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RESEARCH LETTER

A novel nonsense *EIF1AX* mutation identified in a thyroid nodule histologically diagnosed as oncocytic carcinoma

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Introduction

Thyroid nodules are common in the general population (prevalence 16–68% depending on the screening method and population analyzed) [1]. Over 90% of the nodules detected are benign and will never undergo transformation [2, 3]. Accurate preoperative identification of these lesions reduces the risk of unneeded surgery [2]. Preoperative diagnoses of malignancy are based mainly on suspicious ultrasonographic findings verified by cytological examination of fine-needle aspirates (FNA) [4, 5]. For cytologically indeterminate nodules (~25%) [4, 6], molecular analyses of the aspirates can often help to identify or exclude malignancy [7]. A promising tool for this purpose is the ThyroSeq v2 mutation panel [8], which can identify single-nucleotide variants, indels, and gene fusions currently known to drive thyroid carcinogenesis, including several recently identified by The Cancer Genome Analysis (TCGA) network [9]. Using a similar NGS-based approach, we identified a novel *EIF1AX* mutation in a cytologically indeterminate thyroid nodule that ultimately proved to be an angioinvasive oncocytic thyroid carcinoma.

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Methods

Case presentation

A 55-year-old Italian man underwent thyroidectomy for a 2.8-cm nodule in the right lobe that was cytologically indeterminate (TIR 3B—oncocytic nodule in the Italian Reporting System, corresponding to Bethesda class IV—Hürthle cell nodule) (Fig. 1a). The nodule was solid, well-circumscribed, and thickly encapsulated. Histological examination revealed a predominance of oncocytic cells in a microfollicular, trabecular pattern (Fig. 1b). Extensive sampling identified multiple foci of capsular and vascular invasion (Fig. 1c, d, respectively). The final diagnosis was angioinvasive oncocytic thyroid carcinoma (pT2 Nx Mx). It was administered a therapeutic activity of radioactive iodine for adjuvant purpose and post-therapeutic whole-body scan was negative. Sixteen months of follow-up have not revealed any evidence of residual/recurrent disease.

The patient had been diagnosed with myotonic dystrophy type I (DM1, MIM #160900) [10], an autosomal dominant neuromuscular disorder that also affected his mother, brother, two sisters, and two nephews. None of the patient's first-degree relatives, regardless of their DM1 status, had histories of thyroid cancer or other malignancies. Molecular testing for DM1 revealed an abnormal CTG expansion in the 3′ UTR of *DMPK* (130–350 CTG repeats; negative values: 5–35) [11].

Molecular analysis of the thyroid nodule

FNA of the thyroid nodule was processed for cytology with ThinPrep5000™ system (Hologic Co.). The material remaining after cytological analysis was used for molecular profiling. Genetic analysis was performed on the Ion S5 system (Thermo Fisher Scientific) using two custom

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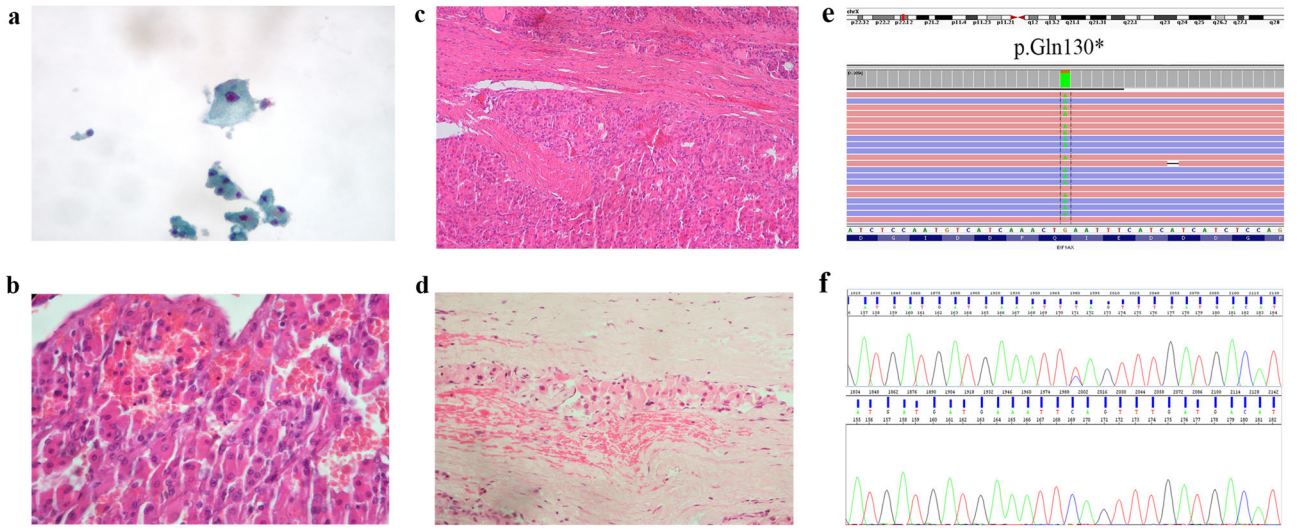

