INVITED REVIEW

Innovative strategies for the treatment of thyroid cancer

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Abstract

Normally, thyroid cancer is a disease with a good prognosis, but about 30% of the tumours dedifferentiate and may finally develop into highly malignant anaplastic thyroid carcinomas with a mean survival time of less than 8 months. Due to the loss of thyroid-specific functions associated with dedifferentiation, these tumours are inaccessible to standard therapeutic procedures such as radioiodide therapy and thyroxine-mediated thyrotrophin suppression. Medullary thyroid carcinomas are also highly aggressive. Here, therapy is limited to surgery, and no alternative is left if patients do not respond to this standard procedure.

Obviously, new approaches would be desirable. Several novel approaches are currently being tested for the treatment of thyroid cancer. Many of them utilise methods of gene therapy, but follow different strategies: (1) reintroduction of the tumour suppressor p53 into a background lacking functional p53; (2) suicide gene therapy with ganciclovir and a transduced gene for herpes simplex virus thymidine kinase controlled by the thyroglobulin promoter; (3) strengthening of the antitumour immune response by expression of an adenovirus-delivered interleukin-2 (IL-2) gene; (4) induction of an immune response by DNA vaccination against the tumour marker calcitonin; (5) transduction of the thyroid sodium/iodide transporter gene to make tissues that do not accumulate iodide treatable by radioiodide therapy; (6) blocking of the expression of the oncogene *c-myc* by antisense oligonucleotides. While these approaches are still tested *in vitro* or in animal models, first results from pilot studies concerning other novel treatment modalities are available: (7) radioimmunotherapy exploits the carcinoembryonic antigen expressed on medullary thyroid carcinomas to target a radiolabelled antibody to the tumour; and (8) retinoic acid is used for a redifferentiation therapy in the case of thyroid cancer. Hopefully, one or the other of these novel strategies may probably extend after some time the current therapeutic repertoire for thyroid cancers and provide a perspective for otherwise untreatable patients.

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Introduction

Thyroid cancer, although the most frequent malignancy of the endocrine system, is a rare disease. It accounts for about 1% of all human cancers, with a higher prevalence in women (5 to 9 of 100 000) as compared with men (2 to 4 of 100 000). Usually, thyroid cancer is a well treatable disease with a good prognosis. Current therapeutic protocols include surgery to remove the thyroid gland in part or totally, followed, if necessary, by application of ^{131}I . This radioisotope is accumulated in remnant or even disseminated thyrocytes by the thyroidal sodium/iodide symporter (NIS) and destroys them by internal radiation. Another widely used therapeutic measure is the administration of thyroxine (T_4) to suppress the production of the thyrotrophic growth factor TSH via hypothalamic and hypophyseal feedback; this aims to reduce the proliferation rate of

residual thyroid tissue or metastases. In contrast, external radiation or chemotherapy, being less potent, are of minor importance (1).

These therapeutic measures are so far the only ones with proven efficiency and prognostic relevance. They are successfully applied for papillary (PTC) or follicular thyroid carcinomas (FTC), i.e. so-called well-differentiated thyroid carcinomas (DTC). However, in about 30% of the cases, dedifferentiation of the cancers is observed (2). This is accompanied by a loss of thyroid-specific functions and properties, which, eventually, makes the tumours inaccessible to conventional therapy. Loss of iodide uptake, at least in part explained by the frequently observed reduction in the expression of the NIS (reviewed in ref. 3), makes radioiodide therapy infeasible. Also, dedifferentiated tumours lose the TSH receptor (TSHr) (4) and thus get insensitive to the growth-regulating effects of varying TSH levels; this

obliterates any benefit from TSH suppression therapy by T₄. It is telling in this context that presence of the TSHr usually correlates with a good prognosis for a thyroid neoplasm (5). Finally, extensive local tumour growth and/or distant metastases often preclude any further surgical intervention. When, after progressive dedifferentiation, the stage of anaplastic thyroid cancer (ATC) is attained, the prognosis is very bad. In fact, this carcinoma is the most malignant solid tumour in the human body, leading to death within a few months after diagnosis (6–8).

