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Original Article

MAP4 and MAP6 expression in breast cancer cell lines

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Abstract: Breast Cancer (BCa) remains one of the most prevalent forms of cancer and is the most common cancer found in women around the world. Multiple steps are involved in the process of tumorigenesis and metastasis, but one of the most important is motility and reorganization. In this process, cytoskeletal elements such as actin, microtubules, and other proteins are important. MAP4 and MAP6 are microtubule-associated proteins that have been related with different mechanisms in the cytoskeletal process; we analyzed the gene expression of MAP4 and MAP6 in cultured cell lines MCF-10A, MDA-MB-231, SKBR3, and T47D by real-time PCR and protein interactions with STRING network analysis. We found mRNA expression of MAP4, but not of MAP6. Gene expression of MAP4 was higher in the MDA-MB-231 than in the MCF-10A cell line. With respect to protein interactions, MAP4 were related with different proteins that are involved in the process, such as tumorigenesis, cell cycle progress, apoptosis and autophagy, ubiquitination, platelet activation and vascular development, formation and elongation of filopodia, and the dynamic process of intracellular movements. In conclusion, regulation of MAP4 could be related with different proteins in different important molecular mechanisms in BCa and could comprise an important anticancer drug target.

Keywords: MAP4, MAP6, patients with breast cancer, microtubule

Introduction

MicroTubules (MT) play important roles in fundamental cellular processes, such as chromosome segregation, intracellular transport, directional migration, and cell morphogenesis [1]. Microtubule-targeting agents have been used for treatment of different types of aggressive cancer [2]. MAP4 and MAP6 are microtubule-associated proteins [3-6]. MAP4 was recognized as a cytosolic MT-binding protein that is ubiquitously expressed in non-neural cells and it possesses an important role in microtubule dynamics [7, 8]. Once it is phosphorylated, MAP4 dissociates from tubulin, resulting in MT instability [9, 10]. Thus, MAP4 may play a major role in the maintenance of vascular integrity [7]; this could be an important role in tumorigenesis. The physiological role of MAP6 proteins is not yet fully understood, but phenotypic and cellular analyses of MAP6-null mice indicated that MAP6 proteins are involved in a number of neuronal functions. MAP6-null mice present defects in synaptic plasticity and neurotransmission associated with severe behavioral disorders [11, 12]. In our study, we showed the genetic expression of MAP4 and MAP6 in Breast Cancer (BCa) cell lines, suggesting that these proteins may be related in different tumor mechanisms, in addition to their potential as a drug target in different tumor types.

Materials and methods

Cell culture

MCF 10A, T47D, SKBR3, and MDA-MB-231 cell lines were obtained from ATCC (Manassas, VA,

Table 1. Primer sets for MAP4 and MAP6 used in real-time PCR. The design was based on ensemble transcript ID of the Human Probe Library

Gene	Nucleotide sequence accession number	Left primer 5'-3'	Right primer 5'-3'
MAP4	NM_002375.4	Ggattcccatttagaatctctgc	ccgttcctgtgacggttt
MAP6	NM_033063.1	gctttt cag ctagcatcatgg	cgctttgtccaactggtt c
18s	NR_003286.2	Cgaacgtctgccctatcaac	ttg gat gtggtagccgtttc
B-Actin	NM_031144	cgt cat ccatggcgaatc	Ccc gcgagtacaaccttc t

USA). MCF-10A (ATCC® CRL-10317™) were cultured in DMEM F12 (GIBCO) supplemented with 4.18 µf/ml insulin, 10 µg/ml HEGF, and 0.4 µg/ml Hydrocortisone. T47D (ATCC® HTB-133™) were cultured in RPMI-1640 (GIBCO). MDA-MB-231 (ATCC® HTB-26™) was cultured in DMEM high glucose (Hyclon, Logan, UT, USA). SKBR (ATCC® HTB-30™) was cultured in McCoy's 5a Modified Medium (Thermo Fisher Scientific). All cell lines were supplemented with 10% FBS (GIBCO) and 1% Penicillin/Streptomycin (SIGMA). Cells were grown as monolayers under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2. The cells were cultured in BD Falcon 250-ml, 75-cm^2 Cell Culture Flasks for gene expression analysis.