Fig. 1 *EIF1AX* mutation in an oncocytic thyroid carcinoma. **a** Nodule cytologically classified as indeterminate TIR 3B (oncocytic nodule) of the Italian Reporting System corresponding to Bethesda class IV—Hürthle cell nodule (Papanicolau-stained slide, ×500) **b**, **c**, **d** Definitive histological diagnosis of angioinvasive oncocytic thyroid carcinoma (H&E-stained sections: (**b**) morphological details, ×250; (**c**) foci of capsular invasion, ×125; (**d**) foci of vascular invasion, ×125). **e** IGV visualization of the *EIF1AX* variant (chrX:20148675G>A; p.Gln130*). Pink and blue bars represent forward and reverse read strands, respectively. **f** Sanger Sequencing validation of the *EIF1AX* variant on tumor (upper panel) and normal (lower panel) thyroid tissues from FFPE samples

NGS multi-gene panels, which tested for single-nucleotide variants/small indels (DNA panel) and gene fusions (RNA panel) involving well-known thyroid cancer-related genes (e.g., *BRAF*, *RAS*, *EIF1AX*, *TERT*, *RET/PTC*, and *PAX8/PPARG* fusion) and others from our in-house database. DNAs and RNAs were isolated simultaneously using the All Prep DNA/RNA kit (QIAGEN) and measured with the Qubit dsDNA/RNA High Sensitivity assays (Thermo Fisher Scientific). Genes for the custom panel were selected based on literature data [8, 9, 12] and our in-house database, and custom primers for their targeted amplification were designed with the Ion AmpliSeq™ Designer (Thermo Fisher Scientific). Two libraries were created from 20 ng of DNA and 20 ng of RNA. The targeted amplification products were partially digested, phosphorylated, and ligated to Ion P1 adapter and Ion Xpress™ barcodes (Thermo Fisher Scientific). Libraries were pooled and clonally amplified on the Ion One Touch2 System. Sequencing was performed using Ion S5 sequencing solution on an Ion 530 chip (Thermo Fisher Scientific). Data were analyzed with Variant Caller v5.2, annotated with Ion Reporter 5.6 and wANNOVAR software and prioritized on the basis of their population frequency (Minor allele frequency <0.005). Variants were called when the position was covered by over 500 reads. The lower detection limits were set at 5% for SNVs and 15% for small indels. Predicted variant deleteriousness was assessed with wANNOVAR (http://wannovar.wglab.org/).

NGS results and the germ line/somatic status of each variant were further validated by Sanger sequencing analysis (as described elsewhere [13]) of formalin-fixed, paraffin-embedded (FFPE) surgical specimens of normal and neoplastic thyroid tissues. Primer sequences are available upon request.

