Mise au point

Recent advances in treatment of medullary thyroid carcinoma ☆

Le carcinome médullaire de la thyroïde : les nouvelles approches thérapeutiques

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Résumé

Le cancer médullaire de la thyroïde représente 5 à 10 % des cancers thyroïdiens. Il est sporadique dans 70 % des cas, mais s’intègre chez 30 % des patients dans un cadre familial. Une mutation germinale du proto-oncogène RET est retrouvée dans plus de 95 % des cancers médullaires familiaux alors qu’une mutation somatique de ce proto-oncogène est présente chez 40 à 70 % des cancers médullaires sporadiques. Le traitement est chirurgical et consiste en une thyroïdectomie totale associée à un curage récurrentiel bilatéral et jugulocarotidien homo- ou bilatéral. Le caractère curatif de la chirurgie dépend du stade anatomoclinique du carcinome médullaire de la thyroïde lors du diagnostic. Ainsi, la chirurgie est curative dans près de 100 % des cas lorsqu’il s’agit de tumeurs millimétriques, dans 90 % des cas lorsqu’il s’agit de tumeurs infracentimétriques, et dans seulement 50 % des cas lorsque la lésion est supracentimétrique. En cas de persistance de la maladie tumorale après chirurgie, les alternatives thérapeutiques sont peu nombreuses et ne sont souvent proposées que dans un but palliatif. Nous décrivons dans cette revue les nouvelles approches thérapeutiques à l’étude actuellement dans le carcinome médullaire de la thyroïde. Nous développerons successivement l’immunothérapie, la radio-immunothérapie, les différentes approches ayant pour cible le gène RET ou sa protéine, les gènes suicides, les inhibiteurs des cyclo-oxygénases et enfin les traitements par iode radioactif après transfert du gène symporteur de l’iode.

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Abstract

Medullary thyroid carcinoma accounts for 5–10% of all thyroid cancers. It is sporadic in 75% of cases and familial in 25% of cases. Germ-line REarranged during transfection (RET) proto-oncogene mutations are detected in more than 95% of patients with familial medullary carcinoma whereas somatic RET mutations are detected in 40–70% of sporadic medullary carcinomas. Surgery is the only curative treatment and should consist of total thyroidectomy with central and ipsilateral or bilateral lateral lymph node dissection. Surgery provides successful cure in almost 100% of patients when tumor size measures a few millimeters, in almost 90% of patients with a tumor measuring less than 1 cm, and in only 50% of patients with a tumor larger than 1 cm. Alternative forms of treatment involving radiotherapy or chemotherapy provide little benefit. A perspective of recent trials and research into novel treatment of medullary thyroid carcinoma is summarized in the following paper. In this review we examine immunotherapy, radioimmunotherapy, therapy targeting the RET gene or protein, suicide gene therapy, cyclooxygenase inhibitors and radioiodine therapy following sodium iodide symporter gene expression.

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Mots clés : Cancer médullaire de la thyroïde ; Immunothérapie ; Radio-immunothérapie ; Inhibiteurs des tyrosines kinases ; Gène suicide

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1. Introduction

Medullary thyroid carcinoma, derived from C cells of the thyroid, accounts for 5–10% of all thyroid cancers. It is sporadic in 70% of cases and familial in 30% of cases (multiple endocrine neoplasia type 2A or 2B, or isolated familial medullary thyroid cancer). RET germ-line proto-oncogene mutations are detected in more than 95% of patients with familial medullary carcinoma whereas RET somatic mutations are detected in 40–70% of sporadic medullary carcinomas [13]. Surgery is the only curative treatment and should consist of total thyroidectomy with central and ipsilateral or bilateral lateral lymph node dissection [34]. Successful surgical treatment depends upon the histopathological stage of the medullary thyroid carcinoma at the time of diagnosis; this approach provides successful cure in almost 100% of patients when tumor size is a few millimeters, in almost 90% of patients with a tumor of less than 1 cm and in only half of patients with a tumor larger than 1 cm [17]. When tumor remnants persist after surgery, there are very few therapeutic alternatives, and these are generally of limited value. The aims of additional therapy are:

- reduction of tumor size (in accordance with the RECIST criteria);
- reduction of calcitonin and/or carcinoembryonic antigen (CEA) levels;
- improved patient survival.

