NOVEL MARKERS FOR EARLY DIAGNOSIS AND PROGNOSTIC CLASSIFICATION IN MEDULLARY THYROID CARCINOMA

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Abstract

Medullary thyroid carcinoma is a neuroendocrine tumour of the parafollicular C cells of the thyroid gland. It is an aggressive tumor that can be cured only by complete resection of the thyroid tumour and any local and regional metastases. Thus, the discovery of novel diagnostic and prognostic markers is very important for early diagnosis and correct management, in order for the survival rates to rise.

New research has emphasized the potential role of various genes, serum and immunohistochemical markers, as well as potential targets for therapeutic agents.

The calcium stimulated calcitonin test has been recently reintroduced in clinical practice, and current medullary thyroid carcinoma guidelines encourage laboratories to set their own criteria defining reference ranges for elevated serum basal and stimulated calcitonin levels.

Key words: medullary thyroid carcinoma, markers, calcitonin, calcium-stimulated test.

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumour of the parafollicular C cells of the thyroid gland. It is an aggressive tumour that can be cured only by complete resection of the thyroid tumour and any local and regional metastases (1), therefore early diagnosis is essential.

In this context, the research of novel markers for early diagnosis and prognosis is very important, for the survival rates to rise.

The diagnosis of MTC is usually made after fine needle aspiration biopsy and additional immunohistochemical staining for calcitonin (CT) (2). Measurement of serum calcitonin was proven to be important in the early diagnosis of MTC, although there is no expert consensus on its role in the evaluation of a thyroid nodule (2). Early diagnosis can also be achieved by RET genetic testing in relatives with familial MTC.

In terms of prognosis, age and stage of disease at the time of diagnosis have been shown to be important factors. Specific germline mutations in RET predict

the aggressiveness of the tumour, although not so clear genotype-phenotype correlations exist for sporadic MTC (2). High preoperative serum carcinoembryonic antigen (CEA) (3), a rising CEA level associated with a stable or declining CT level, immunostaining for CEA associated with scant or absent tissue staining for CT (4), CT and CEA doubling times- provide sensitive markers for progression and aggressiveness of metastatic MTC (5, 6) and requires surgery (7). Other factors that may predict poor prognosis include cellular heterogeneity, prominent tissue immunostaining for galectin-3 (8), an elevated serum procalcitonin: CT ratio (9) or a less than 10-fold increase in preoperative CT levels after stimulation with pentagastrin (10). Several other genetic and molecular markers are currently under investigation regarding MTC prognosis.

In patients with residual, metastatic, progressive disease, classical cytotoxic drugs were proven to be inefficient. Drugs that inhibit different kinases, including RET, such as sorafenib, sunitinib, motesanib, cabozantinib and vandetanib, appear to be a key therapy for these patients (11).

Genetics

Parallel RET mutation analysis in MTC tissues revealed that next-generation sequencing is more sensitive than Sanger sequencing in detecting somatic mutations in tumour tissues (12). According to published literature, in 70% of sporadic MTC somatic RET mutations were identified. The most frequent somatic RET mutation in sporadic MTC was Met918Thr (13).

In a study published in 2016, Tiedje *et al.* attempted to identify prognostic markers for progressive MTC and oncogenic factors associated with response to administration of vandetanib. They concluded that tumour stage and the occurrence of metastases are connected to the expression pattern of tumour-cell receptor tyrosine kinases and endothelial receptor tyrosine kinases in the primary tumour tissues of sporadic MTC. Comparing patients cured by surgery with patients with metastatic

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MTC, the tumour-cell receptor tyrosine kinases FGFR2, FGFR3, the VEGFR ligand VEGFC and the intracellular tyrosine kinase BRAF were significantly downregulated in the latter. Opposed to that, PDGFRA, located at endothelial cells, was significantly upregulated in MTC with metastatic disease. The FLT1, FLT4 and FLT1 ligand VEGFB mRNA expression were significantly higher in vandetanib responders. An aggressive tumour and an elevated risk for metastases are pointed out by a somatic RET Met918Thr mutation and higher PDGFRA and KDR expressions (14).

