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TAZ/WWTR1 is overexpressed in papillary thyroid carcinoma

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ABSTRACT

In this study, we analysed the expression of the transcriptional coactivator TAZ (transcriptional co-activator with PDZ-binding motif), also named WWTR1, in a panel of papillary thyroid carcinoma samples and we observed a significant deregulation of its expression in such tumours. Specifically, by quantitative real-time PCR (qRT-PCR) we evaluated TAZ mRNA levels in tissue specimens ($n = 61$) of papillary thyroid carcinoma (PTC) and herein we show that the PTC samples express much higher TAZ mRNA levels with respect to the normal thyroid tissue ($p < 0.001$). TAZ expression was also evaluated in normal ($n = 10$) and pathological human thyroids ($n = 17$) by immunohistochemical analysis and the increase of TAZ protein levels in PTC was confirmed. To further analyse the molecular mechanisms underlying TAZ overexpression in PTC, we used an inducible system consisting of FRTL-5 rat thyroid cells expressing a conditional RAS oncoprotein and we show that the activation of the RAS signalling pathway is involved in TAZ deregulation. These observations suggest that the activated effectors of the RAS/RAF/MEK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) signalling pathway are involved in the increased expression of TAZ, supporting the idea that this may also occur in thyroid papillary carcinoma. Moreover, we demonstrated that the overexpression of TAZ is able to confer growth advantage to thyroid cells in culture and to induce epithelial–mesenchymal transition. In conclusion, these findings support a potential role for TAZ in the pathogenesis of papillary thyroid carcinomas.

1. Introduction

Thyroid cancer is the most common malignancy of the endocrine system and accounts for approximately 1% of all newly diagnosed cancer cases. The most frequent type of thyroid malignancy is papillary carcinoma (PTC), which constitutes more than 80% of all cases. The overall prognosis of PTC is very good with an average 10-year survival rate of more than 90%. However, approximately 10% of PTC patients may die as a consequence of their disease. Several clinicopathological parameters have been identified to predict a worse prognosis including old age at diagnosis, extrathyroidal invasion, distant metastases and aggressive histological variants.[1] Therefore, the study of the molecular profile of papillary thyroid

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tumours and the identification of new molecular markers are both relevant to improve the diagnosis and the prognosis of the tumour. Consequently, the identification of factors involved in normal thyroid cell differentiation is an essential prelude to the understanding of the aberrations related to thyroid cell transformation.[2]

The differentiation programme of thyroid follicular cells (TFCs) is completed only when the gland reaches its final location in front of the trachea and it depends on the cross-talk between the transcription factors and the transcriptional machinery of the cells.[3] Differentiated TFCs are responsible for thyroid hormone synthesis and are characterised by the expression of a specific set of genes such as thyroglobulin (Tg) and thyroperoxidase (TPO), which are exclusively expressed in this cell type and by the expression of genes expressed only in few tissue other than the thyroid, such as the thyrotropin stimulating hormone receptor (TSHr) and the sodium/iodide symporter (NIS).[4] To date, three transcription factors are known to regulate the expression of these thyroid-specific genes: thyroid transcription factor-1 (TTF-1/NKX2-1), forkhead box protein E1 (FOXE1) and paired box gene 8 (PAX8). TTF-1 is a homeodomain-containing protein expressed in embryonic diencephalon, thyroid and lung[5]; FOXE1 belongs to a family of transcription factors characterised by a forkhead DNA binding domain and it is expressed in pituitary and thyroid[6] and PAX8 is a member of the PAX family of paired domain-containing genes that is expressed in kidney, in the developing excretory system and in the thyroid.[7,8]

Immunohistochemical analysis of thyroid-specific transcription factors in thyroid tumours revealed a progressive decrease of the nuclear staining for TTF-1, FOXE1 and PAX8 from follicular adenoma to differentiated carcinoma and then to anaplastic carcinoma, which parallels the progressive dedifferentiation and increasing malignancy of thyroid tumours.[9,10] Hence, the deregulation of the expression of these transcription factors may play an important role in thyroid tumourigenesis, since they play a decisive role in the determination and maintenance of the differentiated phenotype.[11]

