Advances in Brief

Hepatocyte Growth Factor Receptor, Matrix Metalloproteinase-11, Tissue Inhibitor of Metalloproteinase-1, and Fibronectin Are Up-Regulated in Papillary Thyroid Carcinoma: A cDNA and Tissue Microarray Study

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Abstract

Purpose: To screen and validate the global gene expression in papillary thyroid carcinoma (PTC) using cDNA expression arrays and immunohistochemistry on tumor tissue microarrays in an attempt to find genes that may be of importance in the molecular pathogenesis and malignant progression of PTC.

Experimental Design: Eighteen PTC tissue specimens were compared with three morphologically normal thyroid specimens by applying Atlas Human Cancer 1.2 Array membranes printed with cDNAs of 1176 human genes involved in cancer. Results for selected genes were confirmed by reverse transcription-PCR. Protein expression of selected genes was further studied using a tissue microarray consisting of 107 PTCs and compared with histologically normal thyroid tissue samples.

Results: By cDNA arrays, two genes [c-MET and matrix metalloproteinase (MMP)-11] were expressed only in tumor tissue, where they were present in >50% of cases. Ten genes [macrophage inhibitory cytokine-1, CGD, fibronectin (FN), hypoxia-inducible factor 1, Fc-ε-receptor γ-chain, lactate dehydrogenase A, HLA-DBP1, AH receptor, tissue inhibitor of metalloproteinase (TIMP-1), and glycyl-tRNA-synthetase] were found to be up-regulated >2-fold in 40–100% of cancers, whereas 9 genes (GADD153, polykystic kidney disease-1, CYR61, DPC4, HBA1, gravin, DLG3, protein tyrosine phosphatase δ, and heterochromatin protein 1 homologue-α) were down-regulated to <50% of their normal levels in 40–94% of cases. Conventional reverse transcription-PCR gave consistent results with the cDNA array findings for all four genes selected to be studied (c-MET, FN, TIMP-1, and GADD153). Immunohistochemistry for three selected proteins, FN, MMP-11, and TIMP-1, showed positive staining in 81, 87, and 68% of the tumor samples, respectively.

Conclusions: Several novel and previously undetected tumor promoting/inhibiting genes may be of importance in the molecular pathogenesis and malignant progression of PTC. Transcription of these genes may result in overexpression of proteins, such as c-MET, MMP-11, TIMP-1, and FN, which may contribute to the pathogenesis of PTC.

Introduction

PTC2 is the most common type of the thyroid cancer. It frequently gives rise to cervical lymph node metastases, but distant metastases are relatively rare, and these are most commonly found in the lungs. The primary therapy usually consists of total or subtotal thyroidectomy, followed by radioiodine ablation of the thyroid remnant and possible radioiodine-avid metastases, and thyroxine supplementation. PTC is generally associated with favorable outcome, especially in patients younger than 40 at the time of the diagnosis, but ~10% of patients eventually die from distant metastases, which may appear several years after the diagnosis (1). Many small (<1 cm in diameter) papillary carcinomas appear to be indolent tumors with a low malignant potential. Occult papillary carcinomas are common in the general population and have been found in autopsy studies in as many as 5–36% of the thyroids examined (1, 2).

The molecular pathogenesis of PTC is poorly characterized, and only few chromosomal or genetic abnormalities have been described (3–6). A characteristic intrachromosomal rearrangement, which results in the juxtaposition of sequences encoding the intracellular tyrosine kinase domain of RET with 5′ sequences from one of three unrelated genes, has been identified in ≤30% of PTCs (7). Studies based on CGH have identified DNA copy number changes in 12–48% of papillary carcinomas.

