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Tiam1 promotes thyroid carcinoma metastasis by modulating EMT via Wnt/β-catenin signaling

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ABSTRACT

Aberrant expression of the guanine nucleotide exchange factor Tiam1 is implicated in the invasive phenotype of many cancers. However, its involvement in thyroid carcinoma and downstream molecular events remains largely undefined. Here, we examined the effects of Tiam1 on the invasiveness and metastasis of thyroid carcinoma *in vitro* and *in vivo* and explored the underlying mechanisms by investigating the regulation of Tiam1 expression and the downstream pathways affected. Our results showed that Tiam1 knockdown inhibited the migratory and invasive capacity of thyroid cancer cells, suppressed epithelial-mesenchymal transition (EMT), and inhibited Wnt/β-catenin signaling *in vitro*. Moreover, Tiam1 knockdown suppressed liver metastasis development *in vivo*. The effects of Tiam1 on metastasis and EMT mediated by the Wnt/β-catenin pathway were reversed by Rac1 silencing, suggesting that the prometastatic effect of Tiam1 is mediated by the activation of Rac1. These results indicate that Tiam1 may be a prognostic factor and potential therapeutic target for the treatment of thyroid cancers.

1. Introduction

Thyroid carcinoma is the most common endocrine malignancy worldwide, and its incidence has increased progressively during the last three decades [1]. The most common types of thyroid cancer are papillary thyroid cancer (PTC), which accounts for 80% of all cases, and follicular thyroid cancer, which accounts for 10–20% of cases; both are derived from thyroid follicular cells and are known as differentiated thyroid cancers [2]. Although differentiated thyroid cancers are frequently localized in the thyroid gland, the presence of distant metastasis is the most significant prognostic factor for survival, with a survival rate of 50% in patients with metastatic disease [3]. The most common sites of metastasis are the lungs and bone, whereas metastasis to the brain, breast, liver, kidney, muscle, and skin is rare [4].

Epithelial-mesenchymal transition (EMT) is the process by which polarized epithelial cells that normally interact with the basement membrane undergo a series of changes that lead to the acquisition of a mesenchymal phenotype, including increased migratory capacity, invasiveness, and resistance to apoptosis [5]. EMT can be induced by several signaling pathways including the Wnt/β-catenin signaling pathway, which plays critical roles in the regulation of cell growth, development, and stem cell differentiation; constitutive activation of Wnt/β-catenin signaling is a hallmark of many cancers [6]. In the canonical Wnt pathway, the inactivation of a destruction complex that targets β-catenin for proteasomal degradation results in the translocation of β-catenin to the nucleus, where it displaces Groucho from T cell factor/lymphoid enhancer factor (TCF/LEF) to promote the transcription of Wnt target genes [7]. In colorectal cancer (CRC), however, the Hippo pathway is thought to antagonize Wnt signaling by preventing the nuclear translocation of β-catenin; YAP/TAZ, transcriptional co-activators in the Hippo pathway, are thought to bind to β-catenin thereby preventing its nuclear translocation and suppressing Wnt-target gene expression [8].

T lymphoma invasion and metastasis 1 (Tiam1) is a guanine nucleotide exchange factor (GEF) that plays a role in cellular migration, invasion, and tumor progression. Tiam1 expression is associated with metastasis in several cancers including colorectal, breast, prostate, and lung cancer, renal cell carcinoma, and hepatocellular carcinoma [9,10].

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Tiam1 increases invasion in T-lymphoma cells and migration in fibroblasts, whereas it increases cellular adhesion in epithelial cells, suggesting that the function of Tiam1 may depend on cell type, differentiation status, and the cellular microenvironment [11]. Tiam1 is the GEF for Rac1, a small GTPase of the Rho family that drives actin polymerization and promotes cell-cell adhesion as well as the migration of carcinoma cells [12]. Upon activation by Tiam1, Rac1 regulates an array of downstream factors and triggers several signaling cascades [13,14]. Tiam1 is overexpressed in thyroid carcinoma tissues, but low expression of Tiam1 has been associated with decreasing overall survival of patients with PTC [15].

In the present study, the role of Tiam1 upregulation in thyroid cancer was examined using patient samples and papillary and anaplastic thyroid carcinoma cell lines. The mechanisms underlying the association of Tiam1 with tumorigenesis and metastasis were examined, including the modulation of Tiam1 expression, the Tiam1 signaling axis, the regulation of Wnt/β-catenin signaling, and the effects of these pathways on EMT *in vitro* and metastasis in a tumor xenograft model *in vivo*.

2. Materials and methods

2.1. Ethical statement

The present study was approved by the Ethics Committee of Shanghai Tenth People's Hospital. All animal experiments were performed in compliance with the guidelines of the Animal Experimental Ethics Committee of Tongji University, China, and all animal experiments were performed in compliance with the guidelines provided by the Shanghai Medical Experimental Animal Care Commission.