The transcriptional coactivator TAZ (transcriptional coactivator with PDZ-binding motif) was initially identified through its ability to interact with 14-3-3 proteins.[12] Most human and mouse tissues, except thymus and peripheral blood leucocytes, express TAZ mRNA, with the highest levels in kidney, heart, placenta and lung. Sharing amino acid sequence homology with YAP (yes-associated protein), TAZ contains a conserved WW domain, a 14-3-3 binding site and a C-terminal PDZ-binding motif. Phosphorylation of the serine residue at position 89 is responsible for its interaction with 14-3-3 proteins in the cytoplasm. Differently when dephosphorylated it is translocated in the nucleus where it functions as a transcriptional coactivator.[13] TAZ has been defined for its role in the nucleus, where it functions directly as a transcriptional regulator by interacting with several nuclear factors as Runx2/Cbfa1,[14] NHERF-2,[12] TEF-1,[15] TBX5,[16] PAX3,[17] PAX8[18] and TTF-1.[19] Moreover, it has been recently published that the carboxy-terminal PDZ binding motif of TAZ interacts with the first PDZ domain of zona occludens-1 (ZO-1) and 2 (ZO-2) proteins, suggesting that selected tight junction proteins might control TAZ nuclear translocation and activity.[20] In parallel, TAZ has also been proposed as a mediator of Smad nucleocytoplasmic shuttling that is essential for TGF-beta signalling.[21] Interestingly, it has been reported that TAZ contributes to the tumourigenesis of breast cancer cells by promoting cell migration, invasion and anchorage-independent growth,[22] and the TEAD proteins are the key transcription factors mediating TAZ function in that context.[23,24]

In this study, we analysed TAZ in PTC and we demonstrated an important deregulation of its expression. Moreover, using an inducible system consisting of FRTL-5 rat thyroid cells expressing a conditional RAS oncoprotein[25] we showed that the activation of the RAS signalling pathway is involved in TAZ deregulation.

2. Materials and methods

2.1. Selection of cases and collection of thyroid tissue samples

For quantitative real-time PCR (qRT-PCR) analysis, thyroid tumours were collected at the Service d'Anatomo-Pathologie, Centre Hospitalier Lyon Sud, Pierre Benite, France. Neoplastic human thyroid tissues and normal adjacent tissue or the contralateral normal thyroid lobe were obtained from surgical specimens and immediately frozen in liquid nitrogen. For immunohistochemical analysis a total of 27 thyroid tissue specimens consisting of 10 normal samples, 15 papillary and 2 anaplastic carcinomas were collected at the Pathology Unit of the Istituto Tumori 'Fondazione G. Pascale' (Naples, Italy) from patients undergoing surgery for thyroid cancer. All tissue samples were fixed immediately after surgical removal in 4% paraformaldehyde in phosphate-buffered saline (PBS) w/v.

2.2. RNA extraction, cDNA preparation and q-RT-PCR

Total RNA isolation from human tissues was performed with Trizol (Invitrogen) according to the manufacturer's instructions. The integrity of the RNA was assessed by denaturing agarose gel electrophoresis. One microgram of total RNA of each sample was reverse-transcribed with the QuantiTect®Reverse Transcription (QIAGEN) according to the manufacturer's instructions. To design the qRT-PCR assays we used the Primer Express software and the primers sequences are reported in Supplementary Table 1. qRT-PCR was performed in a 7900HT Applied Biosystems instrument in 384-well plates using a final volume of 20 μl. For the PCR we used 8 μl of 2.5X RealMasterMix™ SYBR ROX (Eppendorf AG, Germany), 200 nM of each primer and the cDNA generated from 50 ng of total RNA. The conditions used for the PCR were: 2 min at 95 °C and then 45 cycles of 20 s at 95 °C and 1 min a 60 °C. Each reaction was performed in duplicate and a melting analysis was performed at the end of the PCR run. To calculate the relative expression levels we used the 2^{-DDCT} method.[26]

2.3. Immunohistochemistry

Immunohistochemical staining was performed on 4 μm-thick serial sections from formalin-fixed paraffin-embedded tissue.

Pre-treatment of sections with heat-induced antigen retrieval in a 650-W microwave oven (three sequential steps of 4 min each, in citrate buffer pH 6.0, 10 mm) was performed. Then, incubation of sections for 20 min at room temperature with 0.3% hydrogen peroxide w/v in methanol to quench endogenous peroxidases and with non-immune horse serum (1:20; Dakopatts, Hamburg, Germany) diluted in PBS–BSA (1% w/v) for 25 min, to prevent non-specific immunostaining was carried out. After three washes with Tris–saline buffer, incubation was carried out overnight, in a moist chamber, at 4 °C with the following primary antibody, anti-TAZ polyclonal rabbit antibody (SC-48805, Santa Cruz).

