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ORIGINAL ARTICLE

EIF1AX mutation in thyroid nodules: a histopathologic analysis of 56 cases in the context of institutional practices

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Abstract

EIF1AX mutation has been identified as a driver mutation for papillary thyroid carcinoma (PTC) by The Cancer Genome Atlas (TCGA) study. Subsequent studies confirmed this mutation in PTC and Anaplastic Thyroid Carcinoma (ATC) but also reported *EIF1AX* mutation in Follicular nodular disease (FND) and benign thyroid nodules. In this study, we review thyroid nodules with *EIF1AX* mutation from two institutions: a tertiary care hospital (YNHH, n = 22) and a major cancer referral center (MSKCC, n = 34) and report the varying histomorphology in the context of additional genetic abnormalities and institutional practices. Pathology diagnoses were reviewed according to the WHO 5th edition and correlated with the type of *EIF1AX* mutation and additional concurrent molecular alterations, if any. Most cases were splice site type mutations. Cases consisted of 9 FND, 7 follicular (FA) or oncocytic adenomas (OA), 2 non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) and 38 follicular-cell derived thyroid carcinomas. Of 8 cases with isolated *EIF1AX* mutation, 7 were FND, FA or OA (88%) and one was an oncocytic carcinoma (12%). Of 12 cases with *EIF1AX* and one additional molecular alteration, 9 (75%) were FND, FA or OA, 2 (17%) were NIFTPs and one (8%) was a poorly differentiated thyroid carcinoma. All 36 cases with *EIF1AX* mutation and ≥ 2 molecular alterations were malignant (100%) and included *TP53* and *TERT* promoter mutations associated with ATC (n = 8) and high-grade follicular cell-derived non-anaplastic carcinoma (HGC, n = 2). Isolated *EIF1AX* mutation was noted only in thyroid nodules seen at YNHH and were predominantly encountered in benign thyroid nodules including FND. Accumulation of additional genetic abnormalities appears to be progressively associated with malignant tumors.

Keywords *EIF1AX* · Thyroid · Tumorigenesis · Molecular alterations · Risk of malignancy (ROM)

Introduction

The Eukaryotic Translation Initiation Factor 1A, X-Linked (*EIF1AX*) gene codes for the eukaryotic translation initiation factor 1A (eIF1A), an essential component of the 43S pre-initiation complex (PIC). eIF1A stabilizes the binding of the ternary complex (Met-tRNAi:eIF2:GTP) to the 40S ribosomal subunit to form the 43S PIC which is then recruited to the 5' end of capped mRNA to form the 48S pre-initiation complex. This complex subsequently starts the scanning process to locate the start codon and initiate protein translation [1, 2]. *EIF1AX* mutations alter the RNA-binding surface of eIF1A and result in defects in 43S and 48S preinitiation complex formation which interfere in protein translation [3]. Deregulation of translation initiation is common in tumorigenesis [4]. Furthermore, overexpression of *E1F1AX* increases the expression of Cyclin D1, a cell cycle regulator, triggering cell proliferation in vitro [5]. Mutations in *EIF1AX* gene were initially discovered in uveal melanomas; they have since been reported in other cancers including low-grade gliomas, lung, uterine, ovarian and papillary thyroid carcinomas (PTC) [6–9]. In thyroid, mutations in the *EIF1AX* gene were deemed to be driver events for thyroid carcinogenesis by the Thyroid Cancer Genome Atlas study [9], which reported *EIF1AX* mutations in 1.5% of well

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differentiated thyroid carcinomas with near-mutual exclusivity with other genetic abnormalities. Subsequent studies have confirmed the presence of *EIF1AX* mutation in around 1–2% of PTCs [9, 10], 5% of FTCS [11] and 10% of PDTCs and ATCs [12, 13]. The TCGA study was however limited by population selection and did not include benign lesions [9]. This mutation has since been confirmed in benign thyroid nodules [10, 14–16].