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2 The abbreviations used are: PTC, papillary thyroid carcinoma; oncFn, oncofetal variant of fibronectin; TMA, tissue microarray; IHC, immunohistochemistry; RT-PCR, reverse transcription-PCR; CGH, comparative genomic hybridization; MMP, matrix metalloproteinase; MIC-1, macrophage inhibitory cytokine-1; HIF, hypoxia-inducible factor; TNM, Tumor-Node-Metastasis; TMA, tumor tissue microarray; HP1-α, heterochromatin protein 1 homologue-α; TIMP, tissue inhibitor of metalloproteinase; PKD1, polykystic kidney disease-1; VEGF, vascular endothelial growth factor; FCER1G, Fc-ε-receptor γ-chain; CGD, chronic granulomatous disease; GARS, glycyl-tRNA-synthetase; AHr, AH receptor; LDH-A, lactate dehydrogenase A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
In one study, only gains were detected, and they were found only in elderly patients, affecting chromosomes 1q, 5q, 7, and 21q, and the gains tended to be associated with the presence of cervical lymph node metastases (4). In another study, DNA copy number losses were detected in chromosomes 1p, 9q, 17, 19, and 22 and gains in chromosomes 4, 5q, 6q, 9q, and 13q (5), and a third study concluded that loss of 9q21.3-q32 or gain of 1q are associated with unfavorable outcome. The presence of distant metastases is associated with gain of 1q (6). These varying results may be explained by different starting materials used or technical factors, and many of the chromosomal changes found in CGH studies may be relatively late events in the natural history of PTC.

Activation of receptor tyrosine kinases, including TRK (nerve growth factor receptor) and c-MET (encoding for the hepatocyte growth factor receptor), has been reported in PTC (8, 9). On the basis of these findings, it has been suggested that hepatocyte growth factor and its cognate receptor tyrosine kinase c-MET play a crucial role in determining the invasiveness of tumor cells in PTC (10, 11). Moreover, increased expression of MET has been detected by IHC and Western blotting in PTCs (12, 13), and it has been associated with a high risk for metastasis (14). The intracellular expression of FN, a component of the extracellular matrix to which cells adhere via the integrin family of transmembrane receptors, has been reported to be stronger in the invading parts of most PTCs than in the central parts (15). Recently, the oncFN mRNA was shown to be expressed in PTC and anaplastic thyroid carcinoma (16), and its presence in preoperatively taken fine needle aspirates has been used to aid the differential diagnosis of these tumors (16, 17).

In the present study, we first investigated the mRNA expression levels of 1176 genes using the cDNA microarray technique in a series of PTCs. Protein expression of a few selected genes identified by the cDNA microarray analysis was further examined using IHC applied on a TMA prepared from a larger series of PTCs. Several genes that were either up- or down-regulated in PTC were found, and these genes might, therefore, have a role in the molecular pathogenesis and malignant progression of PTCs. We are unaware of any similar studies performed on PTC earlier.

Materials and Methods

Subjects and Tissue Samples for cDNA Microarrays.

Fresh tumor samples were taken from 18 patients with primary papillary carcinoma and samples from macroscopically normal thyroid tissue from 3 patients undergoing thyroid surgery for suspected cancer. The samples were immediately snap frozen in liquid nitrogen after surgical removal and stored at −70°C until use at the Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland. Frozen sections were cut, stained with toluidine blue, and inspected for representativity of the tissue. Carcinoma samples were considered as representative when 70–100% of all cells in the tissue sections consisted of cancer cells and the normal tissue samples when the entire sample consisted of macroscopically normal thyroid tissue in all sections examined. The median age of the patients was 43 years (range, 18–78), and 13 (72%) were females. The longest primary tumor diameter ranged from 1 to 5 cm, and 5 patients had cervical nodal metastases. The TNM classification (International Union Against Cancer, 1997) was either T1N0M0 (n = 2), T3N0M0 (n = 5), T3N1M0 (n = 2), T3N2M0 (n = 3), T4N1M0 (n = 4), T3N1M0 (n = 1), or T2N1M0 (n = 1).

Subjects and Tissue Samples for the TMA.

