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

V. Sykorova1, S. Dvorakova1, J. Vcelak1, E. Vaclavikova1, D. Kodetova2, P. Lastuvka2, J. Betcha3, P. Vizek4, P. Sykorova4, P. Bavor5, B. Bendlova1

1Department of Molecular Endocrinology, Institute of Endocrinology, Prague; 2Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague; 3Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague; 4Department of Nuclear Medicine and Endocrinology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague; 5Department of Surgery, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic

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 sequencing (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 TrueSight 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.

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).

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.

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 observe any genetic changes in studied genes, thus the study of other genes potentially involved in carcinogenesis of sporadic MTC will continue.