Differentiated thyroid carcinoma : diagnostic and therapeutic studies

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Chapter 8

Summary & Disscussion

1. Introduction

Differentiated thyroid carcinoma (DTC) has a low incidence and a relatively good prognosis. This relatively favourable prognosis is the result of the biological behaviour of most of these tumors and the efficacy of initial therapy. However, the therapeutic arsenal in DTC is very limited. Once distant metastases have occurred, usually in the lungs or bones, the prognosis is worse, because the results of RaI therapy, which is virtually the only curative treatment, are moderate. A major problem in progressive or metastatic disease is the diminished, or lost, ability of thyroid cancer cells to accumulate RaI, indicated by negative post-therapeutic whole body scintigraphy. In these cases, the prognosis is poor, because alternative treatment options (external radiotherapy or chemotherapy) have only limited success.

Because of the low incidence and favourable prognosis, diagnostic and therapeutic strategies are hard to investigate: the follow-up time is too long and the numbers too small to reach significant endpoints in prospective randomized trials. As a result, many of the current treatment and follow-up protocols are derived from large retrospective studies, mostly from single centers, with many sources of bias. Another aspect is the decentralised approach of the disease. Despite the low incidence, many centers treat patients with DTC. One of the examples of this decentralised approach is the existence of many staging systems, which make comparisons between centers difficult.

DTC is a unique malignant disease in which fascinating biological phenomena are present, like the pathophysiology of iodide transport. This makes DTC an attractive model to study the molecular mechanisms of iodide transport, and to find targets to re-establish iodide transport. This unique position, however, also adds to the somehow isolated situation: DTC is a type of cancer that is less well recognized in the mainstream of novel anti-cancer drugs trials. An example of an unresolved diagnostic dilemma is that the diagnosis of DTC is still largely dependent on conventional histological staining procedures. Despite experimental studies with gene- and protein expression profiles, no important innovation has been introduced in the past decades with respect to diagnosis. This has important implications for the many patients who present with DTC, because in particular the distinction between follicular thyroid carcinoma (FTC) and follicular adenoma (FA) is impossible to make with cytology. As a consequence, many patients will undergo surgery who do not have DTC.

In every follow-up protocol of DTC serum thyroglobulin (Tg) measurements are the backbone of diagnosis of recurrent DTC. However, the many analytical and statistical aspects of Tg measurements are not always reflected in the choice of Tg cut-off values. Indeed, they are defined on some retrospective studies from large centers. An important approach would be to define institutional cut-off values which has been one of the projects in this thesis. Furthermore, the prognostic value of Tg,

in addition to conventional ones like TNM stage, histology and age is an interesting and potentially clinically important issue that has not been addressed extensively in the literature.

A fascinating aspect of DTC is the pathophysiology of iodide transport. The most important molecule for iodide transport, the sodium iodide symporter (NIS) is less functional in DTC. Although decreased expression is important, in this thesis, it was found and confirmed that defective NIS trafficking may be important as well, which may have not only consequences for future research, but also for the diagnosis of DTC.

The introduction of recombinant human TSH (rhTSH) has been important for the patient to avoid the negative aspects of thyroxine withdrawal. The assumption, however, that continuation of thyroid hormone therapy does not directly influence iodide transport has not been properly investigated.

Different approaches to improve iodide transport in DTC can be distinguished. Much attention has been focussed on lithium salts to improve iodide uptake and addition of lithium to radioiodine therapy in metastatic DTC has been recommended in the literature. In the present thesis, these diagnostic and therapeutic dilemmas in differentiated thyroid carcinoma (DTC) are approached from a clinical and experimental perspective.

