *57*: 43-66, 2007.

3 Davies L and Welch HG: Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA *295*: 2164-2167, 2006.

4 Strachan MWJ and Walker BR: Endocrine disease: Davidson's Principles and Practice of Medicine. 20th ed. Philadelphia, PA: Churchill Livingstone Elsevier, 2006.

5 Hölzer S and Dudeck J: Patient care evaluation studies – a comprehensive concept for evaluation of oncologic patient management. Z Arztl Fortbild Qualitatssich *94*: 121-126, 2000.

6 DeGroot LJ and Zhang R: Clinical review 131: Gene therapy for thyroid cancer: where do we stand? J Clin Endocrinol Metab *86*: 2923-2928, 2001.

7 Patel KN and Singh B: Genetic considerations in thyroid cancer. Cancer Control *13*: 111-118, 2006.

8 Hay ID: Papillary thyroid carcinoma. Endocrinol Metab Clin North Am *19*: 545-576, 1990.

9 Shaha AR, Shah JP and Loree TR: Risk group stratification and prognostic factors in papillary carcinoma of thyroid. Ann Surg Oncol *3*: 534-538, 1996.

10 Shaha AR, Loree TR and Shah JP: Prognostic factors and risk group analysis in follicular carcinoma of the thyroid. Surgery *118*: 1131-1138, 1995.

11 Giuffrida D and Gharib H: Anaplastic thyroid carcinoma: current diagnosis and treatment. Ann Oncol *11*: 1083-1089, 2000.

12 Ain KB: Anaplastic thyroid carcinoma: behavior, biology, and therapeutic approaches. Thyroid *8*: 715-726, 1998.

13 Ain KB: Anaplastic thyroid carcinoma: a therapeutic challenge. Semin Surg Oncol *16*: 64-69, 1999.

14 Hölting T, Möller P, Tschahargane C, Meybier H, Buhr H and Herfarth C: Immunohistochemical reclassification of anaplastic carcinoma reveals small and giant cell lymphoma. World J Surg *14*: 291-295, 1990.

15 Kebebew E, Ituarte PH, Siperstein AE, Duh QY and Clark OH: Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. Cancer *88*: 1139-1148, 2000.

16 Ball DW: Medullary thyroid cancer: therapeutic targets and molecular markers. Curr Opin Oncol *19*: 18-23, 2007.

17 Moley JF: Medullary thyroid carcinoma. Curr Treat Options Oncol *4*: 339-347, 2003.

18 Ward LS, Morari EC, Leite JL, Bufalo NE, Guilhen AC, Araujo PP, Tincani AJ, Assumpção LV and Matos PS: Identifying a risk profile for thyroid cancer. Arq Bras Endocrinol Metabol *51*: 713-722, 2007.

19 Knobel M and Medeiros-Neto G: Relevance of iodine intake as a reputed predisposing factor for thyroid cancer. Arq Bras Endocrinol Metabol *51*: 701-712, 2007.

20 Nikiforov Y and Gnepp DR: Pediatric thyroid cancer after the Chernobyl disaster. Pathomorphologic study of 84 cases (1991-1992) from the Republic of Belarus. Cancer *74*: 748-766, 1994.

21 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST,

Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR and Futreal PA: Mutations of the *BRAF* gene in human cancer. Nature *417*: 949-954, 2002.

22 Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B and Velculescu VE: Tumorigenesis: *RAF/RAS* oncogenes and mismatch-repair status. Nature *418*: 934, 2002.

23 Daum G, Eisenmann-Tappe I, Fries HW, Troppmair J and Rapp UR: The ins and outs of Raf kinases. Trends Biochem Sci *19*: 474-480, 1994.

24 Xing M: *BRAF* mutation in thyroid cancer. Endocr Relat Cancer *12*: 245-262, 2005.

25 Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, Tolaney S, Holt EH, Hui P, Umbricht CB, Basaria S, Ewertz M, Tufaro AP, Califano JA, Ringel MD, Zeiger MA, Sidransky D and Ladenson PW: *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab *90*: 6373-6379, 2005.

26 Wu G, Mambo E, Guo Z, Hu S, Huang X, Gollin SM, Trink B, Ladenson PW, Sidransky D and Xing M: Uncommon mutation, but common amplifications, of the *PIK3CA* gene in thyroid tumors. J Clin Endocrinol Metab *90*: 4688-4693, 2005.

27 García-Rostán G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J and Santoro M: Mutation of the *PIK3CA* gene in anaplastic thyroid cancer. Cancer Res *65*: 10199-10207, 2005.

28 Hou P, Liu D, Shan Y, Hu S, Studeman K, Condouris S, Wang Y, Trink A, El-Naggar AK, Tallini G, Vasko V and Xing M: Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer. Clin Cancer Res *13*: 1161-1170, 2007.

29 Takahashi M, Ritz J and Cooper GM: Activation of a novel human transforming gene, RET, by DNA rearrangement. Cell *42*: 581-588, 1985.

