XIAP subcellular localization and its association with chemoresistance in breast cancer

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INTRODUCTION AND OBJECTIVE

Breast cancer is the most common malignancy among women and causes high mortality rates worldwide. Some tumor-associated alterations are related with changes in apoptotic pathways, modifying the balance between proliferation and cell death. XIAP is an anti-apoptotic protein localized predominantly at the cytoplasmic fraction, though studies suggest a possible oncogenic role for nuclear XIAP. Previous data from our group show that nuclear XIAP expression presents adverse impact on overall survival of invasive ductal breast carcinoma patients. Conversely, cytoplasmic expression of XIAP confers favorable prognosis for these patients, suggesting different roles for XIAP depending on its subcellular localization. It is still unknown the role of subcellular localization of XIAP in drug resistance in breast cancer. The aim of this study is to investigate XIAP expression in different cellular compartments and its impact on breast cancer chemoresistance.

MATERIAL AND METHODS

We used five human breast cancer cell lines as models: MCF-7, MCF-7 Dox^R, MCF-7 Tax^R, MDA-MB-231 and BT549. MCF-7 Dox^R and MCF-7 TaxR cell lines were derived from MCF-7 and are resistant to doxorubicin (dox) and taxanes, respectively. Also, we used a non-neoplastic breast cell line: HB4a. To evaluate the cell viability and colony formation, MTT and clonogenic assay were performed, respectively. Subcellular fractioning and Western blotting were used to access the subcellular localization of XIAP.

RESULTS AND CONCLUSION

Our results show that all cell lines present cytoplasmic XIAP, except for MCF-7 Dox^R, which presents nuclear XIAP also **(Figure 1)**. We observed that dox treatment decreased cell viability **(Figures 2 and 3)** and inhibited colony formation **(Figure 4)** in MCF-7 and MDA-MB-231, but not in MCF-7 Dox^R cells, suggesting a possible correlation between nuclear XIAP and dox resistance profile. Besides, treatment with dox did not modulate XIAP subcellular localization in MCF-7 and MCF-7 Dox^R cells **(Figure 5)**. To investigate if the effect extends to others drugs used in the breast cancer treatment, MCF-7 and MCF-7 TaxR were treated with increasing concentrations of paclitaxel. MCF-7 Tax^R cells were resistant to paclitaxel at all concentrations tested compared to MCF-7 cells **(Figure 6)** and, also, presented XIAP nuclear expression **(Figure 7)**. Also, an increase in cytoplasmic XIAP expression was observed in paclitaxel-treated MCF-7 cells **(Figure 7)**, confirming our previous data that associate cytoplasmic XIAP to a favorable prognosis in our patient cohort. These results show that XIAP expression can be found at different subcellular fractions in breast cancer cells and its nuclear localization is correlated to a dox and paclitaxel resistant phenotype **(Figure 8)**.

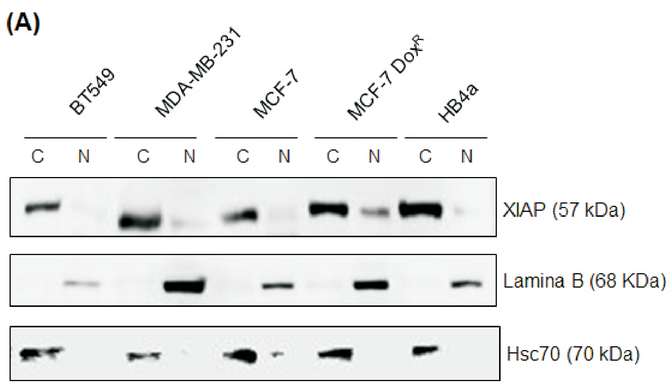

Figure 1: XIAP expression and subcellular localization in a cell line panel. Cells were left to adhere for 24 h and then had their cytoplasmic and nuclear fractions separated by the NE-PER kit (Thermoscientific) **(A)**. Subsequently, XIAP expression was evaluated by Western blotting **(B)**. Lamin B was used as a nuclear constitutive control, while Hsc70 as cytoplasmic constitutive control. C: cytoplasm; N: nucleus.

