Evaluation of genetic background of sporadic medullary thyroid carcinomas (MTC)

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Objectives

Medullary thyroid carcinoma (MTC) occurs in inherited or sporadic form. Although almost all patients with inherited MTC carry the *RET* proto-oncogene germline mutation, somatic mutations in the *RET* gene are only in a half of sporadic MTC cases. In some sporadic MTC, mutations in *RAS* genes are detected. However, the genetic causes of many sporadic MTC cases are still unknown.

The aim of the study was to detect the genetic variants in sporadic MTC not only in known causing genes (*RET* and *RAS*), but also in other cancer genes using next generation sequncing (NGS).

Methods

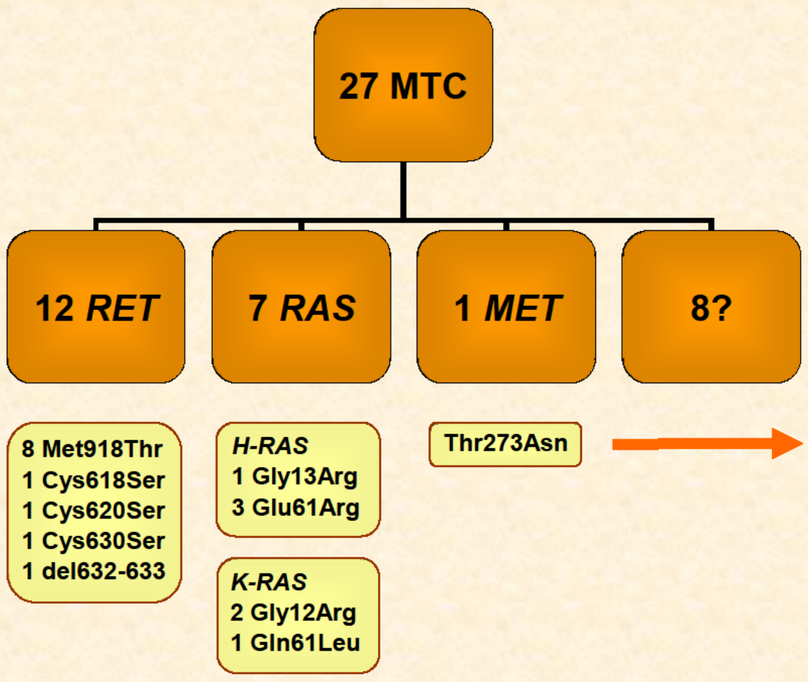
DNAs from fresh frozen thyroid tissues of 27 sporadic MTCs were extracted. The next-generation sequencing (NGS) approach was used to target 175 exonic regions of 26 genes involved in tumors. The samples were prepared using a TruSight Tumor panel (Illumina) and sequenced by a MiSeq sequencer (Illumina). Analysis of variants was performed by MiSeq Reporter software and evaluated by Illumina Variant Studio software. *RET* and *HRAS* genes were analysed separately using direct sequencing by CEQ 8000 (Beckman Coulter), because these two genes were not included in the panel.

AKT1	*EGFR*	*GNAS*	*NRAS*	*STK11*
ALK	*ERBB2*	*KIT*	*PDGFRA*	*TP53*
APC	*FBXW7*	*KRAS*	*PIK3CA*	
BRAF	*FGFR2*	*MAP2K1*	*PTEN*	
CDH1	*FOXL2*	*MET*	*SMAD4*	
CTNNB1	*GNAQ*	*MSH6*	*SRC*	

Genes included in TruSight Tumor panel (Illumina)

Results

Mutations in the *RET* gene were detected in 12 patients. In four patients we found mutations in *HRAS* gene. Using NGS panel, mutations in *KRAS* gene in three patients were detected. In one patient unknown *MET* mutation was found.



***MET* mutation:** In 62-year male patient with 25mm MTC beside the somatic mutation Gln61Arg in *HRAS* gene unknown missense mutation Thr273Asn in gene for the hepatocyte growth factor receptor - *MET* (Figure 1) was detected. Exon 2 was then analyzed in DNA from peripheral blood of the patient and the germline origin of the mutation was found out (Figure 2).