The indications for such additional treatments depend upon the likelihood of progression of the medullary thyroid cancer, which varies widely from one patient to another; in most cases, untreated progression is indolent and without adverse effects on the patient’s general condition, even at the metastatic stage [1,9,22].

External cervical and mediastinal radiotherapy reduces the risk of cervical relapse threefold in patients not cured by surgery [15], although it does not extend survival in this population [2]. Systemic chemotherapy reduces tumor size in 10% of patients but is not beneficial in terms of survival [30]. Chemomobilization of hepatic metastases results in regression or stabilization of metastases in 60% of patients but such therapy becomes ineffective after a few months [16]. In fact this approach only appears effective in the early stages of liver involvement [16]. Finally, somatostatin analogs appear to have no antitumoral efficacy [29].

In this paper, we shall examine therapeutic approaches currently under development in patients with medullary thyroid cancer unsuccessfully treated by surgery. We must nevertheless begin by emphasizing that in most clinical studies:

- very few patients were included due to the rareness of the disease;
- no clinical trials have as yet been completed. Therefore the results of these studies must be interpreted with caution.

2. Immunotherapy

2.1. Physiological overview: dendritic cells

Dendritic cells are responsible for presenting antigens and thus initiating immune response. These cells react to tumors by phagocytosis of tumor cells with degradation of the antigens expressed by these cells to peptides. The dendritic cells then present the resulting antigen to T lymphocytes, which are thus activated, triggering immune response.

2.2. Principles of immunotherapy

Stimulation of physiological immune response to tumoral antigens can in theory reduce tumor size and the immune system had means of combating tumor cells. In practice, however, the immune system is frequently overwhelmed. Therapeutic stimulation of physiological immune response may be achieved by transferring either mature dendritic cells capable of combating a specific antigen (calcitonin or CEA) or immunostimulatory substances.

2.3. Vaccination with mature dendritic cells

The two main studies involving vaccination with mature dendritic cells were performed in 7 and 10 patients presenting metastatic medullary thyroid cancer followed for a mean duration of 13 or 17 months [33,39]. Radiological response (regression of tumor mass by more than 50%) was seen in 1/7 and 4/10 patients. A re-increase in tumor size was seen in one patient after 30 months of follow-up. Biological response (significant reduction of calcitonin and/or CEA) was reported for 3/7 and 7/10 patients although there was a subsequent increase in these parameters and/or in radiological progression of metastases after several months of follow-up in two and three patients. Finally, hormonal and radiological stabilization of the disease was observed in 4/7 patients and 3/10 patients. This treatment had good tolerability (Table 1).

Dendritic cells vaccination could be of value for some patients presenting with medullary thyroid cancer not cured by surgery. However, therapeutic escape occurs in several patients after several months. An international phase I trial is currently underway to investigate intratumoral injection of dendritic cells in hepatic metastases of endocrine cancers and of medullary thyroid cancer in particular.

2.4. Transfer of immunostimulatory substances

Transfer of immunostimulatory substances into tumoral tissue may induce immune response. Two cytokines appear to be of particular interest: interleukin 2 (IL2) and interleukin 12 (IL12). The studies published to date have been conducted in murine models.
2.4.1. IL2

IL2 exerts antineoplastic activity by stimulating the proliferation and differentiation of cytotoxic and memory T cells and of natural killer cells.

The value of systemic administration of IL2 appears limited in current clinical practice due to the severity of associated adverse effects [35]. Use of adenoviral vectors expressing IL2 gene appear more promising [46,50,51]. This approach ensures direct intratumoral delivery of IL2 with improved antineoplastic efficacy and fewer systemic side effects.

Adenoviral vectors expressing IL2 gene were introduced into medullary thyroid cancer cell lines, which were then injected into healthy rats and mice [26,46,49,50]. Tumors were observed in all animals receiving wild type cell lines and in 0–20% of mice receiving cell lines transfected with an adenoviral vector expressing IL2 gene. In addition, in cured rats, injection of wild-type cell lines 60 days after the first injection of the adenoviral vector resulted in the absence of new tumors, thus providing evidence of prolonged immunity [51].