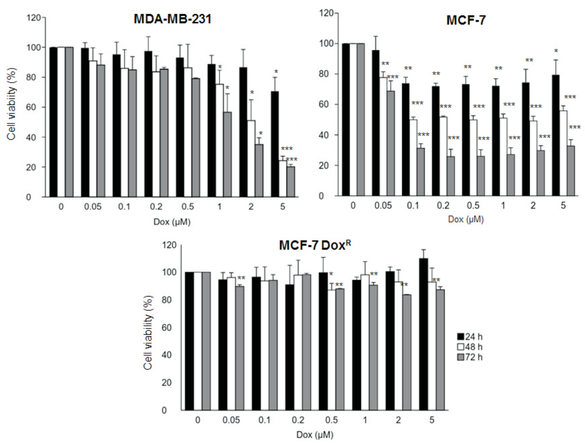

Figure 2: Cell viability changes induced by increasing concentrations of doxorubicin (dox) in MDA-MB-231, MCF-7 and MCF-7 Dox^R cells. Cells were plated in 96 well plates and left to adhere for 24 h. Subsequently, dox was added at increasing concentrations and cells were incubated for 24, 48 and 72 h. Cell lines were compared with their control of untreated cells. The graphs correspond to the mean ± standard deviation of three independent experiments. (Student's t-test: * p <0.05; ** p <0.01; *** p <0.001; it was considered statistically significant).

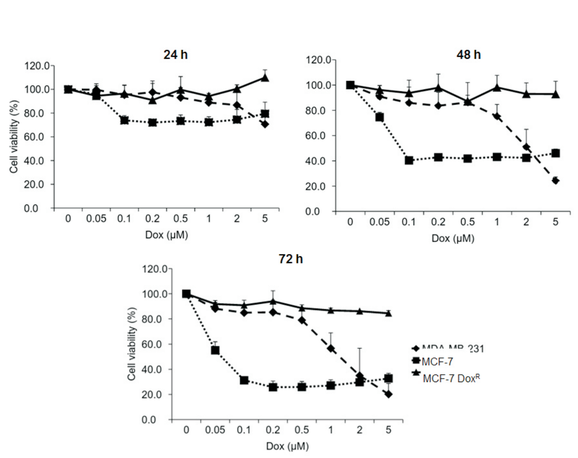

Figure 3: Comparison of MDA-MB-231, MCF-7 and MCF-7 Dox^R cell lines after 24, 48 and 72 h of doxorubicin (dox) exposure. Cells were plated in 96 well plates and left to adhere for 24 h. Subsequently, dox was added at increasing concentrations and the cells were incubated for 24, 48 and 72 h. Cell lines were compared with their control of untreated cells. The graphs correspond to the mean ± standard deviation of three independent experiments. (Student's t-test: * p <0.05; ** p <0.01; *** p <0.001; it was considered statistically significant).

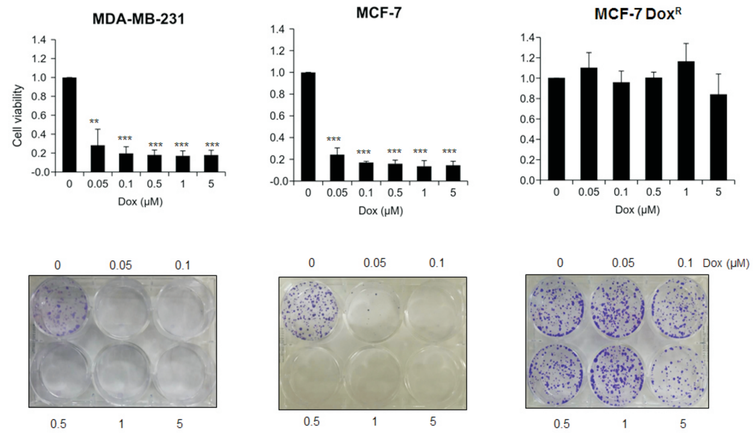

Figure 4: Changes in proliferation levels induced by doxorubicin (dox) in MDA-MB-231, MCF-7 and MCF-7 Dox^R cells. Cells were plated in six-well plates and left to adhere for 24 h. Subsequently, dox was added at increasing concentrations. After 48 h of drug exposure, the medium was changed and the cells were left for 14 days in the CO_2 incubator until colony formation and then stained with crystal violet. The graphs correspond to the mean ± standard deviation of three independent experiments. (Student's t-test: * p <0.05; ** p <0.01; *** p <0.001; it was considered statistically significant).

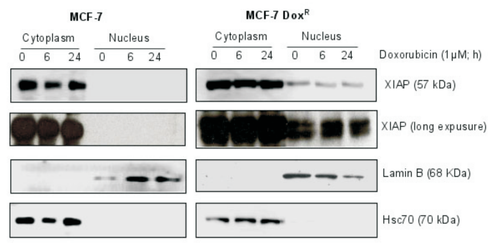

Figure 5: XIAP subcellular localization in doxorubicin-treated MCF-7 and MCF-7 Dox^R cell lines. Cells were left to adhere for 24 h, treated with doxorubicin and then had their cytoplasmic and nuclear fractions separated by the NE-PER kit (Thermoscientific). Subsequently, XIAP expression was evaluated by Western blotting. Lamin B was used as a nuclear constitutive control, while Hsc70 as cytoplasmic constitutive control.

