

The Tumor Suppressive Role of PATZ1 in Thyroid Cancer: A Matter of Epithelial-Mesenchymal Transition

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Abstract

PATZ1 is a chromatin-regulating factor with emerging roles in stemness and cancer. It has been suggested to play a dual oncogene/tumor suppressor role depending on the cellular context, but its function in human tumor biology is still far to be completely elucidated. We have recently identified its tumor suppressive role in thyroid carcinogenesis, possibly through the association between PATZ1 and p53 to oppose epithelial-mesenchymal transition and cell migration. These are major processes in tumor progression, local invasion, metastasis, and therapeutic resistance and play a recognized role in the development of thyroid cancer, particularly anaplastic thyroid carcinoma, but many questions about how they are orchestrated remain opened. Elucidation of the mechanisms regulating epithelial-mesenchymal transition and cell migration can suggest new candidates for antimetastatic drug development that could lead to more effective therapies for highly aggressive and lethal thyroid cancers.

Keywords: Epithelial- Mesenchymal transition; Thyroid cancer; PATZ1

Introduction

Epithelial-mesenchymal transition (EMT) is a major mechanism in embryogenesis, and recent evidence suggests a crucial role in tumor's invasive and metastatic potential, as well as in therapeutic resistance [1,2]. It consists of a transdifferentiation process by which a fully differentiated epithelial cell acquires mesenchymal traits, and therefore, mesenchymal abilities such as motility and invasiveness [3].

A number of transcription factors are master regulators of key events in the EMT both in development and cancer. These factors, which include the Slug/Snail and Twist families, downregulate molecules involved in stabilizing cell-cell junctions (such as E-cadherin) and upregulate components of the migratory machinery in order to allow the tumor to become invasive [4]. In addition, the tumor suppressor p53 protein maintains a transcriptional program to oppose EMT and cell migration to prevent metastasis [5]. Chromatin repressive complexes add a further complexity level of control, which rather than directly decide the transcription programs, modulate the threshold for transcriptional activation or repression [6].

Recent studies in thyroid tumors, showing EMT, with decreased expression of E-cadherin and β -catenin and up-regulation of Twist, Snail and Slug particularly in anaplastic thyroid carcinoma (ATC) specimens and cell lines [7-9], support the role of EMT in the development of ATC.

The POZ-AT hook-Zinc finger (PATZ1) protein is a transcriptional regulatory factor that can either activate or downregulate gene expression depending on the cellular context [10]. It has been recently indicated as a "stemness factor" [11] and shown to play a critical role in reprogramming somatic cells into pluripotent stem cells [12]. PATZ1

expression is regulated by DNA damage [13] and appears deregulated in different human cancers, suggesting a cancer-related role [14-17], which appears oncogenic or anti-oncogenic depending on the tumor type and likely on the presence/absence of a wild-type p53 protein with which it interacts [10,13]. Consistently, silencing of PATZ1 expression in a p53-null osteosarcoma cell line enhanced its sensitivity to the pro-apoptotic chemotherapeutic agent 5-fluorouracil (5FU), while Patz1^{-/-} mouse embryonic fibroblasts show a decreased number of apoptotic cells, either spontaneous or induced by 5FU treatment, compared with wild-type controls [10]. However, in a colon cancer cell line, even in the presence of a wild-type p53, PATZ1 has been shown to inhibit p53 function [13], supporting the concept that PATZ1 may have opposite roles on p53 in different tumors. In this context, our recent studies show that PATZ1 acts as a tumor suppressor in thyroid cancer via targeting p53-dependent genes involved in EMT and cell migration [17]. Indeed, in a wide panel of thyroid cancer tissues, including well differentiated (WDTC), poorly differentiated (PDTC) thyroid carcinomas and ATC, we showed that its expression is downregulated and the protein delocalized from the nucleus to the cytoplasm in association with the acquisition of a less differentiated phenotype. Then, by re-expressing PATZ1 in undifferentiated thyroid cancer cell lines, we showed it inhibits cellular migration *in vitro* and promotes Mesenchymal-Epithelial transition (MET) *in vivo*, supporting a role for PATZ1 in opposing EMT in thyroid carcinogenesis. Importantly, tumors grafted from PATZ1-expressing cells appeared heterogeneous with some patches showing epithelial features (follicular-like structures and E-cadherin expression) and PATZ1 expression, while most of the remaining tissue was characterized by a mesenchymal phenotype and absent expression of PATZ1. Therefore, it is likely that PATZ1 is negatively selected *in vivo* as a way to allow an EMT phenotype. Consistent with the ability of PATZ1 to interact with p53 and regulate transcription of p53 target genes [10,13], we next showed that in PATZ1-expressing cells the EMT and cell migration gene program,

activated by EGF and negatively regulated by p53, was deregulated. In more details, PATZ1 binds the promoter regions of three genes, EpCam, RhoE and Caldesmon, involved in EMT, cellular migration and invasion, respectively, modulating their transcriptional changes associated with EGF stimulation (Figure 1). According to the functional role of these three genes, the resulting biological effect is a potentiation of the transcriptional program opposing EMT, migration and invasiveness in cells carrying a wild-type p53, and only a partial effect in cells carrying a mutant p53.

