The role of sphingolipid metabolism in multidrug resistance (MDR) of breast cancer cells

Maciej Ugorski

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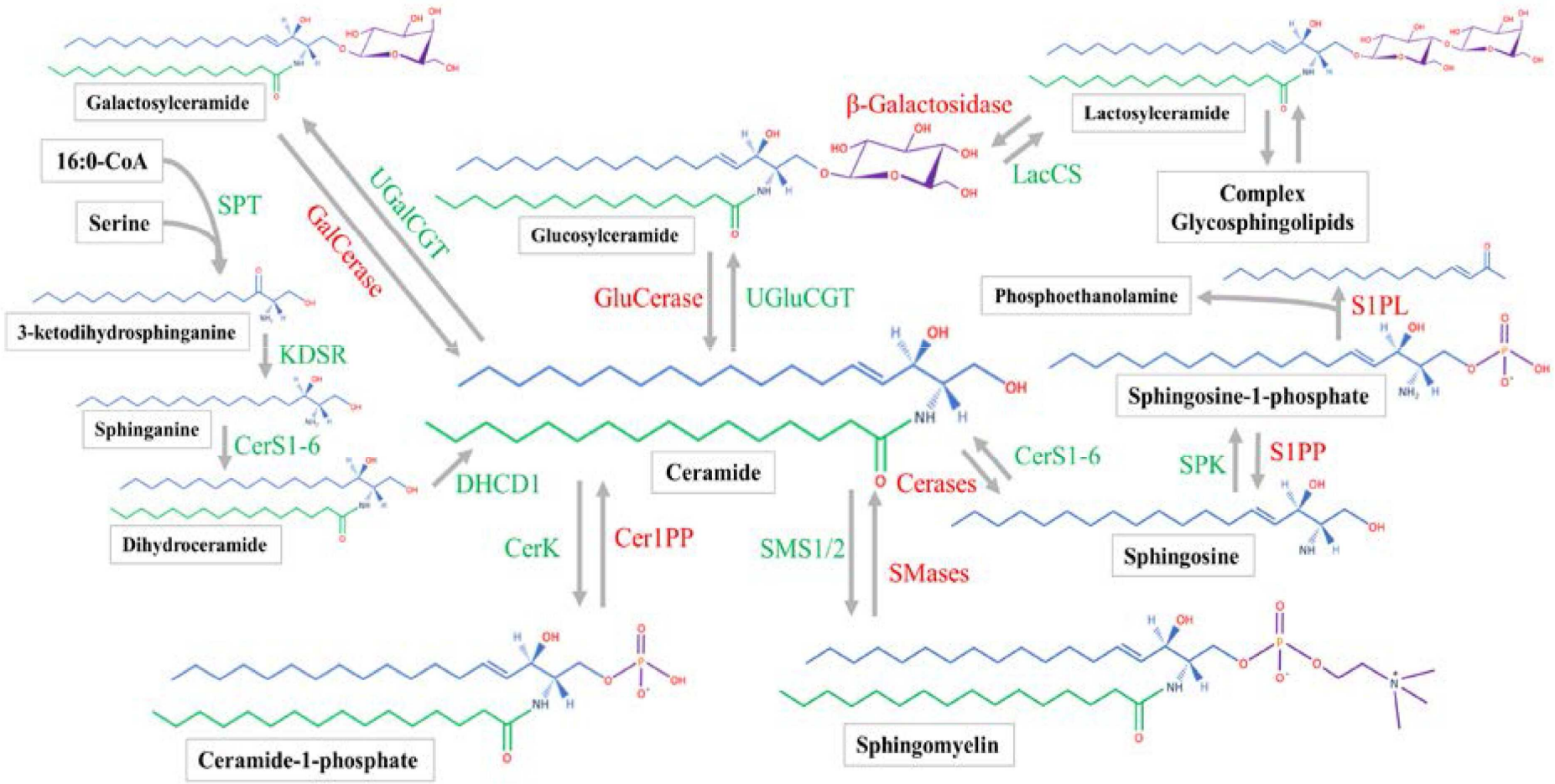
