

## RESEARCH ARTICLE

# Neural Transdifferentiation: MAPTau Gene Expression in Breast Cancer Cells

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### Abstract

**Background:** In tumor cells, aberrant differentiation programs have been described. Several neuronal proteins have been found associated with morphological neuronal-glia changes in breast cancer (BCa). These neuronal proteins have been related to mechanisms that are involved in carcinogenesis; however, this regulation is not well understood. Microtubule-associated protein-tau (MAP-Tau) has been describing in BCa but not its variants. This finding could partly explain the neuronal-glia morphology of BCa cells. Our aim was to determine mRNA expression of MAP-tau variants 2, 4 and 6 in breast cancer cell lines. **Materials and Methods:** Cultured cell lines MCF-10A, MDA-MB-231, SKBR3 and T47D were observed under phase-contrast microscopy for neural morphology and analyzed for gene expression of MAP-Tau transcript variants 2, 4 and 6 by real-time PCR. **Results:** Regarding morphology like neural/glia cells, T47D line shown more cells with these features than MDA-MB-231 and SKBR. In another hand, we found much greater mRNA expression of MAP-Tau transcript variants 2, and to a lesser extent 4 and 6, in T47D cells than the other lines. In conclusion, regulation of MAP-Tau could bring about changes in cytoskeleton, cell morphology and motility; these findings cast further light on neuronal transdifferentiation in BCa.

**Keywords:** Breast cancer - neural transdifferentiation - neuron/glia-like cell - microtubule-associated protein tau

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### Introduction

Breast cancer (BCa) is the most prevalent worldwide oncologic disease in women. BCa incidence and mortality has increased in Mexico in the last years. (Perez-Santos and Anaya-Ruiz, 2013; Anaya-Ruiz et al., 2014; Leon-Hernandez et al., 2014; Soto-Perez-de-Celis and Chavarri-Guerra, 2016). Cell transdifferentiation is a phenomenon of genetic reprogramming and protein expression change; however, transdifferentiated cells could show morphological and phenotypical changes of neuronal lineage despite, their lineage is different (Cho et al., 2005; Bauer et al., 2012; Zhang et al., 2010; Palafox et al., 2012).

It has been shown that BCa cell lines have been found specific markers of neuronal / glial type lineage, indicating that aberrant transdifferentiation is a phenomenon that occurs in this type of cancer (Zhang et al., 2010). Regarding finding to neuronal / glial morphology phenotype, described in the line MDA-MB-435 (lung cell lines derived from lung metastases), has been shown

the presence of polygonal soma (cell body), extended dendrites and axons, and cell-cell communication at synapses (Zhang et al., 2010). Different BCa cell lines show expression of a wide number of neuronal proteins such as: Synaptic Vesicle Protein 2 (SV2A) (Bandala et al., 2012), beta-tubulin class III gene (TuBB3), nestin, (Zhang et al., 2010; Kanojia et al., 2015), SNAP23, vesicle-associated membrane protein 7 (VAMP7), Syntaxin4, 8 (Williams et al., 2014; Bassett et al., 2008), microtubule-associated protein tau (MAP-Tau) (Honig, et al., 2014), synuclein gamma (Ahmad et al., 2007; Guo et al., 2007), synaptotagmin (Sreenath et al., 2005), synapsin 1 (Sadeqzadeh et al., 2011).

Further, cytoskeleton and cytoskeleton-associated proteins are important for cell reprogramming processes (Cheng et al., 2013). There are many cytoskeleton associated proteins, one of them proteins are the Microtubule-associated protein (MAP-Tau) which is a family of proteins that regulate the microtubule dynamics (Zhou et al., 2015). Interestingly, MAP-Tau is widely

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distributed in central nervous system, and recent data suggest that might also play a physiological role in dendrites, but its most abundant in axons. MAP-Tau is a multifunctional protein and its function depends on the protein phosphorylation state. The phosphorylation of MAP-Tau could result in its ability to bind to microtubules, and alteration in these modifications results in neurodegenerative diseases (Goedert et al., 1988; Goedert et al., 1989; Shin et al., 1991; Mietelska-Porowska et al., 2014). In the other hand, MAP-Tau proteins are accepted as marker in paclitaxel response in breast cancer showing that MAP-Tau could repress the activity of taxane drugs (Zhou et al., 2015). MAP-Tau and others molecules have been detected in different cancer types and could be used as molecular markers of cancer and neural transdifferentiation and, like MAP-Tau, could explain transformation, immune-evasion, carcinogenesis and metastasis (Goedert et al., 1988; Goedert et al., 1989; Shin et al 1991; Chapin & Bulinski, 1992; Harada et al., 1994; Paris et al., 2011; Zang et al., 2011; Ksiazkiewicz et al., 2012; Palafox et al., 2012; Kotoula et al., 2013).

