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Fibronectin-1 expression is increased in aggressive thyroid cancer and favors the migration and invasion of cancer cells

Marialuisa Sponziello [a, 1], Francesca Rosignolo [a, 1], Marilena Celano [b], Valentina Maggisano [b], Valeria Pecce [a], Roberta Francesca De Rose [b], Giovanni Enrico Lombardo [b], Cosimo Durante [a], Sebastiano Filetti [a], Giuseppe Damante [c], Diego Russo [b, *], Stefania Bulotta [b]

[a] Department of Internal Medicine and Medical Specialties, "Sapienza" University of Rome, 00161, Rome, Italy
[b] Department of Health Sciences, "Magna Graecia" University of Catanzaro, 88100, Catanzaro, Italy
[c] Institute of Medical Genetics, "S. Maria della Misericordia" University Hospital, 33100, Udine, Italy

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ABSTRACT

In this study we analyzed the expression levels of markers of epithelial-to-mesenchymal transition (EMT) in several papillary thyroid carcinomas (PTCs) and the relation with tumor genotypes and clinicopathological characteristics. The role of fibronectin-1 (FN1) was investigated by analyzing the effects of *FN1* silencing in two human thyroid cancer cell lines.

Most of EMT markers were significantly over-expressed in a group of 36 PTCs. In particular, *FN1* mRNA levels were higher in tumor *vs* non-tumor tissue (117.3, $p < 0.001$) and also in aggressive and $BRAF^{V600E}$ samples. Similar results were observed (and confirmed at the protein level) when FN1 expression was analyzed in a validation group of 50 PTCs and six lymph node (LN) metastases. Silencing of *FN1* in TPC-1 and BCPAP thyroid cancer cells significantly reduced proliferation, adhesion, migration, and invasion in both cell lines.

Collectively, our data indicate that FN1 overexpression is an important determinant of thyroid cancer aggressiveness.

1. Introduction

The incidence of differentiated thyroid carcinoma (DTC) has increased over the past decade (Udelsman and Zhang, 2014; Mehra et al., 2015). Although the majority of patients have an excellent prognosis, the management of invasive tumors and/or distant metastases is still a major clinical challenge, especially those that are unresponsive to radioiodine therapy (Schlumberger et al., 2007; Carneiro et al., 2015). For these tumors, improved understanding of the molecular alterations responsible for their aggressive behavior could lead to the development of new treatment options (Bulotta et al., 2016).

The epithelial-to-mesenchymal transition (EMT) is an essential step in cancer progression: it provides cancer cells with the capacity to migrate from the primary tumor, invade surrounding tissues, and reach distant sites (Thiery and Sleeman, 2006; Huber et al., 2005). Vasko et al. (2007) showed that an expression profile consistent with EMT is common in invasive papillary thyroid carcinomas (PTCs) and highlighted the roles played in this process by vimentin and other extracellular matrix (ECM) proteins. Subsequently, another EMT marker, periostin, was reported to be overexpressed in PTC, and its mRNA levels were positively correlated with extrathyroidal invasion, distant metastasis, and higher grade staging (Puppin et al., 2008). Interestingly, ECM alterations have been described as a consequence of the $BRAF^{V600E}$ mutation (Nucera et al., 2011), the most common genetic alteration detected in human PTCs, and increasing bodies of *in vitro* and *in vivo* data

Abbreviations: ATA, American Thyroid Association; DTC, differentiated thyroid carcinoma; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FNAB, fine needle aspiration biopsy; FN1, fibronectin-1; IR, intermediate risk; LN, lymph node; LR, low risk; PARP1, Poly (ADP-ribose) polymerase 1; PTC, papillary thyroid carcinoma; PTC-CL, classic PTC; PTC-FV, follicular-variant PTC; TLDA, TaqMan Low Density Array.

* Corresponding author. Department of Health Sciences, University "Magna Graecia" of Catanzaro, Campus "S. Venuta", Viale Europa, 88100, Catanzaro, Italy.
E-mail address: d.russo@unicz.it (D. Russo).

[1] Equal contribution.

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suggest that this mutation is associated with invasive properties and aggressive behavior in thyroid cancer (Durante et al., 2007; Puxeddu et al., 2008; Nucera et al., 2009; Knauf et al., 2011).