Significant variations in the risk of malignancy (ROM) conferred by isolated *EIF1AX* mutation are observed across studies, ranging from 13% to approximately 50% [10, 17–19] but tumors are typically low-risk [9, 10]. The ROM is higher in *EIF1AX* splice site mutation and when *EIF1AX* mutation co-exists with other molecular alterations such as *TP53* or *RAS* mutation where it correlates with aggressive phenotypes [4, 9, 10, 12–14, 20]. In light of the new World Health Organization fifth edition classification of endocrine tumors (WHO 5th ed.) [21], we explored the occurrence of *EIF1AX* mutation in thyroid lesions in two settings; we first report our experience with thyroid nodules with an indeterminate cytology and *EIF1AX* mutation as encountered in the general population at a tertiary hospital (YNHH). As the association of *EIF1AX* with malignant tumors has continually been underscored [9–15], we also sought to examine the distribution of *EIF1AX* mutation in various thyroid carcinomas by expanding our study to a separate set of patients with thyroid tumors from a referral cancer center (MSKCC) to see if the genetic abnormalities and their phenotypic correlates are influenced by institutional practices.

Materials and methods

Study patients

This study was approved by the Yale University and Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review boards. Two groups of patients were included in the study. The first group included patients from Yale-New Haven Hospital (YNHH) who had *EIF1AX* mutation detected preoperatively, and thyroid surgery performed between March 2016 and May 2023. This group included patients with molecular testing performed on FNA samples with indeterminate cytology diagnosis which were re-classified according to the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) 3rd edition. Those included Atypia of undetermined significance (AUS) and follicular neoplasm (FN)/oncocytic follicular neoplasm (OFN), i.e. TBSRTC category III or IV diagnosis respectively [22]. In thyroidectomy specimens, we correlated preoperative ultrasound and FNA findings with final pathology which included identification of biopsy site changes in the target nodule. The second group included patients from MSKCC with carcinoma of thyroid origin and *EIF1AX* mutation detected, on primary thyroid tumor, local recurrence, or distant metastasis, diagnosed between 2010 and 2020. The diagnosis was rendered on a core biopsy, surgical resection, or FNA cytology which was also used to perform molecular analysis. Patients' demographics, cytology and surgical diagnosis, molecular results and clinical follow-up were recorded.

All surgical pathology slides were reviewed by 3 pathologists (RA and MLP at YNHH, BX at MSKCC) who were blinded to any additional molecular alterations. The original histopathologic diagnosis and re-classification according to the WHO 5th ed. were recorded for all cases [21]. Cases with multifocal benign follicular-derived nodules with variable architecture, consisting of an admixture of large and small sized follicles, sometimes with papillae formations, were classified as Follicular nodular disease (FND) after identifying the target nodule as described above. Cases with a single or predominant totally encapsulated follicular-patterned tumor distinct from the background were classified as Follicular Adenomas (FA). FAs and the target nodule in FND were reported as having predominantly microfollicular, macrofollicular or mixed growth pattern (Supplemental table). Cases with a single or predominant totally encapsulated follicular cell derived neoplasm composed of > 75% oncocytic cells were classified as Oncocytic Adenomas (OA). Follicular-derived thyroid carcinomas with high grade features, defined as increased mitotic count and tumor necrosis, were classified as high grade follicular cell-derived non-anaplastic carcinomas (HGC). Those included poorly differentiated thyroid carcinomas (PDTC), diagnosed using the Turin proposal (solid, trabecular or insular growth pattern, absence of nuclear features of papillary thyroid carcinoma, presence of at least one of the following 3 features: convoluted nuclei, ≥ 3 mitotic figures/2 mm^2, tumor necrosis) [23] and high grade differentiated thyroid carcinomas (HGDTC) using the MSKCC criteria (≥ 5 mitotic figures/2 mm^2 and/or tumor necrosis) [24]. Follicular thyroid carcinoma (FTC) and oncocytic (Hurthle-cell) carcinoma (OCA) were defined as invasive malignant well-differentiated follicular neoplasm and invasive malignant well-differentiated follicular neoplasm composed of at least 75% of oncocytic cells respectively, without nuclear features of PTC or high grade features. Angioinvasive FTC (A-FTC) was defined as FTC with invasion of vessels within the tumor capsule or beyond. Molecular alterations were correlated with the cytologic and histologic diagnosis of the target nodule.

