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Inhibition of p300 suppresses growth of breast cancer. Role of p300 subcellular localization

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ABSTRACT

There is evidence that p300, a transcriptional co-factor and a lysine acetyl-transferase, could play a role both as an oncoprotein and as a tumor suppressor, although little is known regarding its role in breast cancer (BC). First we investigated the role p300 has on BC by performing pharmacological inhibition of p300 acetyl-transferase function and analyzing the effects on cell count, migration and invasion in LM3 murine breast cancer cell line and on tumor progression in a syngeneic murine model. We subsequently studied p300 protein expression in human BC biopsies and evaluated its correlation with clinical and histopathological parameters of the patients. We observed that inhibition of p300 induced apoptosis and reduced migration and invasion in cultured LM3 cells. Furthermore, a significant reduction in tumor burden, number of lung metastases and number of tumors invading the abdominal cavity was observed in a syngeneic tumor model of LM3 following treatment with the p300 inhibitor. This reduction in tumor burden was accompanied by a decrease in the mitotic index and Ki-67 levels and an increase in Bax expression. Moreover, the analysis of p300 expression in human BC samples showed that p300 immunoreactivity is significantly higher in the cancerous tissues than in the non-malignant mammary tissues and in the histologically normal adjacent tissues. Interestingly, p300 was observed in the cytoplasm, and the rate of cytoplasmic p300 was higher in BC than in non-tumor tissues. Importantly, we found that cytoplasmic localization of p300 is associated with a longer overall survival time of the patients.
In conclusion, we demonstrated that inhibition of the acetylase function of p300 reduces both cell count and invasion in LM3 cells, and decreases tumor progression in the animal model. In addition, we show that the presence of p300 in the cytoplasm correlates with increased survival of patients suggesting that its nuclear localization is necessary for the pro-tumoral effects.

Abbreviations: BC, breast cancer; CREB, cAMP responsive element binding protein; DMSO, dimethylsulfoxide; VV56, 2-(3-(3,4-dichlorobenzyloxy)phenoxy)pentadecanoic acid; VV59, 2-(3-(3,4-dichlorobenzyloxy)phenoxy)hexadecanoic acid; DMEM, Dulbecco's modified Eagle's medium; FBS, Fetal Bovine Serum; PBS, phosphate buffered saline; WB, western blot; IF, immunofluorescence; BSA, bovine serum albumin; PI, propidium iodide; ECL, enhanced chemiluminescence; SC, subcutaneously; PFA, paraformaldehyde; H&E, hematoxylin and eosin; IHC, Immunohistochemistry; IRS, Immuno-Reactive-Score; AV, annexin V; NAT, normal adjacent tissues; HAT, hyperplastic adjacent tissues; ER, estrogen receptor; PR, progesterone receptor; Her2, human epidermal growth factor receptor 2; SVG, status of overall survival; HDAC, histone deacetylase; T, tumor.

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1. Introduction

Breast cancer (BC) is the most frequently diagnosed malignant neoplasia and a leading cause of cancer death in females worldwide (Jemal et al., 2011). It is not a single disease but instead constitutes a variety of lesions with distinct cellular origins, somatic changes, and etiologies (Lanari et al., 2012). In addition, BC patients with the same diagnostic and clinical prognostic profile can have markedly different clinical outcomes. This difference is possibly caused by the limitation of our current taxonomy of BCs, which groups molecularly distinct diseases into clinical classes based mainly on morphology (Sotiriou et al., 2003). This reflects the need to find new molecular markers to assist in the diagnosis, prognosis and treatment of this type of cancer.

Transcriptional coactivator p300 participates in the regulation of a wide range of biological processes such as proliferation, cell cycle regulation, apoptosis, differentiation, senescence and DNA damage response (Chan and La Thangue, 2001; Giles et al., 1998; Giordano and Avantaggiati, 1999; Goodman and Smolik, 2000). This protein functions primarily as a transcription cofactor for a number of nuclear proteins including known oncoproteins such as Jun, Fos, and E2F and for tumor-suppressor proteins such as p53, Rb, Smads, and BRCA1 (Avantaggiati et al., 1997; Chan and La Thangue, 2001; Tomita et al., 2000). In addition, it functions as histone acetyltransferase (Bannister and Kouzarides, 1996; Ogryzko et al., 1996) and is capable of acetylating a number of non-histone proteins, including p53, p73, Rb, E2F, Myb, MyoD, HMG(I)Y, GATA1 and alpha-importin (Bannister and Miska, 2000; Chan and La Thangue, 2001; Costanzo et al., 2002; Tomita et al., 2000).

An increasing body of evidence indicates that p300 may be important in cancer (Iyer et al., 2004). Nonetheless, the role of the protein in this disease remains unclear, since there is evidence indicating that it can function both as a tumor suppressor and as an oncoprotein (Goodman and Smolik, 2000). In this regard, it has been reported that increased expression of p300 correlates with cancer progression and decreased patient survival (Debes et al., 2003; Gao et al., 2014; Ishihama et al., 2007; M. Li et al., 2011; Y. Li et al., 2011; Syrjänen et al., 2010). Contrariwise, it has also been described that p300 overexpression predicted a favorable patient outcome (Huh et al., 2013). Interestingly, decreased expression of nuclear p300 protein levels was associated with disease progression and worse prognosis of melanoma patients (Rotte et al., 2013). Furthermore, the mechanisms that regulate the activity of p300 have not yet been elucidated, although many reports point to the importance of the intracellular localization of p300 for its activity (J. Chen et al., 2007; Mackeh et al., 2014; Sebti et al., 2014; Shi et al., 2009).