Paraffin blocks of 107 patients diagnosed with PTC in 1989–1999 were collected at random from the archives of the Department of Pathology, Helsinki University Central Hospital, for preparation of the TMA blocks. The TMA was constructed as described previously with small modifications (18). The TMA also included 16 of the 18 samples investigated using cDNA arrays. The median age at diagnosis of these 107 patients was 46.5 years (range, 6–84), and 80 (75%) were females. The largest tumor diameter ranged from 0.5 to 6 cm.

Total Tissue RNA Isolation.

Total tissue RNA was isolated from the frozen tissue biopsies, which were first pulverized at liquid nitrogen temperature by a dismembrator (Braun Biotech International, Diessel GmbH), after which the RNeasy kit was applied (Qiagen, Hilden, Germany). Potentially contaminating DNA was removed by treating with RNase-free DNase I (Boehringer-Mannheim, Mannheim, Germany). After phenol treatment and drying, RNA was dissolved in RNase-free H2O. The resulting RNA concentration was measured spectrophotometrically (GeneQuant; Amersham Pharmacia Biotech Ltd., Cambridge, UK), and the quality of the RNAs was checked in agarose gels.

cDNA Probe Preparation.

cDNA was synthesized according to the Atlas cDNA Expression Arrays User Manual (Clontech, Palo Alto, CA). Briefly, 5 μg of DNase-treated total RNA was mixed with 1.5 μl of CDS primer mix (Clontech) in a total volume of 6 μl, heated for 2 min at 70°C, and cooled to 50°C for 2 min (PTC-100; MJ Research, Watertown, MA). Four microinjections of 5 × first-strand cDNA buffer, 2 μl of 10 × deoxynucleotide triphosphate mixture for dA labeling, 1 μl of 100 μM DTT, 1 μl of Superscript II RNase H reverse transcriptase (200 units/μl; Life Technologies, Inc., Gaithersburg, MD), and 5 μl of [α-33P]dATP (2500 Ci/mmol; 10 μCi/μl; Amersham) were added to each tube and incubated for 25 min at 50°C. Finally, 2 μl of 10 × termination mixture were added, and labeled cDNAs were purified by spin column chromatography.

Hybridization and Estimation of Expression Levels on cDNA Arrays.

Atlas Human Cancer 1.2 cDNA array filters consisting of cDNAs of 1176 genes (Clontech; cat. no. 7851-1) were used according to the manufacturer’s recommendations. Briefly, array membranes were prehybridized with 5 ml of ExpressHyb solution (Clontech) for 2 h at 68°C with continuous rotation in a glass hybridization roller (Hybaid Limited, Middlesex, United Kingdom). The whole volume of the purified probe was denatured and added directly into prehybridization solution, and hybridization was continued overnight at 68°C. Posthybridization washes were as follows: (a) four times in 200 ml of wash solution 1 (2 × SSC, 1% SDS) at 68°C for 30 min with agitation; (b) once with wash solution 2 (0.1 × SSC, 0.5% SDS) at 68°C for 30 min; and (c) rinsed once with 2 × SSC for 5 min at room temperature. Finally, damp membranes were sealed in a plastic wrap and exposed to imaging plates (BAS-MP 2040S; Fuji, Nakamura, Japan) for 4–7 days, which were then scanned with a Bio-Imaging Analyzer (BAS 2500; Fuji) to
obtain 16-bit images. AtlasImage 1.5 software (Clontech) was used for analysis. The artifacts were first eliminated by visual inspection, and the intensity of each spot was then analyzed after background subtraction and user-based estimation of a real signal. Mean values of intensity for each gene detected from multiple arrays were generated; the average normal array (three normal samples) and the average tumor array (18 papillary carcinoma samples) were then compared. The data were normalized by using all genes presented on the array filter. Frequency of positive cases was obtained by comparing the average normal array with each individual tumor array. The threshold frequency was set to 40%, and the intensity ratio (tumor: normal) threshold values of 2 for up-regulation and 0.5 for down-regulation were used in an attempt to detect significant expression changes.