Medullary thyroid carcinomas (MTC), arising from the parafollicular, calcitonin-producing C-cells, make up about 3–5% of thyroid cancers. They also represent highly aggressive tumour types occurring in both sporadic (~80%) and familial forms (~20%). The therapeutic options for MTC are limited. Radiation and chemotherapy are of uncertain benefit so that surgery remains the only effective choice. However, after an operation, many patients exhibit elevated levels of calcitonin indicating the presence of a tumour not identifiable by imaging techniques, and eventually develop fatal disease. No alternative is left for the cases that are resistant to standard therapy (1, 9, 10).

Obviously, there is a need for alternative therapeutic concepts. This review will summarise some recent publications on the development of novel strategies that after some time will probably extend the current therapeutic repertoire for thyroid cancers.

Strategies and tools for gene therapy in the case of cancer

Cancer is in essence a disease of genes (11). Genes encoding molecules crucially involved in the regulation of growth, differentiation and the maintenance of intact and functional tissues are lost, mutated or dysregulated. Both activation of growth-promoting genes, proto-oncogenes, and inactivation of growth-limiting genes, tumour suppressors, occurs. There is a hereditary component, contributing varying degrees of predisposition dependent on the type of cancer. The carcinogenic effect of retroviruses can be traced back to the fact that they integrate mutated copies of regulatory genes, which are insensitive to the normal control of gene expression, into the DNA of a host cell together with their own genome. Alternatively, by bringing their strong promoter and enhancer sequences next to a cellular gene they drive its overexpression. However, most cancer-related mutations are somatic, that means they accumulate in cells throughout the lifetime of an organism. Thereby environmental factors such as chemical carcinogens can display their influence. Altogether, it has been well established for some time, that in most instances, cancer is a multigenic disease and that more than one gene must be dysfunctional so that in the end malignant transformation and invasive growth will

result. This is referred to as the 'multiple hit theory' of cancer (12).

The above-mentioned pathogenetic mechanisms, of course, also apply to thyroid cancer. Mutations of the RET oncogene play a prominent role in the pathogenesis of MTC, both in sporadic and in familial forms. It is advised that any patient with MTC should be genetically screened along with his or her relatives, and that family members carrying RET mutations should undergo preventive thyroid removal at early age (13). As for non-medullary thyroid cancers, they seem to arise via two different genetic pathways. FTC may develop from follicular adenomas; the identity of the underlying genetic alterations has not yet been completely clarified. Mutational activation of the RAS proto-oncogene has been observed, but in animal models this same alteration instead causes PTC (14). For PTC, it is estimated that about 3.5–6.2% of the patients have one or more first degree relatives also suffering from this cancer (15). Their precursor is the occult papillary microcarcinoma, and rearrangements of the TRK and RET proto-oncogenes are involved in the pathogenesis. DTC may develop into undifferentiated, anaplastic thyroid carcinomas. A characteristic of these highly malignant diseases are mutations of the tumour suppressor p53, which is found in up to 70% of the cases (16, 17).

On this background a lot of work is done to make cancer curable at the genetic level, namely by gene therapy (reviewed in 18, 19). In fact, most of the trials evaluating the potential of gene therapy do not deal with 'classical' hereditary diseases but with cancer. Several strategies have been tested, each of them devised for a certain range of purposes. A summary is given in Table 1; those techniques that have been used for thyroid cancer are emphasised and will be discussed below.

The cardinal point of gene therapy is the introduction of DNA into target cells. To date, there are two principal strategies to achieve this. Either the cells are manipulated *ex vivo* and afterwards reintroduced into the organism; thereby, only target cells receive the altered genes. Alternatively, the foreign DNA is directly applied, mostly by injecting it into the tumour tissue. As for the methods, there are several possibilities, which are summarised in Table 2. In general, viral vectors are most efficient. This holds true above all for retroviruses. Furthermore, they integrate their genome into that of the host whereby long-term gene expression results. However, for the same reason they may disturb the target cell's gene expression in an unforeseen and unwanted way. Another major disadvantage is that they only infect dividing cells. Adenoviruses, in contrast, do not integrate, but this implies that transduced genes are only temporarily active. Also, adenoviruses do not cause serious diseases, but they do trigger an immune response. Finally, they can be used to transduce non-dividing, differentiated cells. DNA transfection by the aid of lipid complexes is less efficient than

Table 1 Strategies for gene therapy.