Molecular analysis

Molecular analysis on YNHH specimens was performed preoperatively on FNA cytology by next-generation sequencing (NGS)-based ThyroSeq version 2 (v2) (n = 4) or ThyroSeq

version 3 (v3) (n = 10) at the University of Pittsburgh Medical Center (UPMC), or ThyroSure (n = 8) at YNHH. ThyroSeq v2 is a NGS assay assessing 56 thyroid-related genes for single nucleotide variants (SNVs), small insertions/deletion (indels), gene fusions and gene expression analysis. ThyroSeq v3 was expanded on its previous version to include 112 genes which cover additional genetic alterations and copy number variations (CNVs). It also uses a genomic classifier to separate malignant from benign lesions [25, 26]. ThyroSure, a modified thyroid genomic classifier, is a NGS assay developed at the Yale Pathology Molecular Diagnostics Laboratory, which is certified under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988 to perform high complexity clinical laboratory testing. ThyroSure is performed on extracted DNA and RNA from FNA samples and provides analysis of 78 thyroid-related genes to detect, SNVs, indels, gene fusions, gene expression alterations and also uses a genomic classifier to further stratify the cancer risk. Specimens from MSKCC (32 surgical and 2 cytology specimens) were tested by MSK-IMPACT at MSKCC, a deep-coverage targeted NGS technique detecting SNVs, indels, CNVs, and fusion/structural variants in 368 to 505 cancer-related genes, using custom DNA probes designed for targeted sequencing of all exons and selected introns, including canonical and selected non-canonical transcripts [27, 28]. All platforms included testing for *EIF1AX* mutations in exons 2, 5 and 6.

Table 1 Clinicopathologic Data (YNHH patients)

Age, y	
Median	66
Range	44–81
Gender, N (%)	
Female	17 (77%)
Male	5 (23%)
Cytological diagnosis, N (%)	
AUS	15 (68%)
FN	4 (18%)
OFN	3 (14%)
Histopathological diagnosis, N (%)	
FND	9 (40%)
FA	3 (14%)
OA	4 (18%)
NIFTP	2 (9%)
a-FTC	1 (5%)
OCA	1 (5%)
HGC	2 (9%)

AUS: Atypia of undetermined significance, FN: Follicular neoplasm, OFN: Oncocytic follicular neoplasm, FND: Follicular nodular disease, FA: Follicular adenoma, OA: Oncocytic adenoma, NIFTP: Non-invasive follicular thyroid neoplasms with papillary-like nuclear features, a-FTC: Angioinvasive follicular carcinoma, OCA: Oncocytic carcinoma, HGC: high-grade follicular cell-derived non-anaplastic carcinoma [includes poorly differentiated thyroid carcinoma (PDTC) and high grade differentiated thyroid carcinoma (HGDTC)]

Results

YNHH patients

There were 916 TBSRTC category III and IV cytology specimens with molecular testing available for the study period and *EIF1AX* mutation was present in 48 cases, resulting in a mutation prevalence of 5.2% at YNHH. Of these, 22 patients [17 (77%) women, 5 (23%) men, median age 66 (range, 44 – 81) years] (Table 1) underwent surgical resection. Cytology diagnosis included AUS in 15 cases, FN in 4 cases and OFN in 3 cases. 8 cases had *EIF1AX* mutation only and 14 cases had at least one additional molecular alteration. Thyroidectomy specimens were reviewed, and the histopathologic diagnosis of the target nodule was rendered according to the new WHO Classification of Tumors 5th ed [21]. There were 9 FNDs, 3 FAs, 4 OAs, one A-FTC, one OCA and 2 HGCs (one high grade FTC and one PDTC). Two cases with a follicular growth pattern, crowding, nuclear enlargement with clearing and scattered grooves were classified as Non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) (Table 1, Fig. 1). In cases classified as adenomas and NIFTPs, the entire thyroid lobe (n = 2), nodule (n = 1) or capsule (n = 1) had originally been entirely submitted.