2. Improving the diagnosis of DTC

The histological diagnosis of DTC, and in particular follicular thyroid lesions, is an important dilemma evaluated in Chapter 2 with huge implications for general health care is. Although the prevalence of DTC is low, that of thyroid nodular disease is not, and improvements in the current practice in which patients with follicular proliferation are referred for surgery could prevent many surgical procedures. Many genetic and immunohistochemical candidate markers have been identified, but none of those has successfully been introduced in routine diagnostic procedures. We chose to investigate the diagnostic value of a panel of proteins (Galectin-3 (Gal-3), HBME-1, CK-19, CITED-1, Fibronectin-1 (FN-1), the sodium iodide symporter (NIS) and peroxisome proliferator activated receptor (PPAR) in 177 benign and malignant thyroid tissues. Our study differed from earlier ones with regard to the identification of optimal semi-quantitative cut-off levels using ROC analysis and the use of hierarchical cluster analysis.

We found all proteins to be differentially expressed between FA and PTC. The differences between FA on the one hand and FTC and Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) on the other hand were less prominent, but we found a differential expression of PPARγ, HBME-1, Gal-3, cNIS and FN-1.

The accuracies of HBME-1, FN-1 and Gal-3 for the differential diagnosis of FVPTC and FA were fair. For FN-1 the accuracy was 71% for the differentiation between

FA and FTC.

We confirmed cytoplasmic NIS overexpression in PTC and FTC. The differential expression of cNIS between subtypes of thyroid neoplasms makes it a candidate for differentiating between these lesions. Cytoplasmic NIS was also identified by cluster analysis as a potential useful marker in the discrimination between FA and malignant carcinomas.

PPARγ has been found to be downregulated in experimental models of thyroid carcinoma. The importance of the downregulation of PPARγ is also illustrated in the PPARγ/PAX8 rearrangement which was initially observed in a series of FTC. We found decreased PPARγ nuclear staining in malignant tumors, but as the percentage of positive cells varied from 50-100% in benign lesions, the diagnostic accuracies for the differentiation between follicular lesions were limited.

Cluster analysis showed that a diagnostic immunohistochemical panel comprising Gal-3 and FN-1 was 97% sensitive for all thyroid carcinomas, whereas specificity was 100%. However, HBME-1 was found to be a useful marker for the differentiation between FA and FVPTC. Because the number of FVPTC was small, hierarchical clustering did not allow a separate analysis of this group of tumors.

In conclusion, Gal-3, FN-1 and cNIS is a useful diagnostic panel in the differential diagnosis of thyroid lesions. The absence of Gal-3, FN-1 and cNIS is highly suggestive for a benign lesion. HBME-1 may be useful in the specific differentiation of FVPTC from FA.

Perspective

The findings of Chapter 2 need to be confirmed in a follow-up study in which the candidate markers are tested in cytological samples. These samples will be scored according to routine criteria and clinical decisions will be based on those. The scores of the candidate markers will be compared with the final histological diagnosis of those thyroid glands that will be removed and a comparison will also be made with the staining patterns of the candidate markers in the surgical samples.

3. The diagnostic and prognostic value of serum Tg in the follow-up of DTC

Serum Tg levels are the most important diagnostic markers in the follow-up of DTC. Recently, guidelines for the follow-up of DTC have been published by the British Thyroid Association (under the auspices of the Royal College of Physicians), the American Thyroid Association and the European Thyroid Association. In the Netherlands, the medical associations involved in DTC and the Dutch Institute for Healthcare Improvement (CBO) have also completed a consensus paper. The cut-off values for Tg levels that are advised in these documents are often not well defined, and based on retrospective studies from a limited number of large

centers. Indeed, it is advised to define institutional cut-off values. The problem with the definition of Tg cut-off values is, as with any diagnostic procedure, the gold standard that is used to the define presence or absence of disease. In DTC, Tg is considered a better marker than for instance radioiodide scintigraphy, so using iodide scintigraphies as a gold standard may lower the specificity for Tg. In addition, the levels of Tg cut-off values are dependent on what is considered the most acceptable ratio between unnecessary therapies or missed recurrent tumors. This is a subjective choice and may be different in different countries or areas. Therefore, insight into the quantitative relation between sensitivity and specificity of Tg is important, which is the base of receiver operator curve (ROC) analysis. Nevertheless, despite the analytical and methodological problems, in Chapter 3 we investigated the diagnostic and prognostic value of serum Tg measurements for tumor presence, cure and death in the follow-up of DTC by ROC analysis in a homogeneous group of 366 patients with respect to initial therapy.