Few studies related microtubule-associated proteins expressions with BCa (Rody et al., 2007; Bauer et al., 2012; Ikeda et al., 2012; Moleans et al., 2011; Penteroudakis et al., 2011; Sadi et al., 2011; Kolacinska et al., 2012; Leontovich et al, 2012; Endo et al., 2013; Kotoula et al., 2013), that proceed most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk (Sariego 2010). But, in this research we studied 3 different variants of MAP-Tau in different BCa cell lines.

## Materials and Methods

### Cell culture

T47D, SKBR3 and MDA-MB-231 cell lines were obtained from ATCC (Manassa, VA). MCF-10A cells were gifted from Dr. Juan Pedro Luna Arias, CINVESTAV. T47D were cultured in RPMI-1640 (GIBCO). MCF-10A were cultured in DMEM F12 (GIBCO) supplemented with 4.18  $\mu$ f/ml insulin, 10  $\mu$ g/ml HEGF, 0.4  $\mu$ g/ml Hydrocortisone. MDA-MB-231 was cultured in DMEM high glucose (Hyclon, Logan, Utah). 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 humidified atmosphere containing 5 % CO<sub>2</sub> and 95 % air. The cells were cultured in BD Falcon 75 cm<sup>2</sup> Cell Culture Flask, 250 ml for morphologic and genetic expression analysis.

### Cell culture morphologic analysis

The moment that cell cultures reach confluence was

regarded time zero and they stayed in incubation until 6 days. Then cell were observed daily to start to 24 h, and in 6 day they were photographed. The morphology of cells on slides was observed with an inverted Olympus MIC-D Digital Microscope with phase contrast, imaging acquisition system and 40x objective lens.

### RNA extraction and RT-PCR assays

Total RNA from the tissue adipose was isolated using TRIzol Reagent according to the protocol of manufacturer (Life Technologies, USA). Isolated RNA was quantified using the GENESYS 10 Series spectrophotometer (Thermo Scientific, USA) and 5  $\mu$ g of RNA were separated on a 1.0 % agarose gel containing ethidium bromide in MOPS buffer. Running buffer and gel contained 0.2 M formaldehyde. To avoid trace amounts of DNA contamination, RNA samples were treated with amplification grade DNase I (Invitrogen) before reverse transcription. All RNA samples were stored at -70 °C in RNA elution solution until further use. We used 0.5  $\mu$ g of RNA for reverse-transcription with random hexamers in 20- $\mu$ l reaction volume using Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics). The reactions were performed in Eppendorf Mastercycler thermocycler (Eppendorf, Mexico). The amplified cDNA was quantified on a photometer at 260 nm. RT-PCR reactions were carried out using the Human Universal ProbeLibrary (Roche Diagnostics). Specific oligonucleotide primers for MAP-Tau transcript variants 2, 4 and 6 were originally generated by using the online assay design software (ProbeFinder: <http://www.universal-probelibrary.com>) and the primer sequence for each gene that is shown in Table 1. The 20  $\mu$ l-reaction mixture contained 1x LightCycler TaqMan Master reaction mixture (Roche Diagnostics), 200 nM of each primer, 100 nM of Universal ProbeLibrary probe, 0.5 U LightCycler Uracil-DNAGlycosylase and 2  $\mu$ l of standard DNA in appropriate dilution.

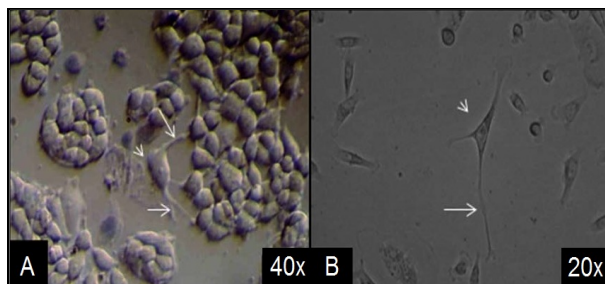
The amplification was performed in borosilicate glass capillaries (Roche Diagnostics). The RT-PCR assay included a standard curve of four serial dilution points for each gene and samples were normalized with the endogenous control 18S. Data were analyzed by LightCycler software.