In FNA cytology samples with TBSRTC category III or IV diagnosis and *EIF1AX* mutation, the overall ROM in the YNHH cohort was 18% (4/22). Eight cases were positive for isolated *EIF1AX* mutation: 4 (50%) were FND, 3 (38%) were FA or OA and one (12%) was an OCA yielding a ROM of 1/8 (12%). There were 12 cases with *EIF1AX* mutation and one additional molecular alteration, 5 (42%) of which were FND, 4 (33%) were FA or OA, 2 (17%) were NIFTP and one (8%) was a PDTC yielding a risk of NIFTP of 17% and a ROM of 8%. There were 2 cases with *EIF1AX* and 2 additional molecular alterations, both of which were FTCs including one high grade FTC conferring a ROM of 100% (Tables 2 and 3). The ROM was 21% in cases with *EIF1AX* mutation and at least one additional molecular alteration. *EIF1AX* mutation consisted of a splice site mutation in 77% (17/22) and missense mutation in 23% (5/22) of the cases. All 8 cases with isolated *EIF1AX* mutations were of the splice site type with a ROM of 12%. All cases with *EIF1AX* missense mutation had additional molecular alterations present and those consisted of CNV in FND with oncocytic changes, *TSHR* in FND and OA, *NRAS* in PDTC, *TERT* and *TP53* in high grade FTC (Supplemental table).

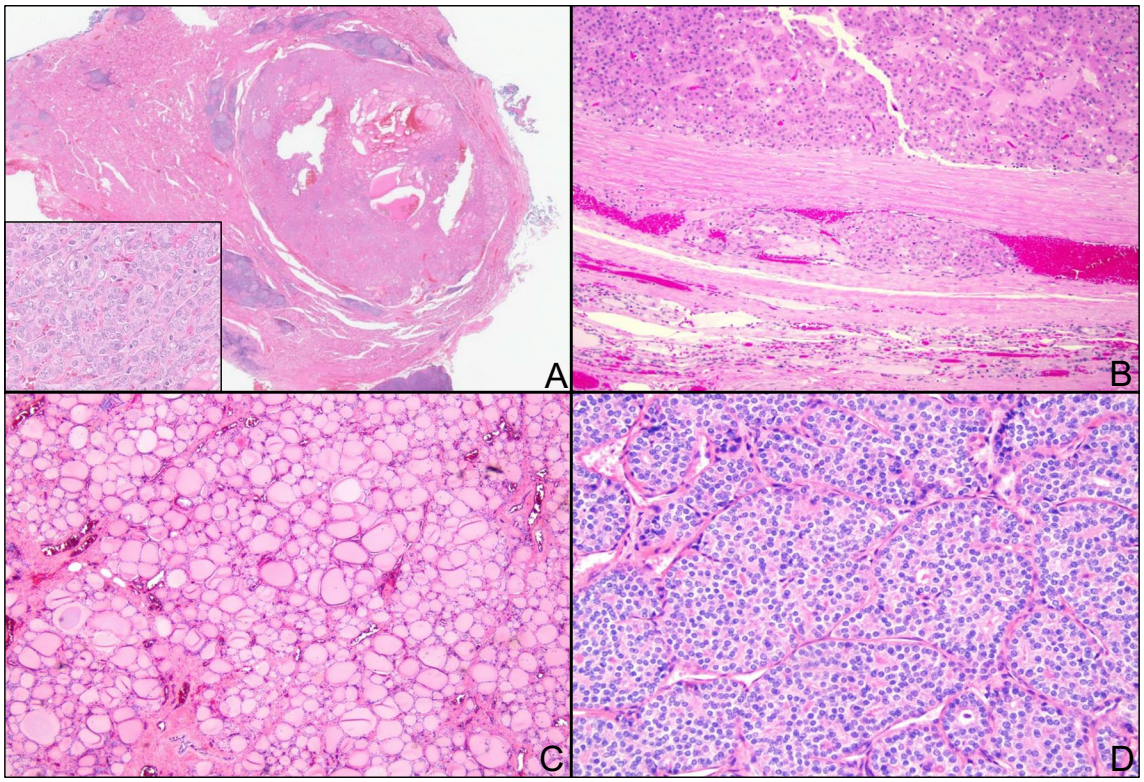

Fig. 1 Histology of representative cases of thyroid lesions classified according to WHO 5.th ed. A- Non-invasive follicular thyroid neoplasm with papillarylike nuclear features (NIFTP) with *EIF1AX* and *KRAS* mutations (H&E, X10), inset showing nuclear enlargement, irregular nuclear membranes and powdery chromatin (H&E, X800), B- *EIF1AX* mutated oncocytic carcinoma with lymphovascular invasion (H&E, X400), C- Follicular nodular disease with *EIF1AX* and *NRAS* mutations (H&E, X400), D- Poorly differentiated carcinoma with insular pattern harboring *EIF1AX* and *NRAS* mutations (H&E, X200)