RNA extraction and RT-PCR assays

Total RNA from the BCa cell lines and tissue human brain was isolated using TRIzol Reagent according to the manufacturer's protocol (Life Technologies, USA). We utilized 0.5 µg of the total simple RNA for reverse-transcription with random hexamers for 10 min at 65°C, for 50 min at 35°C, and for 5 min at 75°C in a 20-µl reaction volume employing Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics). Reactions were performed in an Eppendorf Mastercycler® Thermal Cycler (Eppendorf, México). PCRq reactions were carried out utilizing the Human Universal Probe Library (Roche Diagnostics). Specific oligonucleotide primers for MAP4 and MAP6 were originally generated by employing online assay design software (ProbeFinder: http://www.universal-probelibrary.com) and the primer sequence for each gene that is depicted in **Table 1**. The reaction mixture was prepared according to the manufacturer's instructions (Roche Diagnostics, GmbH Mannheim, Germany). Amplification was performed in borosilicate glass capillaries (Roche Diagnostics) with a LightCycler 2.0 instrument. Amplification conditions for UPL-based assays were initially denaturized for 10 min at 95°C, followed by 45 cycles of 10 sec at 94°C, 20 sec at 60°C, and 5 sec at 72°C. The PCR assay included a standard curve of four serial dilution points for each gene, and mRNA levels were calculated using the comparative parameter threshold Cycle (Ct) method and normalized to the endogenous control: 18S rRNA. Results were calculated as a percentage of the mean level found in the control sample utilizing the ΔCT method.

Protein network analysis

STRING network analysis of protein-protein interactions was performed to identify functionally linked proteins and to determine the potentially affected biological processes [PMID: 12519996]. The network is presented under confidence view, whereby stronger associations are represented by thicker lines or edges and vice versa, whereas proteins are represented as nodes. All gene symbols were derived from the HUGO Gene Nomenclature Committee (HGNC) (http://www.genenames.org).

Statistics

The percentage of cells with different morphology was determined for observed field and was plotted. Results were expressed as the mean ± Standard Deviation (SD). Data were analyzed with one-way ANOVA and the Dunnett test for multiple comparisons, using SigmaPlot ver. 12.0 software (San Jose, CA, USA), and differences were considered statistically significant with $P \leq 0.05$.

Results

MAP gene expression in breast cancer and non-tumor cell lines

We analyzed the MAP4 and MAP6 mRNA transcript in three cancer cell lines compared with MCF-10A (non-transformed epithelial cell lines, derived from human fibrocystic mammary tissue (**Figure 1**). MAP4 (**Figure 1A**) exhibited mRNA expression in all cell lines. In all cases, we found differences between MCF-10A and

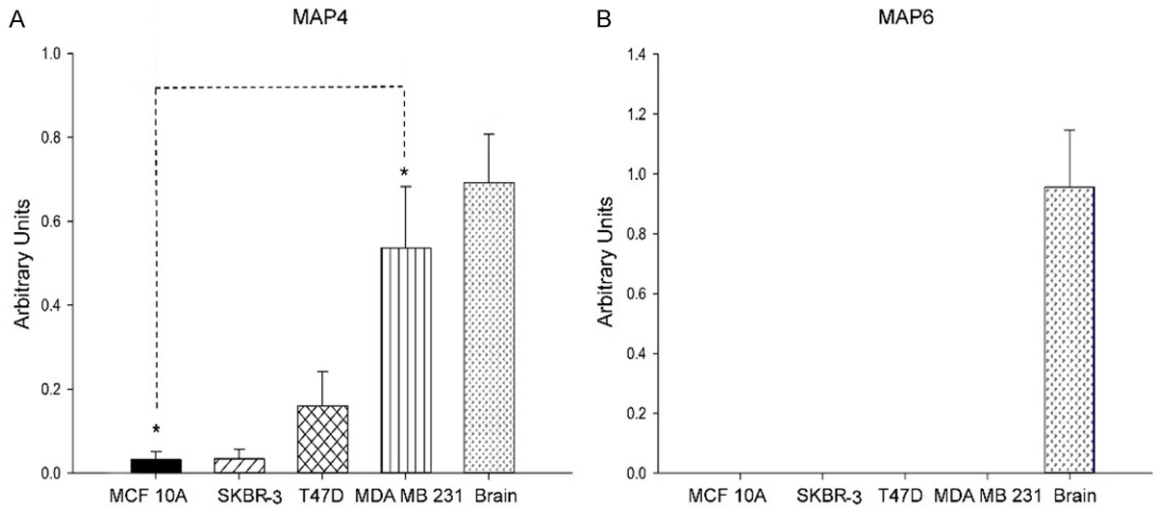

Figure 1. The relative expression level of genes MAP4 (A) and MAP6 (B) was determined after normalization against the 18S internal control for each simple. Data represent the mean ± Standard Deviation (SD). *P<0.01. Human Brain was used as positive control.

