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A molecular expression signature distinguishing follicular lesions in thyroid carcinoma using preamplification RT-PCR in archival samples

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Follicular variant of papillary thyroid carcinoma is a lesion that frequently causes difficulties from a diagnostic perspective in the laboratory. The purpose of this study was to interrogate a cohort of archival thyroid lesions using gene expression analysis of a panel of markers proposed to have utility as adjunctive markers in the diagnosis of thyroid neoplasia and follicular variant of papillary thyroid carcinoma in particular. Laser Capture Microdissection was used to procure pure cell populations for extraction. In addition a novel, multiplex preamplification technique was used to facilitate analysis of multiple targets. The panel comprised: *HLA-DMA*, *HLA-DBQ1*, *CD74*, *CSNK1G2*, *IRF3*, *KRAS2*, *LYN*, *MT1K*, *MT1X*, *RAB23*, *TGFB1* and *TOP2A*, with *CDKN1B* as an endogenous control. Expression profiles for each target were generated using TaqMan® Real-Time PCR. *HLA-DMA*, *HLA-DQB1*, *MT1X*, *CSNK1G2* and *RAB23* were found to be differentially expressed ($P < 0.05$) when comparing follicular adenoma and follicular variant of papillary thyroid carcinoma. Comparison of follicular adenoma and follicular thyroid carcinoma groups showed significant differential expression for *MT1K*, *MT1X* and *RAB23* ($P < 0.05$). Comparison of the papillary thyroid carcinoma group (classic and follicular variants) and the follicular adenoma group showed differential expression for *CSNK1G2*, *HLA-DQB1*, *MT1X* and *RAB23* ($P < 0.05$). Finally, *KRAS2* was found to be differentially expressed ($P < 0.05$) when comparing the papillary thyroid carcinoma and follicular thyroid carcinoma groups. This panel of molecular targets discriminates between follicular adenoma, papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma and follicular thyroid carcinoma by their expression repertoires. It may have utility for broader use in the setting of fine-needle aspiration cytology and could improve the definitive diagnosis of certain categories of thyroid malignancy.
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Thyroid carcinoma is the most frequently occurring endocrine malignancy. Well-differentiated thyroid tumours of follicular cell origin are sub-divided into follicular adenoma, follicular thyroid carcinoma and papillary thyroid carcinoma.[1] Approximately 80% of thyroid carcinomas are papillary carcinomas.[2] Papillary thyroid carcinoma is defined as a malignant tumour showing evidence of follicular differentiation and characterized by distinctive nuclear features.[3] Because papillary structures may occasionally be found in follicular adenoma and follicular thyroid carcinoma, the diagnosis of papillary thyroid carcinoma ultimately depends on its nuclear features, that is ground glass appearance, irregularity of nuclear contours, grooves and pseudo-inclusions.[4] The diagnosis of papillary thyroid carcinoma does not simply depend on the presence of these nuclear features but the extent to which they are present in 'a significant proportion of the neoplasm'.[3] The lack of appreciation of the diagnostic features of a papillary thyroid carcinoma, particularly in encapsulated tumours, may either lead to

the misdiagnosis of a follicular thyroid carcinoma or that of a benign follicular adenoma.[4]

The follicular variant of papillary thyroid carcinoma is found in 9–22.5% of patients with a papillary thyroid carcinoma.[1] Follicular variant of papillary thyroid carcinoma is composed predominantly by follicles, which are lined by cells that have the nuclear features of papillary thyroid carcinoma.[5] Follicular variant of papillary thyroid carcinoma may cause (if the nuclear features of papillary thyroid carcinoma are insufficiently appreciated) severe problems in discrimination from follicular thyroid carcinoma or follicular adenoma. Characteristically, follicular adenoma is encapsulated, cellular and has a relatively uniform follicular pattern.[6] Follicular thyroid carcinoma is unifocal, encapsulated and metastasizes directly to distant organs.[1] Microscopically, follicular thyroid carcinoma most often has a microfollicular pattern and may resemble a cellular follicular adenoma.[6]

Because cytology cannot always accurately discriminate between benign and malignant follicular thyroid tumours, patients displaying a follicular thyroid lesion are advised to undergo surgery, usually a hemithyroidectomy, to verify an accurate diagnosis and to decide additional treatment. Those who ultimately reveal a carcinoma are generally advised to undergo a second operation, a complete thyroidectomy.[7] However, only 10–20% of these undetermined nodules are malignant on histology.[6] Patient management would be greatly enhanced by additional diagnostic tests. There is a role for accurate molecular markers that can further classify problematic groups in thyroid malignancy.