We found an excellent diagnostic accuracy of serum Tg values during TSH stimulation 6 months after initial therapy (sensitivity 100%), albeit with a higher Tg cut-off level than commonly reported. The explanation for this higher cut-off value may be related to a lower initial ablation rate in our institute as compared with others, analytical differences or the use of the ROC technique. We also found that serum Tg levels before RaI ablation are an independent predictor for cure, with a cut-off level of < 27.5 ug/L. TSH stimulated Tg measurements 6 months after initial therapy and at 2 and 5 years after initial therapy were independent predictors of DTC related mortality. Notwithstanding the less than 100% specificity of Tg for DTC, which can indeed be explained by the limitations of gold standards used in our study, we agree with the policy to administer a high dose of RaI to patients with elevated Tg levels. In our opinion, a potential solution to circumvent the debate about specificity of Tg is to consider Tg a risk indicator. The independent prognostic value of serum Tg values for cure and death are arguments to include Tg in the conventional panel of risk factors. The percentage of patients with Tg antibodies (initially 27%) is in line with previous studies. The percentages of active tumor in patients with and without Tg antibodies were comparable, confirming the lack of diagnostic value of Tg antibodies.

In conclusion, our studies illustrate the importance of the definition of institutional Tg cut-off levels. Our analyses allow the definition of groups of patients with an increased risk for residual disease or mortality, in addition to conventionally used prognostic indicators.

Perspective

Given the multiple analytical and methodological aspects that are involved in Tg measurements, we believe that inter-institutional harmonization of Tg measurements should be propagated, preferably in an international context, but certainly at a national

level. In addition, uniformization of treatment protocols and standardized criteria for DTC disease activity should be established, that enable the structured follow up of DTC patients and the definition of Tg cut-off values on a multi-institutional level.

4. Triiodothyronine suppresses in vitro iodide uptake and expression of NIS

The introduction of rhTSH for the diagnosis, initial therapy (ablation) and under certain circumstances the treatment of DTC is without doubt an important innovation for patients with DTC, to avoid the disadvantages of thyroid hormone withdrawal. Although in general the diagnostic properties of rhTSH are similar to thyroxine withdrawal, the iodide uptake kinetics may not be entirely comparable. One of the aspects is that patients with rhTSH are per definition euthyroid. The assumption is that thyroid hormone does not directly affect iodide uptake. We studied in Chapter 4 whether this assumption is correct. We used the rat thyroid cell line FRTL-5 and cultured this cell-line in the presence or absence of physiological concentrations of T3 and studied proliferation, iodide uptake and NIS mRNA and protein expression. We found indeed a decreased uptake of iodide. This decreased uptake was accompanied by decreased NIS mRNA and protein expression.

Although it has been suggested that the iodide content of levo-thyroxine (T4) therapy during rhTSH may dilute the specific activity of radioiodide, and that this may be responsible for decreased radioiodine uptake, we believe that this cannot explain our findings, as the amount of iodide coming from T3 in our experiment is negligible related to the cold iodide concentration of 10 uM. In addition, differences in whole body iodide kinetics, another potential explanation for differences between rhTSH and withdrawal, cannot explain our findings either. From our results, it seems likely that T3 has effects on NIS gene expression resulting in lower functional NIS protein. It has been debated whether the promoter for NIS contains T3 responsive elements. Another explanation can be that the effects of T3 are via repression of the TSHR promoter, but our experiments do not point in that direction.

