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Review Article

Thyroid cancer: are molecular studies making any difference?

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Abstract

'Translational research' encompasses all activities that apply basic scientific research findings to clinical practice. Although it has taken almost 20 years since the first scientific discoveries, the approval of new 'drugs' such as Herceptin and Avastin represents a successful example. There has also been successful translation of science into the clinic in the field of otolaryngology.

In the last decade, we have seen major developments in molecular biology and genetics. Two notable achievements have been the completion of the human genome project and the parallel advances in high-throughput molecular genomic and proteomic technologies. Linked with these events has been the enormous accumulation of new data which offers the promise of important future clinical applications.

This review aims to discuss these major scientific developments, to demonstrate successes in thyroid translational research, and to summarise more recent research findings in thyroid disease which provide hope for the development of future clinical tools.

Key words: Thyroid Cancer; Molecular Biology; Markers

Introduction

Since the elucidation of the structure of deoxyribonucleic acid (DNA) more than 50 years ago, molecular biology has progressed at a remarkable pace, attracting huge research funding from the National Institutes for Health in the USA and from corresponding funding bodies in the UK. Today, basic science research is often given greater precedence than clinical research. Molecular research in the field of thyroid cancer has also been intensive over the last few decades.

This review aims to provide an overview, for the ENT clinician, of the impact of molecular science in clinical medicine and, more specifically, in the management of thyroid cancer. An overview of the most up-to-date research is also presented, thereby setting the scene for the next decade of thyroid cancer research.

Successes in translational research

'Translational research' aims to bridge the gap between basic science research and the clinical setting – so-called 'bench-to-bedside' research. The approval of Avastin (Genetech, San Francisco, USA) and Herceptin (Genetech, San Francisco, USA) by the US Food and Drug Authority and the European Community represents a recent successful example. Avastin and Herceptin are monoclonal antibodies which bind specifically to proteins critical to cancer cell growth, and are examples of novel, targeted therapy.

Herceptin targets human epidermal growth factor receptors, known to be highly expressed in up to 25 per cent of metastatic breast cancers.[1] Its use has been shown to reduce tumour progression and to prolong survival in those patients with human epidermal growth factor R2 positive metastatic cancer.[2] Avastin targets vascular endothelial growth factor, and blocks the binding of this potent, pro-angiogenic factor to tumour vasculature, thereby inhibiting tumour blood supply and growth. When used in patients with metastatic colorectal cancers, it has been shown to inhibit tumour growth and improve survival.[3,4] My research group has recently demonstrated for the first time that thyroid cancer cells also express vascular endothelial growth factor receptor. Although further studies are necessary, Avastin may prove to be important in patients with advanced or metastatic thyroid cancer.

Bench-to-bedside timeline for Herceptin

Human epidermal growth factor receptor was discovered, sequenced and cloned in 1985.[5] Shortly after,

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Slamon *et al.* demonstrated that approximately 25 per cent of breast cancers expressed high levels of human epidermal growth factor.[1] These cancers were more aggressive and associated with a poorer prognosis. Advances in molecular biology, in particular proteomics, led to the synthesis of human anti-human epidermal growth factor antibodies for use in clinical trials. Successful transition through phase II and III trials resulted in its approval for clinical use in 2000.[1,6]

If we trace the development of Herceptin from the discovery of human epidermal growth factor receptor through to its approval for use in metastatic breast cancer, we become aware of several key aspects of basic science and translational research which are important for us to grasp, in order to fully understand the role of basic science research in clinical medicine (see Figure 1). Firstly, it is evident that, from the time of the earliest scientific studies, 15 years of continued and focussed research were necessary in order to produce eventual clinical benefit. Secondly, advances in biological knowledge have provided further understanding of human cancers, without which successful translation into clinical medicine would have been unlikely. Thirdly, parallel advances in molecular biotechnology have been critical, both for the progression in biological knowledge and for the subsequent translation into the clinical setting.

Bench-to-bedside successes in thyroid cancer research

Examples of successful translation of scientific research into the clinical setting, which are more directly relevant to thyroid cancer, include the development of *Ret* genetic testing in patients with medullary cancer for Multiple endocrine neoplasia type 2 (MEN2) syndrome, and the approval of human recombinant thyroid-stimulating hormone (TSH) for use as an adjunctive tool in the follow up of thyroid cancer patients for recurrence.

MEN2 syndrome comprises medullary thyroid carcinoma, pheochromocytoma and parathyroid tumours. Up to 95 per cent of cases are caused directly by mutation in the *Ret* gene.[7] This provides a unique model of cancer prevention by genetic testing developed through molecular studies.

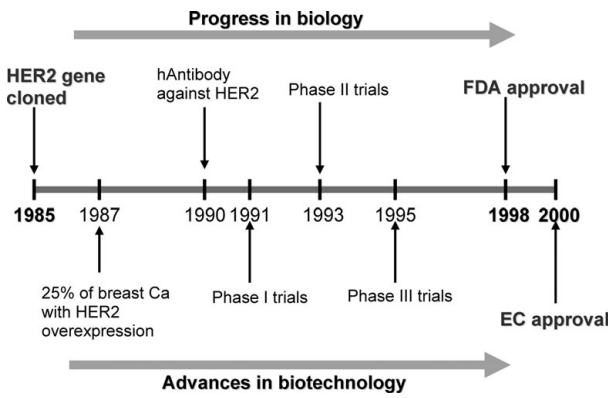

FIG. 1

Timeline outlining development of Herceptin (HER2). FDA = Food and Drug Authority; Ca = cancer; EC = European Community

In 1987, initial studies suggested the MEN2 syndrome to be caused by genetic defects located, rather crudely, in the region of chromosome 10q11.2.[8] Developments in gene-mapping and sequencing technology enabled more precise localisation of the responsible gene and, eventually, the identification of the *Ret* gene as the cause of MEN2.[9] Having successfully sequenced the *Ret* gene, it was possible to identify and describe the various sequence mutations evident in patients with MEN2. Mulligan *et al.* first described a missense mutation in the *Ret* gene as being directly causative of MEN2.[10] Subsequently, further mutations were described. In 1997, a MEN2 international workshop consensus recommended that *Ret* mutational analysis was more accurate than standard calcitonin assay and that it should form the basis for decisions regarding prophylactic thyroidectomy.[11]