Strategy	Intended effect	Example
Oncogene silencing†	Shut-off of genes that support uncontrolled growth and formation of metastases	<i>c-myc</i>
Tumour suppressor replacement†	Reintroduction of a wild-type copy of a lost or mutated gene that protects against cancer	p53
Prodrug therapy†	'Suicide genes' render tumour cells sensitive against toxins applied as non-toxic prodrugs	TK/GCN
Immunotherapy†	Support of the organism's own defence mechanisms against cancer or triggering of immune responses against tumour-specific antigens or tumour markers	IL-2
Genetic immunization†	Generation of an immune response by introduction of 'naked' DNA coding for a cancer-associated antigen	CEA
Antisense therapy†	Sequence-specific oligonucleotides prevent synthesis of pathogenic proteins	<i>c-myc</i>
Radioiodide therapy†	Expression of the NIS gene confers the ability to accumulate radioiodide to non-transporting tissues	NIS
Ribozyme therapy	Sequence-specific 'RNA enzymes' cleave mRNAs coding for pathogenic proteins	
Antibody neutralisation	Introduction of intracellular (truncated) antibodies that neutralise pathogenic proteins	
Chemoprotection	Introduction of genes coding for proteins that protect normal cells from effects of high-dose chemotherapy	

† Strategies tested for the treatment of thyroid cancer.

the virus-based methods but has neither of their disadvantages. This technique may be used for *ex vivo* manipulation as the altered cells can be expanded after transduction, thus compensating for the low efficiency. Liposome-mediated DNA transfection is a widely applied method for pilot experiments *in vitro* using cell culture model systems. Finally, even 'naked' DNA in the absence of any vehicle may be used for vaccination against tumour-associated genes (20–22).

Gene therapy for thyroid cancer

Tumour suppressor replacement: p53

Much work has been done on p53 (23), a protein that acts, under normal conditions, as a tumour suppressor. It is a transcription factor which surveys the integrity of the genome. If genomic DNA is mutated, for example by ionising radiation, chemotherapeutic drugs or hypoxia, p53 arrests the cell cycle to allow for repair. If damage is too severe and DNA alterations are not likely to be

completely removed, p53 induces apoptosis to eradicate cells with genomic alterations and to prevent their propagation.

p53 is the most frequently affected protein in human neoplasms, being altered in 40–45% of the tumours. Usually, one allele is deleted and the other one is mutated and dysfunctional, which contributes to tumour progression towards increasing malignancy and reduced differentiation. This also seems to apply for thyroid cancer. p53 is intact in the healthy thyroid, in benign thyroid disease or in differentiated carcinomas, but there is a high prevalence of p53 mutations in dedifferentiated carcinomas correlating with the most aggressive histologic tumour types. Thus, as already mentioned, p53 mutations seem to be a late event in thyroid carcinomas (24, 25).

The central role of p53 for the pathogenesis of thyroid cancer has made it a potential target for gene therapy. In a couple of studies using thyroid carcinoma cell lines with p53 mutations it has been shown that reintroduction of wild-type p53 causes a more differentiated

Table 2 Vehicles for gene therapy.

Vehicle	Advantages	Disadvantages
Retroviruses	High efficiency; long-term expression of transfected genes	Insert into host cell's DNA and may disturb gene expression; do not infect non-dividing cells
Adenoviruses	Do not cause serious diseases; high capacity for uptake of foreign DNA; do not integrate into host DNA; also infect non-dividing cells	Inserted genes temporarily active only; cause immune response
Adeno-associated viruses	Long-term expression of transfected genes; do not cause serious diseases	Insert into host cell's DNA and may disturb gene expression; low capacity for uptake of foreign DNA
Liposomes	Do not cause diseases or immune response	Gene transfer less efficient than with viral vectors
'Naked' DNA	Applicable for vaccination; does not cause diseases or immune responses	Gene transfer inefficient; unstable in most tissues

phenotype of the cells. Expression of the thyroid-specific differentiation markers thyroperoxidase (TPO), thyroglobulin (Tg), and TSHr, of the thyroid-specific transcription factor Pax8, and of MHC class II antigens is stimulated. Furthermore, responsivity to the physiological stimulator TSH is restored and the tumourigenic potential is decreased. Proliferation is inhibited, both in thyrocyte and C-cell derived tumour lines; however, not apoptosis but cell-cycle arrest seems to be the cause (26–30).