In conclusion, we found evidence for a TSH and iodide independent effect of T3 on NIS gene expression. The mechanism remains to be resolved and also the question whether the effect exists in humans. The clinical implications of this finding are not clear, but they may add to explanations of the suggested differences in iodide accumulation in rhTSH or conventionally treated patients.

Perspective

We believe that the introduction of rhTSH is an important improvement in the impact of DTC for patients. It is, however, important to study relevant aspects that are involved in the physiology of iodide metabolism, including T3. An interesting development is that there is experimental evidence that T3 may also act as a tumor suppressor. In this perspective, continuation of T3 during diagnosis or radioiodine

therapy may be advantageous.

5. Improving radioiodide therapy in DTC

Therapeutic options for metastases of DTC are limited. RaI may be effective in about half of the patients with pulmonary metastases and a small proportion of patients with bone metastases. However, the efficacy of RaI therapy is often limited by decreased iodide uptake of metastatic DTC. Strategies to improve therapeutic options can be distinguished in therapies to improve RaI therapy or identifying other targets.

Improving RaI therapy is a broad theme. Two main subthemes can be distinguished. First tumors that still accumulate iodide, second tumors in which iodide accumulation is lost.

In tumors that still accumulate iodide, improving radioiodide therapy is essentially aimed at increasing the dose of RaI. The dose of RaI is the result of the amount (activity) of RaI administered to the tumor (specific activity), the rate of uptake, the tumor volume and the effective half life of RaI, which on its turn is determined by the physical half life and the biological half life. All these contributing factors can be optimalized: the amount of RaI presented to the tumor can be improved by a low iodide diet that increases the specific activity. The uptake rate can be influenced by high TSH levels. The half-life of RaI in DTC is an important factor. The loss of follicular architecture and probably decreased activity of thyroid peroxidise (TPO) may contribute to a decreased effective half life and thus by a lower tumor dose. We focussed on this problem and studied the potential effects of lithium salts on iodide accumulation in Chapter 5.

5.1 Effects of lithium on iodide uptake and clinical outcome of radioiodide therapy

The relation between lithium salts and the thyroid has been known for long, as many patients with bipolar depression, treated with lithium salts develop hypothyroidism. The mechanism is not clear but some experimental studies have suggested that lithium inhibits the release of thyroid hormone, or in other words, confines iodide to the thyroid. Because of these properties, lithium has been used to increase the dose of RaI in benign and malignant thyroid disease. The problem however is that it is not clear if lithium influences the outcome of RaI therapy in DTC. We therefore studied the clinical effects of RaI without and with lithiumcarbonate in patients with proven metastatic DTC. In addition, controversy exists on the mechanism by which lithium increases RaI dose in DTC. We performed an in vitro study specifically aimed at lithium effects on the NIS.

We studied 12 patients with metastases of DTC that still accumulate RaI. These patients had received previous unsuccessful RaI therapy without lithium (control) that. The patients received 1200 mg lithiumcarbonate/day followed by RaI therapy.

Despite an increased uptake of RaI in 7 patients, no beneficial effect with lithium was observed on the clinical course as assessed by serum Tg measurements and radiographically. An explanation for the lack of success can be that the longer time interval between the 2 RaI therapies may have given rise to changes in biological tumor characteristics or alternatively, that the first therapy may have selected tumor cells that do not accumulate iodide (radioresistant tumor cells). We think that this is unlikely as in all patients; RaI accumulating lesions were present after the second therapy as well. In addition, although Tg levels were progressive in most patients, their long-term increment rates were not altered substantially after the first therapy. Another explanation may be that tumor cells have become resistant to RaI, in other words that apoptotic mechanisms have become defective a phenomenon that is regularly observed in oncology. Of course, it may also be that lithium did not influence the RaI kinetics at all. We studied the effects of lithiumchloride in different physiological concentrations on iodide uptake in the benign rat thyroid cell line FRTL-5, in the polarized non-thyroid MDCK cell-line, stably transfected with hNIS and in the human follicular thyroid carcinoma cell line FTC133-hNIS. Both steady state iodide uptake, initial rate uptake and iodide efflux studies were performed. The aim of these studies was to study whether lithium salts have a direct effect on NIS. No effects of lithiumchloride were found on iodide uptake or efflux, irrespective of the concentrations. We have not studied all steps of iodide physiology. As it is suggested that for the enhancement of iodide trapping by lithium intact organification is necessary we cannot rule out that in the 3-dimensional context of the thyroid, lithium may indeed inhibit the release of organified iodide, which explains the absence of lithium effects in thyroid- or non-thyroid tumors with a short half-life and absence of organification.