The panel of markers employed in this experiment were chosen from a microarray study previously carried out by our group.[8] Significantly expressed targets in the panel included members of the metallothionein family *MT1K* and *MT1X*, casein kinase I *γ2* (*CSNKIG2*) a serine/threonine protein kinase thought to be involved in insulin receptor and platelet-derived growth factor receptor signalling. The panel also included *RAB23*; a small GTPase involved in hedgehog signalling, MHC class II genes *HLA-DMA* and *HLA-DQB1*, and *KRAS* a member of the *RAS* family, whose mutations have been widely demonstrated in thyroid carcinoma.

Materials and methods

Tissue Samples

Thirty-one classic papillary thyroid carcinoma, 14 follicular variant papillary thyroid carcinoma, 10 follicular thyroid carcinoma and 17 follicular adenoma ($n = 72$) cases of formalin-fixed paraffin-embedded thyroid samples were analysed. These cases were accessioned from archives dating from 2000 to 2005 at St James Hospital, Dublin. The study had the approval of the St James Hospital and Adelaide and Meath Hospital research ethics

committee. Haematoxylin and eosin (H&E) sections were reviewed by a histopathologist (SF) and classified according to a recognized system.[5] Corresponding paraffin blocks were then collected from the archives of St James Hospital.

Laser Capture Microdissection

Seven micrometers thick sections were cut using a microtome (Microm HM 325, Medical Supply Co. Ltd, Ireland) from each paraffin block, mounted on uncharged slides, dewaxed and H&E stained (Tissue-Tek DRS 2000 Autostainer, Sakura, CA, USA). Pure populations of thyrocytes were obtained from each section by laser capture microdissection using the PixCell II System (Acturus Engineering Inc., CA, USA). Laser capture microdissection produces low yet accurate yields of RNA (yields in the range of 5–63 ng/μl were achieved in this study). Cells were captured according to a standard protocol: laser spot size = 30 μm, pulse power = 40 mW, pulse width = 1.5 ms, threshold voltage = 285 MV. The total number of pulses in each case was approximately 700 (range = 500–1200). After microdissection the Capsures (Capsure™ Macro LCM caps, LCM 0201, Techno-Path Ltd, Ireland) containing the homogenous cell populations were placed in a sterile microcentrifuge tube ready for RNA extraction.

RNA Extraction

RNA was extracted using the Absolutely RNA® Formalin-Fixed Paraffin Embedded Kit (Stratagene, CA, USA), according to manufacturers' instructions, with the exception of the tissue deparaffinization and dehydration step of the protocol as the sections had been deparaffinized during H&E staining. The protocol recommended the use of two 10 μm thick sections; however, the quantity used in this experiment was sufficient ($\sim$150 cells in some cases). Purified RNA was eluted in 30 μl volumes and the nanogram concentration per microlitre was verified using a NanoDrop spectrophotometer (ND-1000, Labtech International, UK).

cDNA Archiving and PreAmp Synthesis

The RNA was reverse transcribed to single-stranded cDNA using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) in 50 μl reactions. Reactions contained 5 μl of buffer (10 ×), 2 μl of deoxynucleotide triphosphate (25 ×), 5 μl of random primers (10 ×), 5 μl of multiscribe RT enzyme (50 U/μl), 10.5 μl of nuclease-free water and 25 μl of extracted total RNA (0.125 μg). The reactions were incubated at 25°C for 10 min and 37°C for 2 h (Perkin Elmer 9600 GeneAmp PCR system, Applied Biosystems). Laser capture microdissection achieves

low yet adequate yields of RNA, and a range of 5–63 ng/μl was attained.