According to what is known on the function of p53, its re-expression in a p53-deficient tumour might enhance the tumour's sensitivity to radiation or chemotherapy. This has been tested in two recent papers. Narimatsu *et al.* (31) stably transfected the p53-null anaplastic thyroid carcinoma cell line FRO with a temperature-sensitive mutant of the wild-type p53 gene. They did not detect a sensitising effect against the three chemotherapeutics cisplatin, 5-fluorocytosine and doxorubicin. However, they did observe a sensitisation against ionising radiation under the permissive temperature of 32 °C compared with 37 °C; cell viability was decreased by a factor of ~2 at 7.5 Gy and by a factor of ~3.5 at 10 Gy of γ -irradiation. Blagosklonny *et al.* (32) transduced the three anaplastic thyroid carcinoma cell lines BHT-101, SW-1736 and KAT-4, which express mutant p53, with an adenovirus coding for wild-type p53. This induced the p53-responsive proteins p21 and Mdm-2 as well as a cytotoxic effect. Furthermore, the cell lines became more sensitive to adriamycin with an about tenfold increase in IC₅₀ values. However, this approach has so far only been tested *in vitro*, and further studies are needed to show whether it is efficient in experimental animals or, finally, in patients as it is already done in the case of other epithelial tumours (23).

Suicide gene therapy

In this approach, gene transfer is used to introduce into the tumour a vector coding for a 'sensitising enzyme' (33). A chemotherapeutic agent is then applied as a non-toxic prodrug, which is activated only in those cells that express the sensitising enzyme. A positive concomitant is that more cells are killed than have actually been transduced, because the activated drug is secreted and acts on neighbouring cells, too. This is the so-called 'bystander effect'. The most widely used system for suicide therapy is the prodrug ganciclovir (GCN) together with herpes simplex virus type 1 thymidine kinase (HSV1-TK) (33). GCN is a nucleotide analogue, and HSV1-TK, in contrast to normal mammalian TK, preferentially phosphorylates GCN. Monophosphorylated GCN is then converted to its triphosphate by cellular kinases and interferes with cell propagation by competition with normal nucleotides during replication. Growing cells will be destroyed preferentially, which limits the side effects on normal tissue. Therefore,

this technique has above all been tested for brain cancer. Here, minimal damage of brain function is expected as healthy neurones have stopped proliferation and will not be affected. As the thyroid gland is a very slowly growing tissue, gene therapy using GCN and the TK gene might be a method of choice also in this case. Several other prodrug/sensitising enzyme systems have also been tested for thyroid cancer (34).

Nishihara *et al.* (35) transduced WRO and FRO thyroid carcinoma cells with the HSV1-TK gene under the control of the cytomegalovirus promoter using a retroviral vector. They observed a dose- and time-dependent decrease of living cells after GCN treatment accompanied by increased apoptosis. A bystander effect was also observed. Low doses of GCN, which produced only minor cytotoxicity by themselves, resulted in a sensitisation against ionising radiation. Growth inhibition, bystander effect and radiosensitisation were also demonstrated in tumours generated by xenotransplantation of TK-transduced FRO cells to nude mice.