30 LiVolsi VA, Albores-Saavedra J, Asa SL, Baloch ZW, Sobrinho-Simões M, Wenig B, DeLellis RA, Cady B, Mazzaferri EL, Hay I, Fagin JA, Weber AL, Caruso P, Voutilainen PE, Franssila KO, Williams ED, Schneider AB, Nikiforov Y, Rabes HM, Akslen L, Ezzat S, Santoro M, Eng C and Harach HR: Papillary carcinoma. *In*: Pathology and Genetics of Tumours of Endocrine Organs. DeLellis RA, Lloyd RV, Heitz PU and Eng C (eds.). Lyon: IARC Press, pp. 57-66, 2004.

31 Nikiforov YE: *RET/PTC* rearrangement in thyroid tumors. Endocr Pathol *13*: 3-16, 2002.

32 Greco A, Roccato E and Pierotti MA: TRK oncogenes in papillary thyroid carcinoma. Cancer Treat Res *122*: 207-219, 2004.

33 Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM and Fletcher JA: *PAX8-PPARgamma1* fusion oncogene in human thyroid carcinoma. Science *289*: 1357-1360, 2000.

34 Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG and Nikiforov YE: *PAX8-PPARgamma* rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. Am J Surg Pathol *26*: 1016-1023, 2002.

35 Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi T, Hayashi Y, Dohi K, Nakamura N and Akiyama M: Unique association of *p53* mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res *52*: 1369-1371, 1992.

36 Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH and Koeffler HP: High prevalence of mutations of the *p53* gene in poorly differentiated human thyroid carcinomas. J Clin Invest *91*: 179-184, 1993.

37 Dobashi Y, Sakamoto A, Sugimura H, Mernyei M, Mori M, Oyama T and Machinami R: Overexpression of p53 as a possible prognostic factor in human thyroid carcinoma. Am J Surg Pathol *17*: 375-381, 1993.

38 Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G and Pierotti MA: Gene *p53* mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest *91*: 1753-1760, 1993.

39 Matias-Guiu X, Cuatrecasas M, Musulen E and Prat J: p53 expression in anaplastic carcinomas arising from thyroid papillary carcinomas. J Clin Pathol *47*: 337-339, 1994.

40 Salvatore D, Celetti A, Fabien N, Paulin C, Martelli ML, Battaglia C, Califano D, Monaco C, Viglietto G, Santoro M and Fusco A: Low frequency of *p53* mutations in human thyroid tumours; *p53* and *Ras* mutation in two out of fifty-six thyroid tumours. Eur J Endocrinol *134*: 177-183, 1996.

41 Battista S, Martelli ML, Fedele M, Chiappetta G, Trapasso F, De Vita G, Battaglia C, Santoro M, Viglietto G, Fagin JA and Fusco A: A mutated *p53* gene alters thyroid cell differentiation. Oncogene *11*: 2029-2037, 1995.

42 Fusco A and Santoro M: Human thyroid carcinogenesis. Forum *7*: 214-229, 1997.

43 Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL and Tallini G: Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and *CTNNB1* exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol *158*: 987-996, 2001.

44 Cetta F, Montalto G, Gori M, Curia MC, Cama A and Olschwang S: Germline mutations of the *APC* gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. J Clin Endocrinol Metab *85*: 286-292, 2000.

45 Máximo V, Botelho T, Capela J, Soares P, Lima J, Taveira A, Amaro T, Barbosa AP, Preto A, Harach HR, Williams D and Sobrinho-Simões M: Somatic and germline mutation in *GRIM-19*, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hurthle cell) tumours of the thyroid. Br J Cancer *92*: 1892-1898, 2005.

46 Gimm O, Perren A, Weng LP, Marsh DJ, Yeh JJ, Ziebold U, Gil E, Hinze R, Delbridge L, Lees JA, Mutter GL, Robinson BG, Komminoth P, Dralle H and Eng C: Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol *156*: 1693-1700, 2000.

47 Simpson L and Parsons R: PTEN: life as a tumor suppressor. Exp Cell Res *264*: 29-41, 2001.

48 Gimm O: Thyroid cancer. Cancer Lett *163*: 143-156, 2001.

49 Xing M: Gene methylation in thyroid tumorigenesis. Endocrinology *148*: 948-953, 2007.

50 Bell B and Mazzaferri EL: Familial adenomatous polyposis (Gardner's syndrome) and thyroid carcinoma. A case report and review of the literature. Dig Dis Sci *38*: 185-190, 1993.

51 Hover AR, Cawthern T and McDanial W: Cowden disease. A hereditary polyposis syndrome diagnosable by mucocutaneous inspection. J Clin Gastroenterol *8*: 576-579, 1986.

52 Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Miyoshi Y, Miki Y, Nishisho I and Nakamura Y: Identification of FAP locus genes from chromosome 5q21. Science *253*: 661-665, 1991.

53 Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, Utsunomiya J, Baba S and Hedge P: Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science *253*: 665-669, 1991.