Progressive research in this area demonstrated that different mutations were associated with varying degrees of aggressiveness of MEN2.[12] In 2001, a consensus guideline for MEN2 was drafted which recommended that *Ret* mutational analysis was accurate enough to enable stratification of clinical risk and to inform management decisions, based upon the precise mutation detected (see Appendix 1).[7] *Ret* genetic testing is now recommended for all new cases of medullary thyroid cancer, as the test is now relatively simple and cheap, and the identification of the index case of MEN2 has significant implications for the patient and their family.[7]

Human recombinant TSH (Thyrogen; Genetech, San Francisco, USA), is a more recent example of how molecular research provides clinically useful diagnostic tools. After characterisation of the α and β subunits of TSH in 1983,[13] breakthroughs in sequencing and cloning technologies enabled subsequent characterisation of the respective genes a few years later.[14] Modern genetic engineering was employed to enable *in vitro* synthesis of human recombinant TSH.[15] Successful clinical trials using human recombinant TSH resulted in its full approval, in 1998, for clinical use in the follow up of thyroid cancer patients.[16] Modified α and β genes are stably co-transfected into surrogate Chinese hamster ovary cells to enable industrial-scale synthesis and commercial use.[15]

Tracing the development of *Ret* genetic testing and of Thyrogen illustrates, once again, the importance of continued progress in biological knowledge and biotechnological advances in the translation, over a period spanning two decades, of early molecular studies into the clinical setting.

Challenges in thyroid cancer management

Although the treatment of well differentiated thyroid cancers (with surgery and radioiodine therapy) offers a favourable prognosis, there still remain important challenges to overcome.

Thyroid nodules are very common, being palpable in approximately 5–10 per cent of the population.[17] Differentiation between benign and malignant nodules represents an important clinical diagnostic

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challenge. Fineneedle aspiration cytology (FNAC) had been widely established as a reliable and effective procedure in the clinical management of thyroid nodules.[18] Despite the high sensitivity and specificity of FNAC, significant numbers of cases (10–25 per cent) are indeterminate, with FNAC failing to differentiate between benign follicular lesions and follicular thyroid cancers.[19,20] These cases require surgery, in the form of hemi-thyroidectomy, in order to achieve a diagnosis. Only 20–25 per cent of these nodules are ultimately found to be malignant; therefore, 75–80 per cent of patients undergo an unnecessary operation.[21,22]

Despite advances in treatment, recurrence still occurs in approximately 20 per cent of cases of thyroid cancer, most commonly as local nodal metastases and less often as distant haematogenous metastases, leading to substantial morbidity and premature mortality.[23] Although several clinical scoring methods (such as AGES, AMES and EORTC) have been described to identify those at greatest risk, there exists no reliable molecular or histological prognostic indicators. The advent of reliable molecular markers providing diagnostic and prognostic information from FNAC samples would therefore have enormous clinical and economic impact.

In contrast to well differentiated thyroid cancers, poorly differentiated and advanced thyroid cancers have a very poor prognosis. Indeed, anaplastic thyroid cancer represents the most aggressive human cancer, with most patients dying within one year of diagnosis.[24] These cancers are known to be resistant to radiotherapy and modern chemotherapy regimens.[25] More effective, novel treatment modalities are required. A better fundamental understanding of thyroid tumour initiation and progression is necessary in order to develop effective and targeted treatments for these aggressive tumours.

In an attempt to address these issues, molecular scientists have sought to identify discriminatory molecular markers which would provide valuable diagnostic and prognostic information. Others have investigated whether global gene expression patterns in thyroid cancers can provide insight into these cancers' fundamental biology and can therefore assist in their accurate classification and the prediction of their future behaviour. Research into several novel methods of targeting and destroying thyroid cancer cells is ongoing. Early findings are promising, and offer the hope of future, alternative treatments for poorly differentiated thyroid cancers. These areas of research will be briefly discussed.

Molecular markers in thyroid cancer

Numerous genes have been proposed as potential discriminatory markers in thyroid cancer (see Table I). Some have been more extensively studied than others. The present review will limit its discussion to those genes holding the greatest promise, and to the more recently identified genes.

Galectin 3

Galectins, which are b-galactosil-binding proteins involved in regulating cell–matrix interactions, have been implicated in the initiation and regulation of cell growth and malignant transformation.[26,27] The expression of one subtype, galectin 3, appears to be necessary for the maintenance of transformed thyroid papillary cancer cell lines *in vitro*.[28] Numerous studies have evaluated galectin 3 as a marker of thyroid malignancy in immunohistochemistry, and have reported high sensitivity and specificity in differentiating malignant from benign thyroid disease, for both FNAC and conventional histology.[29–33] Two studies showed 100 per cent expression of galectin 3 in malignant thyroid tumours, and no expression in benign lesions and normal thyroid tissues.[26,27] Furthermore, another study specifically examined the accuracy of galectin 3 staining in distinguishing follicular carcinoma from adenomas, based upon FNAC, and reported that galectin 3 accurately predicted final pathology in 100 per cent of cases.[34] Most recently, Bartolazzi *et al.* used a commercially available antibody against galectin 3, in a multicentre study involving 1099 surgical thyroid specimens and 266 FNAC specimens from patients who subsequently underwent surgery. These authors reported galectin 3 expression in only 3 per cent of benign adenomas, but found galectin 3 positivity in 97 per cent of papillary cancers, 92 per cent of minimally invasive follicular cancers and 100 per cent of widely invasive follicular cancers.[32] Furthermore, of the 73 indeterminate FNACs, all malignant nodules were galectin 3 positive but only 5 per cent of the benign nodules expressed galectin 3.