To minimise effects on other organs, the activity of the transduced sensitising enzyme should be limited to the target tissues. This might be achieved by intratumoural injection, but such a strategy would exclude disseminated diffuse metastases from the cytotoxic effect. Braiden *et al.* (36) used a recombinant retrovirus carrying TK under the control of the Tg promoter. With this vector, they transduced the differentiated rat thyrocyte line FRTL-5, the FRTL-5-derived malignant cell line FRTC, and the human anaplastic thyroid carcinoma cell line FRO. They detected a 13 000- and a ~160-fold increase in the sensitivity to GCN in the presence and a ~4- and ~27-fold increase in the absence of TSH in FRTL-5 and FRTC cells respectively. These two cell lines also expressed Tg. However, in the case of the Tg-negative line FRO, there was no difference between parental and transduced cells. TK expression under the control of a foreign promoter is less efficient than under a strong viral promoter. Nagayama *et al.* (37), by exploiting the Cre-loxP system were able to enhance the activity of the Tg promoter. They constructed recombinant adenoviruses with the HSV1-TK gene under the control of the strong cytomegalovirus promoter, but separated by a stuffer fragment, and with the Cre recombinase gene under the control of the Tg promoter. Thereby, Cre activated HSV1-TK gene transcription only in thyroid cell lines expressing Tg (FRTL-5 and FRTC). This resulted in a five- to tenfold higher *in vitro* cytotoxic effect of TK/GCN than with a recombinant adenovirus with the HSV1-TK gene itself fused to the Tg promoter. Enhanced inhibition of tumour growth *in vivo* using FRTC cells xenotransplanted s.c. was also observed in the case of the combined Tg/Cre-loxP system. However, tumour regression was not observed. Obviously, such an approach is limited to those cancers that still maintain Tg expression, which seems to apply for a great part of thyroid carcinomas as Tg is used as a sensitive tumour marker also in advanced stages.

However, one must bear in mind that dedifferentiation is known to coincide with a decreased expression of thyroid-specific transcription factors (reviewed in ref. 38). This will then reduce the efficiency of Tg expression and of any transgene controlled by the Tg promoter.

Immunotherapy: interleukin-2

Although tumours express antigens that may trigger an antitumour immune response, they often evade the surveillance of the immune system. Expression of class I and II MHC antigens may be disturbed, so that neither presentation of processed antigens nor proper T-cell activation occurs. Also, the immune response may be suppressed by products of the tumour or immune tolerance against tumour antigens may develop. Applying immunostimulatory agents to enhance defence reactions by the host should be a promising strategy against cancer. A candidate in this context is the T-cell growth factor interleukin-2 (IL-2). It stimulates proliferation and differentiation of NK, CD4⁺ and CTL effector cells and thereby regulates both specific and non-specific immune responses. Systemic administration of IL-2 produced an antitumoural immunity in animal models. However, the doses required to achieve a therapeutical effect in patients usually cause severe side effects so that this approach is of limited benefit. Local, intratumoural application of the cytokine could provide far higher concentrations and thus circumvent this problem. Alternatively, the IL-2 gene may be introduced into the tumour, an approach that has been tested in animal models for several types of cancers. First results in patients were somewhat disappointing and recent protocols switch to the transduction of combinations of cytokine genes instead of one gene (39).

In three papers by Zhang *et al.* (40–42) the feasibility of this approach in the case of MTC was investigated. They used a replication defective adenovirus to transduce MTC cell lines with the murine IL-2 (mIL-2) gene under the control of the human cytomegalovirus promoter, which established two cell lines (murine and human MTC cells) that secreted large amounts of mIL-2. When the murine cells were injected into syngeneic BALB/c mice, a markedly impaired tumour growth was observed for the mIL-2-positive cells. In two of ten animals growth of the xenografts was detectable after 26 days whereas in the case of mock-transfected and parental mMTC cells nine of ten and ten of ten animals had developed tumours. Similar effects were obtained in immunodeficient BALB/c-SCID mice. This observation together with cytotoxicity assays indicated that both specific and unspecific antitumour activities were involved. Reinjection of the parental cells into tumour-free animals after 60 days did not produce tumours, revealing a long-term immunity against mMTC cells. If the IL-2-encoding adenovirus was injected directly into previously generated tumours, regression was observed in 69% of 'small' tumours (<30 mm³) and stabilisation

in the case of 'large' tumours (>30 mm³); again, long-term immunity was observed as well as a massive infiltration of CD8⁺ and CD4⁺ cells in IL-2 treated tumours. Using a rat model, similar results were obtained. To evaluate the safety of the procedure, adenovirus was also administered i.v. to monitor effects on other tissues. Dissemination to the liver was observed, along with mild to extensive lymphocyte infiltration to the sinusoids and triads of the liver, but this did not affect GPT and GOT enzyme levels. Two of 12 animals showed signs of cell necrosis in the spleen, but there were no specific changes in the lung and the kidneys. Altogether it was concluded from this data that the adenoviral vector used to transduce mIL-2 was safe for gene therapy.