However, antitumoral efficacy appeared to be lower in animals presenting a pre-existing tumor [46,49,51]. This effect thus appears to be dependent upon tumor size, with lower efficacy being seen in larger tumors [46,51]. The tolerability of the treatment appeared good in these murine models [51].

2.4.2. IL12

IL12 stimulates proliferation of natural killer cells and CD8 T lymphocytes, activates macrophages and stimulates differentiation of CD4 T lymphocytes. Like IL2, the systemic route due to its extremely high toxicity cannot give IL12. However, in rats with medullary thyroid cancer xenografts, intratumoral injection of adenoviral vectors expressing IL12 resulted in tumor regression in 86% of cases [48] and in complete disappearance of tumor in 71% of rats [43]. As with IL2, animals treated with IL12-expressing cell lines exhibited prolonged immunity [43]. Tolerability of the treatment appeared good in these animal models [43].

3. Radioimmunotherapy

Medullary thyroid cancer is characterized by intense membrane expression of CEA. Radioimmunotherapy involves the use of antibodies directed specifically against CEA labeled with a radioactive isotope. It should be noted that increased plasma concentration of CEA is not a necessary prerequisite for this type of treatment.

The value of treatment using an anti-CEA antibody labeled with 131 iodine was studied in groups of 9–30 patients presenting metastatic medullary thyroid cancer followed for a mean duration of 12–121 months [7,21,23,24]. This treatment resulted in moderate tumor regression (< 50%) in 7–29% of patients and stable tumor in 35–73% of patients. Plasma calcitonin and/or CEA levels decreased by more than 25% in 23–47% of patients. However, the value of this treatment in terms of patient survival has only been demonstrated for actively progressing medullary thyroid carcinoma with a calcitonin doubling-time of less than 2 years [7]. The toxicity of this treatment was primarily hematologic and hepatic (Table 1).

Treatment combining a dose of myelosuppressant labeled anti-CEA antibodies coupled with autologous graft of hematopoietic cells has also been investigated [20]. In 12 patients presenting rapidly progressing medullary thyroid cancer, two patients exhibited a reduction in tumor size (mean follow-up of 3 months to 1 year) and 10 patients presented with stabilization of tumor size for 1–16 months. Moderate gastrointestinal toxicity was seen in eight patients, with more severe toxicity occurring in one patient.

A number of research teams have studied the effects of combined chemotherapy (dacarbazine or paclitaxel) and radioimmunotherapy. Published studies to date have involved series of athymic mice with medullary thyroid cancer grafts [25,37,38]. Tumor growth was significantly reduced in mice treated with combined chemotherapy and radioimmunotherapy in comparison to animals treated with either therapy alone [25,37,38]. In one study, complete tumor regression was observed in 8 of 10 mice after 7 weeks of treatment with combined radioimmunotherapy and dacarbazine [38]. Toxicity was
mainly hematological, with spontaneous resolution of leukopenia and thrombocytopenia [25,37,38].

Combined chemotherapy and radioimmunotherapy could thus have a synergistic antitumoral effect in patients with medullary thyroid cancer.

4. Treatments targeting the RET gene or protein

4.1. The RET gene and protein

The RET gene is a proto-oncogene located in region 11.2 on the long arm of chromosome 10. The protein encoded by the RET gene is a transmembrane tyrosine kinase receptor. The extracellular domain of this protein comprises four cadherin domains and the cystein-rich region whereas the intracellular domain consists of a domain with tyrosine kinase activity. In order to bind to its ligand, RET protein must exit the endoplasmic reticulum and migrate to the cell membrane. The RET ligands bind to RET protein-independent coreceptors. This ligand-coreceptor binding is responsible for homodimerization of RET protein, which in turn activates tyrosine kinase, with subsequent activation of various signaling pathways.

Germ-line or somatic gain-of-function mutations of RET proto-oncogene are found in 95% of hereditary medullary carcinomas and in 40–70% of sporadic medullary carcinomas. These mutations can lead to homodimerization resulting in RET protein formation in the absence of a ligand or activation of tyrosine kinase formation. In this case, there is constitutive stimulation of tyrosine kinase activity resulting in continuous stimulation of the signaling pathways.