In this study, we investigated the gene expression levels of 27 EMT markers in group of 36 PTCs classified as intermediate or low risk (IR or LR) tumors according to current criteria published by the American Thyroid Association (ATA) (Haugen et al., 2016). *Fibronectin-1* (*FN1*), a fundamental component of the ECM, was the marker most strikingly upregulated in IR-PTCs relative to those classified as LR-PTC, and this finding was validated in a second series of PTCs, which also included metastatic lymph node (LN) tissues. Small-interfering RNA-mediated silencing of *FN1* in two human thyroid cancer cell lines clearly demonstrated the involvement of FN1 in the viability, adhesion, migration, and invasive properties of thyroid cancer cells.

2. Materials and methods

2.1. Collection of thyroid tissues

The study protocol was preapproved by the institutional ethics committee. Tumor tissues and normal thyroid tissues were prospectively collected from consenting 86 patients consecutively subjected to total thyroidectomy for sporadic PTC at the "Sapienza" University Hospital of Rome between 2009 and 2014. Tissue samples were excluded if tumor sample cellularity was less than 60%, and/or if normal thyroid tissue from the uninvolved lobe exhibited signs of inflammation or other types of disease. All 86 PTCs were also used to study the expression of thyroid-specific differentiation markers, and those results have been described elsewhere (Sponziello et al., 2015; Rosignolo et al., 2015).

Samples of tumor tissue (n = 86, 36 in the screening group and 50 in the validation group), LN metastases (n = 6, all from cases in the validation series) and normal thyroid tissue (n = 42, including 18 in the screening group and 24 in the validation group) were collected and immediately frozen after thyroidectomy. For each tumor, the risk of recurrence was classified as LR or IR in accordance with the 2015 ATA Guidelines for the Management of Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer (Haugen et al., 2016). *BRAF* mutational status was determined by Sanger sequencing, as previously described (Passon et al., 2015). PTC histotypes were determined by histopathological examination. The clinical and pathological characteristics of the screening and validation groups are summarized in Table 1.

2.2. Extraction of RNA and gene expression studies

Total RNA was isolated from tissues using TRIzol reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's protocol, quantified with a NanoDrop Spectrophotometer (Thermo Fisher Scientific), and reverse-transcribed with a High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). The expression of 27 EMT-related genes was then assessed using a TaqMan Low Density Array (TLDA), and mRNA levels of *FN1*

Table 1
Clinical data of PTC patients at the time of primary treatment.

Clinicopathological features[a]	Screening group (n = 36)	Validation group (n = 56)
Age at diagnosis		
Patients <45 yr	17 (47.2)	29 (51.8)
Patients ≥45 yr	19 (52.8)	27 (48.2)
Gender		
Male	11 (30.6)	20 (35.7)
Female	25 (69.4)	36 (64.3)
Tumor size		
≥ 1 cm	27 (75.0)	42 (75.0)
< 1 cm	9 (25.0)	14 (25.0)
Multifocality[b]		
Yes	11 (30.6)	10 (17.9)
No	25 (69.4)	35 (62.5)
Extrathyroidal extension[c]		
Yes	14 (38.9)	24 (42.9)
No	22 (61.1)	30 (53.6)
Lymph node metastases[b]		
Yes	11 (30.6)	24 (42.9)
No	25 (69.4)	31 (55.4)
ATA risk[b,d]		
Low	12 (33.3)	13 (23.2)
Intermediate	24 (66.7)	42 (75.0)
BRAF mutational status		
wt	15 (41.7)	16 (28.6)
V600E	20 (55.6)	40 (71.4)
V600_K601 > E	1 (2.8)	0
Histological variant		
PTC-CL	31 (86.1)	45 (80.4)
PTC-FV	5 (13.9)[e]	9 (16.1)[f]
PTC-other	0	2 (3.6)

Validation group includes 6 lymph nodes.
Abbreviations: ATA, American Thyroid Association; PTC, papillary thyroid carcinoma; PTC-CL, classic PTC; PTC-FV, follicular-variant PTC; wt, wild-type.
[a] Results are reported as numbers (%) of patients in the screening and validation groups.
[b] Data not available for one patient.
[c] Data not available for two patients.
[d] American Thyroid Association risk stratification staging system (Haugen et al., 2016).
[e] One sample showed extrathyroidal extension.
[f] Three samples showed extrathyroidal extension.