Initially, normal thyroid tissue and benign nodules were considered not to express galectin 3.[33] However, galectin 3 expression has also been demonstrated in Hashimoto's thyroiditis, using immunohistochemistry, and ubiquitous expression at the

TABLE I

MOLECULAR MARKERS IN THYROID CANCER

Thyroid-specific	Tumour-specific
Thyroglobulin	Telomerase
Thyroid peroxidase	Galectin-3
Na-I symporter	Oncofetal protein
TSH receptor	Cytokeratin-19
Ret/PTC rearrangements	Ki-67
	HBME-1
	p53 mutations
	Ras mutations
	Mucin-1
	Bcl-2
	C-erbB
	PCNA
	VEGF
	C-myc
	B-Raf mutations
	Pax8/PPARλ
	PTTG
	PBF

TSH = thyroid-stimulating hormone; PTC = papillary thyroid cancer; HBME = name of gene/protein for mesothelial cell surface protein HBME1; Bcl = B-cell leukaemia2; C-erbB = epidermal growth; PCNA = proliferating cell nuclear antigen; VEGF = vascular endothelial growth factor; Pax8/PPARλ = peroxisome proliferator-activated receptor-gamma; PTTG = pituitary tumor transforming gene; PBF = PTTG binding protein

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messenger ribonucleic acid (mRNA) level has been seen in benign and malignant thyroid lesions, using reverse transcription polymerase chain reaction.[32,35] Nevertheless, if Hashimoto's thyroiditis is excluded, it still appears that galectin 3 expression (detected via immunohistochemistry) is a potentially valuable marker of thyroid malignancy. Galectin 3 may thus be one of the most promising markers for distinguishing adenomas from carcinomas. However, further studies are needed to evaluate fully whether galectin 3 might be a clinically useful marker, in addition to currently available diagnostic tools for pre-operative identification of malignant thyroid tumours.

Mucin 1

Mucin 1 is a type one transmembrane protein which is involved in cell–cell interactions, signalling and metastasis. Mucin 1 has been shown to be oncogenic, possibly by interfering with critical cell–cell and cell–matrix interactions and thereby promoting cellular dissociation and progression.[36] Mucin 1 has been reported to be upregulated in human malignancies, including aggressive B-cell lymphomas and breast and thyroid cancers.[37–39] Upregulation of mucin 1 has been correlated with increased metastatic potential and poor prognosis of these tumours.[40,41]

A very recent study performed a genome-wide profiling of papillary thyroid cancers and identified mucin 1 as a potential independent prognostic marker. Wreesmann *et al.* reported upregulation of mucin 1 gene copy number and mRNA expression in 100 cases of papillary thyroid cancer.[42] Half the cohort was made up of indolent papillary thyroid tumours and the remaining half of the more aggressive, tall cell variant. Using immunohistochemistry, Wreesmann *et al.* also showed, in keeping with cDNA microarray data, that mucin 1 upregulation was present in 98 per cent of tall cell variants, compared with only 35 per cent of conventional indolent papillary thyroid tumours. Furthermore, mucin 1 overexpression was significantly correlated with decreased relapse-free survival, using univariate analysis.[42] Multivariate analysis showed that this association was independent of histology and established clinical predictors.

The more aggressive tall cell variant, which accounts for approximately 10–15 per cent of papillary thyroid cancer, is often misdiagnosed on routine histopathological analysis.[43] Mucin 1 has been shown to be upregulated in virtually all tall cell variant cells, and this may be used as a adjunct to aid diagnostic classification. Given that mucin 1 is a potential independent prognostic indicator in papillary thyroid cancer, further definitive studies are warranted.

B-Raf proto-oncogene mutation

B-Raf is a serine/threonine kinase and is a member of the mitogen-activated protein kinase pathway, which is involved in the transduction of mitogenic signals from the cell membrane to the nucleus within. B-Raf gene mutations yield elevated kinase activity and are transforming in NIH3T3 cells; they have been shown to be common in human cancers.[44] Several studies have recently identified the most common B-Raf mutation, T1796A transverse mutation, in 29–69 per cent of papillary thyroid cancers.[45–49] Remarkably, to date, this mutation has consistently been reported to be 100 per cent specific for papillary thyroid cancer, with no benign thyroid neoplasms having been found to harbour B-Raf mutations. Consequently, B-Raf mutation has been proposed as a specific molecular marker with relatively good sensitivity for the diagnosis of papillary thyroid cancer. Moreover, B-Raf mutation has been demonstrated to be a novel prognostic biomarker which predicts poor clinicopathological outcomes.[48,49] Namba *et al.* reported a significant association of B-Raf mutation with distant metastases and advanced pathological stages of papillary thyroid cancer.[48] Nikiforova *et al.* reported a significant association of B-Raf mutations with extrathyroidal invasion and advanced pathological stages of papillary thyroid cancer.[49] Xing *et al.* demonstrated, using a novel colorimetric mutation detection method, that B-Raf mutations were readily detectable in thyroid FNAC aspirates. In a series of 48 patients undergoing thyroidectomy for cancer or suspected malignancy, these authors showed that 50 per cent of the nodules that proved to be papillary thyroid cancer on final histology were correctly diagnosed by B-Raf mutation analysis on FNAC samples performed pre-operatively; there were no false positives.[50] They also reported a statistically significant association of B-Raf mutation with neck lymph node metastases and with higher incidence of recurrence. Using multivariate analysis, the presence of B-Raf mutation was shown to be an independent prognostic factor for poor survival in cases of papillary thyroid cancer.

Overall, detection of B-Raf mutation in thyroid FNAC samples could be a useful diagnostic, adjunctive technique in the evaluation of thyroid nodules with indeterminate cytological findings, although this requires further definition by larger studies. Furthermore, detection of B-Raf mutation positivity may help identify those patients who are likely to have a poorer prognosis, allowing appropriate referral and planning for more extensive treatment.

Human genome project

In 2003, after a 13-year period of research, scientists in the human genome project obtained the full DNA sequence of the 3 000 000 000 base-pairs making up approximately 25 000 genes of the human genome (see http://www. Ornl.gov/sci/techresources/Human_Genome/home.shtml). One of the primary aims of the human genome project was to provide an optimal foundation upon which to plan future genetic and molecular studies. Stimulated by the daunting task, there were parallel and intense developments in biotechnology, in order to facilitate not only the massive sequencing challenge but also to enable meaningful handling of the huge data set anticipated. Consequently, one of the major developments in the last 20 years in molecular science has

been the 'omic' technologies. 'Ome' means 'all' in Greek, and these technologies analyse the whole cell, as opposed to the individual genes or proteins.

Genomics, the study of the whole cell genome, was the first in the omics era, and developing in the 1990s with the initiation of the human genome project. Using laser and computing technology, the whole genome is placed onto so-called 'microarrays' or 'gene chips', which are no larger than postage stamps. Microarrays allow the researcher to assay all the genes in the human genome simultaneously and to achieve a global profiling of the gene expression pattern.