Nonaka conducted a study of FoxA1 expression in thyroid tumors. FOXA1 (Forkhead box A1), known as HNF-3A, is noted as an endodermal “pioneer transcription factor,” binding to promoters and enhancers enabling chromatin access for other tissue-specific transcription factors. All 67 MTC in his study (100%), including one calcitonin-negative MTC, presented strong and diffuse FoxA1 nuclear expression. FoxA1 was also strongly expressed in C cell hyperplasia as well as solid cell nests. In comparison, variable intensity of calcitonin, CEA and chromogranin expression was identified in 94.7%, 91.2% and, respectively, 100% of tumours (15). Interestingly, FoxA1 was fully negative in follicular and papillary neoplasms, in poorly differentiated carcinomas, and it was expressed in variable intensity in 55% of anaplastic thyroid carcinomas (33/60). In addition, no FoxA1 expression was present in nodular hyperplasia, Hashimoto thyroiditis, Graves’ disease, neither in paragangliomas or parathyroid lesions. Nonaka concluded in 2017 that FoxA1 discriminates between MTC and tumours derived from follicular cells, with sensitivity and specificity greater than CT and CEA. Thus, considering its reliably uniform quality of staining, it might be a trustworthy marker for the diagnosis of MTC (15).

Chu *et al.* examined the expression of microRNA-21 (miR-21) and lncRNA MALAT1 in MTC and their effects on tumor behavior (2017). They reported a raised expression of miR-21 and MALAT1 in MTC. Their real-time polymerase chain reaction (PCR) expression in primary MTC was significantly higher compared to normal thyroid. Their study showed an *in vitro* pro-oncogenic effect of MALAT1 and miR-21 in MTC. Experiments with small interfering RNAs pointed out inhibition of miR-21 and MALAT1 expression in the MTC-derived cell line, generating significant decreases in cell proliferation and invasion (16).

Serum markers

Calcitonin

Measurement of serum calcitonin was proven

to be important in the early diagnosis of MTC, although there is no expert consensus on its role in the evaluation of a thyroid nodule (2).

Provocative tests

The current revised MTC guidelines do not specify reference ranges of basal serum CT levels for the diagnosis of MTC (2). A provocative test to evaluate stimulated CT is often needed. The stimulation with calcium has recently been reintroduced in clinical practice, to the detriment of pentagastrin, which is more expensive and has more side effects. The guidelines do not specify reference ranges for stimulated serum CT levels either. They recommend that laboratories set their own reference ranges for elevated serum CT based on studies of large numbers of normal patients and patients with MTC (2).

In 2014 Mian *et al.* defined gender-specific basal CT and calcium stimulated CT cutoffs for the identification of C-cell hyperplasia and/or MTC. They reported that stimulated CT levels were found to have the same accuracy as basal CT in the preoperative diagnosis of MTC. The thresholds proposed for the indication of MTC were >26 and >68 for basal CT and >79 and >544 pg/mL for calcium stimulated CT in females and males, respectively (17).

In another report by Papadakis *et al.* (2015), a basal CT level >17.4 pg/mL and a stimulated calcium CT level >452 pg/mL demonstrated the best sensitivity and positive predictive value for discriminating MTC from C-cell hyperplasia. Interestingly, they also noted that a considerable number of patients with stimulated CT levels >100 pg/mL presented differentiated thyroid carcinoma of follicular origin after total thyroidectomy (18).

Kihara *et al.* calculated the reference values for calcium stimulation test using an electrochemiluminescence immunoassay (ECLIA) in patients with non-MTC. They proposed that women and men could be regarded as biochemically cured or as having normal serum calcitonin values when the stimulated CT values obtained by ECLIA were <67.6 pg/mL before surgery and <0.5 pg/mL after total thyroidectomy in women, respectively <83.7 pg/mL before and <0.5 pg/mL after total thyroidectomy in men (19, 20).

Calcitonin doubling time – role in prognosis

It is well known that one can determine the MTC growth rate by measuring serum levels of CT or CEA over a sequential set of points in time to determine the rate at which each marker’s value doubles (2). Doubling times provide sensitive markers for progression and aggressiveness of metastatic MTC (5, 6).

Ito *et al.* analyzed the CT doubling time (CT-DT) of 13 MTC patients with distant recurrence postoperatively and for those with distant metastasis at the initial surgery (2016). 6 died of MTC at 5-93 months after distant metastasis has been identified. Their CT-DTs were ≤ 1.58 years. The other 7 patients have been alive for 73-123 months after the discovery of metastasis, and their CT-DTs were low at -4, -2.25 years and 9.17-33.92 years. Comparable results were attained by calculating the value of $1/CT-DT$ to avoid discontinuity in the DT values among the patients with increasing serum CT values over time and those with decreasing CT values over time. They suggested to use tyrosine-kinase inhibitors (TKI) only for patients with a short CT-DT and a large $1/CT-DT$ with a cutoff at around 1.5 years and 0.67/year (21).