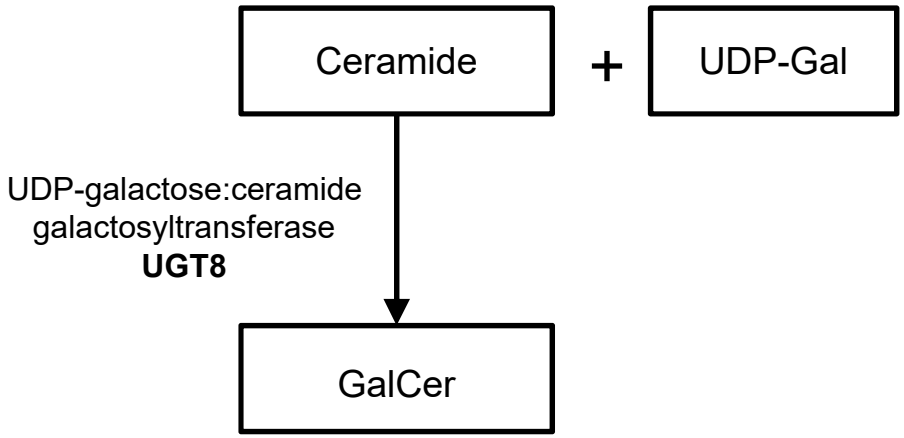
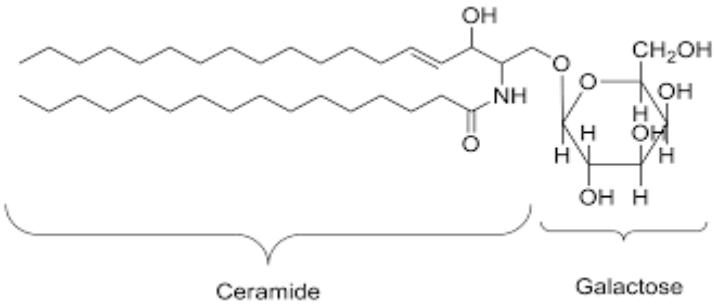
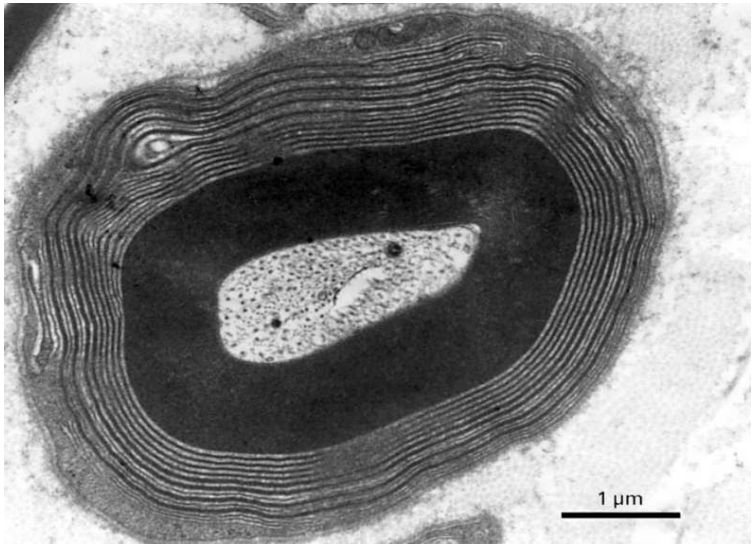
Sphingolipids are a class of lipids containing the aliphatic amino alcohol **sphingosine**.