### Statistics

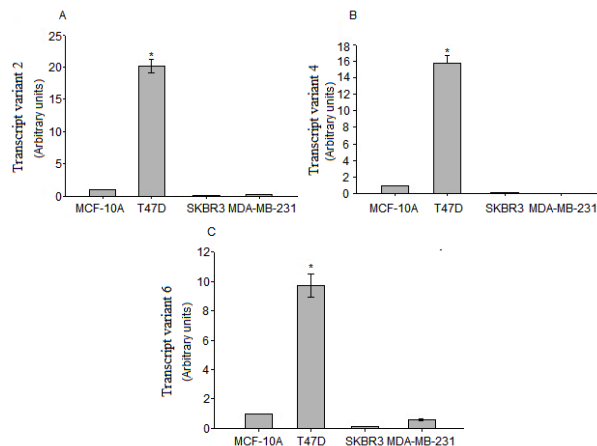
The percentage of cells with different morphology was determined for observed field and was plotted. For compare mRNA expression was utilized Kruskal-Wallis One Way Analysis of Variance on Ranks. Statistical Analysis was performed with SPSS v17 for Windows XP (SPSS, UK, Ltd, Working, UK) and SigmaPlot for Windows v11.0. P<0.05 was regarded as significant.

**Table 1. Primer Sets for MAPT used in Real-time PCR with Designs Based on Ensemble Transcript ID of the Human Probe Library**

Gene	Nucleotide sequence accession number	Left primer 5'-3'	Right primer 5'-3'
Transcript variant 2	NM_005910.5	gatcccagaaggaaccacag	ttttactgaccatcgagctt3
Transcript variant 4	NM_001134364.1	accacagccacctctct	cagccatcctggtcctaagt
Transcript variant 6	NM_001123066.3	tctcttctgtcactcccgaac	gcgatcttctttaccatca



**Figure 1. BCa-Cell Lines Differentiate Into Neuron/Glial-Like Cell.** Phases-contrast microscopy of cell culture. A) T47D, B) MDA-MB-231. Arrows indicate neural-transdifferentiation cell



**Figure 2. The Relative Expression Level of genes Transcript Variants 2, 4 and 6 (A, B, C) of MAP-Tau was Determined After Normalization Against the 18S Internal Control for Each Sample.** Data represent the mean  $\pm$  SD, \*P vs. control (MCF-10A)

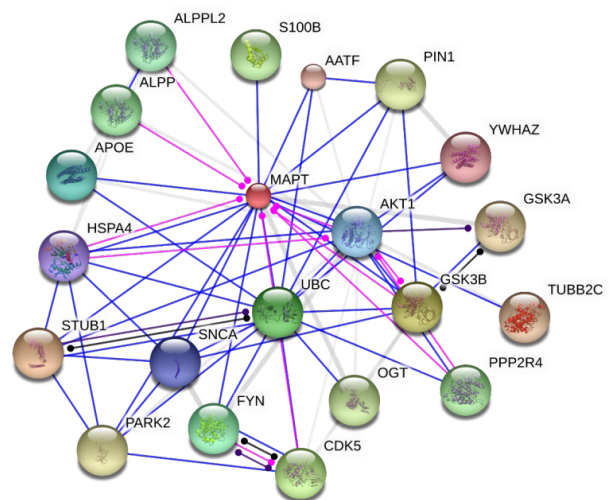
## Results

Presence of cell transdifferentiation in breast cancer

With the aim to know if there is cell transdifferentiation in BCa and that there not is eventual simple dedifferentiation or cell function loss, the cell morphology was explored. It was observed among cancer cells of habitual morphology ones with different aspect, like a neural cells, which were common in TD47D (Figure. 1A) and MDA-MB-231 (Figure. 1B). In each case, the cells showed a uniform morphology until third day of culture, and then cells changed their aspect with extensions like axons of neural cells, feature more appreciable when the cells were dispersed, suggesting a glial/neural transdifferentiation in phenotype of BCa lines. Neuron-like cells formed a subpopulation that reach 4 % of observed total fields in T47D cell line. The transdifferentiation was greater in T47D that MDA-MB-231 (37.8%) and MDA-MB-453 (60.1 %) lines.

