Identification of Novel Oncogenic Mutations in Thyroid Cancer

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BACKGROUND: Thyroid cancer patients frequently have favorable outcomes. However, a small subset develops aggressive disease refractory to traditional treatments. Therefore, we sought to characterize oncogenic mutations in thyroid cancers to identify novel therapeutic targets that may benefit patients with advanced, refractory disease.

STUDY DESIGN: Data on 239 thyroid cancer specimens collected between January 2009 and September 2014 were obtained from the Dana Farber/Brigham and Women's Cancer Center. The tumors were analyzed with the OncoMap-4 or OncoPanel high-throughput genotyping platforms that survey up to 275 cancer genes and 91 introns for DNA rearrangement.

RESULTS: Of the 239 thyroid cancer specimens, 128 (54%) had oncogenic mutations detected. These 128 tumors had 351 different mutations detected in 129 oncogenes or tumor suppressors. Examination of the 128 specimens demonstrated that 55% (n = 70) had 1 oncogenic mutation, and 45% (n = 48) had more than 1 mutation. The 351 oncogenic mutations were in papillary (85%), follicular (4%), medullary (7%), and anaplastic (4%) thyroid cancers. Analysis revealed that 2.3% (n = 3 genes) of the somatic gene mutations were novel. These included *AR* (n = 1), *MPL* (n = 2), and *EXT2* (n = 1), which were present in 4 different papillary thyroid cancer specimens. New mutations were found in an additional 13 genes known to have altered protein expression in thyroid cancer: *BLM*, *CBL*, *CIITA*, *EP300*, *GSTM5*, *LMO2*, *PRAME*, *SBDS*, *SF1*, *TET2*, *TNFAIP3*, *XPO1*, and *ZRSR2*.

CONCLUSIONS: This analysis revealed that several previously unreported oncogenic gene mutations exist in thyroid cancers and may be targets for the development of future therapies. Further investigation into the role of these genes is warranted. (J Am Coll Surg 2016;222:1036–1043.

Thyroid cancer is the most common endocrine malignancy in the United States, with 62,450 new cases estimated to have been diagnosed in 2015, as well as nearly 2000 deaths.[1] Most patients with this disease have favorable outcomes after conventional surgical therapy with or without radioactive iodine ablation and thyroid stimulating hormone suppression. However, a small subset of patients develops aggressive, invasive disease that is refractory to traditional treatments and is associated with significantly increased mortality. The overall 5-year survival for patients with treatment-sensitive, early stage differentiated thyroid cancer is nearly 100%.[2] Meanwhile, patients with more aggressive, advanced treatment-resistant tumors have 5-year survivals that range from 7% to 81%.[2] For these patients, therapeutic options are limited. As a consequence, alternative therapies are needed to treat patients with resistant thyroid tumors.

Characterization of cancer genomes has revealed a vast array of genetic alterations that have diagnostic, prognostic, and therapeutic implications. Medullary thyroid cancer (MTC), for example, is associated with somatic mutations in the *RET* (rearranged during transfection) proto-oncogene.[3-5] Medullary thyroid cancer also represents one of the types of thyroid cancer that is aggressive,

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Abbreviations and Acronyms

ATC = anaplastic thyroid cancer
DF/BWCC = Dana Farber/Brigham and Women's Cancer Center
FTC = follicular thyroid cancer
MTC = medullary thyroid cancer
PTC = papillary thyroid cancer
TCGA = The Cancer Genome Atlas
THPA = The Human Protein Atlas

has increased mortality compared with differentiated thyroid cancer, and is resistant to radioactive iodine and thyroid stimulating hormone suppression.[6] More than 70 mutations have been identified within the *RET* gene sequence, and each of these point mutations is associated with differing levels of aggressiveness, prognostic implications, and treatment recommendations.[7] In the past 5 to 10 years, receptor tyrosine kinase inhibitors targeted at the catalytic domain of *RET,* such as vandetanib or cabozantinib, have been used, but with variable response rates ranging from stable disease to partial response.[8-13] Although some progress has been made in the treatment of MTC, these therapies are not curative. In addition, few if any targeted treatments exist for patients with refractory tumors, such as insular variant of follicular thyroid cancer (FTC), poorly differentiated thyroid cancer, anaplastic thyroid cancer (ATC), and some MTCs. As a result, alternative treatments are needed for these patients, and identifying novel gene targets may play a role in the discovery of new therapies.

Research into genetic alterations in human thyroid cancer has revealed several genes that are known to play a role in oncogenesis. These include mutations of *BRAF*, *RAS*, *PTEN*, *RET*, and others.[14-18] Investigation into the utility of targeting these mutations as therapies is promising and ongoing. However, with the rapidly advancing field of genomics, additional genetic mutations likely exist that play an important role in thyroid cancer oncogenesis. In this study, we sought to characterize novel oncogenic mutations in human thyroid cancers in order to identify potential targets for gene therapies that may benefit patients with refractory advanced disease.

METHODS

We obtained data obtained from the Dana Farber/Brigham and Women's Cancer Center (DF/BWCC) PROFILE clinical research study on 239 consecutively collected thyroid cancer specimens. Consenting adult patients (18 years of age or older), who underwent surgery for pathologically confirmed thyroid cancer between January 2009 and September 2014 at our tertiary referral center, were enrolled in a genotyping mutation detecting study (Institutional review board, IRB #11-104). Molecular diagnostics were performed in the Molecular Diagnostics Division of the Center for Advanced Molecular Diagnostics, which is certified by the Clinical Laboratory Improvement Amendment (CLIA). Data from the DF/BWCC were de-identified and contained information on the mutational status of a panel of oncogenes as well as thyroid cancer type, but not histologic subtypes or variants. All specimens were confirmed by at least 2 separate board-certified pathologists. Our primary outcomes measure was identification of novel oncogenic mutations, which was planned before data collection. Institutional Review Board exemption was obtained from the DF/BWCC Office for the Protection of Research Subjects.

All tumor specimens and normal control thyroid tissue were analyzed with either the OncoMap-4 or OncoPanel platforms, which have been previously described.[19-27] These high-throughput systems use chip-based mass spectrometry technology (Sequenom) designed to detect somatic mutations and variants in DNA extracted from fresh, frozen, or formalin-fixed, paraffin-embedded samples. These platforms do not sequence the entire genome. Before September 30, 2013, tissue samples were analyzed with the OncoMap profile system. The OncoMap assay consists of 460 variants in 33 known oncogenes and tumor suppressors, many of which are known to show response or resistance to targeted therapies. After September 30, 2013, the cancer specimens were examined with the OncoPanel genetic test system. This platform analyzes exonic DNA sequences of 275 cancer genes and 91 introns across 30 genes for rearrangement detection. Variant analysis includes examination of somatic variation, copy number variations, and structural variants. In the OncoMap system, the variants are qualified as either present, absent, or no call; OncoPanel reports variants as present only for somatic variation. For the purpose of this study, only mutations that were classified as present were included.

In order to identify which of the somatic mutations were novel, we compared our results to established gene mutations associated with thyroid cancer in The Cancer Genome Atlas (TCGA) project, cBioPortal for Cancer Genomics, and The Human Protein Atlas (THPA).[28-30] The TCGA is a comprehensive research network that catalogues results of cancer genome analysis and is supported by the US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, and National Human Genome Research Institute. Similarly, the cBioPortal for Cancer Genomics, which is supported by the Center for Molecular Oncology and the Computational Biology Center at Memorial Sloan-Kettering Cancer Center, offers access to large-scale cancer genomics datasets and contains 24,293 samples