Galactosylceramide (GalCer) is synthesized by galactosylceramide synthase (UGT8, CGT)

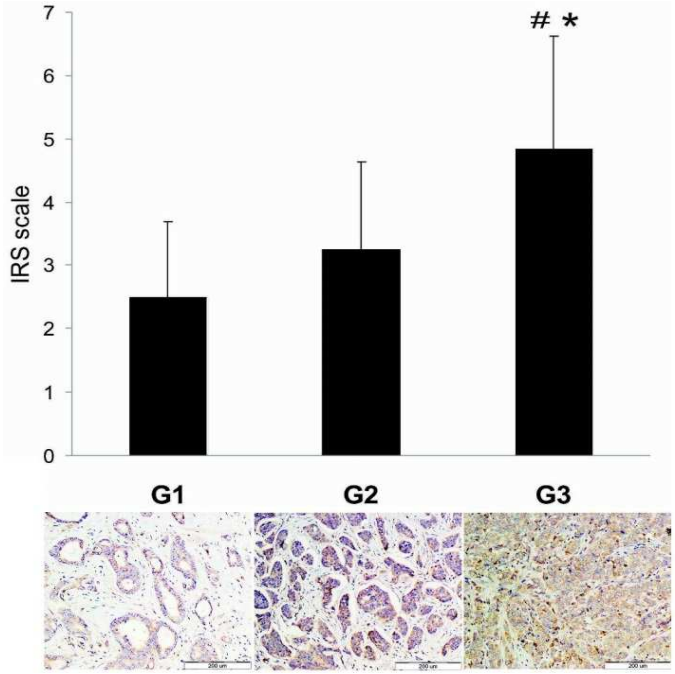




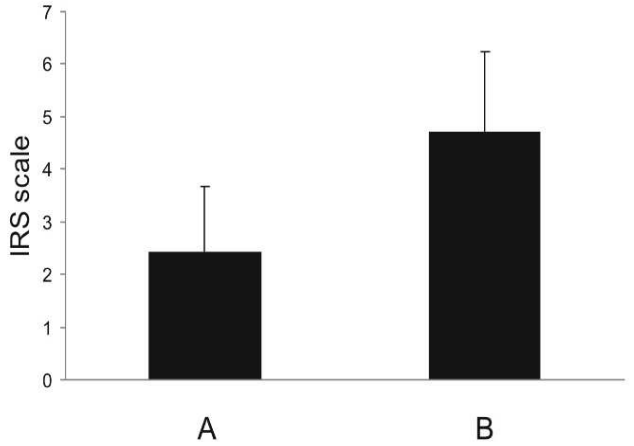


GalCer is the major glycosphingolipid of myelin produced by oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system.

„Our data suggest that UGT8 is a significant index of tumour aggressiveness and a potential marker for the prognostic evaluation of lung metastases in breast cancer” (*Dzięgiel et al., 2010, Br. J Cancer*)

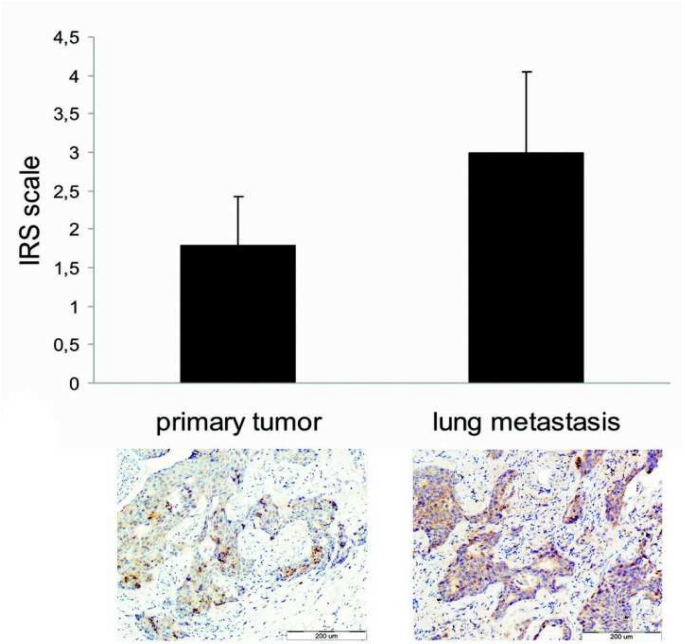


UGT8 expression in tumors with different malignancy grades
Reaction intensities with rabbit polyclonal antibodies for UGT8 (D) are represented as means; *$p < 0.01$ for primary breast tumors of grade G3 compared with primary breast tumor of grade G2 and #$p < 0.001$ for primary breast tumors of grade G3 compared with primary breast tumors of grade G1 (Mann-Whitney *U*-test).