**RT-Differential PCR.** Estimation of the reliability of the cDNA array results was performed by conventional RT-PCR on all tumor and normal samples (18 tumors and three normal controls) for four selected genes displaying a clear change in their expression (c-MET, FN, TIMP-1, and GADD153). In addition, oncFN was tested by RT-PCR for expression levels. The primers were designed with the Primer3 program (Center for Genome Research at Whitehead Institute) and were as follows: GAPDH (219-bp product); sense 5'-CTGACCCACCAACTGCTTAG-3' and antisense 5'-TTCAGCTCAAGGATGCCCT-3'; GAPDH (450 bp): sense 5'-ACCACAGTCTGAGCCATCAC-3' and antisense 5'-TCCACCAACCTGTTGCTGTA-3'; oncFN (215 bp): sense 5'-TCTTCATGAGACAGATTC-3' and antisense 5'-TATGTCTTTGGCCTATAGC-3', or the sequence information was obtained from Clontech (c-MET, TIMP-1, and GADD153; data not shown). The cDNAs were synthesized using the Advantage RT-for-PCR kit (Clontech), using random primers. The cDNAs were diluted to 100 μl by water, and 5 μl of the products were used as a template for the PCR reaction in a volume of 50 μl consisting of 0.25 units of Perkin-Elmer Amplita Gold (Roche, Branchburg, NJ), 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.2 mM each of deoxynucleotide triphosphates (Amersham Pharmacia Biotech, Piscataway, NJ), and 0.6 μM of each primer. PCRs were performed in the PTC-100 cycler (MJ Research) using the cycling profile of 95°C for 10 min, 94°C for 45 s, 56°C for 45 s, 72°C for 2 min (23 cycles), followed by 5 min extension at 72°C. The PCR products were subjected to electrophoresis in 2% agarose gel, and the band intensities were analyzed by a gel documentation system and software (AlphaImager; Alpha Innotech Ltd., Cannock, United Kingdom).

**IHC.** Three-micrometer sections were cut from TMA paraffin blocks containing tissue from 107 papillary carcinomas and histologically normal thyroids, deparaffinized, and rehydrated in a graded ethanol series. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol for 30 min, and the antibodies were incubated as follows: (a) FN (#A-0245; DAKO A/S, Glostrup, Denmark; at dilution 1:6000); (b) TIMP-1 (#IM41L; Oncogene Research, Cambridge, MA; 1:100); and (c) MMP-11 (#MS-1034, clone SL3.05, Neomarkers; 1:500) for 60 min in room temperature. Vectastain Elite kit (Vector Laboratories, Inc., Burlingame, CA) was applied, and the slides were counterstained with hematoxylin. Microscopically normal thyroid tissues were used as controls. A rough qualitative scoring was done without any attempts to quantitate the intensity of the stainings by two authors (V-M.W. and K.F.). Staining result was considered positive whenever increased cytoplasmic staining in the majority of the tumor cells was present as compared with staining of the control tissue consisting of histologically normal thyroid gland. Occasionally, both tumor and normal tissue were present in the same sections, and in such cases, the nontumor thyroid tissue served as an internal control.

**Results**

**Changes in cDNA Expression in PTC.** The analysis of the three normal thyroid tissues by AtlasImage 1.5 software (Clontech) showed that the expression profiles were similar. Thus, the data of the histologically normal tissue samples were pooled to generate an average normal tissue array. The data from the 18 PTC samples were also pooled to form an average tumor array and then compared with the average normal tissue array. The frequency of increased or decreased gene expression changes was determined by comparing the average normal tissue array with each of the 18 tumor tissue arrays. The cutoff values were >2-fold normal for up-regulation. <0.5-fold for down-regulation, and expression alteration frequency of ≥40% of cases. Two genes (hepatocyte growth factor receptor (c-MET) and MMP-11) were only expressed in tumor tissues. Nineteen genes showed significant changes in expression; 10 genes were overexpressed (MIC-1, CGD, FN, HIF1, FCER1G, LDH-A, HLA-DBP1, AHR, TIMP-1, and GARS), and 9 genes were down-regulated (GADD153, PKD1, CYR61, DPC4, HBA1, gravid, DLG3, protein tyrosine phosphatase σ, and HP1-α; Table 1).