Gene therapy using the sodium/iodide-symporter

Cloning of the rat and, shortly afterwards, the human sodium/iodide symporter (rNIS and hNIS, respectively) resulted in a substantial insight into the pathogenesis of thyroid diseases including thyroid cancer. As mentioned above, reduced expression or functionality of this molecule may contribute to the failure of radioiodide therapy due to a lack of iodide accumulation in advanced thyroid carcinomas (3). Shimura *et al.* (43) investigated whether reexpression of this transporter molecule after gene transfer leads to a reinduction of iodide transport in non-accumulating thyroid carcinoma cell lines. They stably transfected rat FRT-Tc cells with rNIS and transplanted them s.c. to Fisher rats. The resulting tumours concentrated radioiodide 27-fold up to a dose of 4 Gy per mCi ¹³¹I. However, there was no growth inhibitory effect after radioiodide treatment as the tumour volume had not changed 2 or 3 weeks post therapy.

Two other publications also focus on the NIS, albeit not in the context of thyroid cancer. They exemplify that this molecule may be useful in the future not only for treating thyroid cancer but many other cancers by radioiodide, too. NIS belongs to a large and widely expressed class of sodium-dependent solute transporters termed after its prototype, a sodium/glucose symporter, the SGLT1 family (44). As NIS shares structural and functional similarity with these molecules, it is no surprise that it should find the prerequisites required for its activity in a great variety of cell types, although its own expression is limited in range (reviewed in ref. 45). This has prompted efforts to express the NIS in cells originating from tissues other than the thyroid, which normally do not transport iodide, to make them amenable to radioiodide therapy. Mandell *et al.* (46) transfected rNIS by the use of retroviruses into human melanoma, hepatocarcinoma, colon carcinoma and ovarian adenocarcinoma cell lines. The cells then accumulated iodide, either in cell culture or in tumours that were generated by xenotransplantation into nude

mice. In an *in vitro* clonogenic assay, they showed that rNIS-transfected cells were selectively killed by radioiodide, although they did not address the question whether there is also a radioiodide-induced growth reduction in the xenografts. Spitzweg *et al.* (47) used a construct with the NIS gene under the control of the androgen-responsive promoter of the prostate specific antigen (PSA) for transient as well as stable transfection experiments. Expression of the NIS protein in transfected cells was demonstrated by Western blot. Radioiodide uptake was androgen responsive, but only in such cells that also express PSA.

It should be added that a recent report suggests that the loss of radioiodide uptake in thyroid carcinomas could be due to reduced transcription caused by an aberrant methylation of the NIS gene (48). Tumour samples did not reveal a specific methylation pattern in correlation with a deficiency in NIS mRNA. However, treatment of seven thyroid carcinoma cell lines lacking NIS mRNA with 5-azacytidine or sodium butyrate restored hNIS mRNA expression in four and iodide uptake in two of them. This was concomitant with demethylation of the NIS gene in the untranslated region of the first exon. Thus, NIS expression and, as a consequence, NIS activity may be restored by manipulating DNA methylation at least in a subset of thyroid carcinomas.

Genetic immunisation: calcitonin

Immune responses can be triggered by simple injection of naked plasmid DNA coding for an antigen (49). In MTC, the tumour marker calcitonin may represent a target for immunotherapy as it is highly specific for C-cells and tumours derived therefrom and most tumours express this molecule. Haupt *et al.* (50) developed a DNA vaccine plasmid encoding preprocalcitonin under the control of the cytomegalovirus promoter. This construct was used to coat gold particles and intradermally injected by the gene gun. An antibody response was detected in four and a significant T-cell proliferation was detected in two out of five animals. Coinjection of granulocyte macrophage colony stimulating factor enhanced the specific immune response.