Immunodetection was performed with sequential 20-min incubations with biotinylated link-antibodies and peroxidase-labelled streptavidin (LSAB-HRP; Dako). As substrate, a chromogen solution of 3,3-diaminobenzidine (DAB; Vector Labs, Burlingame, CA, USA) with 0.3% H_2O_2 w/v was used. After nuclear counterstaining with haematoxylin, sections were coverslipped and mounted with synthetic medium (Entellan; Merck, Darmstadt, Germany). Negative controls have been obtained through not using primary antibody.

The results of immunohistochemical staining were recorded as highly positive when positivity was observed in more than 50% neoplastic cells (+++) and a moderate positivity when positive cells range from 30% to 50% (++), low positivity was recorded when less than 30% of cells was positive. At least 10 high-power fields for each section were randomly selected for microscopic examination.

2.4. Cell culture

Rat thyroid follicular FRTL-5 cells were maintained in Coon's modified F-12 medium (Euroclone, Milano, Italy) supplemented with 5% newborn bovine serum (HyClone, Logan, UT) and a six-hormone mixture (6H) as previously described.[27] 4OHT treatment was performed by addition of 100 nM 4OHT (Sigma–Aldrich) to the culture medium. The FRTL-5/ER™-RAS cells were kindly provided by Prof. R. Di Lauro.

2.5. Protein extracts and immunoblotting

Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed in a buffer containing 10 mM Hepes pH 7.9, 400 mM NaCl, 0.1 mM EGTA pH 7.8, 5% glycerol w/v, 1 mM dithiothreitol (DTT), 1 mM phenylmetilsulfonil fluoride (PMSF). The protein concentration was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA).

For Western blot analysis, proteins were separated on SDS–PAGE, gels were blotted onto Immobilon P (Millipore, Bredford, MA, USA) for 2 h and the membranes were blocked in 5% non-fat dry milk in Tris–buffered saline for 2 h or overnight before the addition of the antibody for 1 h. The primary antibodies used were anti-tubulin (1:500, Santa Cruz, CA) and anti-TAZ (1:1000, Novus Biologicals, CO). The filters were washed three times in Tris–buffered saline plus 0.05% Tween 20 before the addition of horseradish peroxidase-conjugated secondary antibodies for 45 min. Horseradish peroxidase was detected with ECL (Pierce).

2.6. Plasmid constructs, colony-forming and cell proliferation assays

The mouse TAZ cDNA was cloned in the NotI-XbaI sites of the p3xFLAG-CMV-10 cloning vector (Sigma). FRTL-5 cells were transfected with 5 μg of FLAG-TAZ or FLAG-CMV-10 backbone vector and supplemented with geneticin (G418-Invitrogen) 48 h later. Transfections were carried out with the FuGENE6 reagent (Roche Diagnostics). After two weeks of drug selection, the cells were fixed and stained with 1% crystal violet.

To measure cell growth parameters, FRTL5-FLAG-CMV-10 and FRTL5-FLAG-TAZ pools of clones were plated at 350,000 cells per 60-mm plate. The cells were grown in F12 medium with 5% calf serum and six-hormone mixture and growth factors. The medium was changed every 24 h, after which cells were collected and counted.

2.7. RNA interference

SMARTpool interfering RNAs targeting rat TAZ mRNA were obtained from Dharmacon Technologies. For each experiment 8×10^4 cells/well were plated in 24-well plates and transfected with 100 nM siRNA or sclambled control RNA (scRNA) for 72 h using DharmaFECT Transfection Reagent according to the manufacturer's instructions. After 72 h incubation with siRNA the total RNA was prepared using Trizol (Invitrogen).

2.8. Ethics

All the analyses were performed according to the ethical standards of the local ethic committee.

3. Results

3.1. qRT-PCR analysis and immunohistochemical detection of TAZ in normal and neoplastic thyroid tissues