Applied Biosystems TaqMan® PreAmp Master Mix Kit is intended for use with very small quantities of cDNA, and increases the quantity of cDNA for gene expression studies. Before running the preamplification reaction, the intended assays (containing primers and probes) were pooled together, as per the manufacturer's instructions with $1 \times$ Tris–EDTA (TE) buffer in a $0.2 \times$ pooled assay mix. The preamplification reaction was performed in 50 μl reactions containing 25 μl of TaqMan PreAmp master mix ($2 \times$), 12.5 μl of the pooled assay mix ($0.2 \times$), 10 μl of cDNA sample ($2.5 \, \text{ng}/\mu l$) and 10 μl of nuclease-free water. The preamplification reaction was performed for 10 min at 95°C, and 10 cycles of 15 s at 95°C and 4 min at 60°C (PE 9600 GeneAmp PCR system Applied Biosystems).

Taqman Gene Expression Reaction

The thirteen gene expression assays (including one endogenous control) were diluted 1:5 ($20–4 \times$) using $1 \times$ TE buffer. The preamplified products were diluted 1:5 using TE buffer. The gene expression reaction was performed in 20 μl reactions containing 10 μl of TaqMan gene expression master mix w/UNG ($2 \times$), 5 μl of diluted preamp product and 5 μl of TaqMan gene expression assay ($4 \times$). The following thermal cycling specifications were performed on the ABI Prism® 7000 sequence detection system (Applied Biosystems) 2 min at 50°C, 10 min at 95°C and 40 cycles each for 15 s at 95°C and 1 min at 60°C.

Results

TaqMan PCR analysis was performed using the panel of assays (primers and probes) seen in

Table 1 Panel of assays (including *CDKN1B* as the endogenous control) which were used to perform TaqMan® PCR analysis

Gene symbol	Gene name
TOP2A	Topoisomerase II α
HLA-DQB1	Major histocompatibility complex class II DQB1
HLA-DMA	Major histocompatibility complex class II DMA
MT1K	Metallothionein 1M
TGFB1	Transforming growth factor, β-1
CSNK1G2	Casein kinase 1, gamma 2
KRAS2	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
MT1X	Metallothionein 1X
IRF3	Interferon regulatory factor 3
RAB23	RAB23, member RAS oncogene family
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain
LYN	v-yes-1 Yamaguchi sarcoma viral-related oncogene homologue
CDKN1B	Cyclin-dependent kinase inhibitor 1B

Table 1, including *CDKN1B* as the endogenous control.

Analysis of relative gene expression data was performed using the $2^{-\Delta\Delta C_T}$ method with cyclin-dependent kinase inhibitor 1 B (*CDKN1B*) as the endogenous control/reference assay. This algorithm presents data as fold change in gene expression normalized to an endogenous reference gene.[9] Non-parametric Mann–Whitney statistical analysis was performed (Analyse-it® statistical software for Microsoft® Excel®). Table 2 demonstrates targets which significantly discriminated benign from malignant groups. No significant difference was found between classic papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma groups or between follicular variant of papillary thyroid carcinoma and follicular thyroid carcinoma groups. Figure 1 demonstrates the $2^{-\Delta\Delta C_T}$ method data or relative quantification (RQ) data graphically represented as a box plot.

Discussion

The aim of this study was to validate the utility of a panel of molecular markers identified by microarray analysis,[8] which we hypothesized could distinguish papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma, follicular thyroid carcinoma and follicular adenoma by their expression repertoires.

Table 2 Displays targets that significantly discriminated benign follicular adenoma (FA) from follicular variant of papillary thyroid carcinoma (FVPTC) (a) and follicular adenoma from follicular thyroid carcinoma (FTC) (b). (c) Demonstrates targets that significantly distinguished follicular adenoma from the papillary thyroid carcinoma (PTC) group (classic and follicular variants), while (d) displays the assay that showed a significant difference between papillary thyroid carcinoma and follicular thyroid carcinoma