In terms of therapy, the oncogenic activity of the RET gene can be inhibited in a number of pathways. First, dominant-negative mutants of the RET gene may be used to create RET protein without oncogenic activity. Enzymes may be used to destroy the mRNA produced by the mutant RET gene. Inhibitors of the tyrosine kinase of RET protein may also be used and finally, signaling pathways upstream of tyrosine kinase may be inhibited. We shall examine these different possibilities below.

4.2. Use of dominant-negative RET mutants

Adenoviral vectors expressing dominant-negative RET mutants have been used in a number of studies [10,11]. The RET proteins generated by these dominant-negative mutants have altered glycosylation that result in defective transport of the protein from the endoplasmic reticulum to the cell membrane. In addition, these dominant-negative mutants dimerize with endogenous RET protein in the endoplasmic reticulum, thus preventing transfer to the cell membrane. Thus the presence of dominant-negative RET mutants reduces the quantity of RET proteins at the cell surface, thereby reducing the oncogenic capacity of the RET proteins. Use of these negative mutants in athymic mice with medullary thyroid cancer xenografts resulted in reduction of tumor size and increased survival of these animals [11]. However, the main drawback of this treatment is the absence of bystander effect. Inhibition of tumor growth is effectively found only in cells infected with adenoviral vector expressing the dominant-negative mutant and not in non-infected surrounding cells, thus limiting the efficacy of this approach.

4.3. Use of tyrosine kinase inhibitors

A specific inhibitor of RET protein tyrosine kinase could be valuable in the treatment of medullary thyroid cancer by inhibition of the oncogenic activity by RET protein. However, no specific inhibitors of RET tyrosine kinase are as yet available.

Bcr-Abl tyrosine kinase inhibitors such as STI571 (Imatinib, Glivec®) inhibit cell proliferation in chronic myeloblastic leukemia and induce programmed cell death. This treatment inhibited growth of medullary thyroid cancer cell lines with mutation at codon 634 of the RET gene [8] and significantly reduced xenograft tumor size in immunodeficient mice [14]. However, the dose of STI571 needed to achieve these effects is too high for use in clinical practice [36].

More promising results have been obtained with other tyrosine kinase inhibitors [3–5,19,40,41]. Several substances can inhibit tumoral growth in medullary thyroid cancer xenografts in athymic mice at doses suitable for use in clinical practice [3,19,40].

The in vivo side effects associated with such treatments appear limited [12,42].

In addition, tyrosine kinase inhibitors could possibly enhance the efficacy of chemotherapy [41]. Thus in athymic mice with medullary thyroid cancer xenografts, a combination of a tyrosine kinase inhibitor with a topoisomerase inhibitor resulted in complete absence of tumor in 9/9 mice (100%), with 56% of the animals still in remission 160 days after treatment. An international phase I/II study combining imatinib with chemotherapy comprising dacarbazine and cepacitabine is currently underway.

Combination of several tyrosine kinase inhibitors could also be of value. One research team studied the efficacy of combined Bcr-Abl tyrosine kinase inhibitor STI571 (Glivec®) and a tyrosine kinase inhibitor of fibroblast growth factor receptors in immunodeficient mice [14]. This combination of two tyrosine kinase inhibitors resulted in a 60% reduction in tumor size in pre-existing xenografts.

Use of tyrosine kinase inhibitors either alone or in combination could consequently be of interest in medullary thyroid cancer. Two international multicenter phase II studies are currently underway to provide more accurate assessment of the value of these treatments in monotherapy.

4.4. Other alternatives to inhibit RET oncogenic activity

Use of ribozymes directed against mutated RET mRNA has been proposed. Ribozymes are molecules of RNA with a catalytic function. One group built a ribozyme able to cleave
mRNA of RET gene mutated at codon 634 [31]. However, this ribozyme has no effect upon non-mutated RET gene mRNA. Transfection of a ribozyme into cell lines with stable expression of RET gene inhibited cell growth in approximately 80% of cases [31].

Substances acting on the signaling pathways induced by RET gene have also been proposed [18,45]. These agents inhibited cellular proliferation of medullary cancer lines carrying mutated RET gene in 50–75% of cases as well as increasing programmed cell death [18,27,45].