Table 2 Correlation of Molecular Alterations with Surgical Follow-up (YNHH patients)

Histopathologic diagnosis	*EIF1AX* only (N = 8)	*EIF1AX* + other molecular alterations (N = 14)
FND	4	5
FA	2	1
OA	1	3
NIFTP	0	2
a-FTC	0	1
OCA	1	0
HGC	0	2

FND: Follicular nodular disease, FA: Follicular adenoma, OA: Oncocytic adenoma, NIFTP: Non-invasive follicular thyroid neoplasms with papillary-like nuclear features, a-FTC: Angioinvasive follicular carcinoma, OCA: Oncocytic carcinoma, HGC: high-grade follicular cell-derived non-anaplastic carcinoma [includes poorly differentiated thyroid carcinoma (PDTC) and high grade differentiated thyroid carcinoma (HGDTC)]. All MSKCC cases had *EIF1AX* with other molecular alterations

Of 16 benign nodules (FND or adenoma), 7 (44%) had an isolated *EIF1AX* mutation, and 9 (56%) had one additional molecular alteration (Table 2), including CNV, GE, *NRAS*, *HRAS*, *TSHR*, *TERT* promoter and *TP53* mutations (Supplemental table). All but 2 of the FND cases were *EIF1AX* splice site mutation (Table 4). NIFTP cases (n = 2) carried an *EIF1AX* mutation and a *RAS* mutation each. There were 4 malignant cases, 2 of which had 2 molecular alterations in addition to *EIF1AX* mutation: a widely invasive high grade FTC with combined *TERT* promoter, *TP53* and *EIF1AX* missense mutations, and an encapsulated FTC with extensive angioinvasion and combined *TERT* promoter, *HRAS* and *EIF1AX* splice mutation. The other 2 malignant tumors were a PDTC with *NRAS* and *EIF1AX* missense mutation and an OCA case with an isolated *EIF1AX* splice mutation (Supplemental table).

MSKCC patients

Thirty-four patients with thyroid tumors showing *EIF1AX* mutation were included [17 (50%) women, 17 (50%) men, median age 67 (range 43–85) years]. Testing by MSK-IMPACT was performed on 21 tumors from the primary tumor site and 13 local recurrence/regional recurrence/distant metastasis. All tumors were malignant, the

Table 3 Correlation of Molecular Alterations with Histopathologic Diagnoses (YNHH and MSKCC)

	Histopathologic diagnosis				
	FND (N = 9)	FA/OA (N = 7)	NIFTP (N = 2)	a-FTC/OCA/PTC (N = 10)	HGC/ATC (N = 28)
Molecular alterations					
EIF1AX only (n = 8)	4	3	0	1	0
EIF1AX + 1 additional molecular alteration (n = 12)	5	4	2	0	1
EIF1AX + 2 or more additional molecular alterations (n = 36)	0	0	0	9	27

FND: Follicular nodular disease, FA: Follicular adenoma, OA: Oncocytic adenoma, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, a-FTC: Angioinvasive follicular carcinoma, OCA: Oncocytic carcinoma, PTC: Papillary thyroid carcinoma, HGC: high-grade follicular cell-derived non-anaplastic carcinoma [includes poorly differentiated thyroid carcinoma (PDTC) and high grade differentiated thyroid carcinoma (HGDTC)], ATC: Anaplastic thyroid carcinoma

Table 4 Type of *EIF1AX* Mutation by Histopathologic Diagnosis

	YNHH (N = 22)	
	EIF1AX Missense mutation	*EIF1AX* Splice site mutation
FND	2	7
FA	0	3
OA	1	3
NIFTP	0	2
a-FTC	0	1
OCA	0	1
HGC	2	0
	MSKCC (N = 34)	
	EIF1AX Missense mutation	*EIF1AX* Splice site mutation
ATC	5	9
HGC	3	9
a-FTC	0	2
DTC-NOS	0	3
PTC-TCV	1	0
PTC-FV	0	2

