THE LQB-223 COMPOUND EXHIBITS ANTITUMOR ACTIVITY AGAINST BREAST CANCER CELLS WITH DISTINCT PHENOTYPES

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Introduction: Breast cancer is a leading cause of deaths among women worldwide. Although there have been advances in the identification of therapeutic strategies, biomarkers and subgroups, some patients fail to respond to treatment. The development of therapeutic resistance is a major obstacle for successful treatment, resulting in eventual patient relapse and poor survival. Therefore, the identification of novel anticancer compounds that might be able to surpass drug resistance mechanisms is crucial. The compound 11a-N-Tosyl-5-deoxi-pterocarpan, LQB-223, has been previously tested *in vitro* by our group and shown to sensitize breast cancer cells, with little toxicity to non-neoplastic breast cells. LQB223 effects in breast neoplastic cells were associated with decreased cell growth and apoptosis induction. The aim of this study was to investigate the mechanisms underlying LQB-223 antitumor effects in 2D and 3D models of breast cancer cells.

Methods and Results: MCF-7 (non-invasive; luminal) and MDA-MB-231 (invasive and metastatic; triple negative) cell lines were used as models in this study. First, we observed that LQB-223-mediated cytotoxic effects involved the inhibition in FOXM1, Survivin, XIAP, c-IAP1 and Mcl-1 chemoresistance-related proteins (**Fig 1 a and b**), as analyzed by Western blotting. The *wound-healing* assay revealed that LQB-223 treatment reduced the migratory profile of MCF-7 (**Fig 2 a, b and c**) and MDA-MB-231 cells (**Fig 3 a, b and c**). Notably, cell motility was impaired following LQB-223 treatment as assessed by the phagokinetic track motility assay (**Fig 4 a and b**). In 3D conformation, we found a remarkable decrease in tumor volume (**Fig 5 a and b and Fig 6**), cell viability (Fig 7 a and b) and migration (**Fig 8 a and b**) following LQB-223 treatment, as evaluated by photographing and measuring spheroid size, as well as performing the acid phosphatase (APH) and gelatin migration assays.

Conclusion: These results suggest the LQB-223 promotes cytotoxic effects and modulates the migratory profile in 2D and 3D models of breast cancer exhibiting different phenotypes. Ongoing experiments involving the expression analysis of epithelial-mesenchymal transition markers will elucidate the signaling pathways and processes underlying LQB-223 antitumor effects in breast cancer cells. Additionally, *in vivo* models of breast tumors have been carried out to further strenghten our knowledge on the compound efficiency.

Keywords: Breast cancer, LQB-223 compound, chemoresistance.

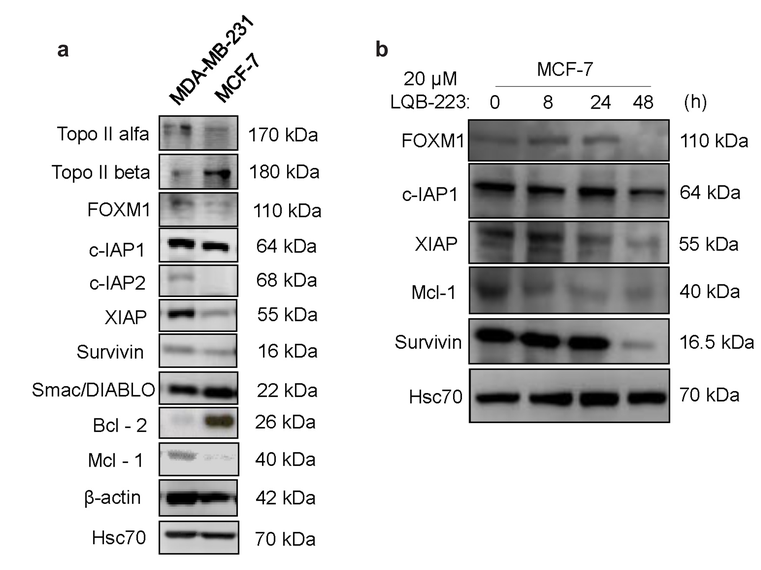

Fig. 1 Evaluation of resistance phenotype related proteins level in breast cancer cells MCF-7 and MDA-MB-231 cells. First, the protein levels were assessed by Western blotting assay of whole-cell lysates. Were identified the expression of Topoisomerases II α and β isoforms, transcriptor factor FOXM1 and apoptosis proteins involved Survivin, XIAP, cIAP-1, cIAP-2, Smac/DIABLO, MCL1 e Bcl-2. The levels were compared between the cell MCF-7 and MDA-MB-231 (a). MCF-7 cells were treated with LQB-223 compound during 0, 8, 24 and 48 h and were observed LQB-223-mediated cytotoxic effects involved the inhibition in FOXM1, c-IAP1, XIAP, Mcl-1 and Survivin proteins (b). β-actin and Hsc70 were used as an internal control. The pictures represent three independent experiment.