The results of our study challenge the reported beneficial effects of lithium in DTC.

Perspective

We believe that lithium salts have no place in RaI therapy of DTC. Alternatively, other mechanisms to improve RaI half-life should be explored. The concept of organification is interesting and it can be hypothesized that when RaI in coupled to another protein, half life may be positively influenced. However, transfection experiments with TPO may be thought of but are difficult from a clinical perspective. Another option may be the blocking of iodide efflux by using chloride channel blockers.

5.2 Effects of redifferentiation therapy with bexarotene on iodide uptake and clinical outcome of radioiodide therapy

The second subtheme of improving RaI therapy involved tumors in which iodide accumulation is lost. The holy grail here is to re-establish functional NIS expression. The pathophysiology of decreased NIS expression in DTC is complex (as indicated

above) and involves both genetic (transcriptional) defects and post-translational (trafficking) defects. Mutations in the NIS gene have infrequently be reported and the most likely explanation is that the transcription of the NIS gene is hampered by either inactivation of the gene itself or more likely decreased promoter activity. The NIS promoter has multiple responsive elements and absence of one or more activators (like TTF-1 or TSH) will cause decreased transcription. An interesting explanation is epigenetic changes in which methylation or histone deacetylation prevents transcription. Interesting studies have been published using pharmacological substances to revert these gene blockades. We have studied the effects of the retinoid receptor X (RXR) agonist Bexarotene (Chapters 6 and 7) on the reestablishment of RaI uptake in patients with metastatic DTC. Retinoids have been used earlier in DTC, but all clinical studies performed so far used 13-cis retinoic acid which binds only to the RAR subtype. Recent studies have elucidated the importance of other retinoic acid receptor subtypes, like the RXR in DTC. We therefore decided to study the effects of 6-weeks treatment with the retinoid receptor RXR activator Bexarotene 300 mg/day on I-131 uptake in 12 patients with metastatic DTC. Prior to, and after this intervention, RaI uptake was measured by whole body scintigraphy and single photon emission tomography (SPECT) 3 days after 185 MBq I-131. Diagnostic imaging was preceded by 2 consecutive injections with rhTSH. Bexarotene treatment induced I-131 uptake in metastases of 8/11 patients. An interesting observation was that in one patient a new lesion became apparent after Bexarotene, which did not accumulate iodide earlier. This is an interesting illustration of the heterogeneity in DTC metastases with respect to iodide metabolism. However, uptake was only discernable at SPECT and had incomplete matching with metastases as visualized by CT scanning.

Although the amount of RaI uptake was limited in the 8 responders, we decided to give them the benefit of the doubt and offer them therapy with high dose RaI. They received 7400 MBq radioiodine preceded by 6 weeks of treatment with Bexarotene 300 mg/day. Six months after RaI therapy 6 patients had progressive disease, whereas 2 patients had stable disease. The explanation for this partial success may be that Bexarotene did not influence other factors involved in RaI accumulation, like the effective half-life (see above). Alternatively, the regulation of NIS may be defective at multiple transcriptional and post-transcriptional levels which can only be partially restored by retinoids. Another possibility could be that the retinoic receptor expression pattern in the patients was not favorable with respect to Bexarotene therapy. To investigate this issue, we performed RAR and RXR staining in a subset of patients. However, we did not find a relation between staining pattern and outcome of therapy. A limitation in this respect is that we only had materials of the primary tumor and it may be that the retinoid receptor expression pattern in the metastases was different. We observed incomplete matching between the metastases identified by radiological imaging and post-therapeutic WBS. This observation illustrates the heterogeneity of iodide metabolism in DTC metastases and is a limitation for

redifferentiation therapy, because even if a subset of metastases becomes susceptible to RaI, this does not prevent the progression of other lesions.