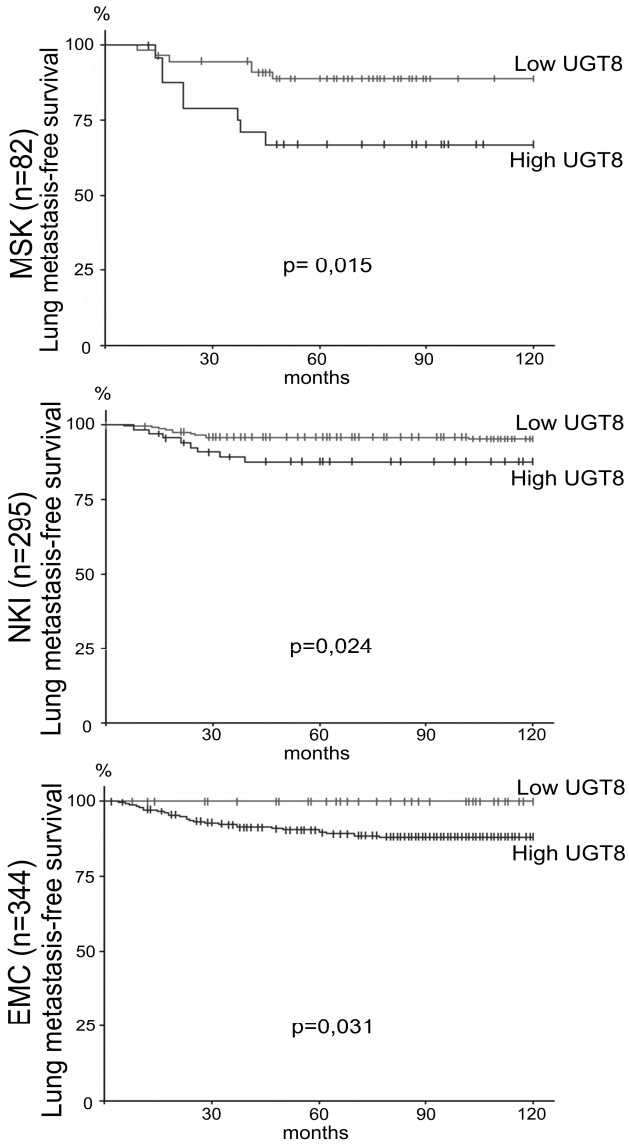


UGT8 expression in node-negative invasive ductal carcinomas and (B) node-positive invasive ductal carcinomas. $p < 0.05$ for UGT8-expressing node-negative primary breast tumors compared with node-positive primary tumors (Mann-Whitney *U*- test).

Reaction intensities were calculated based on the semiquantitative IRS scale of Remmele and Stegner (1987).

