GENETIC SCREENING OF RET CAN IDENTIFY NEW MUTATIONS EVEN AFTER 20 YEARS

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Background/Purpose: In the last 20 years we performed RET genetic screening in more than 1000 hereditary or sporadic MTC patients.

Methods: RET analysis was performed in constitutive and/or somatic DNA by direct sequencing. TA cloning was performed to characterize new mutations and deletions. Site-directed mutagenesis, focus formation and soft agar assays were performed to test in vitro the activity of new mutations. The Align GVGD program was employed for the in silico analysis.

Results: we identified 3 new RET alterations. The first was a 7bp “somatic” in frame deletion in exon 11 encompassing codon 629-631. The second showed the simultaneous presence of a “somatic” E616Q mutation in exon 10 and a “somatic” C630G mutation in exon 11 on different alleles. Moreover, in the same patient, we found an alternative splicing causing the in frame skip of exon 10 in the allele carrying the C630G mutation. The third alteration was a new “germline” mutation (E632K, exon 11) and was found in an apparently sporadic MTC. According to the in vitro and in silico tests, both E616Q and E632K RET mutations were not transforming while the C630G RET mutation showed a high transforming activity.

Discussion & Conclusion: 1) RET genetic screening should be performed by sequencing analysis in all MTC patients to detect also new RET mutations that would be missed when looking only at the “hot spot” mutations; 2) all new mutations must be evaluated by in silico and/or in vitro analysis to define their transforming ability since in some cases they may be inactive mutations.