54 Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C and Parsons R: Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet *16*: 64-67, 1997.

55 Ciampi R and Nikiforov YE: Alterations of the *BRAF* gene in thyroid tumors. Endocr Pathol *16*: 163-172, 2005.

56 Trovisco V, Soares P and Sobrinho-Simões M: *B-RAF* mutations in the etiopathogenesis, diagnosis, and prognosis of thyroid carcinomas. Hum Pathol *37*: 781-786, 2006.

57 Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, Kroll TG and Nikiforov YE: *RAS* point mutations and *PAX8-PPAR gamma* rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab *88*: 2318-2326, 2003.

58 Rosai J: Handling of thyroid follicular patterned lesions. Endocr Pathol *16*: 279-283, 2005.

59 DeLellis RA: Pathology and genetics of thyroid carcinoma. J Surg Oncol *94*: 662-669, 2006.

60 Tallini G and Asa SL: *RET* oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol *8*: 345-354, 2001.

61 Quiros RM, Ding HG, Gattuso P, Prinz RA and Xu X: Evidence that one subset of anaplastic thyroid carcinomas are derived from papillary carcinomas due to *BRAF* and *p53* mutations. Cancer *103*: 2261-2268, 2005.

62 Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE and Fagin JA: High prevalence of *BRAF* mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res *63*: 1454-1457, 2003.

63 Bongarzone I, Vigneri P, Mariani L, Collini P, Pilotti S and Pierotti MA: *RET*/*NTRK1* rearrangements in thyroid gland tumors of the papillary carcinoma family: correlation with clinicopathological features. Clin Cancer Res *4*: 223-228, 1998.

64 Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D and Klugbauer S: Pattern of radiation-induced *RET* and *NTRK1* rearrangements in 191 post-chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. Clin Cancer Res *6*: 1093-1103, 2000.

65 Namba H, Rubin SA and Fagin JA: Point mutations of *ras* oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol *4*: 1474-1479, 1990.

66 Capella G, Matias-Guiu X, Ampudia X, de Leiva A, Perucho M and Prat J: *Ras* oncogene mutations in thyroid tumors: polymerase chain reaction-restriction-fragment-length polymorphism analysis from paraffin-embedded tissues. Diagn Mol Pathol *5*: 45-52, 1996.

67 La Perle KM, Jhiang SM and Capen CC: Loss of p53 promotes anaplasia and local invasion in ret/PTC1-induced thyroid carcinomas. Am J Pathol *157*: 671-677, 2000.

68 Marques AR, Espadinha C, Catarino AL, Moniz S, Pereira T, Sobrinho LG and Leite V: Expression of *PAX8-PPAR gamma 1* rearrangements in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab *87*: 3947-3952, 2002.

69 Máximo V, Soares P, Lima J, Cameselle-Teijeiro J and Sobrinho-Simões M: Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hürthle cell tumors. Am J Pathol *160*: 1857-1865, 2002.

70 Takeuchi Y, Daa T, Kashima K, Yokoyama S, Nakayama I and Noguchi S: Mutations of *p53* in thyroid carcinoma with an insular component. Thyroid *9*: 377-381, 1999.

71 Gimm O, Morrison CD, Suster S, Komminoth P, Mulligan L and Sweet KM: Multiple endocrine neoplasia type 2. *In*: Pathology and Genetics of Tumours of Endocrine Organs. DeLellis RA, Lloyd RV, Heitz PU and Eng C (eds.). Lyon: IARC Press, pp. 211-217, 2004.

72 Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, van Amstel HK, Lips CJ, Nishisho I, Takai SI, Marsh DJ, Robinson BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romei C, Pacini F, Fink M, Niederle B, Zedenius J, Nordenskjöld M, Komminoth P, Hendy GN, Gharib H, Thibodeau SN, Lacroix A, Frilling A, Ponder BAJ and Mulligan LM: The relationship between specific *RET* proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International *RET* Mutation Consortium analysis. JAMA *276*: 1575-1579, 1996.

73 Wohllk N, Cote GJ, Bugalho MM, Ordonez N, Evans DB, Goepfert H, Khorana S, Schultz P, Richards CS and Gagel RF: Relevance of *RET* proto-oncogene mutations in sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab *81*: 3740-3745, 1996.

74 Dvorakova S, Vaclavikova E, Sykorova V, Vcelak J, Novak Z, Duskova J, Ryska A, Laco J, Cap J, Kodetova D, Kodet R, Krskova L, Vlcek P, Astl J, Vesely D and Bendlova B: Somatic mutations in the *RET* proto-oncogene in sporadic medullary thyroid carcinomas. Mol Cell Endocrinol *284*: 21-27, 2008.

75 Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med *319*: 525-532, 1988.

76 Tallini G, Santoro M, Helie M, Carlomagno F, Salvatore G, Chiappetta G, Carcangiu ML and Fusco A: *RET*/*PTC* oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. Clin Cancer Res *4*: 287-294, 1998.