Shortly after this development, advances in robotics (allowing printing of protein molecules onto solid medium, and the arrival of 'protein chips') and mass spectrometry led to the arrival of modern proteomics. Laser desorption of protein molecules and their analysis by mass spectrometry have allowed profiling of all proteins simultaneously within a given tissue.

The most recent addition to omic technology has been metabolomics, which aims to study the small molecule metabolic profile. The metabolome represents the collection of all metabolites in a biological organism, which are the end-products of its gene expression.

A testament to the importance of the omics technologies is the fact that, in the last five years, more than 15 per cent of all publications relating to human cancer research have employed one or more of these analytical modalities. It is believed that these techniques will form a core analytical tool in the majority of cancer research projects in the years to come.

Gene expression signatures in thyroid cancer

In thyroid cancer, it is probable that one molecular marker will be insufficient, and that a panel of markers together may be more likely to provide clinically useful information. To investigate which groups of genes may discriminate cancer and its biological subtypes, many researchers have employed microarray technology. Several studies have proposed gene sets that have been shown to reliably differentiate cancers from benign neoplasms and to identify malignant tumours associated with future recurrence.

Stolf *et al.* compared global gene expression profiles in thyroid follicular adenomas and carcinomas, and described 14 trio-gene sets that correctly classified 100 per cent of their tumour cohort samples.[51] Similarly, Weber *et al.* identified three genes, cyclin D2, protein convertase two and prostate differentiation factor, which allowed accurate molecular classification of follicular adenomas and carcinomas.[52] Using two independent validation cohorts, they demonstrated that the trio-gene set differentiated between follicular adenoma and carcinoma with 100 per cent sensitivity and 94.7 per cent specificity.[52] Puskas *et al.* studied the gene profiles of five types of benign and malignant thyroid nodular tissue (including multinodular goitre, follicular adenoma and carcinoma, and papillary carcinomas), and identified 195 genes the differential expression of which successfully clustered into the clinically relevant groups.[53] Several other groups have reported similarly promising data to support the premise that a molecular classification system for thyroid tumours is feasible and to be expected in the near future.[42,54–56]

Microarrays have also been employed to investigate whether gene expression profile in the primary tumour may predict future biological behaviour. In an attempt to explore the molecular differences between primary human tumours and their metastases, Ramaswamy *et al.* compared the gene-expression profiles of adenocarcinoma metastases of multiple tumour types with unmatched primary adenocarcinomas. A gene expression signature comprising 17 genes distinguishing primary from metastatic adenocarcinomas was described, and those cancers carrying the gene-expression signature were more likely to be associated with metastasis and poor clinical outcome.[57]

However, there appear to be very few genes which are common to these separate studies. Differences in population characteristics, tumour types, selection and microarray methodologies may account for the lack of congruity between these studies. More extensive and well controlled experiments are therefore needed to identify the genesets that will prove to be clinically useful, diagnostically and prognostically.

Novel treatment for poorly differentiated thyroid cancer

Although treatment for early, well differentiated thyroid cancer is very successful and provides a good overall prognosis, more advanced and poorly differentiated tumours fare badly. Up to 30 per cent of well differentiated thyroid cancers dedifferentiate into more aggressive tumours,[58] and these tumours have been shown to respond poorly to currently available chemo-radiotherapy regimens.[25] Besides surgery and radioiodine therapy, there is little alternative when these regimens fail or are unsuitable. To address this challenge, molecular research groups have been investigating several novel methods of cancer treatment.

Gene therapy in thyroid cancer

Gene therapy aims to introduce an exogenous, custom-designed gene which allows the manipulation of the target cells, usually the tumour cells, in order to allow improved cancer cell destruction and/or localisation. In thyroid cancer, several different methods have been investigated to date. Many of these approaches have so far only been tested *in vitro*, and further studies are needed to determine whether they are efficient and effective in animals and finally in patients with thyroid cancers, as has been done in other cancers.

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Corrective gene therapy

Cytoreductive therapy, as the term suggest, aims to restore the normal function of a deleted or mutated gene (usually a tumour suppressor gene). The p53 gene, often referred to as the 'guardian of the genome', plays a critical role as a tumour suppressor gene. This gene either arrests the cell cycle to allow repair of the DNA damage (often caused by carcinogenic factors such as irradiation and chemotherapeutic drugs) or induces apoptosis (programmed cell death) if the DNA damage is deemed too severe for repair. In this way, p53 plays a major role in eradicating cells with mutated genes which could progress into a cancerous phenotype if allowed to propagate further. P53 is the most frequently affected gene in human cancers, being altered in approximately 40–45 per cent of all tumours. There is a high prevalence of p53 mutations in poorly differentiated thyroid carcinomas, correlating with the most aggressive histological tumour types.[59] Mutations in the p53 gene appear to be a late genetic event in thyroid carcinogenesis, being associated with dedifferentiation and being responsible, in part, for the aggressive behaviour of these tumours.[59,60]

In studies using thyroid cells with mutated p53, reintroduction and restoration of wild-type p53 function caused a more differentiated cell phenotype.[61,62] This was associated with the re-emergence of thyroid-specific differentiation markers, including thyroperoxidases, thyroglobulin and sodium-iodide symporter. The increased expression of the latter re-established efficient iodide uptake in the treated cells, which were then amenable to radioiodine treatment. Restitution of p53 function also appeared to enhance tumour sensitivity to radiation and chemotherapy.[63,64]

Immunomodulatory gene therapy

This method introduces a gene that induces or enhances the normal host immune response against cancer cells. Although tumour cells express antigens that may trigger an antitumour immune response, they often evade the immune system. The T-cell growth factor interleukin two (IL-2) stimulates proliferation and differentiation of natural killer and cluster of differentiation four glycoprotein effector cells, and thereby upregulates both specific and non-specific immune responses.[65] Zhang *et al.* published several papers demonstrating the feasibility of this approach in thyroid cancer.[66,67] They demonstrated, using a BALB/c mouse model, that the growth of tumours induced to secrete large amounts of IL-2 was significantly inhibited. Further observations indicated that both specific and non-specific immune mechanisms were involved.[67] Furthermore, when the IL-2 gene transduced tumours cells were re-injected into tumour-free mice, no growth was seen after 60 days, suggesting that long-term immunity had been established against these tumour cells.[66]