**Verification of cDNA Expression Array Results by RT-PCR.** The gene expression profile findings in cDNA arrays were confirmed by semi-quantitative RT-PCR for selected genes (c-MET, FN, TIMP-1, and GADD153; Fig. 1). In general, these analyses showed consistent results with those obtained with cDNA arrays. All 18 PTCs investigated using cDNA array showed increased FN expression, and in line with this, the tumor band intensities in agarose gels as compared with the control band intensity were increased from 3 to 28 times in all tumors for FN in the RT-PCR analyses. Sixteen (89%) of the 18 PTCs had increased TIMP-1 expression in cDNA microarray analysis, and 17 (94%) had from 2 to 16 times increased expression in RT-PCR. Similarly, 14 (78%) of PTCs showed decreased expression for GADD153 in a cDNA expression analysis, and expression ranging from 0.2 to 0.8 of normal was found in 16 (89%) of the 18 cancers in RT-PCR analyses. The greatest difference between the two methods was found in c-MET expression. Ten (56%) of the 18 PTCs showed increased c-MET expression in a cDNA array analysis, whereas samples in RT-PCR analyses as many as 17 (94%) of the 18 tumor samples showed about six times higher expression than the normal controls, which had almost the background level of expression. On the basis of the finding of universally increased FN expression in PTC, we studied also oncFN expression in these tumors by RT-PCR and found the expression pattern of oncFN to be similar to that of FN in all 18 samples studied (data not shown).

**Protein Expression in TMA.** IHC was successful in 85% of the 107 PTC samples investigated in TMA. The unsuccessful cases consisted of unrepresentative, detached, or other-
wise not interpretable samples. FN was expressed in 12 (67%), MMP-11 in 12 (67%), and TIMP-1 in 10 (56%) of the 18 cases studied in a cDNA array. In the entire series of the interpretable PTC samples, FN immunostaining was positive in 81%, MMP-11 in 87%, and TIMP-1 in 68% of the cases. All samples consisting of histologically normal thyroid tissue stained only weakly or not at all for the proteins (Table 2; Fig. 2).

**Discussion**

The molecular genetic pathogenesis of PTC is poorly understood. We first used a gene expression array based on mRNA expression of 1176 genes and subsequently studied protein expression of a few selected genes using IHC on a tumor tissue array to identify novel putative genes that may be associated with the development PTC. In the gene expression array analysis, two genes, c-MET and MMP-11, were found to be expressed only in tumor tissue, and a total of 19 additional genes showed significant changes in their expression levels. Of these, 10 genes showed /H11022/2-fold overexpression, and 9 genes were down-regulated /H11350/40% of the tumors studied as compared with the level found in histologically normal thyroid tissue. Yet, the molecular genetic mechanisms of how these genes might be involved in the genesis of PTC are unknown, and the role of these genes in the pathogenesis of PTC needs to be confirmed in additional studies.

The proteins encoded by the identified genes with altered expression have variable functions. The main categories of putative functions of these genes include: (a) oncogenes/tumor suppressors/cell cycle regulators (c-MET, MMP-11, DPC4, and DLG3); (b) GDP/GTP exchangers and GTPase stimulators/inhibitors or apoptosis-associated genes (HIF-1, AHR, and GADD153); (c) transcription factors/cell signaling and cellular communication-related genes (CGD, FCER1G, PKD1, and HP1-α); (d) cell surface antigens/cell adhesion/receptors