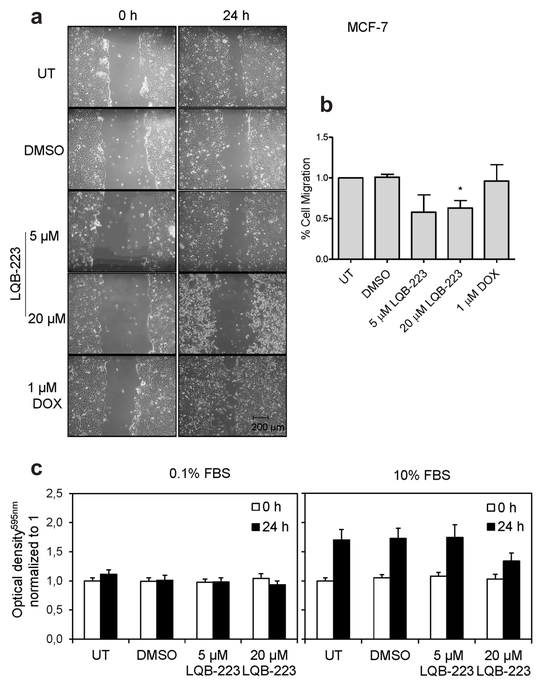

Fig. 2 LQB-223 inhibits the migratory ability of MCF-7 cells treated by LQB-223. For migration assay, MCF-7 cells were cultured in DMEM with 0,1% FBS and treated with 5 and 20 µM LQB-223 for 24 h. The cells wound closure was monitored under microscopy at x10 magnification and analyzed by ImageJ software between 0 and 24 h (**a** and **b**). At the same time the cell proliferation was assessed after the LQB-223 treatment in DMEM with 0.1% and 10% FBS (**c**). The breast cancer cells were fixed and stained with crystal violet after adhesion. The graphs and the pictures of migratory represent from three independent experiments. UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin. Statistical significance was analyzed by the Student´s t test (*p<0.05; **p<0.01; ***p<0.001).

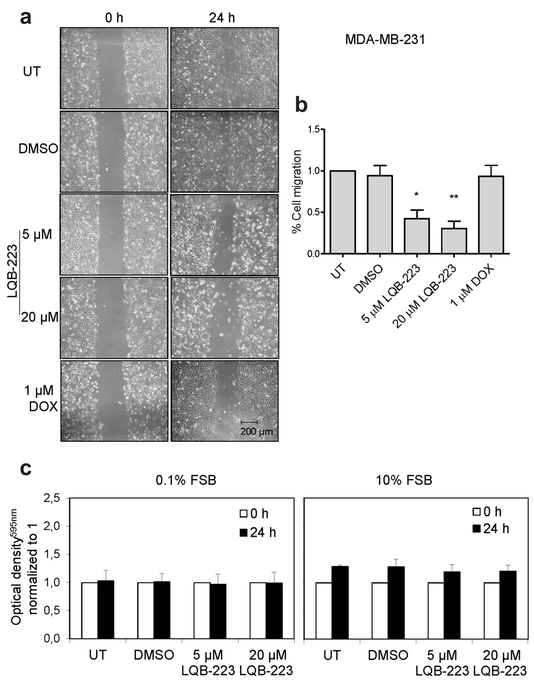

Fig. 3 LQB-223 inhibits the migratory ability of MDA-MB-231 cells treated by LQB-223. (a) For migration assay, MDA-MB-231 cells were cultured in DMEM with 0,1% FBS and treated with 5 and 20 µM LQB-223 for 24 h. The cells wound closure was monitored under microscopy at x10 magnification and analyzed by ImageJ software between 0 and 24 h. **(a** and **b)** At the same time the cell proliferation was assessed after the LQB-223 treatment in DMEM with 0.1% and 10% FBS **(c)**. The breast cancer cells were fixed and stained with crystal violet after adhesion. The graphs and the pictures of migratory represents three independent experiments. UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin. Statistical significance was analyzed by the Student´s t test (*p<0.05; **p<0.01; ***p<0.001)