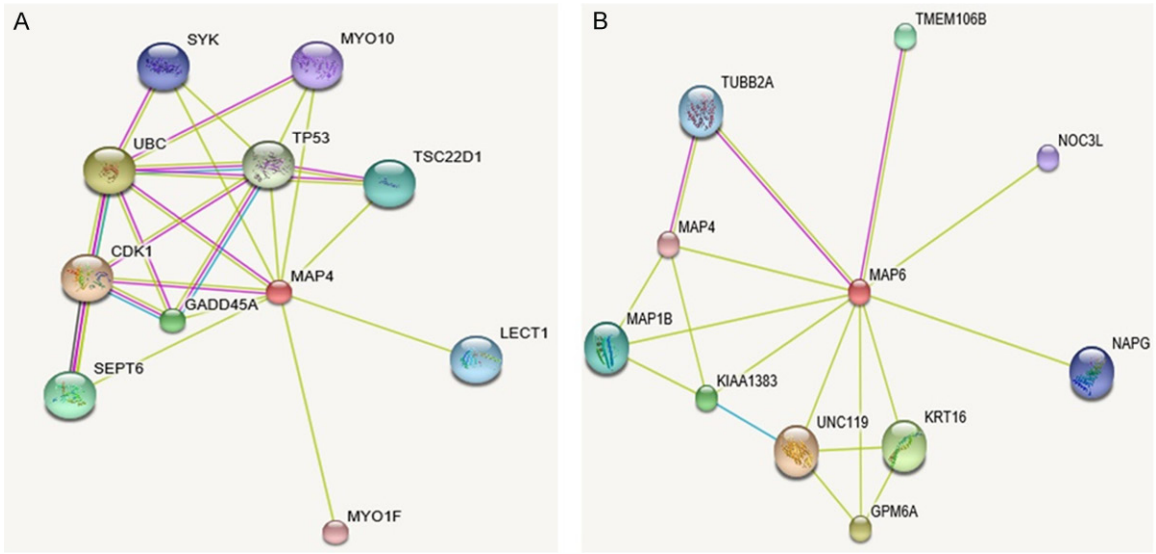

Figure 2. STRING interaction network showing an association between differentially expressed proteins. The interaction of MAP4 and MAP6 was generated using default settings (Medium confidence of 0.4 and 7 criteria for linkage: activation, inhibition, binding, phenotype, catalysis, post-transl. m reaction, expression). Interactions are represented as follows: binding in blue; post-translational modification in pink; expression in green; catalysis in violet, and reaction in black.

MDA-MB-231 cell lines (P<0.01), but did not find MAP6 mRNA expression in MCF-10A or in BCa cell lines (**Figure 1B**).

Protein network analysis

Figure 2 illustrates the interaction between 10 identified proteins and the additional interactions related with MAP4 proteins. We found that MAP4 protein was related with proteins that are involved in ubiquitination, modulation of centrosome cycle, apoptosis, cytokinesis, angiogenesis, cell adhesion, osteoclast maturation, platelet activation, and vascular development and elongation of filopodia, and the dynamical process of intracellular movements (**Table 2**).

Discussion

MAP4 is primarily recognized as a cytosolic MT-binding protein that is ubiquitously expres-

Table 2. MAP4 interaction protein (http://string-db.org/cgi/network.pl?taskId=lAcDMtF1EKwX)