**Table 1** Summary of altered gene expression in papillary thyroid carcinoma by cDNA array

<table>
<thead>
<tr>
<th>Gene name</th>
<th>GenBank no.</th>
<th>Locus</th>
<th>Ratio (T/N)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte growth factor receptor (c-MET)</td>
<td>J02958</td>
<td>7q31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-11</td>
<td>X57766</td>
<td>22q11.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC-1</td>
<td>AF019770</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGD</td>
<td>X04011</td>
<td>Xp21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCER1G</td>
<td>M33195</td>
<td>1q23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIF-1</td>
<td>U22431</td>
<td>14q21-q24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH-A</td>
<td>X02152</td>
<td>11p15.1-p14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>X02761</td>
<td>2q34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major histocompatibility complex, class II, DP β1 (HLA-DPB1)</td>
<td>M83664</td>
<td>6p21.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHR</td>
<td>L19872</td>
<td>7p21-p15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythroid potentiating activity (TIMP-1)</td>
<td>X03124</td>
<td>Xp11.3-p11.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GARS</td>
<td>D30658</td>
<td>7p15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth arrest and DNA damage-inducible protein GADD153 (DDIT3)</td>
<td>S40706</td>
<td>12q13.1-13.2</td>
<td>0.26</td>
<td>78</td>
</tr>
<tr>
<td>Autosomal dominant PKD1</td>
<td>U24497</td>
<td>16p13.3</td>
<td>0.28</td>
<td>94</td>
</tr>
<tr>
<td>Cystein-rich protein 61 (CYP61)</td>
<td>AF031385</td>
<td>1p22-p31</td>
<td>0.31</td>
<td>89</td>
</tr>
<tr>
<td>Homozygous deletion target in pancreatic carcinoma (DPC4/SMAD4)</td>
<td>U44378</td>
<td>18q21.1</td>
<td>0.33</td>
<td>56</td>
</tr>
<tr>
<td>Hemoglobin, α-chain (HBA1)</td>
<td>V00491</td>
<td>16p13.3</td>
<td>0.42</td>
<td>83</td>
</tr>
<tr>
<td>Gravin, a kinase-anchoring protein 250 (AKAP250)</td>
<td>M96332</td>
<td>6q24-q25</td>
<td>0.44</td>
<td>72</td>
</tr>
<tr>
<td>Neuroendocrine-DLG, tumor suppressor protein interacting with the adenomatous polyposis coli protein (DLG3)</td>
<td>U49089</td>
<td>Xq13.1</td>
<td>0.48</td>
<td>40</td>
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<tr>
<td>Protein tyrosine phosphatase σ (PTPRS)</td>
<td>U35234</td>
<td>19p13.3</td>
<td>0.49</td>
<td>67</td>
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<tr>
<td>HP1-α</td>
<td>L07515</td>
<td>12q13.11-q14.3</td>
<td>0.49</td>
<td>83</td>
</tr>
</tbody>
</table>

* Ratio (T/N), intensity ratio of the corresponding signals between tumor and normal thyroid tissue; frequency %, percentage of tumors harboring altered gene expression level.

* Genes detected only in PTC.

**Table 2** Summary of the immunohistochemical stainings of tissue microarray of papillary thyroid carcinoma

<table>
<thead>
<tr>
<th>% of positive staining</th>
<th>cDNA cases (n = 16)</th>
<th>All cases (n = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN</td>
<td>69%</td>
<td>81%</td>
</tr>
<tr>
<td>MMP-11</td>
<td>69%</td>
<td>87%</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>56%</td>
<td>68%</td>
</tr>
</tbody>
</table>

Fig. 1  RT-PCR analysis of c-MET, FN, GADD153, and TIMP-1 genes. ref, reference gene used is GAPDH, and the size of the reference product is 219 bp, except for TIMP-1, which is 450 bp. NTs, normal thyroid tissue samples.
(MIC-1, HLA-DPB1, TIMP-1, and CYR61); and (e) stress response/cell-cell communication-associated genes (FN, LDH-A, GARS, HBA1, and gravin). However, it is not surprising that many cell functions are affected even in a relatively indolent cancer, such as PTC. These findings lend support to the presence of multistep molecular cancer pathogenesis of PTC, although many of the gene expression alterations identified may also result from tumor progression of existing cancer.

