THE LQB-223 COMPOUND IMPAIRS BREAST CANCER CELL GROWTH AND MIGRATION

Lauana Greicy Tonon Lemos[1], Gabriel Mello da Cunha Longo[1], Bruna dos Santos Mendonça[1,2], Marcela Cristina Robaina[1], Mariana Concentino Menezes Brum[2,3], Caíque de Assis Cirilo[1], Etel Rodrigues Pereira Gimba[3], Paulo Roberto Ribeiro Costa[4], Camilla Djenne Buarque[5], Gabriela Nestal de Moraes[1*], Raquel Ciuvalschi Maia[1*]

*These authors share senior authorship.[1]Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular, Instituto Nacional do Câncer (INCA). Rio de Janeiro – RJ. [2]Programa de Pós-Graduação *Strictu Sensu* em Oncologia, INCA. Rio de Janeiro – RJ. [3]Programa de Oncobiologia Celular e Molecular, INCA. Rio de Janeiro – RJ. [4]Laboratório de Química Bioorgânica, Instituto de Pesquisas de Produtos Naturais (IPPN), Universidade Federal do Rio de Janeiro. Rio de Janeiro – RJ. [5]Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro. Rio de Janeiro - RJ

Drug resistance represents a major issue in treating breast cancer, despite the identification of novel therapeutic strategies, biomarkers and subgroups. We have previously identified the LQB-223 11a-N-Tosyl-5-deoxi-pterocarpan as a promising compound in sensitizing doxorubicin-resistant breast cancer cells, with little toxicity to non-neoplastic cells. Here, we investigated the mechanisms underlying LQB-223 antitumor effects in 2D and 3D models of breast cancer. MCF-7 (non-invasive; luminal) and MDA-MB-231 (invasive; metastatic; triple-negative) cells had migration and motility profile assessed by wound-healing and phagokinetic track motility assays, respectively. Cytotoxicity in 3D conformation was evaluated by measuring spheroid size and performing acid phosphatase and gelatin migration assays. Protein expression was analyzed by immunoblotting. Our results show that LQB-223, but not doxorubicin treatment, suppressed the migratory **(Fig. 1)** and motility capacity of breast cancer cells **(Fig. 2)**. In 3D conformation, LQB-223 remarkably decreased cell viability, as well as reduced tumor size **(Fig. 3)** and migration **(Fig. 4)**. Mechanistically, LQB-223-mediated anticancer effects involved decreased proteins levels of XIAP, c-IAP1 and Mcl-1 chemoresistance-related proteins, but not Survivin **(Fig. 5)**. Survivin knockdown partially potentiated LQB-223-induced cytotoxicity **(Fig. 6)**. Additionally, cell treatment with LQB-223 resulted in changes in the mRNA levels epithelial-mesenchymal transition markers, suggesting that it might modulate cell plasticity **(Fig. 7)**. Our data demonstrate that LQB-223 impairs tumor growth and migration in 2D and 3D models of breast cancer exhibiting different phenotypes.

Keywords: Breast cancer, Drug resistance; LQB-223 compound.

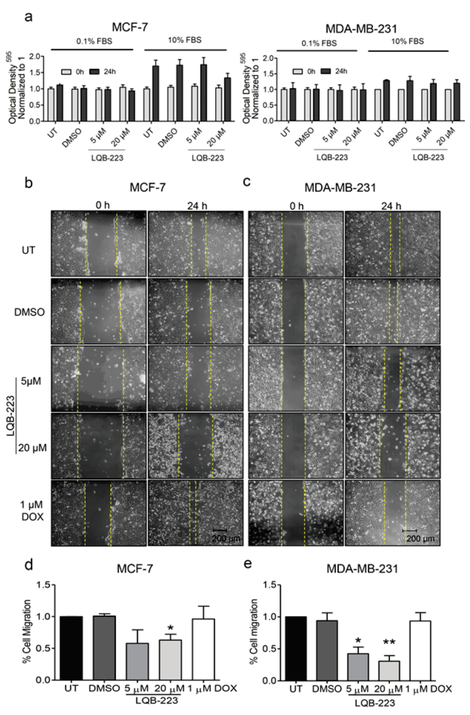

Fig. 1. Assessment of proliferation in serum-deprived conditions and cell migration using the wound healing assay. (a) MCF-7 and MDA-MB-231 cells were cultured in DMEM containing 10% FBS or 0.1% FBS and treated with LQB-223 at the indicated concentrations for 24 hours. Absorbance of cells stained with crystal violet was measured at 595 nm. **(b)** MCF-7 and **(c)** MDA-MB-231 cells were cultured in DMEM 0.1% FBS and treated with 5 µM or 20 µM of LQB-223 for 24 hours. Wound closure was monitored and calculated using the software ImageJ. Migration quantification of **(d)** MCF-7 and **(e)** MDA-MB-231 in percentage of covered area compared to the same region at 0 hours timepoint. Values reported as mean and standard deviation of three independent experiments. Statistical significance was analyzed using the Student´s t test (*p<0.05; **p<0.01; ***p<0.001).

