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• BRIEF REPORTS •

Tiam1 gene expression and its significance in colorectal carcinoma

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Abstract

AIM: To explore the expression of Tiam1 gene in colorectal carcinoma and its correlation with tumor metastasis.

METHODS: Expressions of Tiam1 gene in 8 colorectal carcinoma cell lines were detected by reverse transcriptase-polymerase chain reaction. *In vitro* invasiveness was determined by means of Matrigel invasion assay. The correlation of Tiam1 expression with the invasive ability was also analyzed.

RESULTS: Tiam1 gene was highly expressed in LoVo and SW620, which were established from metastatic colorectal carcinomas in comparison with LS174T, SW480, HCT116, LST, HRT-18 and Hee8693, which were established from primary colorectal carcinomas. *In vitro* cell invasivion demonstrated that LoVo and SW620 had a higher invasive ability than LS174T, SW480, HCT116, LST, HRT-18 and Hee8693. The expression of Tiam1 gene was highly related to the metastatic potential of colorectal carcinoma cells.

CONCLUSION: Tiam1 gene may play an important role in invasion and metastasis of colorectal carcinoma and is a metastasis-related gene.

Key words: Colorectal carcinoma; Tiam1 gene; Gene expression; Tumor metastasis

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INTRODUCTION

Colorectal carcinoma is one of the most common malignancies. The majority of colorectal carcinomas arise from a series of somatic genetic changes that involve activation of oncogenes and inactivation of tumor suppressor genes. The delineation of molecular genetic and biological changes that accompany the pathogenesis of colorectal carcinoma will hopefully improve the outcome of patients in the future. Unfortunately, the overwhelming majority of patients with colorectal carcinoma would die of metastatic disease, that is to say, metastasis is the major cause of mortality in the human population[1]. Unlike the molecular events described for the pathogenesis of primary colorectal carcinomas, genes responsible for metastasis in these tumors have not been well characterized. Exploring metastasis-related genes is significantly important in the prevention of tumor metastasis and prolongation of the life expectancy of patients.

A gene designated Tiam1 was originally identified as the invasion- and metastasis-inducing gene by proviral tagging in combination with *in vitro* selection for invasiveness[2]. Transfection of truncated Tiam1 cDNAs into noninvasive cells can make these cells invasive. Tiam1 protein contains a Dbl homologous (DH) domain and two pleckstrin homologous (PH) domains. DH domain is present in guanine nucleotide exchange factors (GEFs) that activate the Rho-like GTPases[3]. PH domain, present in many signaling proteins, has been reported to be involved in protein-protein and protein-phospholipid interactions and might play a role in translocation of these proteins to membranes[4]. Tiam1 is one of the GEFs and can specifically activate Rac *in vitro* as well as *in vivo*[5]. Tiam1 has been implicated to directly bind to a plethora of different cytoplasmic and membrane-associated proteins, which couple Tima1-Rac activity to specific signaling pathways[6,7]. Interestingly, proteins such as Src[8],Myc[9],CD44[10] and nm23[11], claimed to interact with Tiam1 are well known players in the field of cancer. Tiam1 activation has also been shown to be stimulated by certain serum-derived growth activators such as S1P[12] and LPA[13] during cell spreading and motility.

Recent evidence suggests that Tiam1 could influence Rac GTPase signaling specificity in addition to promoting their activation[14]. That is why Tiam1 has a different effect on different cancers. For example, activation of Tiam1 in breast cancer and T-lymphoma cell lines, produces specific structural changes in plasma membrane-cytoskeleton reorganization leading to membrane ruffling, lamellipodia, filopodia, and stresses fiber formation[15,16]. These changes are prerequisite for invasion and metastasis. Conversely, in renal cell carcinoma cell lines, Tiam1 potentiates homotypic cell-cell adhesion and inhibits invasion[17]. In epithelial MDCK cells, Tiam1-Rac1 signaling plays an invasion-suppressor role in Ras-transformed MDCK cells[18]. A Tiam1 knock-out mouse is relatively resistant to chemical induction of skin tumors but paradoxically more prone to malignant histologic progression of those tumors[19].

Tiam1 has been studied in breast cancer[15], renal carcinoma[17], chondrosarcoma[20], T-lymphoma[16], *etc*. However, it is not known at present whether there is any relationship between Tiam1 and colorectal carcinoma. Here we showed the expression of Tiam1 in 8 colorectal carcinoma cell lines, and analyzed its correlation with tumor metastasis. Our observations suggest that Tiam1 has a close relationship with metastasis of colorectal carcinoma and may be required for colorectal carcinoma cell invasion and migration.