To determine the role of the transcriptional coactivator TAZ in thyroid carcinogenesis, we evaluated TAZ mRNA levels in 61 tissue specimens of PTC by quantitative RT-PCR. As control, we used a pool of six normal thyroid tissues. Relative quantities of TAZ mRNA were normalised to the glucose-6-phosphate dehydrogenase (G6PD) as a reference gene. As showed in Fig. 1, PTC samples express much higher TAZ mRNA levels in comparison to normal thyroids ($p < 0.001$), indicating that a deregulation of TAZ expression occurs in PTC. To further characterise the molecular profiling of our samples, we analysed by qRT-PCR also the expression of the transcription factors TTF-1 and PAX8 and of their target genes Tg and NIS. In agreement with the data present in literature, the mRNA levels of PAX8, TTF-1, Tg and NIS were generally decreased in the PTC samples (data not shown). Subsequently, we evaluated TAZ protein expression and cellular distribution in normal and pathological human thyroids by immunohistochemistry, using a polyclonal antibody raised against TAZ. Specifically, 10 normal thyroid tissues, 15 PTC and 2 anaplastic carcinomas were included in our study. In the normal thyroid tissue we observed a nuclear positivity in most follicular cells, whilst C cells were completely negative; no cytoplasmic staining was observed in positive cells (Fig. 2A). Classical PTC

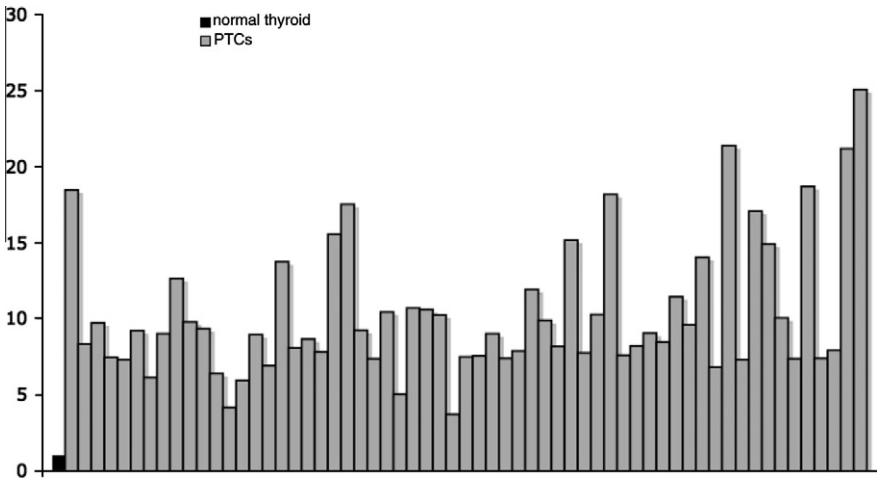

Fig. 1 – Quantitative RT-PCR analysis of TAZ expression. qRT-PCR analysis was performed on total RNA prepared from tissue specimens of 61 papillary thyroid carcinomas (grey bars in the graph). As control, a pool of six normal thyroid tissues (black bar in the graph) was used. Relative quantities of TAZ mRNA were normalised to G6PD as reference gene. Each amplification was performed in duplicate and the data are expressed as fold change with respect to the control, whose value was set at 1.0. Statistical analysis uses *t*-test ($p < 0.001$).

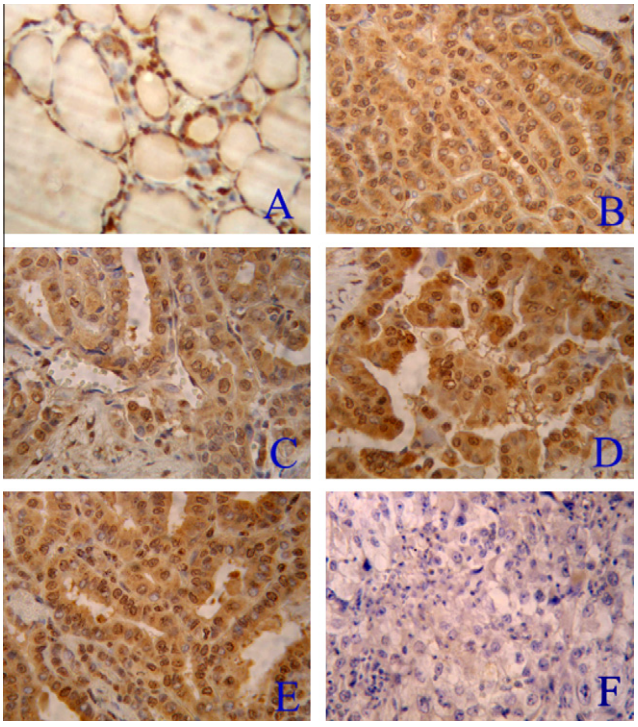

Fig. 2 – Immunohistochemical analysis of TAZ protein in normal and malignant thyroid tissues. Paraffin sections from normal and neoplastic thyroid tissues were analysed by immunohistochemistry using an antibody raised against TAZ protein. (A) Immunostaining of normal thyroid (40×). Mainly nuclear positivity was observed in most follicular cells, whilst no cytoplasmic staining was observed. (B–E) Immunostaining of PTC samples (40×). Diffuse and intense nuclear and cytoplasmic positivity was observed. (F) Immunostaining of an anaplastic thyroid carcinoma (40×). No immunoreactivity was observed.

showed overexpression of the TAZ protein with intense nuclear positivity in almost all the neoplastic cells constituting the papillae, with a slight staining of the cytoplasm (Fig 2B–E). Conversely, TAZ expression was undetectable in all the analysed anaplastic thyroid carcinoma samples (Fig. 2F).