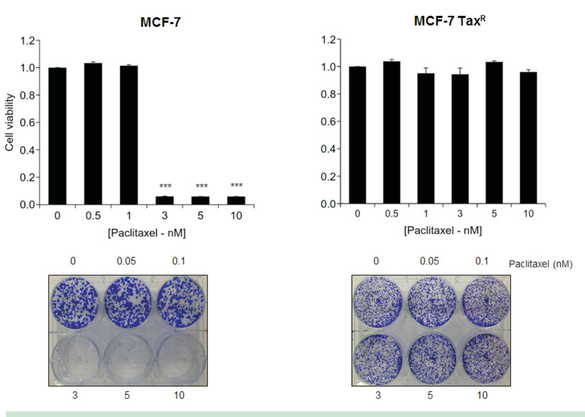

Figure 6: Changes in proliferation levels induced by paclitaxel in MCF-7 and MCF-7 Tax^R cell lines. Cells were plated in six-well plates and left to adhere for 24 h. Subsequently, paclitaxel was added at increasing concentrations. After 48 h of drug exposure, the medium was changed and cells were left for 14 days in the CO_2 incubator until the colonies formation and then stained with crystal violet. The graphs correspond to the mean ± standard deviation of three independent experiments. (Student's t-test: * p <0.05; ** p <0.01; *** p <0.001; it was considered statistically significant).

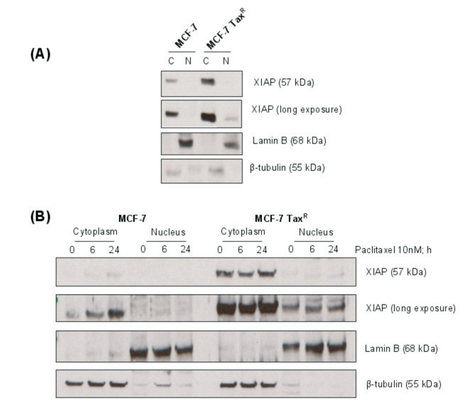

Figure 7: XIAP subcellular localization in MCF-7 and MCF-7 Tax^R cell lines. (A) Cells were left to adhere for 24 h and then had their cytoplasmic and nuclear fractions separated by the NE-PER kit (Thermoscientific). Subsequently, XIAP expression was evaluated by Western blotting. (B) Nuclear and cytoplasmic XIAP expression was evaluated after treatment with 10 nM of paclitaxel for 0, 6, and 24 h by Western blotting. Lamin B was used as a nuclear constitutive control, while β-tubulin as cytoplasmic constitutive control. C: cytoplasm; N: nucleus.

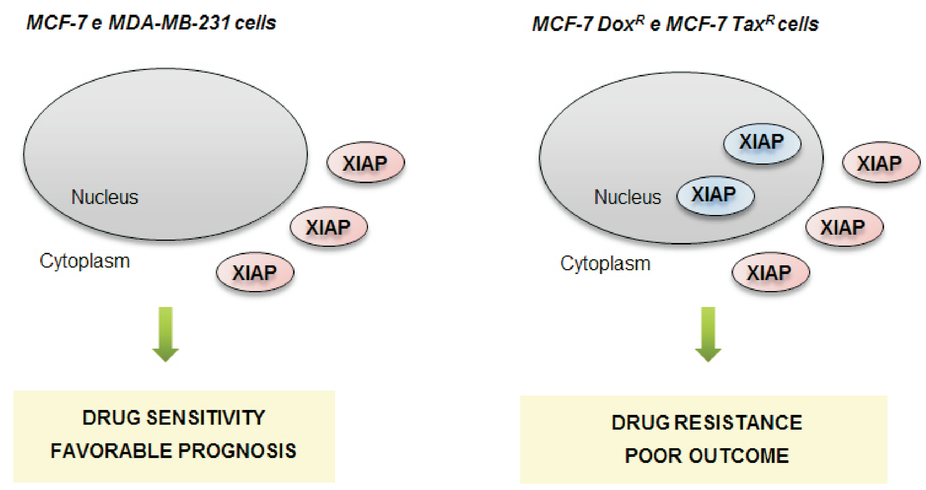

Figure 8: Schematic figure of the association between the nuclear localization of XIAP and its oncogenic role in breast cancer. XIAP expression can be found in different subcellular compartments in breast cancer cells. Cytoplasmic XIAP expression is associated with drug sensitivity in cell lines and an increased overall survival in ductal invasive breast carcinoma patients. In opposite way, nuclear XIAP correlates with poor prognosis and doxorubicin and paclitaxel resistance *in vitro*, pointing to an oncogenic role in breast tumors.

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Keywords: Breast cancer; chemoresistance; XIAP subcellular localization.

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