MATERIALS AND METHODS

Cell lines and culture conditions

HRT-18 and Hee8693 cell lines were a kind gift from Cancer Research Institute in Xiangya Medical School of Central South University. LST cell line was a generous gift from Digestive Department in Nanfang Hospital. LoVo, LS174T, HCT116, SW480

and SW620 were obtained from America Type Culture Collection. LoVo, LS174T, LST, HRT-18, HCT116 and Hee8693 cell lines were cultured in RPMI-1640 medium. SW480 and SW620 were cultured in Dulbecco's modified Eagle's medium. All mediums supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin. Cultured cells were grown in a 37 ℃ humidfied incubator with 50 mL/L CO_2.

Total RNA extraction

Total RNA was extracted from the cell lines using TRIzol reagent (Invitrogen Corporation). Then DNase I was used to remove genomic DNA from total RNA. Concentration and purity of the RNA samples were examined by a spectrophotometer. Two oligonucleotides, 5'-AATCCCATCACCATCTTCCA-3' and 5'-CCTGCTTCACCACCTTCTTG-3', designated GAPDH (a housekeeping gene), were utilized as primers to perform polymerase chain reaction (PCR) in order to check whether the RNA was contaminated with trace genomic DNA.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Reverse transcriptase (RT) was performed using an access RT-PCR system (Promega Corporation) according to the manufacturer's protocol. Total RNA in the amount of 1 μg was used in each RT reaction to synthesize cDNA. For PCR, we used the forward primer 5'-AAGACGTACTCAGGCCATGTCC-3'and reverse primer 5'-GACCCAAATGTCGCAGTCAG-3', which were designed by primer premier 5.0 software, to amplify human Tiam1 (NM_003253 from GeneBank). And human GAPDH primers were utilized as an internal control. Thirty cycles of reactions were carried out, each at 94 ℃ for 45 s, at 58 ℃ for 45 s, at 72 ℃ for 45 s. PCR products were electrophoresed in 2% agarose gel and sequenced by Bioasia Biological Corporation. Electrophorectogram was taken by a pickup camera under UV light and analyzed by 1-D advanced software.

In vitro invasion assay

This assay was based on the principle of Boyden chamber[21]. The top and bottom of Boyden chamber (corning company) were separated by a polycarbonate filter with 8 μm pore size. The top chamber was prepared by coating the filter with 50 μg of diluted Matrigel and incubated for 30 min. A single cell suspension of 10 000 tumor cells in serum-free medium was inoculated in the upper chamber, after 5% FBS was added into the bottom chamber as a chemoattactant. After 24 h incubation, noninvasive cells were removed with a cotton swab. The cells migrated through the membrane and stuck to the lower surface of the membrane were fixed with methanol and stained with hematoxylin. Tumor cell invasiveness was determined by counting all tumor cells in five randomly selected counting fields at ×200.

Statistical analysis

Relationship between expression of Tiam1 and invasive ability was analyzed by bivariate correlation using SPSS 10.0 Software.

RESULTS

Expression of Tiam1 in colorectal carcinoma cell lines

Total RNA extracted from eight colorectal carcinoma cell lines was confirmed to have no degradation by agarose gel electrophoresis (Figure 1). In order to detect whether the RNA was contaminated with gDNA, we used the RNA digested with DNase I and RNA not digested with DNaseIas templates to amplify the housekeeping gene, GAPDH. The results showed that the RNA we extracted was not contaminated with gDNA (Figure 2).

RT-PCR detection of Tiam1expression in eight colorectal carcinoma cell lines was heterogeneous (Figure 3). Analyzed by 1-D Advanced software, relative Tiam1 expression was calculated as the ratio of the densitometry of the Tiam1 band: GAPDH band on RT-PCR. We found that Tiam1 was abundantly expressed in metastastic colorectal carcinoma cell lines, LoVo and SW620, 1.19 and 0.96 respectively. In primary colorectal carcinoma cell lines, SW480 (0.57), LS174T (0.32), LST (0.44), HCT116 (0.60), HRT-18 (0.52) and Hee8693 (0.55) were moderately and lowly expressed. The product of RT-PCR, Tiam1, was confirmed by sequence analysis (date was not shown).

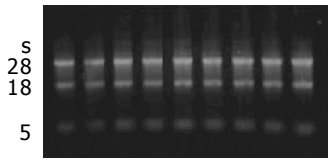

Figure 1 Agarose gel electrophoresis of total RNA from colorectal carcinoma cell lines.