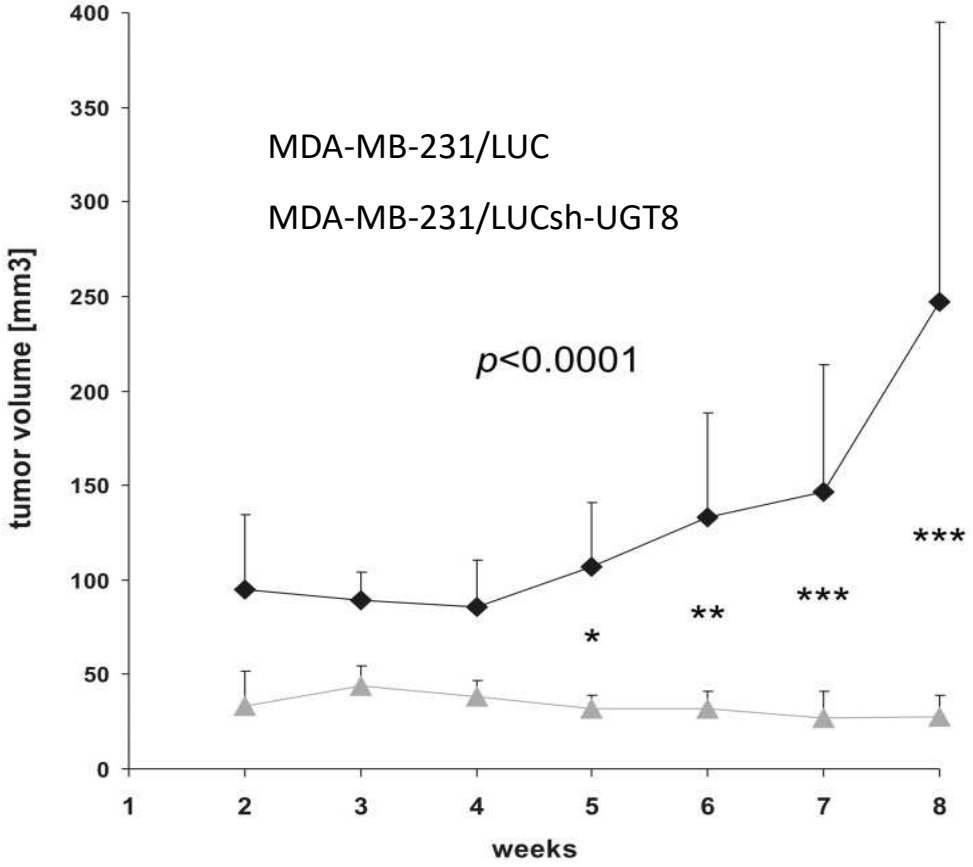


UGT8 expression in primary tumors and lung metastases. Reaction intensities with rabbit polyclonal antibodies for UGT8 are represented as means. $p < 0.05$ for UGT8-expressing primary breast tumors compared with matched lung metastases (Mann-Whitney *U*-test).



Validation of UGT8 expression as a predictive marker of lung metastasis in three independent series of breast cancer patients

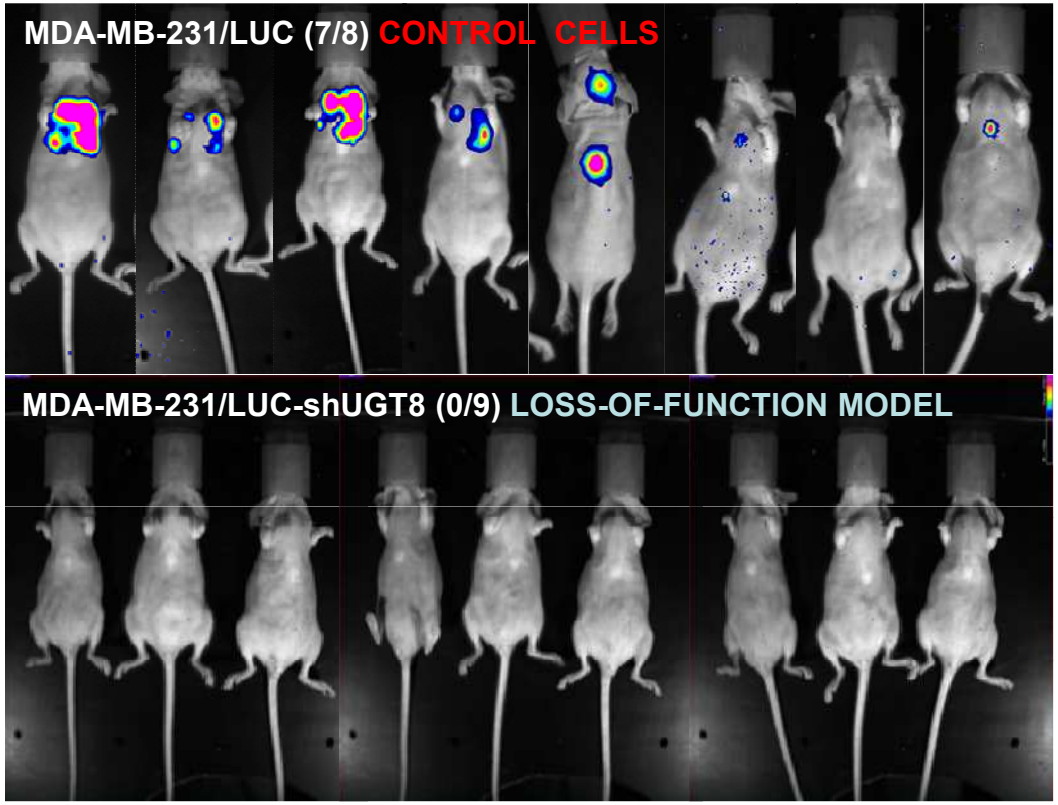
„Galactosylceramide affects tumorigenic and metastatic properties of breast cancer cells as an anti-apoptotic molecule" (*Owczarek et al., 2013, PLOS ONE*)