	Score	Protein description
CDK1	0.98	Cyclin-dependent kinase 1; Plays a key role in the control of the eukaryotic cell cycle by modulating the centrosome cycle as well as mitotic onset; promotes G2-M transition, and regulates G1 progress and G1-S transition via association with multiple interphase cyclins. Required in higher cells for entry into S-phase and mitosis.
UBC	0.92	Ubiquitin C
TP53	0.87	Tumor protein p53; Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases
GADD45A	0.85	Growth arrest and DNA-damage-inducible, alpha; In T-cells, functions as a regulator of p38 MAPK by inhibiting p88 phosphorylation and activity (by similarity). Might affect PCNA interaction with some CDK (cell division protein kinase) complexes; stimulates DNA excision repair *in vitro* and inhibits entry of cells into S phase
SEPT6	0.85	Septin 6; Filament-forming cytoskeletal GTPase. Required for normal organization of the actin cytoskeleton. Involved in cytokinesis. May play a role in HCV RNA replication
TSC22D1	0.81	TSC22 domain family, member 1; Transcriptional repressor. Acts on the C-type Natriuretic Peptide (CNP) promoter
LECT1	0.80	Leukocyte cell-derived chemotaxin 1; Bifunctional growth regulator that stimulates the growth of cultured chondrocytes in the presence of basic Fibroblast Growth Factor (FGF) but inhibits the growth of cultured vascular endothelial cells. May contribute to the rapid growth of cartilage and vascular invasion prior to the replacement of cartilage by bone during endochondral bone development. Inhibits *in vitro* tube formation and mobilization of endothelial cells. Plays a role as antiangiogenic factor in cardiac valves to suppress neovascularization
SYK	0.80	Spleen tyrosine kinase; Non-receptor tyrosine kinase which mediates signal transduction downstream of a variety of transmembrane receptors including classical immunoreceptors such as the B-Cell Receptor (BCR). Regulates several biological processes including innate and adaptive immunity, cell adhesion, osteoclast maturation, platelet activation, and vascular development.
MYO10	0.75	Myosin X; Myosins are actin-based motor molecules with ATPase activity. Unconventional myosins serve in intracellular movements. MYO10 binds to actin filaments and actin bundles and functions as plus end-directed motor. The tail domain binds to membranous compartments containing phosphatidylinositol 3,4,5-trisphosphate or integrins, and mediates cargo transport along actin filaments. Regulates cell shape, cell spreading, and cell adhesion. Stimulates the formation and elongation of filopodia. May play a role in neurite outgrowth and axon guidance.
MYO1F	0.75	Myosin IF; Myosins are actin-based motor molecules with ATPase activity. Unconventional myosins serve in intracellular movements.

sed in non-neural cells and that is possesses an important role in microtubule dynamics [7]. Therefore, it has been mentioned that MAP4 may play a major role in the maintenance of vascular integrity, and that it could be involved in the modulation of inflammation [7] and in invasion through microtubule dynamics. In bladder cancer, it has been described that overexpression of MAP4 appears to be positively correlated with tumor stage and degree of malignancy [13]. With respect to with STRING protein analysis, MAP4 were related with the Ubiquitin C protein. Ubiquitin Carboxyl-terminal Hydrolase L1 (UCH-L1) is an abundant neuronal protein; it was overexpressed in wide-type tumors and was related with invasion in some solid tumors, including BCa [14-16]; Goto et al. demonstrated that Ubiquitin C-terminal Hydrolase-L1 (UCHL1) abrogates von Hippel-Lindau-mediated ubiquitination of HIF-1α, the regulatory subunit of HIF-1, and consequently promotes metastasis, and that overexpression of UCHL1 increases tumor metastases in an HIF-1-dependent manner in murine models of pulmonary tumor. Meanwhile, blockade of the UCHL1-HIF-1 axis could suppress the formation

of metastatic tumors. Goto et al. also found that the expression levels of UCHL1 correlate positively with HIF-1α, and that they were related with poor prognosis of patients with breast and lung cancer [15]. In addition, these authors' findings are similar to those reported by others. In colorectal and pancreatic tumors, overexpression of UCHL1 was associated with higher incidence of tumor recurrence and shorter survival time [17, 18]. Thus, UCHL1 could be a cancer drug target in wide-type tumors [19-21]. Another important protein related with MAP4 was Tumor Protein p53 (TP53). TP53, the guardian of the genome, possesses a tumor suppressor function through the maintenance of genetic integrity, cell-cycle machinery, apoptosis, and DNA repair [22, 23]. In order to check genetic errors, p53 accumulates in the nucleus in response to cellular stress, such as DNA damage, hypoxia, and nucleotide deprivation [23, 24]. Once p53 is transported into the nucleus, it transactivates its target genes, involved either in cell-cycle arrest or in apoptosis [25]. Some studies have demonstrated that G-actin is guides through p53 transport toward the nucleus; the p53 cargo reaches the perinuclear region and interacts importantly in receptors [23, 26]. Microtubules have been targeting several drugs that are important in the treatment of a wide variety of tumor types [27, 28], but proteins associated with microtubules are acquiring importance within this context in different cancer types [29, 30].

Conclusions

In conclusion, this study shows the gene expression of MAP4, but not of MAP6, in BCa cell lines and its *in silico* relationship among proteins involved in different cancer processes. However, further studies are needed to clarify our results.

Disclosure of conflict of interest

None.

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