The mRNA expression alterations of the selected genes (c-MET, FN, TIMP-1, and GADD153) were well in line with results obtained with RT-PCR. Although IHC cannot be regarded as a quantitative technique, the corresponding expression levels of the protein products of these genes were also in line with the gene expression profiles in immunostainings for FN, MMP-11, and TIMP-1. In line with the present findings, MET, MMP-11, and FN overexpression has been found earlier to be present in PTC (15). Interestingly as well, DNA copy number increase has been found by CGH in the chromosomal regions 1q23-ter, 2, 7p, 11p15-ter, 14q, 19q, and X in PTC, which regions harbor the genes MIC-1, CGD, FCER1G, HIF-1-α, LDH-A, FN, AHR, TIMP-1, and GARS (4–6), suggesting that mRNA overexpression of these genes may result from gene amplification.

Overexpression of c-MET and MMP-11 in PTC is in agreement with IHC studies performed in PTC and also in other carcinomas (8, 11, 12, 14, 19–22), and c-MET has also been found to be up-regulated in ovarian carcinomas in a study based on cDNA microarrays (23). The hepatocyte growth factor and its receptor MET are physiological regulators of cell migration and have also been implicated in tumor progression and metastasis (24). It has been suggested that the hepatocyte growth factor/MET signaling plays a crucial role in determining the invasiveness of tumor cells in PTC (10, 11). MET is overexpressed in PTC (12), and its expression has been found to be associated with a high risk for recurrence metastases in children and young adults with PTC (14). Among the MMPs, MMP-11 appears to have a unique role, because it is not able to degrade any major extracellular matrix component, and yet it has been associated with tumor progression and poor patient outcome. In vivo studies in a syngeneic tumorigenesis mouse model for MMP-11 suggest that MMP-11 acts during the early steps of tumor development and favors cancer cell survival in the stromal environment (25).

TIMP-1 is a human tissue inhibitor of metalloproteinases, especially of MMP1, but it is known to act on most of collagenases, including MMP-11. TIMP-1 also has erythroid potentiating activity. Overexpression of TIMP-1 could contribute to the restricted tendency of PTCs to give rise to distant metastases, but its overexpression may also be secondary to that of other genes, such as MMP-11. In earlier studies, TIMP-1 mRNA

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Fig. 2  Immunohistochemical staining of tissue microarray of PTC in a case with both tumor (T) and normal (N) tissue compartments in the same section. Note the cytoplasmic staining of the tumor cells in Panels 2–4. 1, H&E staining; 2, staining of FN (also note the faint positivity of the normal thyroid follicle epithelial cells); 3, MMP-11; 4, TIMP-1. Original magnification: ×200.
levels have been associated with increased thyroid carcinoma aggressiveness, but most TIMP-1 mRNA is probably derived from tumor stroma rather than thyroid carcinoma cells (26). Using IHC, Maeta et al. (27) detected MMP2, MMP9, TIMP-1, and TIMP-2 expression in PTC, and expression of these proteins significantly correlated with several unfavorable prognostic features, such as a large tumor size, presence of lymph node metastasis, and high intrathyroidal and vascular invasion. Thus, the combined overexpression of MMP-11 and TIMP-1 found in the present study might be associated with the invasive features of PTCs.

FN and its receptors are important in mediating cell adhesion, migration, and signal transduction and possibly in prevention of apoptosis in some tissues (28). FN is a component of the extracellular matrix, and cells adhere to FN via the integrin transmembrane receptors. Stimulation of both integrins and growth factor receptors is needed to enhance cell cycle progression through the G1 phase, and mitogenic signaling is weak and transient in the absence of integrin-mediated cell adhesion (29).