Perspective

We have shown that redifferentiation therapy in a clinical setting is indeed able to partially restore iodide uptake in DTC. A conceptual problem with redifferentiation therapy is that this therapy is aspecific: also other genes unrelated to the disease may be influenced by these compounds. This may be a serious problem, unless a specific molecular defect in the target pathway is identified, like promyelocytic leukemia, where a translocation in the RAR is the base of retinoid therapy. The advent of designer drugs aimed at specific molecular defects in well dissected pathways, like the class of tyrosine kinase inhibitors, is an important development in cancer, and may prove to be successful in DTC as well.

6. Conclusions of the studies described in this thesis

The low incidence, high survival and the specific clinical and biological aspects of DTC complicate the diagnosis, treatment and follow-up of patients with DTC. This the way in which research and patient care for DTC patients are organized should be seriously evaluated and improved. The recent publication of multi-national guidelines is an important development. The studies in this thesis have addressed some of the clinical and fundamental questions in DTC. Although they have resolved some of the questions, many new questions have risen, warranting ongoing research.

The diagnosis of DTC, which is currently performed using conventional histological techniques, could be improved considerably by applying new markers. Our studies offer interesting perspectives for the introduction of a panel of new immunohistochemical markers that may improve the diagnosis.

Although serum Tg measurements are recommended as the most important diagnostic procedure in the follow-up of DTC, multiple analytical and statistical aspects are involved. Our studies illustrate the importance of defining institutional cut-off levels for Tg, using ROC techniques, to offer insight in the quantitative relationship between sensitivity and specificity. We found Tg to be an independent prognostic marker for cure and mortality as well, which may help the clinician to identify high-risk patients.

The ability of DTC cells to accumulate iodide is the core of radioiodine diagnostic and therapeutic procedures. The introduction of rhTSH has been a great advantage for patients, to avoid thyroid hormone withdrawal. However, among others, the assumption that continuation of thyroid hormone treatment has no effects on iodide metabolism is likely not true. We found that T3 directly influences the expression and function of NIS. Although the clinical implication of this finding is not clear, we believe that a careful evaluation of the effects of any new regimen for radioiodide

therapy on well identified aspects of thyroid physiology should be investigated as good as possible.

The most important problem in the treatment of DTC is the ineffectiveness of radioiodide therapy in a considerable proportion of patients with metastases. Strategies to improve radioiodide therapy should be based on the identification of the molecular defects in iodide physiology in DTC. In patients in whom iodide uptake is still present but ineffective, interventions aimed at improving iodide kinetics are worthwhile. We demonstrated that the frequently propagated strategy to treat patients with lithium salts did not result in a better outcome of radioiodide therapy. Nor did we observe any effect of lithium on the in vitro iodide uptake. Other approaches to improve iodide kinetics are warranted.

In patients in whom DTC metastases do not accumulate radioiodide, research is focusing on the re-expression of functional NIS. We found that the redifferentiating RXR ligand Bexarotene re-established radioiodide uptake in a group of patients. The intensity of radioiodide uptake was however low and only present in a subset of metastases. Subsequent therapy with a high activity of radioiodine was unsuccessful. Many mechanistic and conceptual questions with respect to redifferentiation therapy remain and the advent of a novel class of designer drugs aimed at well identified molecular defects, like the class of tyrosine kinase inhibitors, may prove to be more promising.