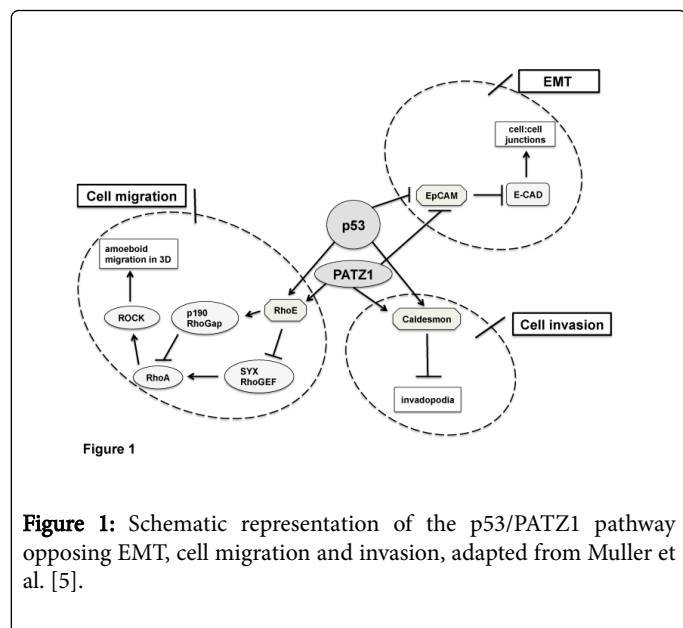


Figure 1: Schematic representation of the p53/PATZ1 pathway opposing EMT, cell migration and invasion, adapted from Muller et al. [5].

The tumor suppressive role of PATZ1 and its function as negative modulator of EMT appears not limited to thyroid cancer. Previous results from our laboratory showed that Patz1-ko mice have enhanced susceptibility to spontaneously develop different tumors in advanced age, including B cell lymphomas, sarcomas, hepatocarcinomas and rare lung and thyroid adenocarcinomas [18]. Regarding B Cell lymphomas, we have shown that a critical mechanism for their development relies on the negative control that PATZ1 has on BCL6 gene transcription [18], a gene tightly associated with the development of B Cell lymphomas [19], and recently shown to be involved in EMT induction in breast cancer [20]. In a meta-analysis of public datasets (GOBO and TGCA), PATZ1 expression is significantly downregulated in basal (specifically basal B type) vs luminal breast tumors and vs normal breast. Since the basal B type exhibits gene expression patterns associated with EMT, we can hypothesize that PATZ1, as in thyroid cancer, may be required by breast cancer cells for keeping the differentiated phenotype and avoid to go towards EMT and the basal B type phenotype, which is characteristic of the most aggressive triple-negative breast cancer subtype [21]. In this context, PATZ1 could have also a role in the cross-talk between stroma and cancer cells. Indeed, we found that luminal breast cancer cells conditioned by Patz1-ko mouse embryonic fibroblasts showed gene expression changes, including upregulation of fibronectin, Sox2, twist and EpCam, and downregulation of E-cadherin, associated with EMT (Crescenzi, E. and Vitiello, M., unpublished observations).

Coming back to thyroid cancer, although most cases of WDTC are associated with an excellent prognosis, a small percentage of patients with WDTC as well as most patients with poorly differentiated carcinomas and ATC have recurrent and/or metastatic disease that is

often fatal [22]. Further, ATC is refractory to conventional chemotherapy, radiotherapy, and radioiodine (¹³¹I) therapy [23], rendering imperative to develop new targeted strategies able to control ATC, diminish toxicity and delay the onset of patient resistance to chemotherapy.

Notably, blocking EMT is not yet been used in clinical practice [24] and this may be consequence of the still debated implication of EMT in the metastatic process. This is essentially due to the difficult of discriminating *in vivo* the mesenchymal tumor cells from neighboring stromal cells, and also because metastatic lesions are mostly epithelial. Even if the latter may be due to the hypothesized MET reverse process, it is unproven that disseminated mesenchymal tumor cells can complete the metastatic cascade producing secondary lesions. Nevertheless, emerging evidence coming from *in vitro* studies and clinical prognostic data clearly support the contribution of EMT to chemoresistance in various different cancers. Moreover, by using multiple transgenic mouse models of breast cancers, it has been recently shown that while EMT is not required for metastasis formation, it strongly contributes to chemoresistance thus suggesting that specifically targeting EMT tumor cells will be synergistic with conventional chemotherapy [25]. In this context, the close relationship between EMT and ATC may suggest more effective therapies based on reversing this plastic process in order to combat chemoresistance.

Based on our observations, it would be helpful to acquire insights on the cellular mechanisms regulating PATZ1 expression, to find a way to re-express it in cancer cells and possibly block EMT. We recently found that PATZ1 is a ras oncogene-downregulated gene in thyroid carcinogenesis, crucial for the RAS-induced transformed phenotype, including enhanced cell proliferation and capacity to migrate (Vitiello et al. manuscript submitted). A direct regulator of PATZ1 expression in RAS-transformed thyroid cells may be the miR-29b, which is specifically up-regulated upon ras oncogene induction, and specifically targets PATZ1. However, the RAS-miR-29b-PATZ1 axis seems to be active only early upon ras oncogene activation. Then, other late regulatory events, that still need to be elucidated, may occur to keep PATZ1 downregulated. Indeed, it is known that downstream of ras oncogene activation there are different gene regulatory mechanisms, some of which occur early and are often reversible (i.e. miR-21 upregulation), while others, such as DNA methylation and histone acetylation, represent more stable modifications [26]. Further experiments, using demethylation reagents and/or deacetylation inhibitors, will clarify this issue eventually providing the way to re-express PATZ1 in thyroid cancer cells.

Conclusion

Despite the EMT is considered a critical process for the development of metastases and the acquisition of chemoresistance, blocking EMT is not yet been used in clinical practice and only preclinical studies have been so far carried out. In our opinion, these studies should be pursued, especially as possible therapeutic approach for the ATC, the more aggressive and less therapy-responsive thyroid cancer. In this context, a valuable target may be PATZ1, whose re-expression in ATC cells has been shown to revert EMT tumors grafted in animal models [17].

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