FND: Follicular nodular disease, FA: Follicular adenoma, OA: Oncocytic adenoma, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features, a-FTC: Angioinvasive follicular carcinoma, OCA: Oncocytic carcinoma, HGC: High-grade follicular cell-derived non-anaplastic carcinoma [includes poorly differentiated thyroid carcinoma (PDTC) and high grade differentiated thyroid carcinoma (HGDTC)], ATC: Anaplastic thyroid carcinoma, DTC-NOS: Differentiated thyroid carcinoma, not otherwise specified, PTC-TCV: Papillary thyroid carcinoma, tall cell variant, PTC-FV: Papillary thyroid carcinoma, follicular variant

histopathologic subtype was ATC in 14 cases, HGC in 12 cases, A-FTC in 2 cases and differentiated thyroid carcinoma (DTC) in 6 cases (differentiated thyroid carcinoma – not otherwise specified: DTC-NOS; n = 3, tall cell variant of papillary thyroid carcinoma: PTC-TCV; n = 1, follicular variant of papillary thyroid carcinoma: PTC-FV; n = 2). All 3 cases classified as DTC-NOS were follicular-patterned distant metastasis (bone, kidney, liver) without high grade features diagnosed on core biopsies. The nature of core biopsies prevented definite classification. All MSKCC cases harbored *EIF1AX* mutation with additional molecular alterations, and those included *NRAS* (n = 21), *HRAS* (n = 4), *KRAS* (n = 2), *TERT* promoter (n = 25) and *TP53* (n = 13) mutations. Other molecular alterations included *ATM, AXIN2, BBC3, BRAF, BREBBP, CUL1-EZH2, CDKN2A, CDKN2C, DAXX, GNAS, MLL1, MLL2, NF1, PAK7, PBRM1, PIK3CA, PTPRT, RAC1, SMAD2, TET2, TSHR, DDR2, PRKAR1A, RAD21, RBM10, SDHA, SDHB, TP53BP1, JAK2, NEGR1, SF3B1, PTCH1, TGFBR1, KLF4, TGFBR2, ZFHX3, NOTCH1* rearrangement, *CCNE1* amp, *PIK3CD del*, *CD79B* gain and loss of *FANCC* (Supplemental table). *EIF1AX* mutation consisted of a splice site mutation in 26 and missense mutation in 8 cases (Table 4). *TERT* promoter mutation occurred in 64% (9/14) of ATCs, 83% (10/12) of HGCs, 100% (2/2) A-FTCs and 67% (4/6) of DTCs. *TP53* mutation occurred in 79% (11/14) of ATCs, 0% (0/12) of HGCs, 50% (1/2) of A-FTCs and 17% (1/6) of DTCs. All 34 cases had at least 2 molecular alterations in addition to *EIF1AX* mutation including *TERT* promoter and/or *TP53* mutations in 29 (85%) ATC and 22 (85%) HGC. *RAS* mutation was present in 27/34 (79%) cases (Table 3 and Supplemental table).

Discussion

Our study confirms previous reports that *EIF1AX* mutations occur in both benign and malignant thyroid nodules. They were originally reported to confer a ROM of 20% approximately [10]. However, subsequent studies revealed different ROM estimates depending on the type of *EIF1AX* mutation and the presence of additional molecular alterations [17, 19, 29]. In cases with indeterminate cytology and isolated *EIF1AX* mutation, the ROM ranged from 13% to 47.6% but higher ROM/NIFTPs, ranging between 70 and 80%, have been reported in cases with *EIF1AX* mutation co-existing with other driver mutations, reaching 100% in cases with *EIF1AX* splice site mutation and one additional molecular alteration for instance [10, 17–19, 29]. The findings suggest that the presence of an additional molecular alteration could represent a step into a malignant progression.