Overall, the data obtained by qRT-PCR and immunohistochemistry revealed that the increase of TAZ expression is a marker of papillary thyroid carcinoma. Particularly, the immunohistochemical staining observed in the cytoplasm suggests that a dislocation of TAZ might be one of the causes of the malignant phenotype.

To further strengthen our observations, we evaluated TAZ expression in thyroid neoplasia developed in transgenic mouse lines expressing different oncogenes under the transcriptional control of the thyroglobulin promoter. In particular, we analysed transgenic mice carrying TRK and RET/PTC3 oncogenes which develop PTC[28,29] and N-ras mice that develop thyroid follicular tumours that undergo dedifferentiation, predominantly follicular thyroid carcinoma (FTC).[30] As shown in Fig. 3, qRT-PCR analysis revealed that the levels of TAZ mRNA are significantly increased in PTC from Tg-TRK and Tg-PTC3 mice and in FTC from Tg-N-ras mice.

3.2. *TAZ overexpression is induced by RAS activation*

PTC frequently has genetic alterations leading to the activation of the mitogen-activated protein kinase (MAPK) signalling pathway. The most common mutations are point mutations of the *BRAF* and *RAS* genes and *RET/PTC* rearrangement. These genetic alterations are found in >70% of PTC and they rarely overlap in the same tumour.[31]

To investigate whether the activation of the MAPK signalling pathway is involved in the deregulation of TAZ expression, we used an inducible system consisting of FRTL-5 cells expressing a conditional RAS oncoprotein, obtained by fusing H-RAS^{V12} downstream of a tamoxifen (40HT)-sensitive mutant of the oestrogen receptor ligand binding domain

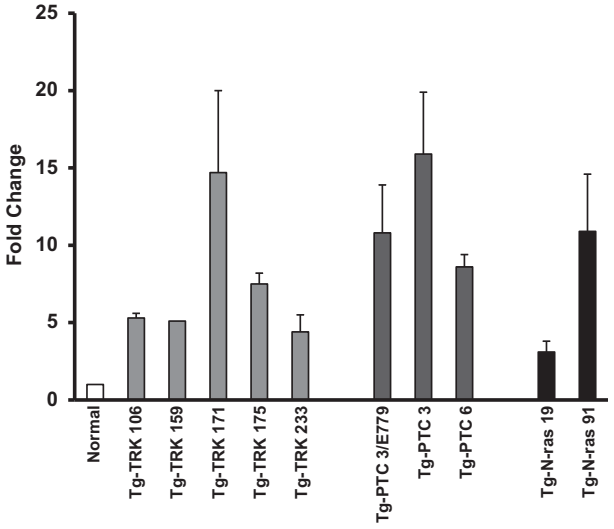

Fig. 3 – TAZ expression in experimental mouse models of thyroid carcinogenesis. qRT-PCR analysis was performed on total RNA prepared from thyroid carcinomas developed in Tg-TRK, Tg-PTC3 and Tg-N-ras transgenic mice expressing, respectively, TRK, RET/PTC3 and N-ras under the transcriptional control of the Tg promoter. Relative quantities of TAZ mRNA were normalised to the cyclophilinA as reference gene. Each amplification was performed in duplicate and the data are expressed as fold change with respect to the normal mouse thyroid tissues used as a control, whose value was set at 1.0. Statistical analysis uses t-test ($p < 0.01$).