77 Mitsutake N, Knauf JA, Mitsutake S, Mesa C Jr, Zhang L and Fagin JA: Conditional *BRAF*V600E expression induces DNA synthesis, apoptosis, dedifferentiation, and chromosomal instability in thyroid PCCL3 cells. Cancer Res *65*: 2465-2473, 2005.

78 Dwight T, Thoppe SR, Foukakis T, Lui WO, Wallin G, Höög A, Frisk T, Larsson C and Zedenius J: Involvement of the *PAX8*/*peroxisome proliferator-activated receptor gamma* rearrangement in follicular thyroid tumors. J Clin Endocrinol Metab *88*: 4440-4445, 2003.

79 Farid NR, Shi Y and Zou M: Molecular basis of thyroid cancer. Endocr Rev *15*: 202-232, 1994.

80 Takano T and Amino N: Molecular-based diagnosis of thyroid carcinomas by detecting cancer-specific mRNAs. Rinsho Byori *48*: 149-154, 2000.

81 Takano T: Fetal cell carcinogenesis of the thyroid: a hypothesis for better understanding of gene expression profile and genomic alternation in thyroid carcinoma. Endocr J *51*: 509-515, 2004.
82 Messina M, Yu DM, Learoyd DL, Both GW, Molloy PL and Robinson BG: High level, tissue-specific expression of a modified calcitonin/calcitonin gene-related peptide promoter in a human medullary thyroid carcinoma cell line. Mol Cell Endocrinol *164*: 219-224, 2000.
83 Goldman CK, Rogers BE, Douglas JT, Sosnowski BA, Ying W, Siegal GP, Baird A, Campain JA and Curiel DT: Targeted gene delivery to Kaposi's sarcoma cells *via* the fibroblast growth factor receptor. Cancer Res *57*: 1447-1451, 1997.
84 Böckmann M, Drosten M and Pützer BM: Discovery of targeting peptides for selective therapy of medullary thyroid carcinoma. J Gene Med *7*: 179-188, 2005.
85 Böckmann M, Hilken G, Schmidt A, Cranston AN, Tannapfel A, Drosten M, Frilling A, Ponder BA and Pützer BM: Novel SRESPHP peptide mediates specific binding to primary medullary thyroid carcinoma after systemic injection. Hum Gene Ther *16*: 1267-1275, 2005.
86 Nagayama Y, Nishihara E, Iitaka M, Namba H, Yamashita S and Niwa M: Enhanced efficacy of transcriptionally targeted suicide gene/prodrug therapy for thyroid carcinoma with the Cre-loxP system. Cancer Res *59*: 3049-3052, 1999.
87 Nishihara E, Nagayama Y, Mawatari F, Tanaka K, Namba H, Niwa M and Yamashita S: Retrovirus-mediated herpes simplex virus thymidine kinase gene transduction renders human thyroid carcinoma cell lines sensitive to ganciclovir and radiation *in vitro* and *in vivo*. Endocrinology *138*: 4577-4583, 1997.
88 Braiden V, Nagayama Y, Iitaka M, Namba H, Niwa M and Yamashita S: Retrovirus-mediated suicide gene/prodrug therapy targeting thyroid carcinoma using a thyroid-specific promoter. Endocrinology *139*: 3996-3999, 1998.
89 Zhang R, Straus FH and DeGroot LJ: Adenoviral-mediated gene therapy for thyroid carcinoma using thymidine kinase controlled by thyroglobulin promoter demonstrates high specificity and low toxicity. Thyroid *11*: 115-123, 2001.
90 Zhang R and DeGroot LJ: Gene therapy of established medullary thyroid carcinoma with herpes simplex viral thymidine kinase in a rat tumor model: relationship of bystander effect and antitumor efficacy. Thyroid *10*: 313-319, 2000.
91 Zhang R, Baunoch D and DeGroot LJ: Genetic immunotherapy for medullary thyroid carcinoma: destruction of tumors in mice by *in vivo* delivery of adenoviral vector transducing the murineinterleukin-2 gene. Thyroid *8*: 1137-1146, 1998.
92 Zhang R, Straus FH and DeGroot LJ: Effective genetic therapy of established medullary thyroid carcinomas with murineinterleukin-2: dissemination and cytotoxicity studies in a rat tumor model. Endocrinology *140*: 2152-2158, 1999.