Cytoreductive gene therapy

This strategy delivers an exogenous gene which either causes cell death itself or allows the application of selective cytotoxic agents. The most common method employed is to introduce into the tumour cells a gene coding for a 'sensitising enzyme'. A chemotherapeutic agent is then applied as a non-toxic prodrug, which is activated only in those tumour cells that express the enzyme. The most widely used system for such 'suicide' therapy is the prodrug ganciclovir together with herpes simplex virus (type one) thymidine kinase.[68] Ganciclovir is a nucleotide analogue, and herpes simplex virus type one thymidine kinase (in contrast with normal human thymidine kinase) preferentially phosphorylates and activates ganciclovir. Phosphorylated ganciclovir competes with normal nucleotides during replication and therefore inhibits cell propagation. Nishihara *et al.* transduced two thyroid cancer cell types with herpes simplex virus type one thymidine kinase, and then observed dose- and time-dependent death of cancer cells after ganciclovir treatment.[69]

Gene silencing

This technique exploits the development of antisense or silencing RNA segments, which are able to specifically target a complementary gene and cause abrogation of its expression. Often, these target genes are oncogenes that are critical to tumour initiation and progression.

The c-myc proto-oncogene codes for a nuclear protein that acts as a transcription factor stimulating cell proliferation.[70] In several human tumours, including thyroid cancers, the gene is amplified and constitutively activated by a mutation.[71] In thyroid cancer, c-myc overexpression is highest in the most malignant cell types.[72] Cerutti *et al.* demonstrated that c-myc expression was raised in cancers and that blocking its expression with antisense oligonucleotides significantly reduced the growth rate of the thyroid cancer cells.[72]

Redifferentiation therapy

De-differentiation of thyroid cancers is observed in up to 30 per cent of cases.[58] As noted above, this is accompanied by the loss of thyroid-specific functions and properties, which eventually makes the tumours inaccessible to conventional therapy.[24,25] Most notably, loss of iodide uptake due to reduction in expression of sodium iodide symporter makes radioiodide therapy infeasible.[73] Also, dedifferentiated tumours lose their TSH receptors and thus become insensitive to the growth-regulating effects of varying TSH levels; this obliterates any benefit from TSH suppression therapy by thyroxine (T4).[74] Redifferentiation therapy aims to reverse these deleterious changes, making these cancers more amenable to radioiodine and T4 treatment.

Retinoid analogues

Retinoic acids are the biologically active metabolites of vitamin A. They have been shown to inhibit tissue growth and to possess differentiating properties.[75] Retinoids have been used for therapy and

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chemoprevention in many different human cancers.[76] The most dramatic effects of retinoids are seen in acute, promyelocytic leukaemia, in which up to 90 per cent remission can be achieved.[77] As for thyroid cells, several *in vitro* studies have demonstrated the inhibition of growth and metastatic potential of thyroid cancer cells, and stimulation of thyroid-specific functions, including NIS expression and iodide uptake.[78–80]

These studies indicated that poorly differentiated thyroid cancers may redifferentiate sufficiently to become amenable to radioiodide therapy again. This finding prompted multicentre clinical studies aiming to assess the potential of retinoids for redifferentiation therapy in thyroid cancer. One study investigated a total of 75 patients with poorly differentiated, inoperable thyroid cancers with absent or minimal iodide uptake.[81] Radioiodide uptake was increased in 40 per cent of the patients. In 11 per cent of patients, tumour size regressed, and in a further 31 per cent no further growth was detectable. Another study demonstrated comparable positive effects of retinoids in thyroid cancer.[82]

Deoxyribonucleic acid methyltransferase inhibitors

Gene methylation is a common mechanism employed by the cell to control the expression of various genes, effectively 'turning off' those genes until required. This process can also be exploited during tumourigenesis in order to switch off critical tumour suppressor genes and genes associated with end-differentiation.[83] Indeed, a high degree of gene methylation has been documented in thyroid cancers,[84] and methylation has been shown to be responsible for silencing the gene for NIS, causing loss of iodide uptake.[85] Using demethylation agents such as five-azacytidine and sodium butyrate *in vitro*, thyroid cancer cells lacking the ability to uptake iodide have been stimulated to reacquire this function through stimulation of NIS expression.[85] Furthermore, sodium butyrate treatment of thyroid cancers has been shown to inhibit tumour growth in animal models.[86]

Histone deacetylation inhibitors

Histone deacetylation is another common mechanism used by the cell to regulate gene expression. Histones are nuclear proteins intimately associated with DNA, and their acetylation is required for efficient transcription of genes necessary for differentiation function.[83] Inhibitors of histone deacetylation, such as depsipeptide, have been shown to increase NIS expression and iodide uptake in poorly differentiated and undifferentiated thyroid cells.[87] Phase II human trials are currently underway for depsipeptide.

Summary and concluding remarks

This review has discussed the impact molecular research has had on clinical medicine in general and on thyroid cancer management in particular, by providing examples of successes in bench-to-bedside translation of molecular science. By tracing the time-line in the development of each of these successful examples, it is evident that there are two underlying and critical themes, without which translational research is infeasible, namely, progress in fundamental biological knowledge and parallel developments in biotechnology.

The completion of the human genome project marks one of the major achievements in the last 50 years, and represents an invaluable scientific foundation upon which to base future genomic research in thyroid cancer. The omics biotechnological revolution, triggered by the human genome project, is another major breakthrough in molecular research and provides powerful tools with which to achieve a better understanding of thyroid cancer.

Despite excellent treatment available for early, well differentiated thyroid cancers, other treatment modalities are urgently required to manage poorly differentiated and metastatic cancers, which are still associated with a very poor prognosis. There are presently many new avenues being explored, including redifferentiation therapy and gene therapy. Although it is early days, studies investigating many of these novel treatment methods have provided exciting and promising data. Other, equally challenging issues exist today in the management of thyroid cancer. To overcome these issues, many groups are investigating potential molecular markers that may provide the critical diagnostic and prognostic information needed for more optimal treatment of thyroid cancer.

In conclusion, molecular studies have made a significant impact in clinical medicine over the last few decades, not only in terms of the end-product in the bench-to-bedside process (such as novel cancer treatments), but also by advancing our fundamental knowledge of thyroid cancer and by enabling the development of exciting biotechnology hardware critical to future research activities and to the translation of scientific principles into the clinical setting. Presently, numerous research groups are investigating potential tumour markers and evaluating novel treatments in thyroid cancer.