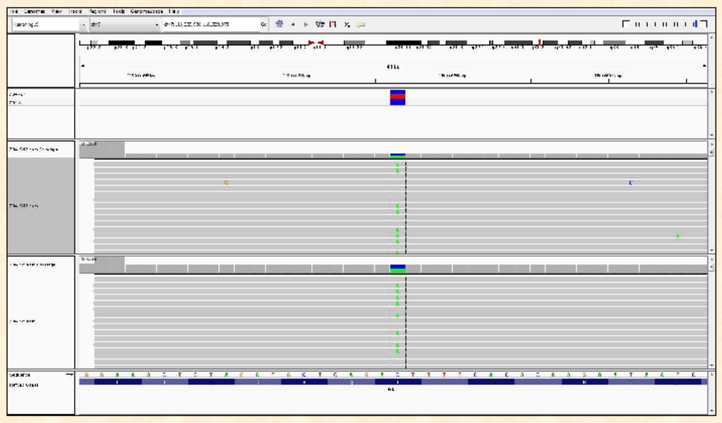


Figure 1. The Thr273Asn mutation in *MET* gene - NGS sequencing of DNA from tissue (displayed in IGV browser). The coverage of the region was 31 606 reads with 15 685 reads of altered allele.

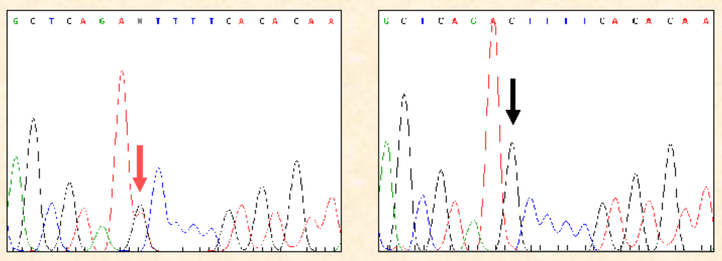


Figure 2. Mutation Thr273Asn in *MET* gene – Sanger sequencing of DNA from peripheral blood of patient (left) and Sanger sequencing of exon 2 in patients without the mutation (right).

The *MET* gene is located on chromosome 7 and consists of 21 exons. Codon 273 is located in exon 2 in extracellular SEMA domain (Figure 3). The missense mutation Thr273Asn was identified as deleterious and possibly damaging in software SIFT and PolyPhen-2, respectively.

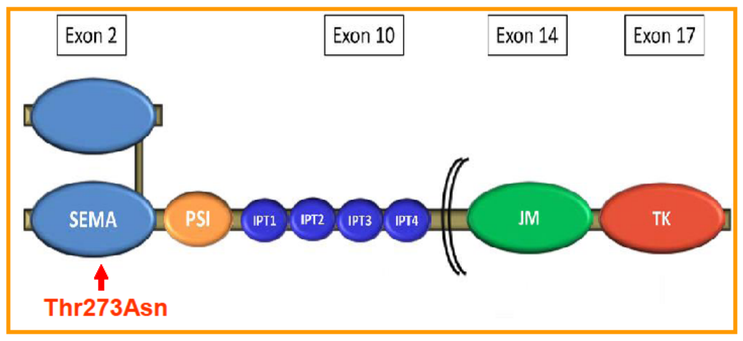


Figure 3. Structure of c-MET receptor and identified Thr273Asn mutation. *SEMA - Semaphorin domain; PSI - plexins-semaphorin-integrin domain; IPT1-4, four immunoglobulin plexins transcription domains; JM - juxtamembrane domain; TK – tyrosine kinase domain (Gelsomino et al 2014).*

Reference: Gelsomino F, Rossi G, Tiseo M. MET and Small-Cell Lung Cancer. Cancers (Basel). 2014 Oct 13;6(4):2100-15.

Conclusion

In our cohort of sporadic MTC tissues, mutations in 19 patients (70.3%) were detected – *RET* mutations in 44.4% and *RAS* mutations in 25.9%. Except of known mutations in *RET* and *RAS* genes, the unknown variant in conserved sequence of *MET* gene was revealed which was identified as deleterious and possibly damaging in software SIFT and PolyPhen-2, respectively. In 8 patients we did not observed any genetic changes in studied genes, thus the study of other genes potentially involved in carcinogenesis of sporadic MTC will continue.

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