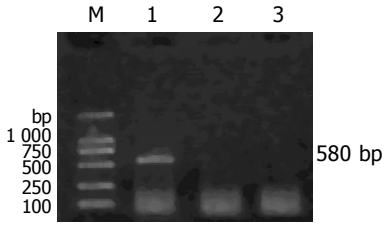

Figure 2 2% agarose gel electrophoresis of the product of PCR (580 bp). M: Marker; lane 1: Templates were the RNAs not digested with DNase I, GAPDH was amplified; lanes 2, 3: Templates were the RNAs digested with DNase I, no product was amplified.

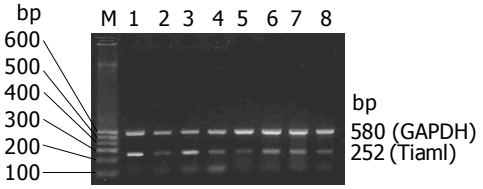

Figure 3 Expression of Tiam1 in 8 colorectal carcinoma cell lines by RT-PCR. M: Marker; lane 1: LoVo; lane 2: SW480; lane 3: SW620; lane 4: HCT116; lane 5: LS174T; lane 6: LST; lane 7: HRT-18; lane 8: Hee8693.

In vitro invasive assay

In vitro cell invasive assay was performed based on the principle of the Boyden chamber assay. Matrigel matrix served as a reconstituted basement membrane *in vitro*. The number of cells migrating through the Matrigel matrix was counted. The result indicated that LoVo (145) and SW620 (130) had a higher invasive ability than SW480 (91), HCT116 (89), LS174T (64), LST (79), HRT-18 (87) and Hee8693 (90).

Correlation analysis

We used the SPSS 10.0 to analysis the correlation between the expression of Tiam1 and invasive ability. The results showed that the both are highly correlative($r = 0.995$, $P<0.01$).

DISCUSSION

Tumor invasion is a complex biological process, during which tumor cells detach from the primary tumor and infiltrate its surrounding tissues. This process requires loss of cell contacts between tumor cells, active cell migration, adhesion to the extracellular matrix (ECM) and proteolytic degradation of

ECM[22]. At the molecular level, a number of different molecules, including cadherins, integrins, proteases, and growth factors, have been implicated in the regulation of tumor cell invasiveness[23].

It has been found that Tiam1 is capable of activating Rac1 as a ubiquitous guanine nucleotide exchange factor and inducing membrane cytoskeleton-mediated cell shape changes, cell adhesion, and cell motility[24-26]. Rac1 could act on downstream of Tiam1 signaling and regulate the function of several cell adhesion molecules such as laminin receptor, integrin[27], E-cadherin[18], and the hyaluronan receptor, CD44.

In the past, to search new metastasis-associated genes, we prepared cDNA microarray which is highly sensitive and applicable in examining gene expression profile. Then metastatic colorectal carcinoma microarray gene expression data were mined by literature profiling, based on the analysis of literature profiles generated by extracting the frequencies of certain terms from MEDLINE. We found that Tiam1 gene had a potential relation to metastatic colorectal carcinoma.

In this study, we investigated the relation between Tiam1 expression and metastasis in colorectal carcinoma. LoVo cell line was derived from human colonic adenocarcinoma established from the metastatic nodule. SW480 was isolated from a high-grade primary colonic tumor, and SW620 was isolated from a metastatic lymph node from the same patient 1-year later at the time of clinical relapse. LS174T, LST and HCT116 were isolated from primary colonic carcinomas. HRT-18 was isolated from primary rectal cancer and Hee8693 from primary cecal cancer. We found that the expression of Tiam1 in cell lines established from metastatic colorectal carcinomas was much higher than that in cell lines established from primary colorectal carcinomas. We also found that the invasiveness and metastasis in cell lines established from metastatic colorectal carcinomas were much stronger than those in cell lines established from primary colorectal carcinomas. We identified that Tiam1 was highly correlated with invasiveness and metastasis ($P<0.01$). These data indicate that enhanced expression of Tima1 is associated with increased invasive ability.

In a word, our results suggest that Tiam1 might be a metastasis-related gene in colorectal carcinoma. In the future research, we will study the effect of Tiam1 gene on invasion and metastasis in colorectal carcinoma *in vivo* and *in vitro* through stable Tiam1 gene transfection or RNA interfering. The two-dimensional electrophoresis and mass spectrum analysis will be used to study the possible mechanism of Tiam1 gene and its signal transduction.

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