Evaluation of therapeutic efficiency

During the follow-up of MTC patients treated with TKI, an initial decline in CT or CEA is seen in almost all patients, followed in one-third of them by transient fluctuations including spikes above baseline, despite the absence of radiological progression. Therefore, no correlation has been noted between CT and CEA fluctuations and tumour response, outlining the necessity for a new tumour marker in MTC (11).

Novel serum markers

Jabbari *et al.* investigated the possible significance of serum visceral adipose tissue-derived serine protease inhibitor (vaspin) and retinol binding protein-4 in MTC, and discovered that patients with MTC had significantly higher serum vaspin levels compared to the controls. The authors suggested that vaspin might be a promising biomarker for diagnosis of MTC in conjunction with other specific tumour markers (22).

Ramos-Vara *et al.* (2016) evaluated the immunoreactivity of Pax8 and napsin A in canine thyroid tumours and compared the expression of these markers with that of CT, thyroid transcription factor-1 (TTF-1) and thyroglobulin. They examined 25 tumours of medullary origin. All 25 C-cell lesions expressed CT and 22 (88%) were positive for TTF-1, 13 (57%) for Pax8 and 24/24 for napsin A. The authors noted that napsin A was as sensitive as CT and more sensitive than TTF-1 and Pax8 for tumours of medullary differentiation and, with the exception of pulmonary, renal and thyroid follicular tumours, it can be used to identify metastatic MTC (23).

Parra-Robert *et al.* studied the release of progastrin releasing peptide (proGRP) from thyroid tumours and its utility as a serum tumour marker in the MTC (2016). The authors noted that proGRP release was related to the origin of the tumour and also to the

tumour stage. Remarkably, all patients with abnormal proGRP had MTC. The median concentration of proGRP in advanced MTC was significantly higher (1398.4 pg/mL) when compared with non-MTC, either in advanced disease (24.9 pg/mL) or no evidence of disease (14.6 pg/mL). In non-MTC patients, the median concentration of proGRP was below its cutoff level (50 pg/mL). Comparable with CT, proGRP was able to identify 88.9% of MTC, but with a little lower specificity (76.9%). Using both proGRP and CT, the sensitivity raised to 100% (11).

Other studies have shown the relationship between GRP and TKI treatment in small cell lung cancer, suggesting the use of proGRP as a marker of drug resistance (24, 25). Further studies are needed for TKI used for the treatment of MTC.

Immunohistochemical analysis

The use of CD133 and CD44 epitopes as markers for cancer stem cells, which may contribute to the tumour genesis, recurrence and chemoradioresistance, has been actively investigated in thyroid cancers (26, 27).

In a study published in 2016, Bi *et al.* analyzed the expression of cancer stem cells markers CD133 and CD44 in a cohort of MTC patients, and their prognostic values during 10-year follow-up. Higher expression of CD133 and CD44 was present in MTC compared to control, and was positively correlated with capsule invasion. Each other, and their co-expression was significantly correlated with capsule invasion, tissue invasion and metastases at surgery. Both were unfavorable prognostic predictors for overall survival, while only CD44 was a significant predictor for disease free survival (28).

Werner *et al.* published in 2017 their investigation on the role of the C-X-C chemokine receptors type 4 and 7 (CXCR4/7) in MTC. High CXCR4 expression was linked to large tumour size and presence of metastasis. CXCR4 antagonists significantly reduced tumour cell invasiveness, while the treatment with recombinant human SDF1 α stimulated invasive growth, caused cell cycle activation and induced epithelial-mesenchymal transition. The authors proposed the CXCR4/CXCR7/CXCL12 axis as an important functional determinant in the tumour biology of MTC. Considering its strong association with disseminated disease, CXCR4 can be considered as a novel and viable therapeutic target in advanced MTC (29).

In conclusion, recent studies are very promising, indicating potential markers for an early diagnosis of MTC, as well as potential targets for treatment agents. Further research is needed to discover novel markers and techniques, as well as new therapeutic molecules, for the survival rates of this aggressive cancer to rise.

Conflict of interest

The authors declare that they have no conflict of interest.

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