The most prominent finding in the present study was overexpression of FN mRNA, which was present in all PTCs, whereas none of the normal thyroids expressed FN significantly over the background level. Thus, FN might work in concert with the hepatocyte growth factor and other growth factors in the molecular pathogenesis of PTCs and in stimulating the cell cycle. Either up-regulation (23, 30) or down-regulation (10, 31, 32) of FN has been reported in several human cancers. Down-regulation of FN in cancer has been suggested to support the hypothesis of the tumor-suppressing role of FN in sarcomas (32), but several features of the putative functions of FN, such as the ability to promote epithelial cell migration and suppression of apoptosis, suggest that its overexpression may be tumor promoting in some tumor types, such as PTC, hepatoblastoma (30), and ovarian cancer (23). The spatial distribution of the oncFN and FN proteins has been found to be distinct, and the former appears to be associated with the propensity to metastasis formation in colon cancer (33). Overexpression of FN and oncFN has also been described in PTC using either mRNA detection or IHC (15, 16, 34, 35). In the present study, we found a similar pattern of expression for both FN and oncFN mRNAs in PTC, with a minimal expression in normal thyroid tissue.

Apart from the genes discussed above, several of the other genes with altered expression may contribute to the genesis of PTC. In the present study, we found overexpression of LDH-A in 50% and HIF-1 in 72% of the PTCs investigated. LDH-A expression is increased frequently in human cancers attributable to c-MYC or HIF-1 binding to the LDH-A promoter cis-acting elements (36). The reasons for its up-regulation in cancer are unknown, but tissue hypoxia might induce LDH-A transcription. DPC4 (SMAD4), PKD1, and DLG3 showed decreased expression in PTC, and these genes are putative tumor suppressors that appear to be lost in several types of human cancer (37, 38). HIF-1 activates transcription of hypoxia-inducible genes, such as glucose transporters, glycolytic enzymes, and VEGF. It is overexpressed, e.g., in human primary bladder cancers (39, 40), and increased levels of HIF-1 have been associated with increased cell proliferation and expression of estrogen receptor and VEGF in genesis of breast carcinoma (41). However, in the present study, overexpression of HIF-1 was detected without concomitant up-regulation of VEGF. AHR is a ligand-activated transcription activator that also responds to transcription factors involved in the hypoxia response. GADD153/CHOP, in turn, is induced by a variety of cell stresses, e.g., growth arrest and DNA damage, and it may act as a negative regulator of CAAT/enhancer binding protein transcription factors, induce apoptosis (42), and be associated with metastasis formation (43). Decreased expression of GADD153 found in the present study might, thus, shift the balance from apoptosis toward cell proliferation. Mutations in the CYBB/CGD gene are common in CGD (44), and overexpression of CGD might render PTC cells more capable in surviving in a low pH. FCER1G might exert its malignancy promoting action by activating the tyrosine kinase LYN and HP1-α by alterations in chromatin organization and suppression of apoptosis (45), MIC-1 by increased propensity to metastases formation (36, 46), CYR61 by reduced cell adhesion (47), and Gravin (AKAP250) by loss of organization of the G protein-linked signaling complexes (48). Yet, the functions of these genes in the molecular genetic pathogenesis of PTC remain hypothetical and require further study.

In sum, the results of the present study suggest that several genes are either up- or down-regulated in PTC. Some of the alterations identified, such as the up-regulation of c-MET, MMP-11, TIMP-1, and FN or down-regulation of GADD153, may lead to tumor invasiveness and metastasis formation and shift the balance from apoptosis to cell survival and proliferation, but decreased expression of some potential tumor suppressor genes, such as PDK1, DPC4 (SMAD4), and DLG3, may also contribute to the genesis and promotion of PTC. The role of these genes in the pathogenesis of PTC needs to be confirmed in other similar studies on PTC, and the molecular mechanisms involved need to be studied in detail.

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References


Hepatocyte Growth Factor Receptor, Matrix Metalloproteinase-11, Tissue Inhibitor of Metalloproteinase-1, and Fibronectin Are Up-Regulated in Papillary Thyroid Carcinoma: A cDNA and Tissue Microarray Study