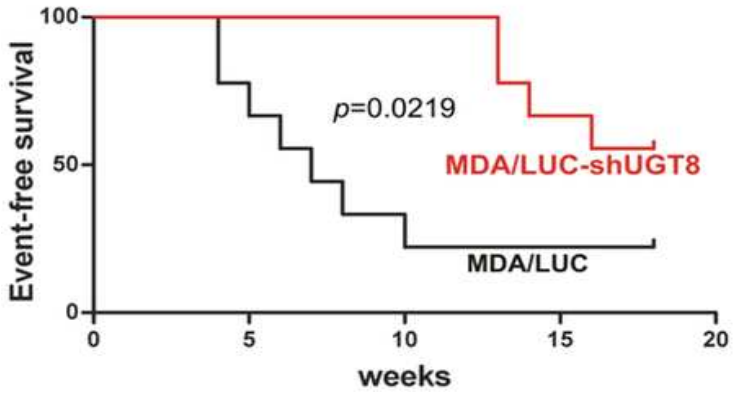
Xenograft tumor growth into athymic Crl:NU-Foxn1 female mice.
Tumor growth was recorded once a week. Data are shown as the mean tumor volume for each group of mice (n = 7) ±SE at each indicated time point. (***p<0.0001).

Breast cancer cells with silenced expression of UGT8 have significantly lower tumorigenic potential in comparison with cells with a high expression of UGT8 and GalCer.

Metastatic potential of breast cancer cells with different expression of UGT8 and GalCer



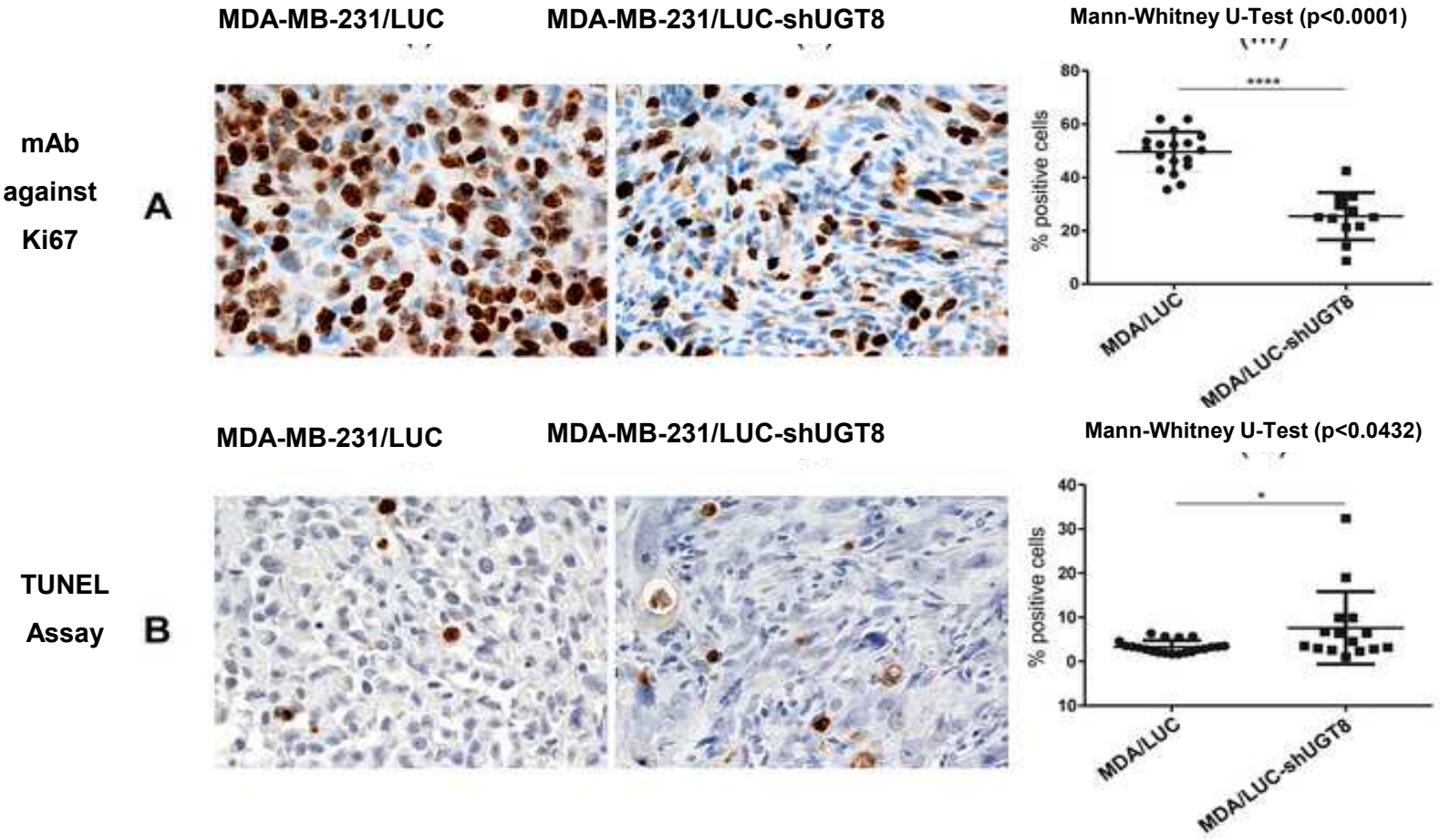
9 week after intracardiac inoculation of cell variants (2.5×10^{5}control MDA/LUC and MDA/LUC-shUGT8) , athymic mices Crl:NU-Foxn1^{nu} were monitored by bioluminescence imaging.