References

1 Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong DS, Keith D *et al*. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;**244**:707–12
2 Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L *et al*. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999;**17**:2639–48
3 Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny W, Lieberman G *et al*. Phase II, randomised trial comparing bevacizumab plus florouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003;**21**:60–5
4 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W *et al*. Bevacizumab plus irinotecan, florouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42
5 Coussens L, Yang-Feng TL, Liao Y-C, Chen E, Gray A, McGrath J *et al*. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with NEU oncogene. *Science* 1985;**230**:1132–9

6 Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D *et al*. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 1998;**16**:2659–71
7 Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C *et al*. Guideline for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab* 2001;**86**:5658–71
8 Mathew CG, Chin KS, Easton DF, Thorpe K, Carter C, Liou GI *et al*. A linked genetic marker for multiple endocrine neoplasia type 2A on chromosome 10. *Nature* 1987; **328**:527–8
9 Ishizaka Y, Itoh F, Tahira T, Ikeda I, Sugimura T, Tucker J *et al*. Human ret proto-oncogene mapped to chromosome 10q11.2. *Oncogene* 1989;**4**:1519–21
10 Mulligan LM, Eng C, Healey CS, Calyton D, Kwok JBJ, Gardner E *et al*. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN2A and FMTC. *Nat Genet* 1994;**6**:70–4
11 Lips CJM. Clinical management of the multiple endocrine neoplasia syndromes: results of a computerized opinion poll at the Sixth International Workshop on Multiple Endocrine Neoplasia and von-Hippel-Lindau disease. *J Intern Med* 1998;**243**:589–94
12 Frank-Raue K, Hoppner W, Frilling A, Kotzerke J, Dralle H, Hasse R *et al*. Mutations of the ret proto-oncogene in German multiple endocrine neoplasia families: relation between genotype and phenotype. German Medullary Thyroid Carcinoma Study Group. *J Clin Endocrinol Metab* 1996;**81**:1780–3
13 Naylor SL, Chin WW, Goodman HM, Lalley PA, Grzeschik K-H, Sakaguchi AY. Chromosome assignment of the genes encoding the alpha and beta subunits of the glycoprotein hormones in man and mouse. *Somat Cell Genet* 1983;**9**:757–70
14 Wondisford FE, Usala SJ, DeCherney GS, Castren M, Radovick S, Gyves PW *et al*. Cloning of the human thyrotropin beta-subunit gene and transient expression of biologically active human thyrotropin after gene transfection. *Mol Endocrinol* 1988;**2**:32–9
15 Szkudlinski MW, Thotakura NR, Bucci I, Joshi LR, Tsai A, East-Palmer J *et al*. Purification and characterization of recombinant human thyrotropin (TSH) isoforms produced by Chinese hamster ovary cells: the role of sialylation and sulfation in TSH bioactivity. *Endocrinology* 1993;**133**: 1490–503
16 Mazzaferri EL, Kloos RT. Using recombinant human TSH in the management of well-differentiated thyroid cancer: current strategies and future directions. *Thyroid* 2000;**10**: 767–78
17 Mazzaferri EL. Management of a solitary thyroid nodule. *N Eng J Med* 1993;**328**:553–9
18 Gharib H. Fine-needle aspiration biopsy of thyroid nodules. Advantages, limitations, and effect. *Mayo Clin Proc* 1994;**69**:44–9
19 Gharib H, Goellner JR, Zinsmeister AR, Grant CS, Van Heerden JA. Fine-needle aspiration biopsy of the thyroid. The problem of suspicious cytologic findings. *Ann Intern Med* 1982;**101**:25–8
20 Bloch MA, Dalley GE, Robb JA. Thyroid nodules indeterminate by fine needle biopsy. *Am J Surg* 1983;**146**: 72–8
21 Yang GC, Liebeskind D, Messina AV. Should cytopathologists stop reporting follicular neoplasms on fine-needle aspiration of the thyroid? *Cancer* 2003;**99**:69–74
22 Vini L, Harmer C. Management of thyroid cancer. *Lancet Oncol* 2002;**3**:407–14
23 Loh KC, Greenspan FS, Gee L, Miller TR, Yeo PPB. Pathological tumor-node-metastases (pTNM) staging of papillary and follicular thyroid carcinomas: a retrospective anaysis of 700 patients. *J Clin Endocrinol Metab* 1997;**82**: 3553–62
24 Are C, Shaha AR. Anaplastic thyroid carcinoma: biology, pathogenesis, prognostic factors, and treatment approaches. *Ann Surg Oncol* 2006;**13**:453–64
25 Ain K. Management of undifferentiated thyroid cancer. *Baillieres Best Pract Res Clin Endocrinol Metab* 2000;**14**: 615–29
26 Fernandez PL, Merino MJ, Gomez M, Campo E, Medina T, Castronovo V *et al*. Galectin-3 and laminin expression in neoplastic and non-neoplastic thyroid tissue. *J Pathol* 1997;**181**:80–6
27 Xu XC, el-Naggar AK, Lotan R. Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications. *Am J Pathol* 1995;**147**:815–22
28 Yoshii T, Inohara H, Takenaka Y, Honjo Y, Akahani S, Nomura T *et al*. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. *Int J Oncol* 2001;**18**:787–92
29 Orlandi F, Saggiorato E, Pivano G, Puligheddu B, Termine A, Cappia S *et al*. Galectin-3 is a presurgical marker of human thyroid carcinoma. *Cancer Res* 1998;**58**:3015–20
30 Inohara H, Honjo Y, Yoshii T, Akahani S, Yoshida J, Hattori K *et al*. Expression of galectin-3 in fine-needle aspirates as a diagnostic marker differentiating benign from malignant thyroid neoplasms. *Cancer* 1999;**85**:2475–84
31 Lloyd RV. Distinguishing benign from malignant thyroid lesions: galectin 3 as the latest candidate. *Endocr Pathol* 2001;**12**:255–7
32 Bartolazzi A, Gasbarri A, Papotti M, Bussolati G, Lucante T, Khan A *et al*. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. *Lancet* 2001;**357**:1644–50
33 Saggiorato E, Cappia S, De Giuli P, Mussa A, Pancani G, Caraci P *et al*. Galectin-3 as a presurgical immunocytodiagnostic marker of minimally invasive follicular thyroid carcinoma. *J Clin Endocrinol Metab* 2001;**86**:5152–8
34 Gasbarri A, Martegani MP, Del Prete F, Lucante T, Natali PG, Bartolazzi A. Galectin-3 and CD44v6 isoforms in the preoperative evaluation of thyroid nodules. *J Clin Oncol* 1999;**17**:3494–502
35 Niedziela M, Maceluch J, Korman E. Galectin-3 is not a universal marker of malignancy in thyroid nodular disease in children and adolescents. *J Clin Endocrinol Metab* 2002;**87**:4411–15
36 Hilkens J, Ligtenberg MJ, Vos HL, Litvinov SV. Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem Sci* 1992;**17**:359–63
37 Bieche I, Ruffet E, Zweibaum A, Vilde F, Lidereau R, Franc B. MUC1 mucin gene, transcripts, and protein in adenomas and papillary carcinomas of the thyroid. *Thyroid* 1997;**7**:725–31
38 Waltz MR, Pandelidis SM, Pratt W, Barnes D, Swallow DM, Gendler SJ *et al*. A microsatellite within the MUC1 locus at 1q21 is altered in the neoplastic cells of breast cancer patients. *Cancer Genet Cytogenet* 1998;**100**:63–7
39 Dyomin VG, Palanisamy N, Lloyd KO, Dyomina K, Jhanwar SC, Houldsworth J *et al*. MUC1 is activated in a B-cell lymphoma by the t(1;14)(q21;q32) translocation and is rearranged and amplified in B-cell lymphoma subsets. *Blood* 2000;**95**:2666–71
40 Ho SB, Niehans GA, Lyftogt C, Yan PS, Cherwitz DL, Gum ET *et al*. Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res* 1993;**53**:641–51
41 McGuckin MA, Walsh MD, Hohn BG, Ward BG, Wright RG. Prognostic significance of MUC1 epithelial mucin expression in breast cancer. *Hum Pathol* 1995;**26**:432–9
42 Wreesmann VB, Sieczka EM, Socci ND, Hezel M, Belbin TJ, Childs G *et al*. Genome-wide profiling of papillary thyroid cancer identifies MUC1 as an independent prognostic marker. *Cancer Res* 2004;**64**:3780–9
43 Prendiville S, Burman KD, Ringel MD, Shmookler BM, Deeb ZE, Wolfe K *et al*. Tall cell variant: an aggressive form of papillary thyroid carcinoma. *Otolaryngol Head Neck Surg* 2000;**122**:352–7
44 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54
45 Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, 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* 2003;**63**:1454–7

