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Review

Clinical implications of genotype-phenotype correlation in multiple endocrine neoplasia type 2

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Abstract

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant cancer syndrome caused by missense gain-of-function mutations of the RET proto-oncogene with a strong penetrance of medullary thyroid carcinoma (MTC) and a intense genotype–phenotype correlation. Recommendations on the timing of early thyroidectomy are based on RET mutations concerning the age of onset of MTC. Impact of RET-mutation predicting aggressiveness and prognosis is limited. Carriers of different RET mutations reveal a broad spectrum of MTC aggressiveness during follow up. The RET mutation seems to be the first step responsible for the age of onset of MTC, while presumably, different regulatory events determine the long term behavior of the tumour.

Keywords: multiple endocrine neoplasia type 2, RET mutation, medullary thyroid carcinoma, pheochromocytoma, tyrosine kinase inhibitors

Introduction

Genotype-Phenotype Correlation in MEN 2

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant cancer syndrome caused by gain of function mutations in the RET proto-oncogene. It has a strong penetrance of medullary thyroid carcinoma (MTC) and can be associated with bilateral pheochromcytomas and primary hyperparathyroidism as well with other disorders. Over 100 RET point mutations have been identified in patients with MEN 2 [1]. The codon specific RET mutation suggests a predilection toward a particular phenotype with strong genotype–phenotype correlations [2]. Different mutations in the RET gene produce varying phenotypes with different penetrance and age of onset of MTC, and the presence or absence of other endocrine neoplasms. Three different clinical variants, MEN2 A with the subgroup familial medullary thyroid carcinoma (FMTC) and MEN 2B, can be described. In 95% of patients with MEN 2A, RET mutations occur in codon 634 in exon 11 or in codons 609, 611, 618, and 620 in exon 10. The presence of any germline mutation at codon 634 is highly associated with the development of pheochromocytoma and also hyperparathyroidism. The large majority of patients with MEN2B have mutations in exon 16 (M918T) and less often exon 15 (A883F). FMTC is seen in all exons, mainly in exon 13-15. Age at pheochromocytoma diagnosis significantly varied with mutations in the different RET exons, with the earliest diagnosis associated with mutations in exon 16 codon 918 (MEN 2B), followed by exons 11, codon 634 (MEN 2A) and exon 10 [3]. MEN 2 disease genotype-phenotype correlations can also predict the risk of individual patients developing MTC. RET mutations are classified into three RET mutation risk levels regarding the age of onset and the potential aggressiveness of MTC [4].

Prophylactic Thyroidectomy-Cure of Patients from Medullary Thyroid Carcinoma

The RET dependent age of onset of MTC is the basis for clinical decision making when managing patients and families with MEN 2. Recommendations for the timing of prophylactic thyroidectomy and the extent of surgical resection are based on a model that utilizes genotype-phenotype correlations to stratify mutations into risk three risk-levels, namely moderate risk mutations (exon 10,

Table 1. Risk stratification and management of patients with different RET mutations [4].

ATA risk level[1] (2015)	**moderate**	**high**	**highest**
mutated codon	533, 609, 611, 618, 620, 630, 631, 768, 790, 804, 891,	634 / 883	918
MEN 2 subtype	FMTC/MEN 2	MEN 2A / MEN 2B	MEN 2B
MTC age of onset	5 years or older	before the age of 5 years	first year of life
Timing of prophylactic thyroidectomy	when calcitonin rises/ age 5 or 10 years	before the age of 5 years	first months of life
Screening for Pheo	start at 16 years, periodically	start at 11 years, annually	start at 11 years, annually
Screening for HPT	start at 16 years, periodically	start at 11 years, annually	-

exon 13-15), high risk mutations (exon 11 codon 634, the classical MEN 2A mutation and exon 15 codon 883) and highest risk risk mutation (exon 16 codon 918) (Table 1). For children with codon 918 mutations (found in >95% of MEN 2B patients), the highest risk group, operation is suggested as early as possible, preferably within the first year after birth despite the higher operative morbidity. In patients with the high risk (classical MEN 2A group) thyroidectomy is recommended before the age of five years. Timing of thyroidectomy in the moderate risk-group is challenging: there is a lower age related penetrance of MTC and a substantial variability in the age at which MTC develops, even within different families with MEN 2 due to the same RET mutation. Within each risk group defined by the RET mutation, the individual risk of developing C cell hyperplasia/ micro-MTC can be assessed by determining the calctonin level; when calcitonin exceed the upper limit of the referrence range, a thyroidectomy should be planned. Prophylactic thyroidectomy should be performed before MTC development or while the tumour is confined to the thyroid gland [5,6]. The early identification and risk classification of asymptomatic infants and young children as RET carriers is crucial for the 'window of opportunity', within which total thyroidectomy alone represents adequate therapy [4]. The age related onset of MTC across the RET-mutation risk categories suggests an age related progression of MTC development [7].

Aggressiveness of Hereditary MTC: Does the age at which MTC Develops Predict its Long-term Behavior?