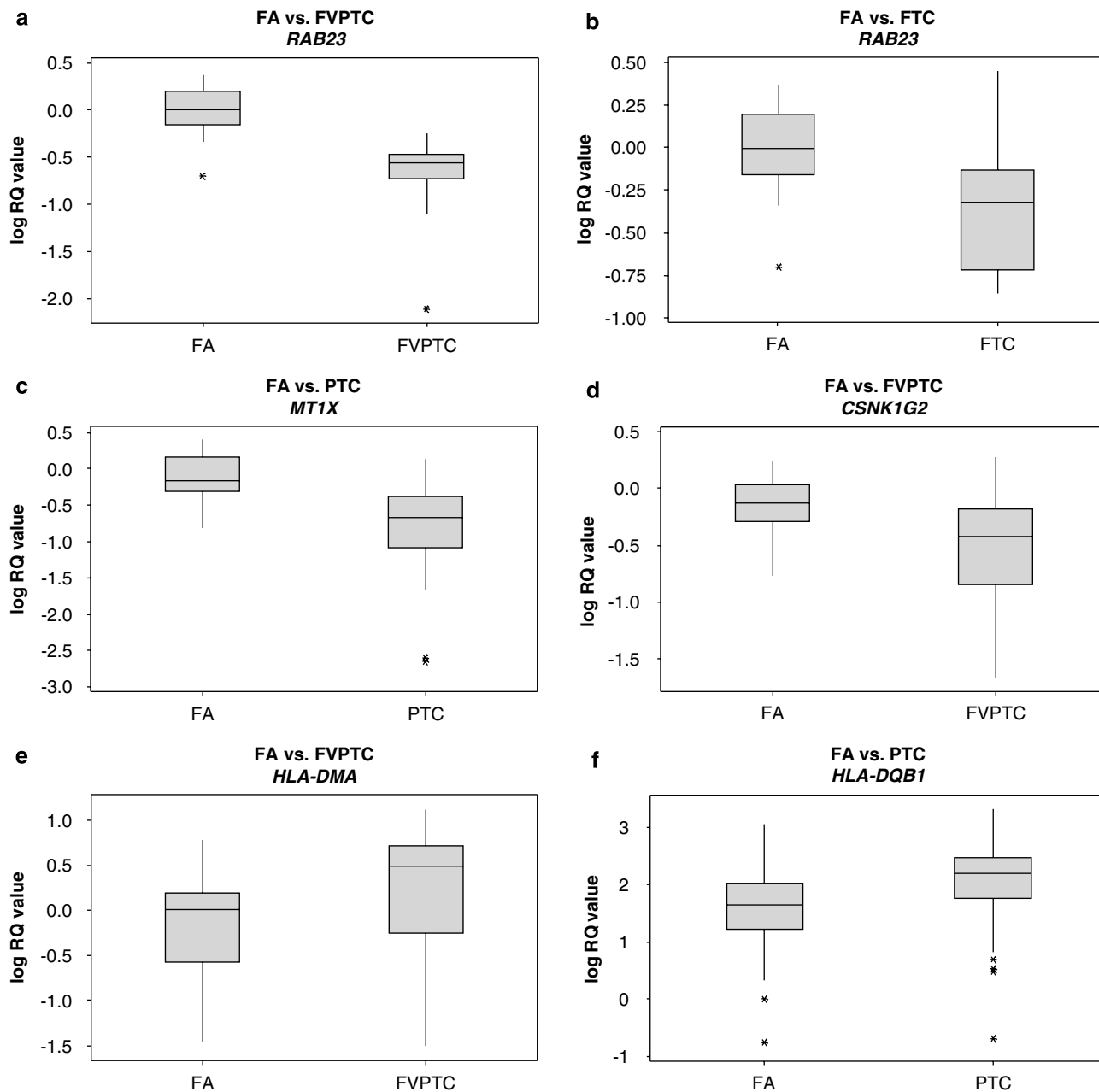| Target	Two-tailed P-value
(a) P-values for FA vs FVPTC	
MT1X	0.0015
CSNKIG2	0.0124
RAB23	<0.0001
HLA-DMA	0.0390
HLA-DQB1	0.0390
(b) P-values for FA vs FTC	
MT1X	0.05
MT1K	0.0446
RAB23	0.0149
(c) P-values for FA vs PTC	
CSNK1G2	0.0010
HLA-DQB1	0.0086
MT1X	<0.0001
RAB23	<0.0001
(d) P-value for FTC vs PTC	
KRAS2	0.0382