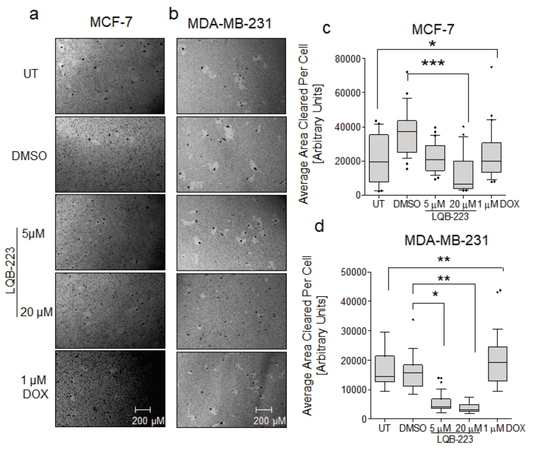

Fig. 2. LQB-223 impairs motility of MCF-7 and MDA-MB-231 cells. (a) MCF-7 and **(b)** MDA-MB-231 cells were seeded onto 24-well plates coated with colloidal gold and treated with 5 µM or 20 µM of LQB-223 or 1 µM DOX for 24 h. The motility tracks were monitored under microscopy at x10 magnification and analyzed using the ImageJ software. Average area cleared per cell is shown for **(c)** MCF-7 and **(d)** MDA-MB-231 from three independent experiments. Statistical significance was analyzed using the One-way ANOVA test (*p<0.05; **p<0.01; ***p<0.001).

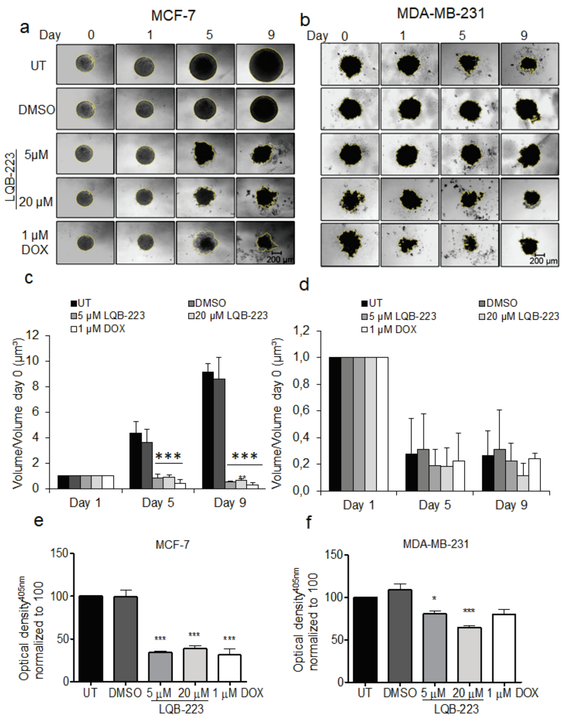

Fig. 3. Cell viability and relative growth kinetics of 3D cultures after treatment with LQB-223 or DOX. (a) 3D structures of breast cancer cells were formed in non-adherent conditions. MCF-7 and MDA-MB-231 cells were seeded onto 96-well plates coated with 1.5% agarose and cultured for 3 days (day 0). The 3D cultures were cultivated for 9 days and imaged at the timepoints indicated in each experiment **(b)** MCF-7 spheroids and **(c)** MDA-MB-231 cell aggregates were treated with LQB-223 or DOX and volume growth was analyzed on day 1, 5 and 9 after treatment. **(d)** Volume of MCF-7 spheroids and **(e)** MDA-MB-231 aggregates. The volume measurements were normalized to the volume of spheroids on day 0, and values are reported as mean and standard deviation of the fold change. Viability was assessed on day 9 using the APH assay. **(f)** Viability of MCF-7 spheroids after treatment with LQB-223 or DOX. (g) Viability of MDA-MB-231 aggregates after treatment with LQB-223 or DOX. Values reported as mean standard deviation of three independent experiments with at least 8 replicates each. Statistical significance was analyzed using the Student´s t test (*p<0.05; **p<0.01; ***p<0.001).

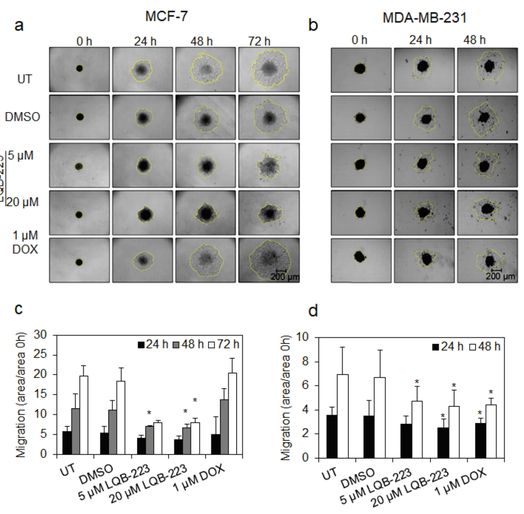

Fig. 4. Analysis of cell migration cultivated as 3D cultures after treatment with LQB-223. (a) Fully formed spheroids of MCF-7 or **(b)** aggregates of MDA-MB-231 were plated onto 24-well plates coated with gelatin and migration was assessed over time upon exposure to LQB-223 or DOX. Area of cells migrated outwards the 3D structure was calculated for **(c)** MCF-7 and **(d)** MDA-MB-231 aggregates was calculated and divided by the area of the 3D cultures on day 0. Values reported as mean and standard deviation of three independent experiments. Statistical significance was analyzed using the Student´s t test (*p<0.05; **p<0.01; ***p<0.001).