Only few studies have focused on the aggressiveness of MTC in different risk groups in the follow up. The natural course or growth rate of MTC of patients with RET mutation cannot be measured directly. Tumour diameter at the surgical pathology level and age at the time of MTC diagnosis can be compared between asymptomatic carriers and index patients. By this procedure tumour growth can be approximated. A tumour growth of lymph node-negative MTC, was 0.4–0.5 mm/year with no difference between carriers of high risk (RET634) and moderate risk (exon 13-15) mutations (8). Node-positive carriers revealed an annual rate of lymph node metastasis of 0.6–0.7 nodes independent of the risk group. In an additional study of hereditary MTC age related progression of MTC within histopathological groups (normal/C-cell hyperplasia, node-negative, node-positive MTC) was significantly different between different risk groups, (7) but the development of lymph node negative into lymph node positive occurred at similar time intervals in all risk groups (8 to 12 years). This is also in line with the observation that patients with high- and moderate -risk RET mutation had similar overall survival and development of distant metastatic disease after initial onset of MTC (9). Once MTC developed, the clinical course was statistically equivalent in terms of distant metastases and survival. These findings support the hypothesis that all hereditary MTC progressed with comparable aggressiveness after the initial signs of MTC, independent of risk classification.

The only and most important prognostic factor in MEN 2 patients is tumour stage at diagnosis, the percentage of advanced tumour stage is higher in higher risk groups, the survival rates are lower in higher risk groups. In a study that included a group with the highest risk mutation,

among 73 patients with MEN 2B patients, 20% were cured and, astonishingly the 5-, 10-, and 20-year cancer-specific survival rates were 85%, 74%, and 58%, respectively [10]. These findings were comparable to those from the recent SEER Study of 2400 patients with MTC that were not differentiated between sporadic and hereditary showing a 5 and 10 year survival of 83% and 72 % respectively [11]. In comparing the survival data of patients with MEN 2 or sporadic MTC, the disease course and tumour stage dependent survival appeared similar [12-14]. In MEN 2 MTC onset is largely driven by the respective RET mutation. Thereafter equally aggressive tumours become apparent across all RET mutations risk levels. This raises important questions about the molecular events, aside from the RET mutation, that occur in the different risk groups after manifestation of MTC.

Tumourbiology of MTC

Germline dominant-activating mutations in the RET protooncogene, a receptor tyrosine kinase, have been identified as primary initiating event and the most common driver gene in MTC, that causes MTC and other components of MEN 2. The role of RET is further supported by somatic point mutations in the RET proto-oncogene in up to 43% of sporadic MTC, mostly the RET M918T mutation [15,16]. Oncogenic RET mutation induces its ligand-independent constitutive trans-autophosphorylation, stimulates multiple downstream pathways that promote cell growth, proliferation, survival and differentiation, and seems to be a prerequisite for cellular transformation. Independent of the type of activating mutation, the final effect is uncontrolled activation of the MAPK and the PI3K pathways that results in uncontrolled growth and cell de-differentiation. Sporadic MTC cases typically have few mutations beside RET mutations. The only other mutations identified in sporadic MTC are NRAS, KRAS and HRAS and are almost mutually exclusive with RET mutation supporting the belief that RET -mediated oncogenetic transformation occurs separately from RAS [17]. RAS mutations in sporadic RET negative MTC patient itself had no significant prognostic value in predicting tumour aggressiveness [18].

Inactivation or activation of regulatory pathways as potential mediators of C-cell transformation was found in MTC tissue: inactivation of Rb1 regulatory pathway [19], inhibition of the pro-apoptotic transcription factor ATF4 was associated with tumour progression and aggressiveness [20]. Dysregulation of miRNAs have been shown in MTC, participating in tumour progression [21-23]. There is a significant up-regulation of miR-182 in MTC tissues harboring mutated RET in codons 918 and 634 compared to normal thyroid tissue, leading to a more malignant phenotype [24]. When comparing the whole-gene expression profile of MTC with regard to the type of RET gene mutation and the cancer genetic background (hereditary vs sporadic), no distinct differences were found in the gene expression profiles of hereditary and sporadic MTCs [25]. More work is needed on the molecular pathogenesis of MTC and on the genetic defects and altered cellular signaling pathways involved in the tu¬morigenesis of human MTC.

The knowledge of molecular pathogenesis has led to effective targeted therapies in clinical use, like the RET tyrosine kinase inhibitors vandetanib and carbozantinib [26,27]. Several new RET inhibitors have been reported with increased potency and increased specificity like Alectinib, BLU-667, LOXO-292, which are in phase I clinical trails. New strategies are being developed to inhibit the RAS proteins, which are potential therapeutic targets in MTC. The inhibition of prenyl-binding chaperone protein PDE6δ, which is necessary for RAS membrane localization, and the farnesyltransferase inhibitors (FTIs), (tipifarnib), and antisense oligonucleotides, which inhibt KRAS are studied in preclinical trails [28]. The uncovered regulation between RET, miR-182 and HES1 clearly points toward new therapeutic options by using components of the RET-NF- κB/miR-182-HES1/Notch1 axis as potential key targets to restrict cancer progression [24]. Identification of additional targets, particularly ones that play a prominent role in regulation of both proliferation and apoptosis, would lead to novel and much-needed therapeutic options for MTC malignancy.

Disclosure

The authors have nothing to disclose.

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