93 Lausson S, Fournes B, Borrel C, Milhaud G and Treilhou-Lahille F: Immune response against medullary thyroid carcinoma (MTC) induced by parentaland/or interleukin-2-secreting MTC cells in a rat model of human familial medullary thyroid carcinoma. Cancer Immunol Immunother *43*: 116-123, 1996.
94 Zhang R and DeGroot LJ: Genetic immunotherapy of established tumours with adenoviral vectors transducing murine interleukin-12 (mIL12) subunits in a rat medullary thyroid carcinoma model. Clin Endocrinol (Oxf) *52*: 687-694, 2000.
95 Yamazaki M, Zhang R, Straus FH, Messina M, Robinson BG, Hashizume K and DeGroot LJ: Effective gene therapy for medullary thyroid carcinoma using recombinant adenovirus inducing tumor-specific expression of interleukin-12. Gene Ther *9*: 64-74, 2002.
96 Zhang R and DeGroot LJ: Gene therapy of a rat follicular thyroid carcinoma model with adenoviral vectors transducing murine interleukin-12. Endocrinology *144*: 1393-1398, 2003.
97 Soler MN, Milhaud G, Lekmine F, Treilhou-Lahille F, Klatzmann D and Lausson S: Treatment of medullary thyroid carcinoma by combined expression of suicide and interleukin-2 genes. Cancer Immunol Immunother *48*: 91-99, 1999.
98 Zhang R and DeGroot LJ: An adenoviral vector expressing functional heterogeneous proteins herpes simplex viral thymidine kinase and human interleukin-2 has enhanced *in vivo* antitumor activity against medullary thyroid carcinoma. Endocr Relat Cancer *8*: 315-325, 2001.
99 Yamazaki M, Straus FH, Messina M, Robinson BG, Takeda T, Hashizume K and DeGroot LJ: Adenovirus-mediated tumor-specific combined gene therapy using Herpes simplex virus thymidine/ganciclovir system and murine interleukin-12 induces effective antitumor activity against medullary thyroid carcinoma. Cancer Gene Ther *11*: 8-15, 2004.
100 Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Franchin E, Del Vecchio C, Giaretta I, Boscaro M and Palù G: Gene therapy of thyroid cancer *via* retrovirally-driven combined expression of human interleukin-2 and herpes simplex virus thymidine kinase. Eur J Endocrinol *148*: 73-80, 2003.
101 Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Gnatta E, Parolin C, Boscaro M and Palù G: Transcriptionally targeted retroviral vector for combined suicide and immunomodulating gene therapy of thyroid cancer. J Clin Endocrinol Metab *87*: 5304-5311, 2002.
102 Barzon L, Pacenti M, Taccaliti A, Franchin E, Bruglia M, Boscaro M and Palù G: A pilot study of combined suicide/cytokine gene therapy in two patients with end-stage anaplastic thyroid carcinoma. J Clin Endocrinol Metab *90*: 2831-2834, 2005.
103 Messina M, Yu DM, Both GW, Molloy PL and Robinson BG: Calcitonin-specific transcription and splicing targets gene-directed enzyme prodrug therapy to medullary thyroid carcinoma cells. J Clin Endocrinol Metab *88*: 1310-1318, 2003.
104 Albrechtsen N, Dornreiter I, Grosse F, Kim E, Wiesmüller L and Deppert W: Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53. Oncogene *18*: 7706-7717, 1999.
105 Lakin ND and Jackson SP: Regulation of p53 in response to DNA damage. Oncogene *18*: 7644-7655, 1999.
106 McCormick F: Cancer gene therapy: fringe or cutting edge? Nat Rev Cancer *1*: 130-141, 2001.
107 Nagayama Y, Shigematsu K, Namba H, Zeki K, Yamashita S and Niwa M: Inhibition of angiogenesis and tumorigenesis, and induction of dormancy by p53 in a *p53*-null thyroid carcinoma cell line *in vivo*. Anticancer Res *20*: 2723-2728, 2000.
108 Moretti F, Farsetti A, Soddu S, Misiti S, Crescenzi M, Filetti S, Andreoli M, Sacchi A and Pontecorvi A: p53 re-expression inhibits proliferation and restores differentiation of human thyroid anaplastic carcinoma cells. Oncogene *14*: 729-740, 1997.