Results

Our amplicon-based NGS analysis of the cytologically indeterminate thyroid nodule FNA revealed a single somatic mutation involving exon 6 of the eukaryotic translation initiation factor 1A (*EIF1AX*) gene (NM_001412, c.C388T, p.Gln130*) (Fig. 1e), which is located on chromosome X. No mutations were detected in the well-known thyroid cancer-related genes, including *BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARG*, and *TERT*. The *EIF1AX* mutation was found in 82% of the reads. Sanger sequencing confirmed its presence in FFPE tumor slices but not in normal tissue from the unaffected lobe (Fig. 1f). The high allele frequency (82%) can be attributed to *EIF1AX*'s localization on the X chromosome. The presence of two alleles probably reflects contamination from non-tumor cells. The deleteriousness of the *EIF1AX* stopgain variant was predicted by MutationTaster, CADD, DANN, Fathmm-MKL, GenoCanyon, GERP++, phyloP100way_mammalian, pastCons20way_mammalian, and SiPhy_29way_logOdds (data not shown). Sequencing of DNA from the patient's normal thyroid tissue also revealed a germ line missense mutation in *CHEK2* (NM_007194.3, c.1067C>T, p.Ser356Leu), which controls cell cycling and DNA repair.

Discussion

EIF1AX mutations have been documented in papillary, poorly differentiated, and anaplastic thyroid carcinomas—with or without known driver mutations affecting *RAS* or *TP53*—and, more recently, in benign thyroid nodules [14] and a Hürthle cell carcinoma [15]. Most of these mutations affected the sequence encoding the N-terminal tail of eIF1A. [9, 12, 14], whereas the novel mutation we found, p.Gln130*, involves the sequence encoding eIF1A's C-terminal. Few cancer-related mutations have been described in this area, and the only one reported in thyroid cancer is the A113_splice mutation [9, 12, 14], which seems to be the most prevalent *EIF1AX* mutation in thyroid malignancy [14]. Moreover, p.Gln130* is a nonsense mutation that results in a truncated protein. Nonsense *EIF1AX* mutations have been reported in cancers of the colon (p.Gly8*), esophagus (p.Glu99*), breast (p.Glu117*), and vagina (p.Glu139*) (http://www.cbioportal.org/, accessed January 2018). However, the p.Gln130* variant is the first eIF1A nonsense mutation reported in thyroid cancer and the second *EIF1AX* mutation of any type in an oncocytic thyroid carcinoma. Importantly, because *EIF1AX* is located on the X chromosome, our male patient's tumor harbored only truncated eIF1A proteins. The potential impact of this alteration is unclear. However, studies in yeast indicate that the C-terminal is necessary for eIF1A's interaction with EIF5B, which is essential for its wild-type translation in vivo. Yeast cells harboring eIF1A lacking the final 24 residues display decreased rates of translation initiation in vivo [16].

The possible role of our patient's germ line missense mutation in *CHEK2* is also unclear. Germ line mutations involving *CHEK2* have been implicated in inherited susceptibility to cancers of the breast, ovary, and prostate [17]. The p.Ser356Leu variant itself is annotated in ClinVar (rs121908703) as having "uncertain clinical significance." However, none of the patient's first-degree relatives had histories of any type of cancer, including that of the thyroid. Moreover, the germ line *CHEK2* p.Ser356Leu was also found in a brother of the patient's with DM1, which is reportedly associated with an increased risk of thyroid cancer [18–20]. However, it was also harbored by a second sibling without DM1 (data not shown), which also argues against the possibility of an association between *CHEK2* and *DMPK* gene.

In summary, we identified a novel *EIF1AX* mutation (c.388C>T) in a nodule ultimately diagnosed as oncocytic thyroid carcinoma. Functional studies are needed to better define its role in thyroid tumorigenesis. The possibility that it is a new driver of thyroid carcinogenesis is suggested by the absence of other somatic driver mutations in the FNA specimen we analyzed. It is also consistent with the fact that the mutation results in a truncated eIF1A protein lacking the C-terminal, which appears to play a key role in eukaryotic translation.

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Compliance with ethical standards

Ethics approval All procedures involving humans were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all subjects included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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