Figure 1 The $2^{-\Delta\Delta C_T}$ method data or RQ data graphically represented as a box plot. (**a**) Downregulation of *Rab23* in the malignant follicular variant of papillary thyroid carcinoma (FVPTC) group in contrast to the benign follicular adenoma (FA) group ($P < 0.0001$). (**b**) Downregulation of *Rab23* in the malignant cohort (follicular thyroid carcinoma (FTC)) compared to the benign follicular adenoma cohort ($P < 0.0149$). (**c**) Downregulation of *MT1X* in the papillary thyroid carcinoma group (PTC) (classic and follicular variants) when compared to benign follicular adenoma group ($P < 0.0001$). (**d**) Downregulation of *CSNK1G2* in the follicular variant of papillary thyroid carcinoma group in contrast to the follicular adenoma category ($P < 0.0124$). (**e** and **f**) Upregulation in expression of the MHC class II targets in the malignant papillary thyroid carcinoma cohorts. (**e**) Upregulation of *HLA-DMA* in follicular variant of papillary thyroid carcinoma in contrast to the benign follicular adenoma group ($P < 0.0390$). (**f**) Upregulation of *HLA-DQB1* in the papillary thyroid carcinoma group (classic and follicular variants) compared to the follicular adenoma group ($P < 0.0086$). Asterisks (*) refer to outliers.

Preamplification and Endogenous Control

The panel was applied to a series ($n = 72$) of formalin-fixed paraffin-embedded archival tissue samples using laser capture microdissection as a means to retrieve homogenous cell populations of follicular epithelial cells. Although an accurate and precise technique, laser capture microdissection procures a small yield of RNA (yields in the range of 5–63 ng/μl were achieved in this study). Relative quantitative analysis was performed to determine relative gene expression levels in each sample. To this end, equal quantities of RNA were used in each sample. Consequently, only 5 ng/μl (or 0.125 μg in

25 μl) of RNA was available from each sample for use in each individual assay. In the past, gene expression studies using archival material have been limited to analysis of few targets (<4) in any given experiment.[10–13] Limited quantities available from archival material become a greater issue when microdissection is introduced. While it is possible to repeat the preparation and microdissection using serial sections, this is time consuming and labour intensive, and requires pooling of extracts to achieve uniformity in analysis.

In this study, we successfully performed TaqMan® RT-PCR analysis on a panel of 13 assays using a total of 0.125 μg of input RNA per sample. This increased sensitivity was achieved using a novel preamplification technique (TaqMan® PreAmp Master Mix, Applied Biosystems). The technique is intended for use with very small quantities of cDNA and facilitates multiplex preamplification up to 100-plex. Moreover, it generates unbiased amplicons of specific regions of the starting cDNA to be analysed. In a separate study (data not shown) our group has compared parallel extracts from fresh-frozen paraffin-embedded and snap-frozen cell preparations using a normal thyroid cell line model applying the preamplification technique. The application of PreAmp lowered C_T values in snap-frozen and fresh-frozen paraffin-embedded material when compared to snap-frozen and fresh-frozen paraffin-embedded tissues that were not preamplified. This increased sensitivity was consistent and reproducible indicating that preamplification does not distort relative expression levels rather it enhances sensitivity.

In selecting the endogenous control to be used as a normalizer in the study, two controls were initially proposed: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and cyclin-dependent kinase inhibitor 1B (*CDKN1B*). Preliminary data correlating preamplified with un-preamplified cDNA (data not shown) suggested that endogenous controls with an amplicon size similar to that of the assays to be utilized produced more reproducible expression data when parallel fresh and formalin-fixed samples were analysed. The pilot study compared amplicons over a range from 62 to 122 bp. In general, bias was introduced into the system when the amplicon size of the reference gene exceeded that of the target to be analysed. Given the commercially available *GAPDH* is 122 bp, it was decided to revert to *CDKN1B* (71 bp) for calculation of relative expression, given its unchanging expression levels across samples tested.