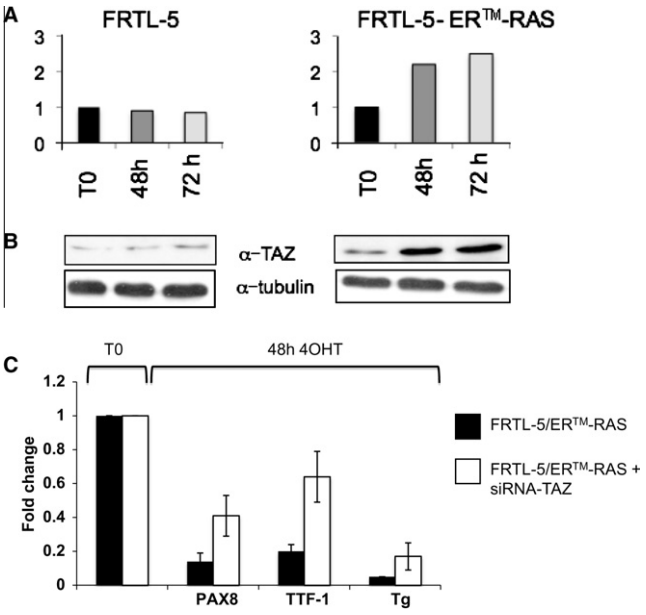

Fig. 4 – RAS oncogene activation induces TAZ overexpression. (A) qRT-PCR analysis was performed on total RNA prepared from FRTL-5 and FRTL-5/ER™-RAS cells. The expression of TAZ mRNA was measured after 48 h and 72 h of 4OHT treatment. The values are means ± SD of three independent experiments in duplicate, normalised by the expression of β-actin and expressed as fold change with respect to the T0, whose value was set at 1.0. Statistical analysis uses t-test ($p < 0.01$). (B) Total cell lysates were prepared and analysed by Western blot with an anti-TAZ antibody. Wild-type FRTL-5 cells treated with 4OHT for 48 h and 72 h are shown as control. (C) qRT-PCR analysis was performed on total RNA prepared from FRTL-5/ER™-RAS cells and FRTL-5/ER™-RAS cells transfected with siRNA against TAZ. The expression of Pax8, TTF-1 and Tg mRNA was measured after 4OHT treatment for 48 h. The values are means ± SD of three independent experiments in duplicate, normalised by the expression of β-actin and expressed as fold change with respect to the T0, whose value was set at 1.0. Statistical analysis uses t-test ($p < 0.01$).

(ER™-RAS). Previously, it has been reported that in this RAS-transformed cell line there is a clear loss of differentiation with a decrease of all markers of thyroid differentiation.[25]

We used one representative high expressing FRTL-5/ER™-RAS clone and we measured the expression of TAZ by qRT-PCR 48 h and 72 h after RAS induction by tamoxifen. As control, we used FRTL-5 wild-type cells treated at same extent. Fig. 4A shows the results of the qPCR analysis with expression values normalised for β-actin expression and reported as fold change with respect to the value in the absence of 4OHT (T0). After 48 h and 72 h of tamoxifen treatment, FRTL-5/ER™-RAS cells express significantly higher levels of TAZ mRNA in comparison to untreated cells. At the same time, FRTL-5 wild-type cells do not show any difference upon tamoxifen treatment.

We further analysed whether the increase of TAZ mRNA levels corresponds to an increase of TAZ protein expression. After tamoxifen treatment, cells were lysed and proteins were subjected to Western blot analysis with a polyclonal antibody recognising TAZ. In agreement with the qRT-PCR results, we observed an increase of TAZ protein levels only in FRTL-5/ER™-RAS cells treated with tamoxifen (Fig. 4B). The same protein extracts were also analysed for the expression of Tg, PAX8 and NIS proteins with specific polyclonal antibodies and a marked down-regulation of the thyroid differentiation markers caused by Ras activation was observed (data not shown), as previously described.[25] All together, these findings indicate that the activated effectors of the RAS/RAF/MEK/ERK signalling pathway are involved in the increased expression of TAZ, suggesting that this may also occur in PTC.

To test whether TAZ overexpression contributes to the loss of differentiation caused by RAS, we knocked-down TAZ in FRTL-5/ER™-RAS cells and we determined the intracellular mRNA levels of the thyroid differentiation markers after the tamoxifen treatment. Specifically, we transfected FRTL-5 cells with a pool of siRNA (Dharmacon RNAi Technologies) that targets the TAZ coding region and 24 h after transfection we performed the tamoxifen treatment in order to activate RAS. qRT-PCR was performed 48 h after the 4OHT treatment to analyse the expression of PAX8, TTF-1 and Tg. As expected, all markers analysed showed a decrease in their mRNA level after the induction of the RAS activity, but the reduction appeared less impressive when TAZ was silenced (Fig. 4C). Differently, FRTL-5/ER™-RAS cells transfected with a scrambled siRNA did not show any difference upon tamoxifen treatment (data not shown).