Treatments that inhibit the oncogenic activity of RET protein may thus be able to slow down or stabilize the progression of medullary thyroid cancer.

5. Use of suicide genes

This approach is based on the transfer in tumor cells of a gene allowing transformation of a non-active drug to a toxic drug. The suicide gene system most commonly used involves a combination of herpes-simplex virus type 1 thymidine kinase (HSV-TK) and ganciclovir. The latter substance acts as a non-toxic prodrug for cells. However, HSV-TK is able to transform ganciclovir to ganciclovir monophosphate. Physiologically, intracellular kinases can convert ganciclovir monophosphate to ganciclovir triphosphate, a substance toxic for cells. A specific promoter of thyroid C cells can be associated with HSV-TK, allowing selective expression of HSV-TK in C cells. During in vitro studies of thyroid carcinoma cell lines, this treatment resulted in the death of 75% of cells [28]. Nevertheless it appears to be of more limited interest in the case of pre-existing tumors, probably due to the absence of bystander effect [47,49]. Thus the efficacy of this treatment thus seems dependent upon the size of the pre-existing tumor [47].

Combination of a suicide gene with immunotherapy appears to enhance the efficacy of each treatment used alone. Thus, combination of a suicide gene and an adenoviral vector expressing IL2 resulted in significant size reduction or even disappearance of pre-existing tumors in murine models in 63% of tumors [49]. Combined use of these two therapies appears to be superior to that of each individual therapy used alone.

6. Treatment with cyclooxygenase 1 and 2 (COX-1 and -2) inhibitors

Use of COX-1 and -2 inhibitors such as indomethacin could inhibit tumor growth and reduce calcitonin secretion by medullary thyroid cancer cell lines [32]. In vivo, oral administration of indomethacin in nude mice with medullary thyroid cancer xenografts resulted in reduction of tumor size by 49–77% and reduction of calcitonin secretion by 55–86% [32].

COX-2 inhibitors could also sensitize medullary thyroid carcinoma cells to chemotherapeutic agents. Medullary thyroid cancer cell lines were treated with combined doxorubicin and COX-2 inhibitor [44]. Inhibition of cell proliferation and reduced cellular viability were noted in cell lines treated with the two agents despite absence of efficacy for each treatment when used alone.

Nevertheless it should be noted that COX-2 inhibitors may increase long-term cardiovascular risk. Doxorubicin is also associated with cardiac side effects. In vivo safety studies involving combined use of these two agents in animal models are thus essential before clinical studies may be undertaken in humans.

7. Radioiodine therapy following iodine symporter gene expression

Iodine symporter, which is physiologically absent in C cells, is a transmembrane glycoprotein responsible for transport of iodine from the extracellular medium to follicular thyroid cells. A research team transfected medullary thyroid cancer cell lines with a vector containing the gene expressing iodine symporter [6], which after radiation therapy, resulted in the death of 84% of transfected cells. However, these results are extremely preliminary and require confirmation data with in vivo studies.

8. Conclusion

Promising new therapies are currently under investigation in the field of medullary thyroid cancer. Nevertheless further studies are necessary in order to demonstrate the ability of these new therapies to improve patient survival. As a result, and given the rarity of patients with medullary thyroid cancer, the number of therapeutic trials conducted simultaneously must be restricted in order to allow recruitment of adequate numbers of patients and to provide statistically valuable results, in view of the specific difficulties involved in organizing of such clinical trials.

Finally, it is essential to recall that first-line therapy for medullary thyroid cancer continues to be the most complete surgical removal of tumor mass. In order to undertake such surgery under good conditions and in a single procedure, accurate preoperative evaluation of patients with medullary thyroid cancer is essential. Consequently, calcitonin determination is a necessary prerequisite prior to thyroid surgery. A preoperative diagnosis of medullary thyroid cancer requires total thyroidectomy with central bilateral neck dissection and at least ipsilateral lateral lymph node dissection. It is also essential to bear in mind the need to identify familial forms of the disease with RET determination in all cases of medullary thyroid carcinoma to ensure early detection of medullary cancer in relatives.

9. French version

A French version of this article is available at doi: 10.1016/j.ando.2007.03.005.
References


