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Original Article

Using Ion Torrent sequencing to study genetic mutation profiles of fatal thyroid cancers

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Background/Purpose: Surgery followed by radioiodine is a mainstay of treatment for thyroid cancers of follicular origins. However, about 5% of the thyroid cancers are non-operable and/or radioiodine-refractory diseases, which are either locally advanced or metastatic and result in a survival of less than 5 years. How to treat this population of thyroid cancer patients becomes a critical issue requiring further understanding of the tumor's genetic information.
Methods: We used formalin-fixed paraffin-embedded specimens of 22 fatal thyroid cancers and their corresponding non-tumor parts, if available, to yield genomic DNA, and applied the Ion Torrent™ Personal Genome Machine (IT-PGM) System (Life Technologies), a next generation

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sequencing technology, to interrogate 740 mutational hotspots in 46 oncogenes. We further validated the results by conventional direct sequencing.
Results: We confirmed 21 mutations of 11 oncogenes in the 22 fatal thyroid cancer samples. Among them, the *MET* p.N375S and *MLH1* p.V384D mutations, each was detected in two cases, and has rarely been found to be involved in thyroid cancer pathogenesis before. We also identified homozygous *PDGFRA* p.V824V mutation in eight out of the 22 cases, while the non-tumor counterparts carried heterozygous *PDGFRA* p.V824V mutation. We noted that the Ion Torrent technique unfortunately showed high false positive rates for detecting *EGFR* mutations in thyroid cancers.
Conclusion: The extensive genetic studies provide new insights to future targeted therapy in these patients. IT-PGM proved to be valuable for comprehensively searching genetic mutations in potentially fatal thyroid cancers.

Introduction

Thyroid cancer is the most common endocrine malignancy derived from thyroid follicular epithelial cells or parafollicular C cells. The histologic types of thyroid cancers comprise those derived from thyroid follicular epithelial cells, including well differentiated papillary thyroid cancer (PTC) (88%) and follicular thyroid cancer (FTC) (8%), and undifferentiated anaplastic thyroid carcinomas (ATC) (1%); while medullary thyroid carcinoma (MTC) (1.5%) is derived from parafollicular C cells (Cancer Registry Annual Report, Taiwan 2008).[1] Although most of the thyroid cancers have favorable prognosis, around 5% of them initially manifest as or will progress to surgically inoperable and radioactive iodine-refractory diseases, which persist, recur or metastasize to distant sites and result in mortality within 5 years.[2] Besides, a significant recurrence rate about 20% at 10 years and 30% at 20 years of follow-up is observed after initial treatment.[3,4] How to identify patients with increased risk of advanced diseases and disease recurrence after initial treatment becomes an important issue for reducing the recurrence rate as well as providing curative treatment for these advanced thyroid cancers.

Advancement in our knowledge of the molecular taxonomy underlying the development of cancer has opened the way for patient-specific therapy by targeting the mutated genes that are causally responsible for oncogenesis of thyroid cancers. Besides, molecular analyses can largely aid pre-operative decision making in patients with unsatisfactory cytological results.[5] In papillary thyroid cancers, non-overlapping mutations of *RET*, *NTRK*, *RAS* and *BRAF*,[6] all of them activate a downstream effector MAPK, account for about 50–70% of cases.[7,8] Rare cases of *AKT1*, *PIK3CA* and *PTEN* mutations are also found in thyroid cancers.[9] On the other hand, follicular thyroid cancers carry almost exclusively *RAS* mutations in Southern Taiwan,[10] as well as in our case series (unpublished data). Previously, there are about 30–50% of thyroid cancers harboring unknown driver mutations.[9,11] Fortunately, the recent analysis of the papillary thyroid cancer by The Cancer Genome Atlas (TCGA) Research Network has brought down the percentage of PTCs with unknown genetic drivers to 3.5%.[12]

The exceptional success of the ABL tyrosine kinase inhibitor, imatinib, in chronic myeloid leukemia patients with *BCR-ABL* translocations has revolutionized treatment of this disease.[13] Similarly, about 10–20% of thyroid cancer patients harbor *RET* and *NTRK1* (two tyrosine kinases) gene rearrangements that are potentially treatable.[14,15] In our preliminary data, about 56% of papillary thyroid cancer, and 26% of anaplastic thyroid cancer patients have *BRAF* V600E mutations, to which there are effective inhibitors, vemurafenib (PLX 4032, Roche)[16] and dabrafenib,[17] approved for melanoma treatment by the US FDA. Besides, oncogenes encoding signaling effectors in MAPK and PI3K pathways are supposed to be "druggable", since compounds targeting oncoproteins in the MAPK and PI3K pathways are currently in preclinical and clinical development.[18–20] All of the above genetic alteration-harboring thyroid cancers will be potentially treatable once the underlying mutations are identified.

In this report, we aim to find somatic point mutations in coding regions of selected oncogenes in surgically incurable, radioiodine refractory, thus fatal thyroid cancer tissues.[21] The ultimate goal of the study is to find the drug targets of the advanced thyroid cancers.

Patients and methods

Sample collection

We analyzed 22 tumor tissues and their corresponding non-tumor parts from patients with fatal thyroid cancers diagnosed and treated from 2004 to 2011 at National Taiwan University Hospital, an affiliated teaching hospital of the National Taiwan University College of Medicine. Five of the patients were male (70 ± 10.1 y/o, 3 ATC and 2 PTC), and 17 were female (71.1 ± 8.2 y/o, 13 ATC, 3 PTC, and 1 MTC) patients. All the fatal thyroid cancer patients died of their thyroid cancers. Two of these patients died within 10 days after diagnosis (Table 1). The median and mean survival

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