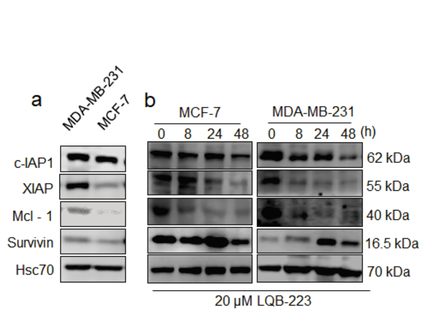

Fig. 5. Modulation of chemoresistance-related proteins by the compound LQB-223. Protein levels were assessed by Western blotting assay of whole-cell lysates in MCF-7 and MDA-MB-231 cells. (a) Basal levels of apoptosis-associated proteins Survivin, XIAP, cIAP-1 and MCL1 were compared between the MCF-7 and MDA-MB-231 cells. **(b)** MCF-7 or MDA-MB231 cells were treated with the LQB-223 compound for 8, 24 and 48 hours and expression of c-IAP1, XIAP, Mcl-1 and Survivin proteins was analyzed by Western blotting. Hsc70 was used as an internal control. Blots representative of three independent experiments.

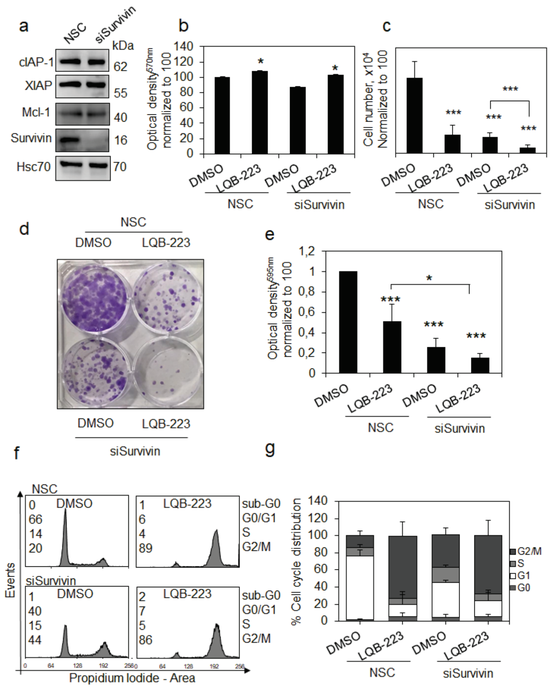

Fig. 6. Analysis of the role of survivin in MDA-MB-231 cells sensitivity to LQB-223. (a) Modulation of chemoresistance-related proteins upon survivin inhibition in MDA-MB-231 Cells. **(b)** Cell viability of MDA-MB-231 cells after exposure to LQB-223 or/and Survivin inhibition. **(c)** Cell count using trypan blue after exposure to LQB-223 or/and Survivin inhibition. **(d)** Clonogenic assay after treatment with LQB-223 or/and Survivin inhibition. **(e)** Absorbance of colonies of MDA-MB-231 stained with crystal violet shown in **(d).** Values reported as mean and standard deviation and statistical analysis performed using Statistical significance was analyzed using the Student´s t test (*p<0.05; **p<0.01; ***p<0.001). **(f)** Analysis of cell cycle phases distribution after treatment with LQB-223 or/and survivin inhibition by means of flow cytometry. **(g)** Cell cycle distribution of MDA-MB-231 cells after treatment with LQB-223 or/and Survivin inhibition. Values reported as percentage of cells in each cell cycle phase. Mean and standard deviation of three independent experiments. Statistical significance was analyzed using the Student´s t test (*p<0.05; **p<0.01; ***p<0.001).

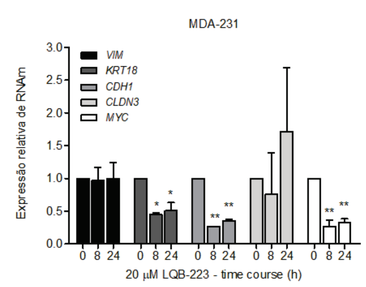

Fig. 7. Effect of LQB-223 on epithelial-mesenchymal transition markers expression. MDA-231 cells were treated with 20 µM LQB-223 for 8 and 24 h. VIM, KRT18, CDH1, CLDN3 and C-MYC mRNA levels were measured using qPCR and 2−ΔΔCT method was used to calculate relative expression. The cells incubated with 20 µM LQB-223 for 8 and 24 h were compared with the cells without LQB-223 (0 h). ACTB was used as the endogenous control. Mean and standard deviation from three independent experiments are shown. One-way ANOVA followed by Bonferroni post-test (*p<0.05; **p<0.01; ***p<0.001).

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