Expression Patterns

MT1K and *MT1X* are members of the metallothionein family. Metallothioneins are a group of low-molecular weight, cystein-rich intracellular proteins. *MT1K* and *MT1X* were downregulated in the follicular thyroid carcinoma group compared to follicular adenoma, while *MT1X* was downregulated in the follicular variant of papillary thyroid carcinoma group compared to follicular adenoma. *MT1X* was downregulated in the papillary thyroid carcinoma group as a whole (classic and follicular variants) in contrast to the benign follicular adenoma group. To date, a number of microarray studies have shown metallothioneins to be downregulated in thyroid carcinoma.[7,14,15] Huang *et al*[14] expanded on their initial microarray experiment by trying to identify novel tumour suppressor genes involved in thyroid carcinogenesis. They found that *MT1G* was downregulated in papillary thyroid carcinoma consequent to hypermethylation. In this study, metallothioneins were observed to be under-expressed in all of the malignant cohorts. This finding fortifies their candidacy as tumour suppressor genes in the setting of thyroid neoplasia.

Casein kinase I γ2 (*CSNKIG2*) was downregulated in the follicular variant of papillary thyroid carcinoma group compared to the follicular adenoma group and was also downregulated in the combined papillary thyroid carcinoma group (classic and follicular variants) in contrast to the follicular adenoma group. Casein kinase I represents a unique group of serine/threonine protein kinases that are ubiquitously expressed in eukaryotic organisms. Studies have shown that rat *CSNKIG2* directly interacts with Nck adaptor protein[16,17] and human *CSNKIG2* has 94% homology with the rat homology.[17] Nck is believed to act as an adaptor protein mediating signal transduction initiated by receptor tyrosine kinases, such as the insulin receptor, which has implications in the context of thyroid tumorigenesis as activation of the insulin receptor and other tyrosine kinase receptors are thought to play a role in thyroid cancer progression[18,19] It is feasible that *CSNKIG2* is the isoform responsible for attenuating the insulin-mediated signalling pathway by modifying the receptor directly.[20] *CSKNIG2* has also been shown to phosphorylate the ligand-activated platelet-derived growth factor receptor, a membrane protein–tyrosine kinase and result in its inactivation.[21] Platelet-derived growth factor and the insulin receptor are essential for the regulation of cell growth and differentiation, factors which if were lost may result in cellular transformation. Marin *et al*[22] showed that casein kinase I phosphorylates Ser-45 of β-catenin priming the subsequent phosphorylation by glycogen synthatase 3 that signals the degradation of β-catenin and prevents its function in triggering cell division in thyroid carcinoma. Furthermore, work by Garcia-Rostan *et al*[23,24] has correlated mutations of the casein kinase I target phosphorylation site in β-catenin with thyroid tumours.

Downregulation of *Rab23* was seen in three malignant cohorts; follicular thyroid carcinoma, papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma when compared with

the benign follicular adenoma group. Highly significant *P*-values of <0.0001 were observed for the follicular variant of papillary thyroid carcinoma and papillary thyroid carcinoma groups when compared with follicular adenoma. *Rab23* is a member of the Ras super family of small GTPases that function in the regulation of vesicular trafficking. In vertebrate neural systems, *Rab23* acts as a negative regulator of hedgehog signalling.[25,26] The hedgehog signalling cascade is pivotal to embryonic development, and is involved in patterning a diverse range of vertebrate structures.[27] Aberrant activation of this pathway in postnatal life has been associated with a range of cancers including lung, prostate, colorectal and basal cell carcinomas of the skin.[28–31] It is interesting to speculate that the absence of *Rab23* and therefore the absence of a negative regulator of hedgehog signalling is implicated in the development of thyroid carcinoma.

KRAS2 was upregulated in the follicular thyroid carcinoma group when compared with the papillary thyroid carcinoma group as a whole (classic and follicular variants). Numerous studies have demonstrated *RAS* mutations in thyroid tumours.[32] Activating mutations of all three *RAS* oncogenes (*KRAS*, *HRAS* and *NRAS*) have historically been associated with a higher frequency in follicular than in papillary tumours.[33,34] A recent study by Carta *et al*[35] found an absence of *RAS* mutations in an Italian papillary thyroid carcinoma cohort but the presence of *RAS* oncogenic mutations in 75% of follicular thyroid carcinomas.