### Neural gene expression in breast cancer and non-tumor cell lines

We analyzed the MAP-Tau mRNA transcript variants in three cancer cell lines and compared with epithelial cells, non-transformed derived from human fibrocystic mammary tissue, MCF-10A (Figure 2). The expression of different transcript variants was founded in each cell line. There was not expression difference between SKRB3,



**Figure 3. STRING Interaction Network Showing Association between Differentially Expressed Proteins.** The interaction map 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 and non-tumor cell line for MAP-Tau transcript variants 2, 4 and 6, however, the expression was higher in T47D respect to the others cells ( $P < 0.05$ ). Furthermore, variant 2 shown more mRNA expression the variant 4 and 6.

### Protein Network Analysis

STRING network analysis of protein-protein interactions was performed to identify functionally linked proteins and determine the potential biological processes affected [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>). Figure 3 shows the interaction between 20 identified proteins and the additional interactions. Fourteen proteins were found to be linked for binding with MAP-Tau and six proteins for Post-translational modification. The interaction patterns will be helpful to have a better understanding of the protein functional activities. As the identified proteins are involved in the modulation of neuronal apoptosis process and cell growth (AATF and FYN), but also but also proteins associated with phosphorylation, ubiquitination and increase levels of tau protein (S100B, PIN1, STUB1, CDK5, AKT1, TUBB2C, and GSK3A).

## Discussion

Cancer tumors usually are apparently dedifferentiated cell growths, which form a mosaic of disorganized, undifferentiated cell subpopulations that express non-characteristic proteins of original tissue. Interestingly, the BCa cell underwent a differentiation process into neuron/glial-like cell, apparently changing its phenotypic

aspect, and perhaps this could be regarding with epigenetic processes (McPherson et al., 2000; Battula et al., 2012; Palafox et al 2012). Neuronal / glial phenotype morphology was described in the MDA-MB-435 line (lung cell lines derived from lung metastases): such as the presence of polygonal soma (cell body), extended dendrites and axons, and cell-cell communication at synapses (Zhang et al., 2010), are similar to ours results in MDA-MB-231 line, MDA-MB-453, T47D.

We found MAP-Tau expression 2, 4 and 6 transcript variants, which ones, interestingly are essential in neurogenesis process, stabilizing microtubules growth (Shin et al., 1991; Goedert et al., 1988; Goedert et al., 1989; Lim & Halpain, 2000; Ray et al., 2011; Barneda-Zahonero et al., 2012). These proteins suggest a neural nature of Neural-like cell founded in this study, because products of paralogous genes in rat and mouse were be associated with neuron-specific cytoskeletal proteins that are enriched in dendrites, implicating a role in determining and stabilizing dendritic shape during neuron development (Barneda-Zahonero et al., 2012). The regulation of MAP-Tau could bring changes in cytoskeleton and cell morphology and motility, which result unless in transdifferentiation look (Bengtsson et al., 1993; Goedert et al., 1988; Goedert et al., 1989; Yang et al., 2008; Barrachina and Ferrer, 2009). Our results showed that MAP-Tau transcript variants are related with BCa, but may be associated with positive estrogen alpha receptors (Endo et al., 2013), in the other hand, recently has been found that MAP-Tau could be associated to kinesin-1 which is a molecular motor that transport different molecules across the microtubules, and interestingly, this Kinesin-1 binds to the same microtubule region were paclitaxel binds and act, and may be, because of that MAP-Tau proteins are associated with poor prognosis and paclitaxel tumor resistance (Seeger and Rice, 2010), but other results are controversial (Honing et al., 2014). Cell transdifferentiation could have an important role, joint to RANK (Palafox et al 2012), ganglioside GD2 (Battula et al., 2012) or epigenetic causes (Barneda-Zahonero et al., 2012) and such as in case of human adult peripheral blood mononuclear cells could be induced to transdifferentiate into neural precursor cells and retinal progenitor cells in vitro (Liu et al., 2011). In conclusion, regarding with the expression of MAP-Tau transcripts and with its function in neurogenesis, we suggest an apparently transdifferentiation, or a reprogramming process in BCa cell lines; MAP-Tau variant 2 has been more expressed than variant 4 and 6 in BCa cell lines.

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