2.2. Clinical specimens

Based on WHO criteria of histological classification, total 45 primary thyroid cancer tissues (26 papillary thyroid carcinoma (PTC)), 19 follicular thyroid carcinoma (FTC) and 28 distant metastatic patients (their histology was as follows: 16 PTC and 12 FTC) were obtained from patients undergoing resection of thyroid carcinoma or thyroid nodules at Shanghai Tenth People's Hospital (Tongji University, Shanghai, China) between 2015 and 2017. Normal thyroid tissues were taken from tissue a distance from solitary thyroid adenomas (n = 25). None of the patients received local or systemic treatment prior to the operation. All participants provided informed consent to participate in this study and all human specimens were approved by the Research Ethics Committee of Shanghai Tenth People's Hospital (Shanghai, China). All tissues were frozen in liquid nitrogen at the time of surgical removal.

2.3. Immunohistochemistry

Paraffin-embedded sections (3 μm thick) were incubated overnight at 4 °C with primary antibodies against Tiam1 (sc-393315, 1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were washed thoroughly, stained with diaminobenzidine, and examined using an Olympus BX41 microscope (Olympus Corporation, Japan). Six randomly selected fields of each section were examined by two pathology specialists blinded to patient diagnosis and outcome. Tiam1 immunostaining was analyzed using intensity and distribution measurements as previously described [16,17]. The staining intensity was scored as 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The staining distribution was measured as the percentage of positive tumor cells (0% to 100%). Tiam1 expression was scored by multiplying the intensity and distribution. A final score of 0 corresponded to no staining and a high score of 300 indicated 100% of cells with a staining intensity of 3. Cells were further divided according to Tiam1 expression into “low” (Tiam1 low) and “high” (Tiam1 high) groups, according to a cutoff point. The cutoff point for Tiam1 expression was calculated using X-tile software program as previously described [18].

2.4. Cell culture

The human PTC cell lines TPC-1 and anaplastic thyroid cancer (ATC) cell lines 8505c cell were obtained from the Chinese Academy of Medical Sciences (Shanghai, China) and cultured in RPMI-1640 containing 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin and grown at 37 °C in 5% CO_2.

2.5. Extraction of total RNA

RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions.

2.6. Real time-PCR

Reverse transcription was performed using the miScript Reverse Transcription kit (Qiagen) and real-time PCR was performed using the SYBR Premium Ex Taq II kit (Takara, Dalian, China) in an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). All reactions were performed in triplicate and the mean value was used to calculate expression levels after normalization to GAPDH. Primers were as follows: Tiam1 forward: 5′-AAGACGTACTC AGGCCATGTCC-3′ and reverse:5′-GACCCAAATGTCGCAGTCAG-3′; GAPDH forward: 5′-ACCA CAGTCCATGCCATCAC-3′, and reverse: 5′-TCCACCACCCTGTTGCT GTA-3′.

2.7. Western blot analysis

Protein lysates (60 μg) were separated by SDS-PAGE using an 8% polyacrylamide gel and transferred to 0.45 μm PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk in PBS containing 0.05% Tween-20, and blotted with anti-Tiam1 antibody (sc-393315, 1:300 dilution), anti-E-cadherin (sc-71009,1:500 dilution), anti-β-catenin (sc-376841, 1:500 dilution), anti-vimentin (sc-373717, 1:1000 dilution), anti-Snail (ab167609, 1:1000 dilution, Abcam) anti-Twist-1 (ab175430, 1:2000, Abcam), anti-YAP1 (sc101199, 1:500 dilution) and anti-TAZ (sc293183, 1:500 dilution). After primary antibody incubation, the membranes were washed with buffer and incubated with rabbit anti-mouse IgG conjugated with HRP (horseradish peroxidase) for 1 h at room temperature. Signals were detected with an Immobilon Western chemiluminescence HRP substrate, and β-actin was used as an internal control. The images were scanned using Odyssey software (version 2.1; LI-COR Biosciences).

2.8. Establishment of stable thyroid cancer cell lines

RNAi against Tiam1 (Sigma) [19,20], pLKO.1-shTiam1, was used to deplete endogenous Tiam1 in thyroid cancer cells. p-EZ-M02-Tiam1 [21]was used to overexpress endogenousTiam1 in thyroid cancer cells. Specifically, thyroid cancer cells were transfected with pLKO.1-shTiam1 and p-EZ-M02-Tiam1 using Turbofect (Fermentas). The siRNA sequence targeting Rac1 corresponded to the coding region5′-TGAAG AAGAGGAAGAGAAA-3′ and was generatedfrom a siRNA duplex of the primers 5′-UGAAGAAGAGGAAGAGAAAdTdT-3′(sense) and 3′-dTdTA-CUUCUUCUCCUUCUCUUU-5′(antisense) [22]. Transfected cells were incubated and selected in DMEM supplemented with 10% FBS containing 0.3 mg/mL puromycin for at least 2 weeks until colonies were selected.

2.9. Migration and invasion assays

Migration and invasion of TPC-1 and 8505c cells were assessed using Transwell plates (Millipore). Cells were plated on uncoated upper