46 Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B *et al*. BRAF mutation in papillary thyroid carcinoma. *J Natl Cancer Inst* 2003;**95**:625–7
47 Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A *et al*. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. *Oncogene* 2003;**22**:4578–80
48 Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI *et al*. Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. *J Clin Endocrinol Metab* 2003;**88**:4393–7
49 Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F *et al*. BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *J Clin Endocrinol Metab* 2003;**88**: 5399–404
50 Xing M, Tufano RP, Tufaro AP, Basaria S, Ewertz M, Rosenbaum E *et al*. Detection of BRAF mutation on fine needle aspiration biopsy specimens: a new diagnostic tool for papillary thyroid cancer. *J Clin Endocrinol Metab* 2004;**89**:2867–72
51 Stolf BS, Santos M, Simao DF, Diaz JP, Cristo EB, Hirata R *et al*. Class distinction between follicular adenomas and follicular carcinomas of the thyroid gland on the basis of their signature expression. *Cancer* 2006;**106**:1891–900
52 Weber F, Shen L, Aldred MA, Morrison CD, Frilling A, Saji M *et al*. Gene classification of benign and malignant thyroid follicular neoplasia based on a three-gene combination. *J Clin Endocrinol Metab* 2005;**90**:2512–21
53 Puskas LG, Juhasz F, Zarva A, Hackler L, Farid NR. Gene profiling identifies genes specific for well-differentiated epithelial thyroid tumors. *Cell Mol Biol* 2005;**51**:177–86
54 Chen KT, Lin JD, Chao TC, Hsueh C, Chang CA, Weng HF *et al*. Identifying differentially expressed genes associated with metastases of follicular thyroid cancer by cDNA expression array. *Thyroid* 2001;**11**:41–6
55 Barden CB, Shister KW, Zhu B, Guiter G, Greenblatt DY, Zieger MA *et al*. Classification of follicular thyroid tumors by molecular signature: results of gene profiling. *Clin Cancer Res* 2003;**9**:1792–800
56 Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K *et al*. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. *Proc Natl Acad Sci U S A* 2001;**98**:15044–9
57 Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nature Genetics* 2003;**33**:49–54
58 Goretzki PE, Simon D, Frilling A, Witte L, Reiners C, Grussendorf M *et al*. Surgical reintervention for differentiated thyroid carcinoma. *Br J Surg* 1994;**80**:1131–4
59 Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *J Clin Invest* 1993;**91**:179–84
60 Salvatore D, Celetti A, Fabien N, Paulin C, Martelli ML, Battaglia C *et al*. Low frequency of p53 mutations in human thyroid tumours; p53 and Ras mutation in two out of fifty-six thyroid tumours. *Eur J Endocrinol* 1996; **134**:177–83
61 Fagin JA, Tang SH, Zeki K, Di Lauro R, Fusco A, Gonsky R. Reexpression of thyroid peroxidase in a derivative of an undifferentiated thyroid carcinoma cell line by introduction of wild-type p53. *Cancer Res* 1996;**56**:765–71
62 Battista S, Martelli ML, Fedele M, Chiappetta G, Trapasso F, De Vita G *et al*. A mutated p53 gene alters thyroid cell differentiation. *Oncogene* 1995;**11**:2029–37
63 Blagosklonny MV, Giannakokou P, Wojtowicz M, Romanova LY, Ain KB, Bates SE *et al*. Effects of p53-expressing adenovirus on the chemosensitivity and differentiation of anaplastic thyroid cancer cells. *J Clin Endocrinol Metab* 1988;**83**:2516–22
64 Narimatsu M, Nagayama Y, Akino K, Yasuda M, Yamamoto T, Yang TT *et al*. Therapeutic usefulness of wild-type p53 gene introduction in a p53-null anaplastic thyroid carcinoma cell line. *J Clin Endocrinol Metab* 1998;**83**:3668–72
65 Nanni P, Forni G, Lollini P-L. Cytokine gene therapy: hopes and pitfalls. *Ann Oncol* 1999;**10**:261–6
66 Zhang R, Baunoch D, DeGroot LJ. Genetic immunotherapy for medullary thyroid carcinoma: destruction of tumours in mice by in vivo delivery of adenoviral vector transducing the murine interleukin-2 gene. *Thyroid* 1998; **8**:1137–45
67 Zhang R, Minemura K, DeGroot LJ. Immunotherapy for medullary thyroid carcinoma by a replication-defective adenovirus transducing murine interleukin-2. *Endocrinology* 1998;**139**:601–8
68 Moolten FL and Wells JM. Curability of tumours bearing herpes thymidine kinase genes transferred by retroviral vectors. *J Natl Cancer Inst* 1990;**82**:297–300
69 Nishihara E, Nagayama Y, Mawatari F, Tanaka K, Namba H, Niwa M *et al*. 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* 1997;**138**:4577–83
70 Schmutzler C, Koehrle J. Innovative strategies for the treatment of thyroid cancer. *Eur J Endocrinol* 2000;**143**: 15–24
71 Weinberg RA. How cancer arises. *Sci Am* 1996;**271**:62–75
72 Cerutti JTF, Battaglia C, Zhang L, Martelli ML, Visconti R *et al*. Block of c-myc expression by antisense oligonucleotides inhibits proliferation of human thyroid carcinoma cell lines. *Clin Cancer Res* 1996;**2**:119–26
73 Filetti S, Bidart JM, Arturi F, Caillou B, Russo D, Schlumberger M. Sodium/iodide symporter: a key transport system in thyroid cancer cell metabolism. *Eur J Endocrinol* 1999;**141**:443–57
74 Brabant G, Maenhaut C, Koehrle J, Scheumann G, Dralle H, Hoang-Vu C *et al*. Human thyrotropin receptor gene: expression in thyroid tumours and correlation to markers of thyroid differentiation and dedifferentiation. *Mol Cell Endocrinol* 1991;**82**:R7–12
75 Hofman C, Eichele G. *Retinoids in Development*. New York: Raven, 1994
76 Hong WK, Itri LM. Retinoids and human cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. In: *The Retinoids: Biology, Chemistry and Medicine*. New York: Raven, 1994;597–630
77 Chomienne C, Fenaux P, Degos L. Retinoid differentiation therapy in promyelocytic leukemia. *Faseb J* 1996;**10**:1025–30
78 Schmutzler C, Winzer R, Meissner-Weigl J, Koehrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochem Biophys Res Commun* 1997;**240**:832–8
79 Schmutzler C, Brtko J, Bienert K, Koehrle J. Effects of retinoids and role of retinoic acid receptors in human thyroid carcinomas and cell lines derived therefrom. *Exp Clin Endocrinol Diabetes* 1996;**104**:16–19
80 van Herle AJ, Agatep ML, Padua DNI, Totanes TL, Canlapan DV, van Herle HML *et al*. Effects of 13 cis-retinoic acid on growth and differentiation of human follicular carcinoma cells (UCLA RO 82 W-1) in vitro. *J Clin Endocrinol Metab* 1990;**71**:755–63
81 Simon D, Koehrle J, Reiners C, Groth P, Boerner AR, Gruenwald F *et al*. Redifferentiation therapy in thyroid cancer ± results of a multicenter pilot study. International Symposium 'Thyroid Cancer: Pathogenesis, Diagnosis including PET, and Treatment'. *Thyroid* 1998;**8**:1217
82 Gruenwald F, Menzel C, Bender H, Palmedo H, Otte R, Fimmers R *et al*. Redifferentiation therapy-induced radioiodine uptake in thyroid cancer. *J Nucl Med* 1998;**39**: 1903–6
83 Haugen BR. Redifferentiation therapy in advanced thyroid cancer. *Curr Drug Targets Immune Endocr Metabol Disord* 2004;**4**:175–80
84 de Capoa A, Grappelli C, Volpino P, Bononi M, Musolino A, Ciardi A *et al*. Nuclear methylation levels in normal and cancerous thyroid cells. *Anticancer Res* 2004;**24**:1495–500
85 Venkataraman GM, Yatin M, Marcinek R, Ain K. Restoration of iodide uptake in dedifferentiated thyroid carcinoma: relationship to human Na + /I-symporter gene methylation status. *J Clin Endocrinol Metab* 1999;**84**: 2449–57