109 Fagin JA, Tang SH, Zeki K, Di Lauro R, Fusco A and Gonsky R: Reexpression of thyroid peroxidase in a derivative of an undifferentiated thyroid carcinoma cell line by introduction of wild-type *p53*. Cancer Res *56*: 765-771, 1996.
110 Imanishi R, Ohtsuru A, Iwamatsu M, Iioka T, Namba H, Seto S, Yano K and Yamashita S: A histone deacetylase inhibitor enhances killing of undifferentiated thyroid carcinoma cells by *p53* gene therapy. J Clin Endocrinol Metab *87*: 4821-4824, 2002.
111 Moretti F, Nanni S, Farsetti A, Narducci M, Crescenzi M, Giuliacci S, Sacchi A and Pontecorvi A: Effects of exogenous *p53* transduction in thyroid tumor cells with different p53 status. J Clin Endocrinol Metab *85*: 302-308, 2000.
112 Narimatsu M, Nagayama Y, Akino K, Yasuda M, Yamamoto T, Yang TT, Ohtsuru A, Namba H, Yamashita S, Ayabe H and Niwa M: Therapeutic usefulness of wild-type *p53* gene introduction in a p53-null anaplastic thyroid carcinoma cell line. J Clin Endocrinol Metab *83*: 3668-3672, 1998.
113 Nagayama Y, Yokoi H, Takeda K, Hasegawa M, Nishihara E, Namba H, Yamashita S and Niwa M: Adenovirus-mediated tumor suppressor *p53* gene therapy for anaplastic thyroid carcinoma *in vitro* and *in vivo*. J Clin Endocrinol Metab *85*: 4081-4086, 2000.
114 Blagosklonny MV, Giannakakou P, Wojtowicz M, Romanova LY, Ain KB, Bates SE and Fojo T: Effects of p53-expressing adenovirus on the chemosensitivity and differentiation of anaplastic thyroid cancer cells. J Clin Endocrinol Metab *83*: 2516-2522, 1998.
115 Kim SB, Ahn IM, Park HJ, Park JS, Cho HJ, Gong G, Suh C, Lee JS, Kim WK and Kim SH: Growth inhibition and chemosensitivity of poorly differentiated human thyroid cancer cell line (NPA) transfected with *p53* gene. Head Neck *23*: 223-229, 2001.
116 Drosten M, Frilling A, Stiewe T and Pützer BM: A new therapeutic approach in medullary thyroid cancer treatment: inhibition of oncogenic RET signaling by adenoviral vector-mediated expression of a dominant-negative RET mutant. Surgery *132*: 991-997, 2002.
117 Drosten M, Stiewe T and Pützer BM: Antitumor capacity of a dominant-negative *RET* proto-oncogene mutant in a medullary thyroid carcinoma model. Hum Gene Ther 14: 971-982, 2003.
118 Chun YS, Saji M and Zeiger MA: Overexpression of TTF-1 and PAX-8 restores thyroglobulin gene promoter activity in ARO and WRO cell lines. Surgery *124*: 1100-1105, 1998.
119 Shimura H, Suzuki H, Miyazaki A, Furuya F, Ohta K, Haraguchi K, Endo T and Onaya T: Transcriptional activation of the thyroglobulin promoter directing suicide gene expression by thyroid transcription factor-1 in thyroid cancer cells. Cancer Res *61*: 3640-3646, 2001.
120 Chung HK, Yi YW, Jung NC, Kim D, Suh JM, Kim H, Park KC, Kim DW, Hwang ES, Song JH, Ku BJ, Han HJ, Ro HK, Kim JM and Shong M: Gadd45gamma expression is reduced in anaplastic thyroid cancer and its reexpression results in apoptosis. J Clin Endocrinol Metab *88*: 3913-3920, 2003.
121 Hennige AM, Lammers R, Höppner W, Arlt D, Strack V, Teichmann R, Machicao F, Ullrich A, Häring HU and Kellerer M: Inhibition of *Ret* oncogene activity by the protein tyrosine phosphatase SHP1. Endocrinology *142*: 4441-4447, 2001.
122 Qiao S, Iwashita T, Furukawa T, Yamamoto M, Sobue G and Takahashi M: Differential effects of leukocyte common antigen-related protein on biochemical and biological activities of RET-MEN2A and RET-MEN2B mutant proteins. J Biol Chem *276*: 9460-9467, 2001.
123 Iuliano R, Trapasso F, Le Pera I, Schepis F, Samà I, Clodomiro A, Dumon KR, Santoro M, Chiariotti L, Viglietto G and Fusco A: An adenovirus carrying the rat protein tyrosine phosphatase eta suppresses the growth of human thyroid carcinoma cell lines *in vitro* and *in vivo*. Cancer Res *63*: 882-886, 2003.
124 Scala S, Portella G, Fedele M, Chiappetta G and Fusco A: Adenovirus-mediated suppression of HMGI(Y) protein synthesis as potential therapy of human malignant neoplasias. Proc Natl Acad Sci USA *97*: 4256-4261, 2000.
125 Hassan I, Wunderlich A, Burchert A, Hoffmann S and Zielke A: Antisense p53 oligonucleotides inhibit proliferation and induce chemosensitivity in follicular thyroid cancer cells. Anticancer Res *26*: 1171-1176, 2006.
126 Parthasarathy R, Cote GJ and Gagel RF: Hammerhead ribozyme-mediated inactivation of mutant RET in medullary thyroid carcinoma. Cancer Res *59*: 3911-3914, 1999.
127 Du ZX, Zhang HY, Gao da X, Wang HQ, Li YJ and Liu GL: Antisurvivin oligonucleotides inhibit growth and induce apoptosis in human medullary thyroid carcinoma cells. Exp Mol Med *38*: 230-240, 2006.
128 Nagayama Y, Nishihara E, Namba H, Yokoi H, Hasegawa M, Mizuguchi H, Hayakawa T, Hamada H, Yamashita S and Niwa M: Targeting the replication of adenovirus to p53-defective thyroid carcinoma with a p53-regulated Cre/loxP system. Cancer Gene Ther *8*: 36-44, 2001.
129 Portella G, Scala S, Vitagliano D, Vecchio G and Fusco A: ONYX-015, an *E1B* gene-defective adenovirus, induces cell death in human anaplastic thyroid carcinoma cell lines. J Clin Endocrinol Metab *87*: 2525-2531, 2002.
130 Crompton AM and Kirn DH: From ONYX-015 to armed vaccinia viruses: the education and evolution of oncolytic virus development. Curr Cancer Drug Targets *7*: 133-139, 2007.
131 Parato KA, Senger D, Forsyth PA and Bell JC: Recent progress in the battle between oncolytic viruses and tumours. Nat Rev Cancer *5*: 965-976, 2005.
132 Kruyt FA and Curiel DT: Toward a new generation of conditionally replicating adenoviruses: pairing tumor selectivity with maximal oncolysis. Hum Gene Ther *13*: 485-495, 2002.
133 Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff DD and Kirn DH: ONYX-015, an *E1B* gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med *3*: 639-645, 1997.
134 Abbosh PH, Li X, Li L, Gardner TA, Kao C and Nephew KP: A conditionally replicative, Wnt/beta-catenin pathway-based adenovirus therapy for anaplastic thyroid cancer. Cancer Gene Ther *14*: 399-408, 2007.
135 Reddi HV, Madde P, Reichert-Eberhardt AJ, Galanis EC, Copland JA, McIver B, Grebe SK and Eberhardt NL: ONYX-411, a conditionally replicative oncolytic adenovirus, induces cell death in anaplastic thyroid carcinoma cell lines and suppresses the growth of xenograft tumors in nude mice. Cancer Gene Ther *15*: 750-757, 2008.
136 Lowe SW, Ruley HE, Jacks T and Housman DE: p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell *74*: 957-967, 1993.