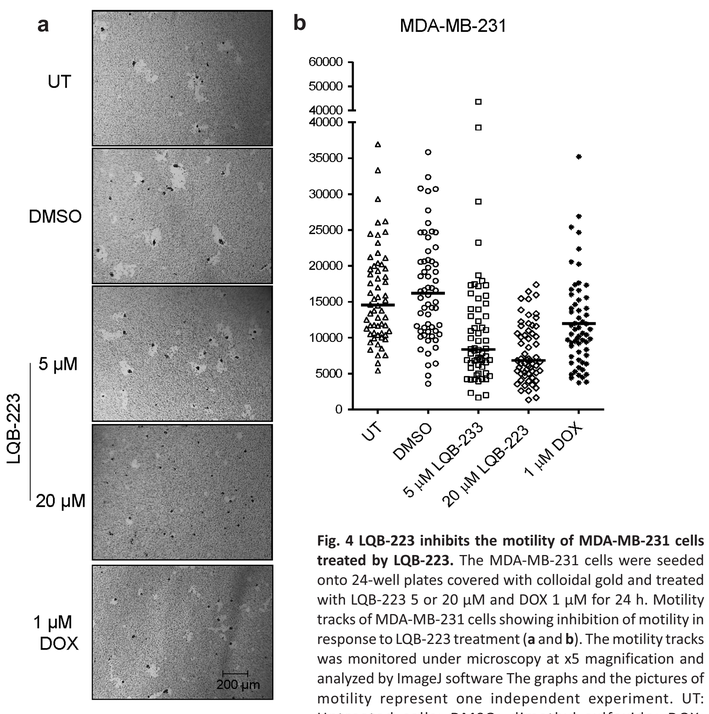

Fig. 4 LQB-223 inhibits the motility of MDA-MB-231 cells treated by LQB-223. The MDA-MB-231 cells were seeded onto 24-well plates covered with colloidal gold and treated with LQB-223 5 or 20 µM and DOX 1 µM for 24 h. Motility tracks of MDA-MB-231 cells showing inhibition of motility in response to LQB-223 treatment (**a** and **b**). The motility tracks was monitored under microscopy at x5 magnification and analyzed by ImageJ software The graphs and the pictures of motility represent one independent experiment. UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin. Statistical significance was analyzed by the One-way ANOVA test (*p<0.05; **p<0.01; ***p<0.001)

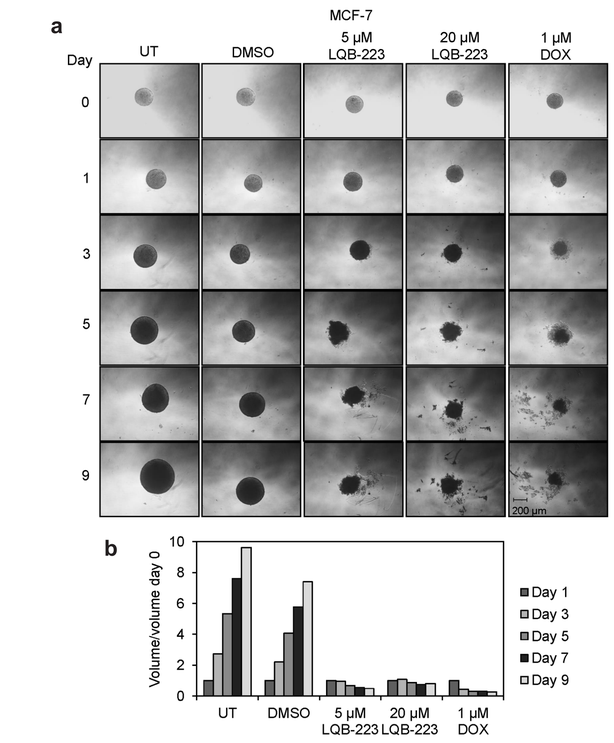

Fig. 5 LQB-223 induces morphologic alterations in MCF-7 multicellular spheroid. The MCF-7 cells were seeded onto 96-well plates coated with 1.5% agarose and cultured for 3 days. After generation, the spheroids was treated with LQB-223 5 or 20 µM and DOX 1 µM. The drug exposure time were 9 days and images of all spheroids were taken on day 0, 1, 3, 5, 7 and 9 for morphology **(a)** and growth determination **(b)**. The growth was determined as from spheroids volume monitored under microscopy at x5 magnification and analyzed by ImageJ software. The graphs and the pictures represents three independent experiments. UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin.