Most tumors with isolated *EIF1AX* mutation are well-differentiated carcinomas or NIFTPs, but co-occurrence of *EIF1AX* and *RAS* mutations correlates with larger tumors, aggressive behavior, advanced disease and predicts for shorter survival [4, 9, 10, 12, 13, 20]. Furthermore, the presence of *TERT* promoter or *TP53* mutation with *EIF1AX* mutation confers a 100% ROM with a higher risk of a more aggressive malignancy such as FTC and OCA but has mostly been associated with PDTC and ATC [4, 5, 12, 14, 17–19, 29, 30]. Indeed, PDTCs and ATCs are characterized by distinct genomic profiles with multiple molecular alterations; although *BRAF* V600E and *RAS* mutations are the main drivers, PDTCs and ATCs were also reported to be enriched for *EIF1AX* mutations, frequently associated with mutations in the *TERT* promoter, *TP53* or genes encoding *PI3K/AKT/mTOR* pathway effectors or chromatin modifiers, which are major drivers of tumor progression [4, 10, 12, 31–33]. In a series of PDTCs and ATCs with concurrent well differentiated PTC and/or nodular hyperplasia components, Simões-Pereira et al. reported a case of PDTC case with co-existing *EIF1AX* and *RAS* mutations in the PDTC component, isolated *RAS* mutation in the well differentiated PTC component and no molecular alteration detected in the nodular hyperplasia component, suggesting the dedifferentiation may be driven by accumulation of multiple genetic events [30].

Our results are consistent with the literature. In the general population at our tertiary care center, the overall ROM was 18% and this risk increased when multiple molecular alterations were present, with *TP53* and/or *TERT* mutations being associated with aggressive phenotypes. To reflect daily practice, we have limited our YNHH study cohort to pre-operative *EIF1AX* mutation detected in TBSRTC category III and IV thyroid cytology cases where molecular testing is performed [17–19, 29]. We should however note the limitation of including the indeterminate cytology samples only for the purpose of assessing the relationship of *EIF1AX* mutation with malignancy, since TBSRTC category III and IV carry a high prevalence of *RAS* mutations in general, which are mutations encountered in both benign, low-risk and malignant neoplasms, and that the ROM in those categories is only 22% and 30% respectively [22]. We therefore included a separate set of patients with known malignancy from a cancer referral center. All malignant cases from MSKCC harbored at least 2 molecular alterations in addition to *EIF1AX* mutation and were associated with aggressive clinicopathologic characteristics, supporting that co-occurrence of multiple molecular alterations with *EIF1AX* mutation is associated with an aggressive phenotype and contributes to an unfavorable clinical course.

The WHO 5th ed. has defined multifocal benign proliferation of thyroid follicular cells resulting in multiple clonal and non-clonal nodules with variable architecture as FND and discouraged the use of the previous designation of adenomatoid hyperplasia, goiter, diffuse goiter or colloid nodule suggesting that clonal nodules represent true neoplasms [21]. Molecular alterations identified in the clonal nodules include genetic variants of a few genes including *RGS12*, *GRPEL1*, *CLIC6* and *WSF1* in familial goiters [34] and somatic alterations including *SPOP*, *EZH1* and *ZNF148* genes [35]. Thus, with the new classification, FND falls under benign tumors [21, 36–39]. It is possible that the presence of *EIF1AX* mutation in both NIFTPs and adenomas, as well as in the FND cases in our study represents the molecular alteration in a clonal nodule, and the "hyperplastic" nodules harboring *EIF1AX* mutation, reported in many studies, are probably clonal nodules and should be designated FND. In this setting, an isolated *EIF1AX* mutation in nodules comprising FND could represent an early genetic event in the multistep process of thyroid neoplasia. It would not be sufficient for malignant transformation when occurring in isolation and requires other mutations for progression to overt malignancy. In fact, the progressive accumulation of molecular alterations from benign to malignant tumors, with suggestions that FAs may be precursors to FTCs are known [40]. As evidenced by other studies [10] and supported by ours, the fact that *EIF1AX* is encountered as the sole molecular alteration in benign neoplasms supports this theory. There was one OCA case with an isolated *EIF1AX* mutation in this study; *EIF1AX* mutation might have occurred as a late event in this case or it might have been the driver mutation with an additional undetected molecular alteration that could have been responsible for the malignant process.