To our knowledge there is only one report showing the association of p300 expression with tumor recurrence and prognosis of breast cancer patients (Xiao et al., 2011) and no investigations that explore the role of the subcellular localization of p300 in BC progression. In this study we present the first findings to investigate the mechanisms through which p300 influences BC progression evaluating the possibility that p300 and its subcellular localization can be important factors in the progression of this disease.

2. Materials and methods

2.1. Reagents

Curcumin (C.I.75300, Biopack), a novel p300/cAMP responsive element binding (CREB) protein specific inhibitor of acetyltransferase (Balasubramanyam et al., 2004; Y. Chen et al., 2007) was dissolved in dimethyl sulfoxide (DMSO, Sigma) to produce a 200 mM stock solution. 2-(3-(3,4-Dichlorobenzyloxy)phenoxy)pentadecanoic acid (VV56 or Cpd 4k) and 2-(3-(3,4-dichlorobenzyloxy)phenoxy)hexadecanoic acid (VV59 or Cpd 4l), inhibitors of p300 acetyl-transferase activity (Eliseeva et al., 2007) were dissolved in DMSO to produce a 100 mM stock solution.

2.2. Cell culture

LM3 is a tumor cell line derived from a murine mammary adenocarcinoma that spontaneously arose in BALB/c mice (Urtreger et al., 1997) and was a generous gift from E. Bal de Kier Joffé (Instituto de Oncología Ángel Roffo, Buenos Aires, Argentina). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 5% (v/v) Fetal Bovine Serum (FBS, Gibco), L-glutamine (5 mM, Gibco), penicillin (Gibco, 100 U/ml), and streptomycin (Gibco, 100 μg/ml) at 37 °C in a humidified 5% CO_2 air atmosphere.

2.3. Cell count

The cells were plated at a density of 1500 cells/well into 96 multi-well dishes in complete medium. They were treated with 25, 50, 75 and 100 μM of VV56, VV59 or vehicle (DMSO) for 12, 24, 48, and 72 h. They were washed with phosphate buffered saline (PBS) 1×, trypsinized, suspended in 100 μl complete medium and counted manually using a hemocytometer, as previously described (Gandini et al., 2014). Additionally, cell viability was assessed by the WST colorimetric assay (Roche). For this purpose, following treatment with p300 inhibitor, the cells were incubated for 1 h at 37 °C with the tetrazolium salt WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) and the absorbance of the formazan product was read at 450 nm. The results were depicted as percentage of vehicle-treated cells.

2.4. Cell migration

Cell migration was studied by employing the "wound healing" assay as previously described (Petit et al., 2000). Briefly, the cells were seeded in 35 mm Petri dishes and cultured until confluence. The cells were treated with VV59 (12.5 μM), VV56 (12.5 μM) or DMSO and they were scraped with a 200 μl micropipette tip, denuding a strip of the monolayer. Then they were observed and photographed every 4 h for

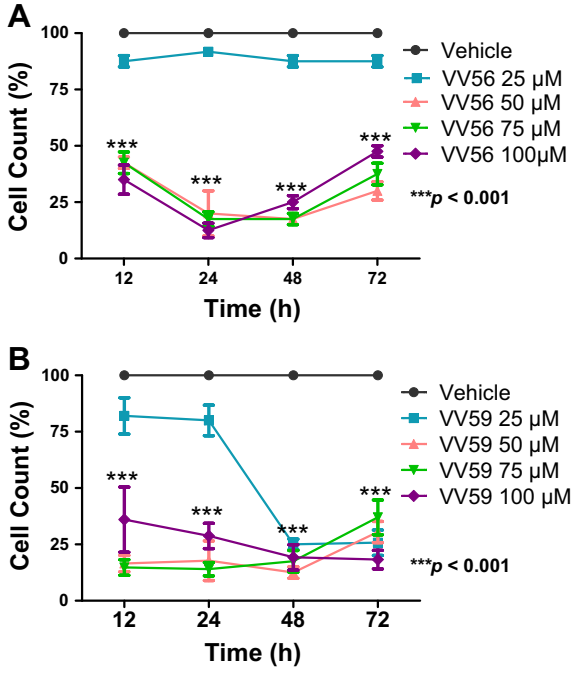

Fig. 1. p300 inhibition decreases LM3 cell count. Cell count was assessed in LM3 cells following different concentrations and times of VV56 (A) or VV59 (B). Concentrations and times used were 25, 50, 75, 100 μM and 12, 24, 48 and 72 h, respectively. Data show the percentage of cells in relation to vehicle-treated cells and are the means (±SD) of triplicate experiments; $p < 0.001$, from Anova test.