Antisense therapy

The *c-myc* proto-oncogene codes for a nuclear protein that acts as a transcription factor transducing mitogenic stimuli. The gene is amplified and constitutively active in a couple of tumours (11). In thyroid cancer, overexpression of *c-myc* seems to be related to an unfavourable prognosis. In the thyroid gland, *c-myc* is involved in the growth regulation via the cAMP pathway (51). Cerutti *et al.* (52) found an increase in *c-myc* mRNA expression in seven human thyroid carcinoma cell lines as compared with normal thyroid primary cells, with the highest levels in the most

malignant cell lines ARO and FRO. They also examined 50 thyroid tumour samples and detected elevated *c-myc* mRNA levels in 4 of 24 PTC, in one of eight FTC, and in five of six ATC. Blocking *c-myc* protein expression with antisense oligonucleotides against the translation initiation region of the mRNA significantly reduced the growth rate of the thyroid carcinoma cell lines. Although these experiments were primarily done to clarify the biological role of *c-myc* in transformation of thyrocytes, they may also have relevance for gene therapy of thyroid cancer.

Other novel approaches for the treatment of thyroid cancer

Radioimmunotherapy

Carcinoembryonic antigen (CEA) is a tumour-associated antigen which occurs among a wide range of cancers, among them MTC. Mouse monoclonal anti-CEA antibodies are available that can be used in their radioiodinated form for radioimmunotherapy. Juweid *et al.* (53) initiated a Phase I/II study to determine the safety, maximum tolerated dose (MTD), and therapeutic potential of radioisotope-labelled ¹³¹I-MN-14 F(ab)₂ anti-CEA MAbs for patients with metastatic MTC. Radioantibody doses of 140, 180 and 220 Gy to the bone marrow were applied to 3, 11 and 1 patient, respectively. This resulted in a mean tumour dose of 23.7 ± 27.5 cGy/mCi (range 1.3–124 cGy/mCi; median 16.6 cGy/mCi), which was 37.7-fold higher than the whole body dose. Myelosuppression was detected as the only treatment-related dose limiting toxicity, and the maximal tolerated dose appeared to be 180 Gy to the bone marrow. In seven patients, a 55% median reduction in the tumour marker calcitonin was detected at a median time of 3 months after therapy. In one patient, a 45% reduction of the tumour burden in the neck region was observed. The disease was radiologically stable for 3–26 months in 11 of 12 assessable patients. The therapy was well tolerated and showed evidence of biochemical and radiological antitumour activity. However, after 2–6 weeks, anti-mouse antibodies had developed in 8 of the 15 patients, so that the use of humanised antibodies for future radioimmunotherapy was recommended.

Retinoic acid redifferentiation therapy

Another novel anticancer strategy – at least in the context of the thyroid gland – may exploit the growth-inhibiting and differentiation-inducing properties of retinoic acids. These are the biologically active metabolites of vitamin A, and their effects in the organisms of vertebrates range from early embryonic differentiation over limb morphogenesis to the maintenance of functional tissues in the adult (54–60). The paradigm of retinoic acid (RA) therapy is acute promyelocytic leukaemia, where up to 90% remission can be achieved (61). However,

retinoids have also been used for therapy and chemoprevention in the case of many other cancer entities (62). As for thyroid cells, several *in vitro* studies indicated that retinoids may indeed have effects. Components relevant for growth, thyroid-specific functions and metastatic potential of thyrocytes are influenced by RA in a way that can be interpreted as a partial redifferentiation. Examples are type I 5' deiodinase (63, 64), NIS mRNA and iodide uptake (65, 66), ICAM-1 (67), and CD97 (68).

These data, above all those on the NIS and on iodide uptake, make it probable that thyroid tumours may be redifferentiated at least to such a degree that established treatment protocols, especially radioiodide therapy, may be applicable again. They have prompted a multicentric clinical study to test the potential of retinoids for a redifferentiation therapy of thyroid cancer. So far, 75 patients with poorly differentiated, inoperable thyroid cancer and absent or insufficient iodide uptake have been included (69, 70). They were treated with oral doses of 1.0–1.5 mg 13-*cis*-RA per kg body weight and day. Monitoring was performed by imaging (CT, MR, PET), radiosciintigraphy (therapeutic doses of 3–10 GBq), and determination of the tumour marker Tg. Side effects were usually well tolerated; only in one case the treatment had to be stopped due to an increase in liver enzymes. Fifty-six of the patients could be evaluated. Of 54 patients, 11 (20%) showed a decrease and 18 (33%) a stable level of Tg during follow-up. Radioiodide uptake increased in 21 of 52 patients (40%), F-18-fluorodeoxyglucose uptake decreased in six and remained unchanged in 25 of 32 patients (19 and 78% respectively). In 4 (11%) of 36 patients, tumour size regressed, and in 11 (31%) no further growth was detectable. In another study comprising 12 patients, Grünwald *et al.* (71, 72) reported comparable results.