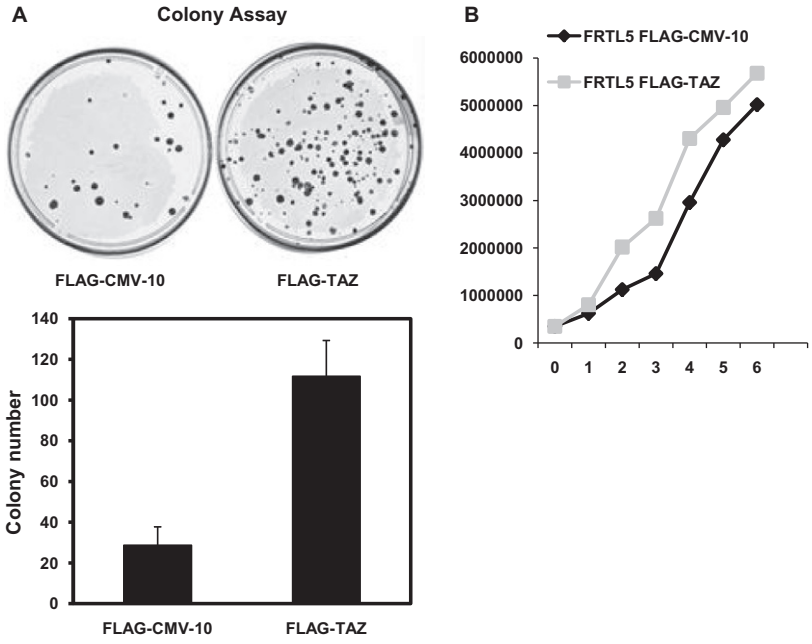

Fig. 5 – Effect of TAZ expression on thyroid cell growth. (A) Colony-forming assays were performed with FRTL-5 cells transfected with a vector expressing TAZ cDNA (FLAG-TAZ) or with the empty vector (FLAG-CMV-10). The results are the mean of two independent experiments. Cells transfected with the TAZ gene generated a higher number of colonies than cells transfected with the backbone vector. (B) Growth curve of FRTL-5 pools of clones either expressing TAZ or transfected with the empty vector. The results are the mean of two independent experiments.

These data indicate that TAZ overexpression induced by RAS contributes to generate a dedifferentiated phenotype of the FRTL-5/ER™-RAS cells.

3.3. *TAZ overexpression affects cell growth and induces epithelial–mesenchymal transition (EMT)*

To determine whether TAZ is able to confer a growth advantage to thyroid cells we evaluated the growth rate of FRTL-5 cells in which TAZ has been overexpressed. To this purpose, we transfected the cells with a TAZ expression vector (FLAG-TAZ) or with the empty vector (FLAG-CMV-10) and we carried out a colony-forming assay. As shown in Fig. 5A, cells transfected with FLAG-TAZ were able to form a higher number of colonies compared to cells transfected with the empty vector, demonstrating that the expression of TAZ represents an advantage in cell growth. Moreover, by growth curve experiments we compared the proliferation rate of wild-type FRTL-5 cells with that of a TAZ stable-transfected pool of clones. The analysis shows that the proliferation rate of the TAZ clones is significantly higher than that of the backbone vector-transfected FRTL-5 cells (Fig. 5B).

In addition, we examined whether the overexpression of TAZ could promote EMT in FRTL-5 cells. By using qRT-PCR we show that the mRNA levels of the epithelial markers E-cadherin (E-cadh) and occludin (ocln) are downregulated, whilst the expression of the mesenchymal markers fibronectin-1 (Fn-1) and vimentin (VIM) is upregulated in the TAZ stable-transfected pool of clones with respect to the FRTL-5-FLAG-CMV-10 pool (Fig. 6).

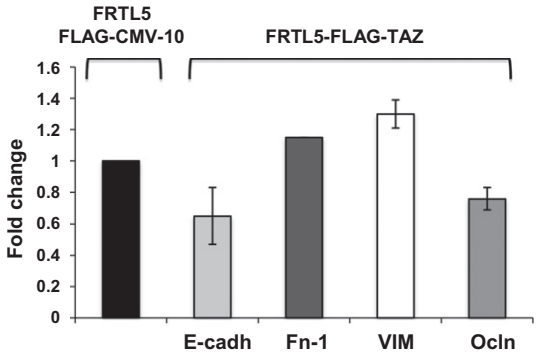

Fig. 6 – Effect of TAZ expression on EMT of thyroid cells. qRT-PCR analysis was performed on total RNA prepared from FRTL-5 pools of clones either expressing TAZ or transfected with the empty vector. The expression of E-cadherin, fibronectin-1, vimentin and occludin was measured. The values are means ± SD of three independent experiments in duplicate, normalised by the expression of β-actin and expressed as fold change with respect to the expression in FRTL-5 control cells, whose value was set at 1.0. Statistical analysis uses t-test ($p < 0.1$).