137 Teodoro JG, Shore GC and Branton PE: Adenovirus E1A proteins induce apoptosis by both p53-dependent and p53-independent mechanisms. Oncogene *11*: 467-474, 1995.

138 Zhou Z, Jia SF, Hung MC and Kleinerman ES: E1A sensitizes HER2/neu-overexpressing Ewing's sarcoma cells to topoisomerase II-targeting anticancer drugs. Cancer Res *61*: 3394-3398, 2001.

139 Kim KI, Kang JH, Chung JK, Lee YJ, Jeong JM, Lee DS and Lee MC: Doxorubicin enhances the expression of transgene under control of the CMV promoter in anaplastic thyroid carcinoma cells. J Nucl Med *48*: 1553-1561, 2007.

140 Portella G, Pacelli R, Libertini S, Cella L, Vecchio G, Salvatore M and Fusco A: ONYX-015 enhances radiation-induced death of human anaplastic thyroid carcinoma cells. J Clin Endocrinol Metab *88*: 5027-5032, 2003.

141 Libertini S, Iacuzzo I, Ferraro A, Vitale M, Bifulco M, Fusco A and Portella G: Lovastatin enhances the replication of the oncolytic adenovirus dl1520 and its antineoplastic activity against anaplastic thyroid carcinoma cells. Endocrinology *148*: 5186-5194, 2007.

142 Graaf MR, Richel DJ, van Noorden CJ and Guchelaar HJ: Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer. Cancer Treat Rev *30*: 609-641, 2004.

143 Chan KK, Oza AM and Siu LL: The statins as anticancer agents. Clin Cancer Res *9*: 10-19, 2003.

144 Wang CY, Zhong WB, Chang TC, Lai SM and Tsai YF: Lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, induces apoptosis and differentiation in human anaplastic thyroid carcinoma cells. J Clin Endocrinol Metab *88*: 3021-3026, 2003.

145 Zhong WB, Wang CY, Chang TC and Lee WS: Lovastatin induces apoptosis of anaplastic thyroid cancer cells *via* inhibition of protein geranylgeranylation and *de novo* protein synthesis. Endocrinology *144*: 3852-3859, 2003.

146 Lin SF, Gao SP, Price DL, Li S, Chou TC, Singh P, Huang YY, Fong Y and Wong RJ: Synergy of a herpes oncolytic virus and paclitaxel for anaplastic thyroid cancer. Clin Cancer Res *14*: 1519-1528, 2008.

147 Carrasco N: Iodide transport in the thyroid gland. Biochim Biophys Acta *1154*: 65-82, 1993.

148 Dai G, Levy O and Carrasco N: Cloning and characterization of the thyroid iodide transporter. Nature *379*: 458-460, 1996.

149 Smanik PA, Liu Q, Furminger TL, Ryu K, Xing S, Mazzaferri EL and Jhiang SM: Cloning of the human sodium iodide symporter. Biochem Biophys Res Commun *226*: 339-345, 1996.

150 Hertz S and Roberts A: Radioactive iodine as an indicator in thyroid physiology. V. The use of radioactive iodine in the differential diagnosis of two types of Graves' disease. J Clin Invest *21*: 31-32, 1942.

151 Thomas-Morvan C, Carayon P, Schlumberger M, Vignal A and Tubiana M: Thyrotrophin stimulation of adenylate cyclase and iodine uptake in human differentiated thyroid cancer. Acta Endocrinol (Copenh) *101*: 25-31, 1982.