86 Massart C, Denais A, Gibassier J. Effects of all-trans retinoic acid and sodium butyrate in vitro and in vivo on thyroid carcinoma xenografts. *Anticancer Drugs* 2006;**17**: 559–63
87 Furuya F, Shimura H, Suzuki H, Taki K, Ohta K, Haraguchi K *et al*. Histone deacetylase inhibitors restore radioactive uptake and retention in poorly differentiated and anaplastic cancer cells by expression of the sodium/iodide symporter thyroperoxidase and thyroglobulin. *Endocrinology* 2004;**145**:2865–75

Appendix 1. Surgical management of MEN2A, based upon international MEN consensus, 2001

Risk level three

Highest risk of development of an aggressive and early form of medullary thyroid carcinoma. Children with multiple endocrine neoplasia 2B, or those with mutation carriers in codons 883, 918 or 922, should be submitted to total thyroidectomy during the first six months of life, preferentially the first month as microscopic medullary thyroid carcinoma with metastases may occur in the first months of life. Total thyroidectomy should be performed, in association with an extensive resection of the neck lymph nodes, mainly including central neck lymph nodes.

Risk level two

High risk of presenting with an aggressive form of medullary thyroid carcinoma. Children carrying *RET* mutation in codons 611, 618, 620 or 634 should be submitted to total surgery before five years of age. Total thyroidectomy should be performed, in association with removal of thyroid posterior capsule and dissection of central lymph nodes.

Risk level one

Moderate risk of developing aggressive forms of medullary thyroid carcinoma. Children carrying mutations in codons 609, 768, 790, 791, 804 or 891 should also be submitted to total thyroidectomy. There are three, age-related alternatives for surgical procedures. Some authors indicate that patients should be operated upon before the age of five years, as in risk level two. Others suggest 10 years of age as a cut-off for surgical indication. A third alternative is to wait for abnormal basal or stimulated calcitonin values as an indication for surgery. The biological behaviours of these tumours are variable, but frequently present a late or indolent evolution.

(Adapted with permission.)[7]

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