Similar to the results of the TCGA and a few other studies, our study shows that isolated *EIF1AX* mutation was most likely encountered in follicular and oncocytic type

neoplasms such as FA, OA and OCA. *EIF1AX* mutated tumors, with or without *RAS* mutation, were reportedly PTCs with a follicular phenotype, typically encapsulated, as well as FAs, OAs, FTCs and OCAs [5, 9–11, 14, 18, 29, 41–43]. Contrary to other studies, there were no PTCs with isolated *EIF1AX* mutation in our study.

EIF1AX mutations appear to cluster into different regions of the gene depending on the tumor type [6, 7]. In thyroid carcinomas, all mutations are single nucleotide substitutions that are clustered in two specific areas of the gene: either in codons 6–15 near the N-terminal domain in exon 2 as a missense mutation as seen in uveal melanomas [6] or more commonly, in codon 113 at a hotspot splice acceptor site between exons 5 and 6 in the C-terminal domain of *EIF1AX* (*X113_splice* mutation) resulting in a 12 amino acid deletion [10, 12]. *EIF1AX* splice mutation is specific to thyroid cancer and confers a higher ROM compared to exon 2 mutation, but this finding has not been universally confirmed with both mutation types being reported in malignant and benign thyroid lesions [9, 10, 14, 17–20]. We have found similar results; both *EIF1AX* splice site and missense mutations were encountered in carcinomas, adenomas and FND; *EIF1AX* splice site mutation was associated with a ROM of 12% while all missense mutated tumors had at least one molecular alteration in addition to *EIF1AX* mutation. It is unclear whether *EIF1AX* missense mutation was the driver gene in these cases, since many of the additional molecular alterations have been associated with thyroid pathogenesis [4, 12, 31, 44]. There were no cases of isolated *EIF1AX* missense mutation in our study, limiting the assessment of its clinical significance.

There are multiple drawbacks to our study inherent to its retrospective nature and the selection bias at the two institutions. The use of multiple molecular testing platforms represents one shortcoming of this study. While NGS panels are relatively sensitive, the difference in sensitivity among the different platforms may alter the detection of *EIF1AX* and other molecular alterations which could be missed if a lower sensitivity platform is used. Furthermore, we have documented the presence of *EIF1AX* mutation in FND and various thyroid lesions in the general population, but its true prevalence in each of those categories is difficult to assess. Only a subset of patients with indeterminate cytology diagnosis, probably those with concerning clinical and radiologic features, underwent surgery. Additionally, molecular studies are not performed on Bethesda Category II. Therefore, the prevalence of *EIF1AX* mutation in FND remains unknown, but is likely to be underestimated. Similarly, the assessment and distribution of *EIF1AX* mutation in malignant thyroid tumors is largely unknown since no category V or VI were included in the YNHH patients. To review the histology and molecular findings of *EIF1AX*-mutated aggressive cancers, we included a set of patients with known thyroid carcinomas from a cancer referral center. While the inclusion of patients with advanced disease reflects a selection bias towards aggressive malignancies characteristic of referral practice, it nevertheless reveals a different distribution of molecular alterations in *EIF1AX*-mutated cancers compared to *EIF1AX*-mutated nodules with indeterminate cytology. In addition, the pathologic and molecular characteristics of the tumors in this group confirm the findings from the general population. It is important to stress that this study does not investigate the prevalence of *EIF1AX* in thyroid or investigate its distribution across thyroid lesions. It rather shows that *EIF1AX* mutations are present in thyroid carcinomas as well as benign nodules including FND and reflects the interpretation of *EIF1AX* mutation in thyroid FNA specimens classified as Bethesda category III or IV as encountered in daily practice.

In conclusion, *EIF1AX* mutation is present in benign and malignant thyroid nodules. An isolated *EIF1AX* mutation detected pre-operatively in Bethesda categories III and IV confers a low ROM while the accumulation of additional molecular alterations is associated with increased ROM and aggressive forms of thyroid cancers.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00428-024-03914-5.

Author Contributions All authors contributed to the study concept and design. R.A. and M.L.P performed study concept and design; R.A., B.X. and M.L.P. performed development of methodology, acquisition, analysis, interpretation of data, writing, review and revision of the paper; S.G. and R.G. performed writing, review and revision of the paper. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing financial interests.

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