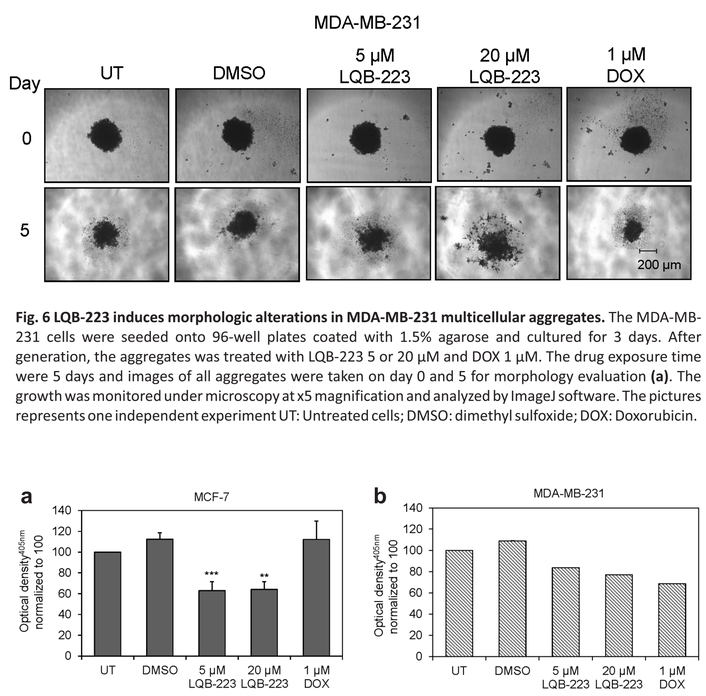

Fig. 6 LQB-223 induces morphologic alterations in MDA-MB-231 multicellular aggregates. The MDA-MB-231 cells were seeded onto 96-well plates coated with 1.5% agarose and cultured for 3 days. After generation, the aggregates was treated with LQB-223 5 or 20 µM and DOX 1 µM. The drug exposure time were 5 days and images of all aggregates were taken on day 0 and 5 for morphology evaluation **(a)**. The growth was monitored under microscopy at x5 magnification and analyzed by ImageJ software. The pictures represents one independent experiment UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin.

Fig. 7 LQB-223 treatment induces cell toxicity in 3D culture assessed by APH levels. The MCF-7 **(a)** and MDA-MB-231 **(b)** cells were seeded onto 96-well plates coated with 1.5% agarose and cultured for 3 days. After generation, the 3D culture was treated with LQB-223 5 or 20 µM and DOX 1 µM for 9 or 5 days. The cell viability was assessed by acid phosphatase (APH) assay. The graphs and the pictres represents three or one independent experiments. UT: Untreated cells; DMSO: dimethyl sulfoxide; DOX: Doxorubicin.

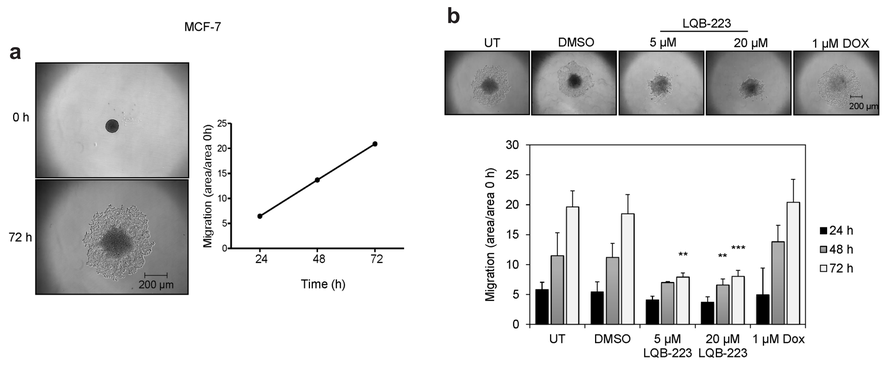

Fig. 8 Cell migration in a 3D conformation of MCF-7 cell lines. The MCF-7 **(a)** cell lines were seeded onto 96-well plates coated with 1.5% agarose and cultured for 3 days. After generation, the 3D culture were transferred to a 24 well plate coated with 0.1% gelatin and 2% FBS. Tumor 3D culture migration was imaged over 24, 48 and 72 h under microscopy at x10 magnification and analyzed by ImageJ software. A migration index (b) was calculated from the images obtained leading edge zone for each time. The graphs and the pictures represents three independent experiments.

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