4. Discussion

TAZ sits at the convergence point of multiple signalling pathways that regulate the switch between proliferation and differentiation of mesenchymal stem cells.[13] In addition,

TAZ has been shown to promote cell proliferation and to induce epithelial–mesenchymal transition.[32] Accordingly, it has been recently demonstrated that TAZ behaves like an oncogene in its ability to transform MCF10A and that its transforming ability is mediated by the TEADs proteins.[23]

On these assumptions, we analysed the expression of TAZ in papillary thyroid carcinoma (PTC) and we demonstrated that TAZ is overexpressed in all samples analysed. By qRT-PCR we measured a ten-fold increase of TAZ mRNA in PTC samples and in parallel by immunohistochemical analysis we observed a significant increase of TAZ protein levels in the nucleus of papillary thyroid carcinoma cells with some signal also in the cytoplasm.

The C-terminus of TAZ contains a highly conserved PDZ-binding motif that localises TAZ into discrete nuclear foci and is essential for TAZ-stimulated gene transcription. On the other hand, TAZ binding to 14-3-3 proteins requires its phosphorylation on a single serine residue, resulting in the inhibition of TAZ transcriptional coactivation through 14-3-3-mediated nuclear export. Competition between PDZ domain-mediated nuclear targeting along with 14-3-3 cytoplasmic sequestrations provides a mechanism for the spatial control of TAZ function.[12]

The unusual cytoplasmic localisation of TAZ in PTC cells suggested us that some cytoplasmic function of TAZ might be involved in the initiation and/or the progression of thyroid neoplasia. It is well-known that the subcellular localisation and the loss of the expression of the transcriptions factors PAX8, TTF-1 and FOXE1 play an important role in the thyroid tumourigenesis.[11] In the context of this study, it is possible that TAZ dislocation might contribute to the loss of differentiation that occurs in the development of the malignant phenotype. However, more experimental evidences are needed to strengthen such hypothesis.

At difference from PTC cells, anaplastic carcinoma cells show a marked reduction of TAZ expression as well as of all the other differentiation markers indicating that the up-regulation of TAZ expression can be considered a specific marker of papillary thyroid carcinoma.

Thyroid tumours, especially those of the papillary type, frequently have genetic alterations leading to activation of the MAPK signalling pathway.[31] Molecular alterations found in papillary carcinomas involve gene encoding the receptor tyrosine kinases, RET and NTRK1 and two intracellular effectors of the MAPK pathway, the GTP-binding protein RAS and the serine-threonine kinase BRAF. Mutation of one of these genes can be found in more than 70% of papillary carcinomas and they rarely overlap in the same tumour, suggesting that the activation of these pathways is essential for tumour initiation.

We demonstrated that in the RAS/FRTL-5 cell line there is an increase of TAZ mRNA levels and a corresponding increase of TAZ protein levels, revealing that the activated effectors of the RAS/RAF/MEK/ERK signalling pathway are involved in the increased expression of TAZ. In addition, we showed that TAZ overexpression likely contributes to the loss of thyroid differentiation induced by RAS. In fact, we demonstrated that the TAZ confers a growth advantage to thyroid cells *in vitro* and induces epithelia-mesenchymal transition.

The finding that TAZ is overexpressed in papillary thyroid cancers might be useful to evaluate TAZ as a potential biomarker. We believe that our results show the way for future studies aiming to reveal additional insights into the molecular mechanisms responsible of thyroid carcinogenesis, and the TAZ protein might be a novel target for the treatment of thyroid carcinoma.

An extensive analysis of TAZ expression in a large number of thyroid carcinoma, considering prevalence, clinical outcome and response to treatments might be considered. Papillary thyroid carcinomas that invade into local structures are associated with a poor prognosis. Recently it has been demonstrated that EMT is associated with PTC invasion *in vivo*[33] and a possible role of TAZ in this process might be considered.

Conflict of interest statement

None declared.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.11.008.

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