152 Smanik PA, Ryu KY, Theil KS, Mazzaferri EL and Jhiang SM: Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. Endocrinology *138*: 3555-3558, 1997.

153 Arturi F, Russo D, Schlumberger M, du Villard JA, Caillou B, Vigneri P, Wicker R, Chiefari E, Suarez HG and Filetti S: Iodide symporter gene expression in human thyroid tumors. J Clin Endocrinol Metab *83*: 2493-2496, 1998.

154 Lazar V, Bidart JM, Caillou B, Mahé C, Lacroix L, Filetti S and Schlumberger M: Expression of the Na^+/I^- symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab *84*: 3228-3234, 1999.

155 Caillou B, Troalen F, Baudin E, Talbot M, Filetti S, Schlumberger M and Bidart JM: Na^+/I^- symporter distribution in human thyroid tissues: an immunohistochemical study. J Clin Endocrinol Metab *83*: 4102-4106, 1998.

156 Saito T, Endo T, Kawaguchi A, Ikeda M, Katoh R, Kawaoi A, Muramatsu A and Onaya T: Increased expression of the sodium/iodide symporter in papillary thyroid carcinomas. J Clin Invest *101*: 1296-1300, 1998.

157 Dohán O, Baloch Z, Bánrévi Z, Livolsi V and Carrasco N: Rapid communication: predominant intracellular overexpression of the Na^+/I^- symporter (NIS) in a large sampling of thyroid cancer cases. J Clin Endocrinol Metab *86*: 2697-2700, 2001.

158 Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, Greco RS, Dohán O and Carrasco N: Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. J Clin Endocrinol Metab *88*: 1880-1888, 2003.

159 Dohán O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS and Carrasco N: The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocr Rev *24*: 48-77, 2003.

160 Spitzweg C, Zhang S, Bergert ER, Castro MR, McIver B, Heufelder AE, Tindall DJ, Young CY and Morris JC: Prostate-specific antigen (PSA) promoter-driven androgen-inducible expression of sodium iodide symporter in prostate cancer cell lines. Cancer Res *59*: 2136-2141, 1999.

161 Mandell RB, Mandell LZ and Link CJ Jr: Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. Cancer Res *59*: 661-668, 1999.

162 Spitzweg C, Baker CH, Bergert ER, O'Connor MK and Morris JC: Image-guided radioiodide therapy of medullary thyroid cancer after carcinoembryonic antigen promoter-targeted sodium iodide symporter gene expression. Hum Gene Ther *18*: 916-924, 2007.

163 Boland A, Ricard M, Opolon P, Bidart JM, Yeh P, Filetti S, Schlumberger M and Perricaudet M: Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. Cancer Res *60*: 3484-3492, 2000.

164 Dingli D, Russell SJ and Morris JC 3rd: *In vivo* imaging and tumor therapy with the sodium iodide symporter. J Cell Biochem *90*: 1079-1086, 2003.

165 Shimura H, Haraguchi K, Miyazaki A, Endo T and Onaya T: Iodide uptake and experimental 131I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na^+/I^- symporter gene. Endocrinology *38*: 4493-4496, 1997.

166 Smit JW, Shröder-van der Elst JP, Karperien M, Que I, van der Pluijm G, Goslings B, Romijn JA and van der Heide D: Reestablishment of *in vitro* and *in vivo* iodide uptake by transfection of the human sodium iodide symporter (hNIS) in a hNIS defective human thyroid carcinoma cell line. Thyroid *10*: 939-943, 2000.

167 Petrich T, Knapp WH and Pötter E: Functional activity of human sodium/iodide symporter in tumor cell lines. Nuklearmedizin *42*: 15-18, 2003.
168 Lee WW, Lee B, Kim SJ, Jin J, Moon DH and Lee H: Kinetics of iodide uptake and efflux in various human thyroid cancer cells by expressing sodium iodide symporter gene *via* a recombinant adenovirus. Oncol Rep *10*: 845-849, 2003.
169 Hsieh YJ, Ke CC, Liu RS, Wang FH, Tang KT, Chi CW, Chen FD and Lee CH: Radioiodide imaging and treatment of ARO cancer xenograft in a mouse model after expression of human sodium iodide symporter. Anticancer Res *27*: 2515-2522, 2007.
170 Ward LS, Santarosa PL, Granja F, da Assumpção LV, Savoldi M and Goldman GH: Low expression of sodium iodide symporter identifies aggressive thyroid tumors. Cancer Lett *200*: 85-91, 2003.
171 Riesco-Eizaguirre G, Gutiérrez-Martínez P, García-Cabezas MA, Nistal M and Santisteban P: The oncogene *BRAF* V600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of Na^+/I^- targeting to the membrane. Endocr Relat Cancer *13*: 257-269, 2006.

Received September 20, 2009
Revised December 23, 2009
Accepted December 24, 2009