To explore more precisely whether there was an actual therapeutic benefit, out of the 75 patients a subgroup of 20 patients with well documented and comparable courses of disease was selected for a closer analysis. Here, Tg was monitored at least once before and after RA treatment under stimulating conditions (T_4 withdrawal; $TSH > 30 \mu U/ml$) and a radioiodide scan as well as data on tumour size (CT, MR) had to be available. Using these categories the patients were assigned by three independent examiners to three groups: responders, where either radioiodide uptake and at least one other parameter had improved; stable disease, where radioiodide uptake might have improved but no change of any other parameter was observed; and non-responders, where no effect on radioiodide uptake was observed and either tumour size or Tg levels were progressive. There were four responders, four patients with stable disease, and 11 non-responders; one patient could not be classified. This means that 20% of the patients showed an improvement, and another 20% at least did not experience a further progression of their disease. Follow-up was from 3 to 35 months. In

evaluating these data it should be noted that all the patients had a history of year-long progressive disease and of unresponsiveness to any other treatment modality for thyroid cancer.

Perspectives

Since gene therapy was first used in 1990 for adenosine deaminase deficiency, more than 160 clinical gene-therapy trials have been registered by the National Institutes of Health. So far, the actual success of gene therapy in terms of a benefit for the patients seems to be a little disappointing (19, 39). However, research in the field is rapidly going on. Projects focus on the development of more efficient targeting adjuvants, viral or non-viral, (19–21) and on modifying viral antigens to achieve more efficient infection or enhancing the specificity of the transduction of foreign genes by utilising tissue-specific promoters. The latter strategy has been followed also in the thyroid system (36, 37). Other perspectives come from the observation that combination therapies are more efficient than the treatment with one agent alone. In the context of thyroid cancers, this was tested for genetic immunotherapy (39), for suicide therapy (73), and also for radioimmunotherapy (74).

As for RA treatment, first results are encouraging to further investigate the potential of a redifferentiation therapy for thyroid cancer. It will have to be clarified what may be described as a therapeutic success in this context. For example, a decrease in the level of the tumour marker Tg in some patients is not an unambiguous finding. An increase might be interpreted on the one hand as resulting from further tumour growth, on the other hand as a consequence of differentiation leading to an enhanced synthesis and secretion of this thyroid-specific protein by thyrocytes not correctly organised in follicles. Tumour size would be the most valuable parameter, but is often difficult to determine, especially when diffuse metastases are involved. FDG-PET could play an important role for diagnosis in this context. Also, the right time point for RA therapy has to be determined. In *in vitro* studies, responsivity to RA decreased with progressive malignancy of thyroid carcinoma cells, as exemplified by the stimulation of type I 5' deiodinase and NIS mRNA in follicular, but not anaplastic, lines (63, 66). Moreover, in patients, a remaining, but insufficient iodide uptake can be enhanced by RA treatment to therapeutically useful levels (72). These observations suggest that patients with less advanced tumours might respond better to RA redifferentiation, which should be considered in further studies. Another point is to decide whether a reduction in tumour growth is achieved by the action of the ^{131}I applied afterwards for diagnostic or therapeutic purposes after RA therapy or the consequence of the growth-inhibiting or pro-apoptotic effects of RA itself, as different modes of RA application to optimise the desired

outcome might be required. In the latter case, the use of synthetic RA derivatives that display limited ranges of effects, for example, growth reduction by interaction with AP-1 (75) might be particularly potent. Finally, concepts of combination therapy may be considered also for thyroid carcinomas. For other cancers, a wide variety of compounds has been evaluated in combination with RA (61), above all interferon α in the case of solid tumours (76). The observation that in pilot studies a subgroup of patients definitely showed a positive response together with the well known fact that RA causes only comparatively mild and well tolerable side effects certainly warrants further investigation in this direction.

Altogether, there seem to be multiple options and a growing potential in the new approaches reviewed in this article, and it is conceivable that patients will soon have the benefit from their introduction into the therapeutic repertoire against thyroid cancer.

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