Next-Generation Sequencing Enables Identification of RET Rearrangements in Papillary Thyroid Cancer

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Background

- RET rearrangements have emerged as a new actionable alteration in papillary thyroid cancers (PTC).
- These fusions may involve many partner genes, are thought to be mutually exclusive with other oncogenic drivers and their screening is recommended based on fluorescence in situ hybridization (FISH), RT-PCR or next-generation sequencing (NGS).
- The aim of this study is to evaluate the use of NGS as a standard molecular technique to characterize RET gene fusions.

Methods

Sixty PTC cases without the BRAF v600E and/or TERT promoter mutations were selected retrospectively in two institutions. Molecular alterations were evaluated using the Oncomine Focus Assay, and all samples were analyzed by RT-PCR using the Idylla GeneFusion Assay. Fusion positive samples were confirmed with FISH with break-apart probes (Figure 1).

Results

All DNA samples were acceptable for analysis, whereas 4 RNA samples failed quality control NGS parameters. RET fusions were detected in 11 cases (18%): CCDC6::RET (C1-R12) in 8 samples, NCOA4::RET (N6-R12) in 2 samples, and 1 RET expression imbalance positive sample without gene partner characterization. All 11 cases were validated by both FISH and RT-PCR (Figure 2).

Besides, 4 more cases presented NGS RET expression imbalance but none of them were confirmed neither by RT-PCR nor by FISH and considered false positive results. There were no false negative NGS cases identified in parallel by RT-PCR. Overall, a positive predictive value of 89% and a negative predictive value of 90% were estimated, reaching a concordance of 89% compared to NGS (Figure 3).

Regarding DNA analysis, NRAS p.Q61R/K was the most prevalent alteration in 13 cases (22%) followed by 2 cases with Kras mutations (p.G12S and p.Q61R). Other potentially actionable fusions found in our series were 2 ETV6::NTRK3 (E4:N14), 2 STRN::ALK (S3:A20) and 1 EML4::ALK (E6:A20) (Figure 4).

Conclusions

- Molecular screening in non-BRAF PTC patients is useful to identify patients harboring RET fusions who may benefit from targeted therapies.
- As other potentially actionable gene fusions are also found in these patients, routine implementation of NGS analysis warrants a comprehensive biomarker study.

Figure 1. Schematic summary of the study design for detection of cases with RET gene fusions in PTC. Positive cases for RET gene rearrangements detected by NGS and RT-PCR were validated by FISH, including NGS RET expression imbalance results.

Figure 2. Results of the RET positive cases by NGS, RT-PCR and FISH techniques. Cases with concordant results among the three techniques are displayed in green (n=11), whereas discordant are shown in red (n=4).

Figure 3. H&E and FISH images of three representative papillary thyroid cancers included in the study. These cases showed typical split signals considered positive by FISH for the presence of RET gene rearrangement.

Figure 4. NGS Oncomine Focus Assay results. *Only 1 of the 5 RET expression imbalance cases detected by NGS was confirmed by RT-PCR and FISH. **The other 4 cases were not confirmed and therefore added to the ‘None detected’.