HLA Markers

The pattern of expression in MHC class II assays in follicular variant of papillary thyroid carcinoma was noteworthy. Upregulated expression was established for *HLA-DMA* and *HLA-DQB1* genes in the follicular variant of papillary thyroid carcinoma group compared to follicular adenoma and upregulation of *HLA-DQB1* was observed in the papillary thyroid carcinoma (classic and follicular) group compared to follicular adenoma. This finding corroborates an earlier observation by Finn *et al*[8] who also found increased expression of class II MHC genes in follicular variant of papillary thyroid carcinoma. Furthermore, Hwang *et al*[36] have demonstrated *ret/PTC* bearing papillary thyroid carcinoma cells that strongly express MHC class II (*HLA-DRA*) genes, although surrounding normal tissue did not. *ret/PTC* (rearranged in transformation/papillary thyroid carcinoma) is a constitutively active tyrosine kinase frequently found in papillary thyroid carcinoma and autoimmune Hashimoto's thyroiditis.[37,38] This observation suggests that overexpression of MHC class II genes may represent a novel feature of malignant thyroid follicular epithelium, and may play a role in the evasion of the immune system.

Applications

Fine-needle aspiration, histology and cytology are widely used in the initial diagnosis of thyroid malignancy. However they are impaired by limitations, such as the inability to definitively differentiate follicular adenomas from follicular carcinomas. Sangalli *et al*'s[39] investigation into the efficacy of 5469 thyroid fine-needle aspirations found a worryingly high false-negative rate of 6.2%, due to the failure to recognize follicular variant of papillary thyroid carcinoma. Inclusion of targets tested in this experiment into the routine testing protocol may provide useful adjunctive data in this setting. A number of groups have suggested molecular markers to assist in the diagnosis of papillary thyroid carcinoma from fine-needle aspiration specimens.[40,41] Sapio *et al*[42] recently proposed detection of *ret/PTC*, *TRK* and *BRAF* (V600E) in fine-needle aspiration specimens as a diagnostic adjunctive tool in the evaluation of thyroid nodules. They found that the discovery of oncogenes in fine-needle aspiration specimens suspicious for malignancy guided the extent of surgical resection, changing the surgery from diagnostic to therapeutic in five cases. The application of these markers to the diagnosis of follicular lesions in thyroid carcinoma may hopefully provide another approach in the challenging diagnosis of these lesions. Targets such as *RAB23* (Figure 1a, $P < 0.001$) and *MT1X* (Figure 1c, $P < 0.001$) showed better discriminatory power than some of the HLA markers (Figure 1e, $P = 0.0390$) when displayed as box plots (Figure 1). While some overlap was observed in expression of certain HLA markers (*HLA-DMA*) comparing follicular adenoma and follicular variant of papillary thyroid carcinoma cohorts, it should be noted that the data are presented using a log scale. It is also noteworthy that there is a significant difference between medians in these groups for these markers. The presence of outlying data, while potentially diminishing the statistical significance of the data should not detract from the biological significance of aberrant HLA expression in the follicular variant of papillary thyroid carcinoma population.

In this experiment, we set out to validate the efficacy of a panel of markers identified through microarray analysis[8] by its application to a large series of archival benign and malignant thyroid lesions. Laser Capture Microdissection was employed to obtain homogenous populations of follicular epithelial cells. Any impediment due to low RNA yields from small cell numbers was surmounted using a novel preamplification technique that allowed interrogation of a large panel of targets in the study cohort. This study not only demonstrates the ability to draw on the substantial archive of formalin-fixed paraffin-embedded tissue available and to analyse samples with unbiased preamplification, but it also highlights seven gene targets that have robustly discriminated between subgroups of

thyroid lesions. There is scope for this panel to be further refined to include other gene targets proposed by microarray analysis. Future directions for this work may include further insight into the proposed tumour suppressor genes, in particular casein kinase I γ2 as it appears to play a role in the activation of the insulin receptor and other tyrosine kinase receptors which are thought to be involved in thyroid cancer progression. Also further rationale is needed to explain the aberrant expression of the MHC II genes *HLA-DMA* and *HLA-DQB1*, as these could stand to represent a novel feature of malignant thyroid follicular epithelium.

Nonetheless, the study has established proof of principle that expression analysis may be reliably performed using a panel of markers in archival samples and this strategy may prove valuable as an adjunctive analytical tool in the diagnostic setting.

Disclosure

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