Event-free survival , defined as bio-luminescence-free (metastases free) period after inoculation of breast cancer cells.
(*p = 0.0219, Mentel-Cox test).

Supression of UGT8 expression resulting in the absence of GalCer has a profound effect on metastatic potential of breast cancer MDA-MB-231

Proliferation potential and apoptosis level of tumor xenografts

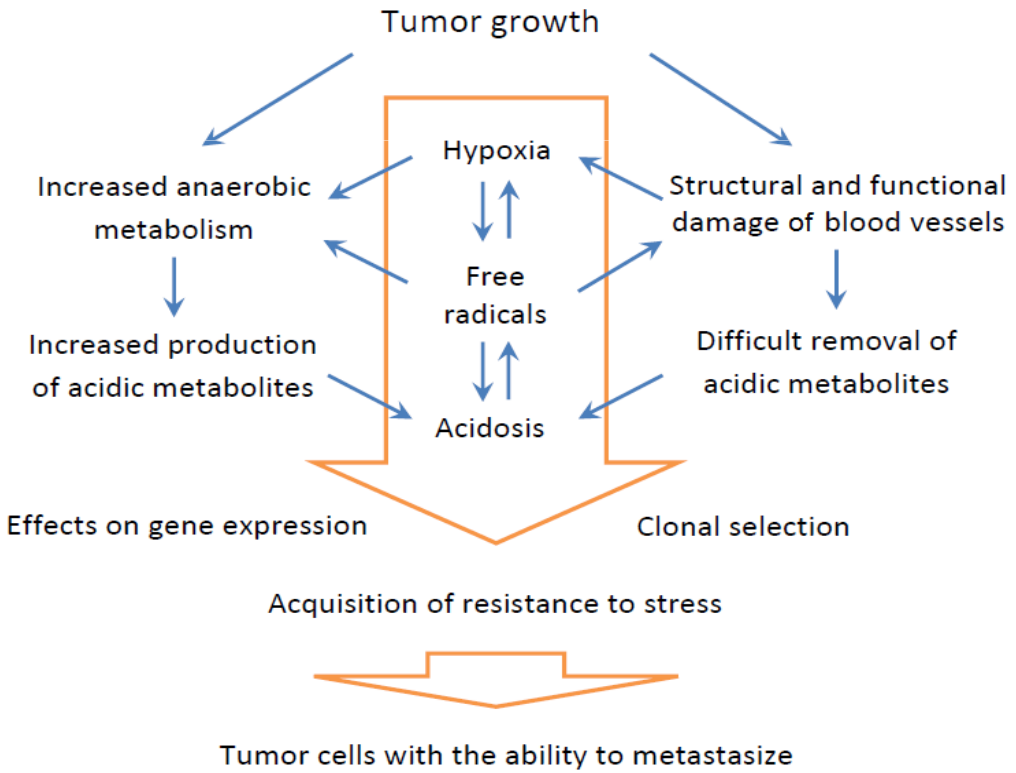


High expression of UGT8 accompanied by accumulation of GalCer is associated with much higher proliferative index and lower number of apoptotic cells.

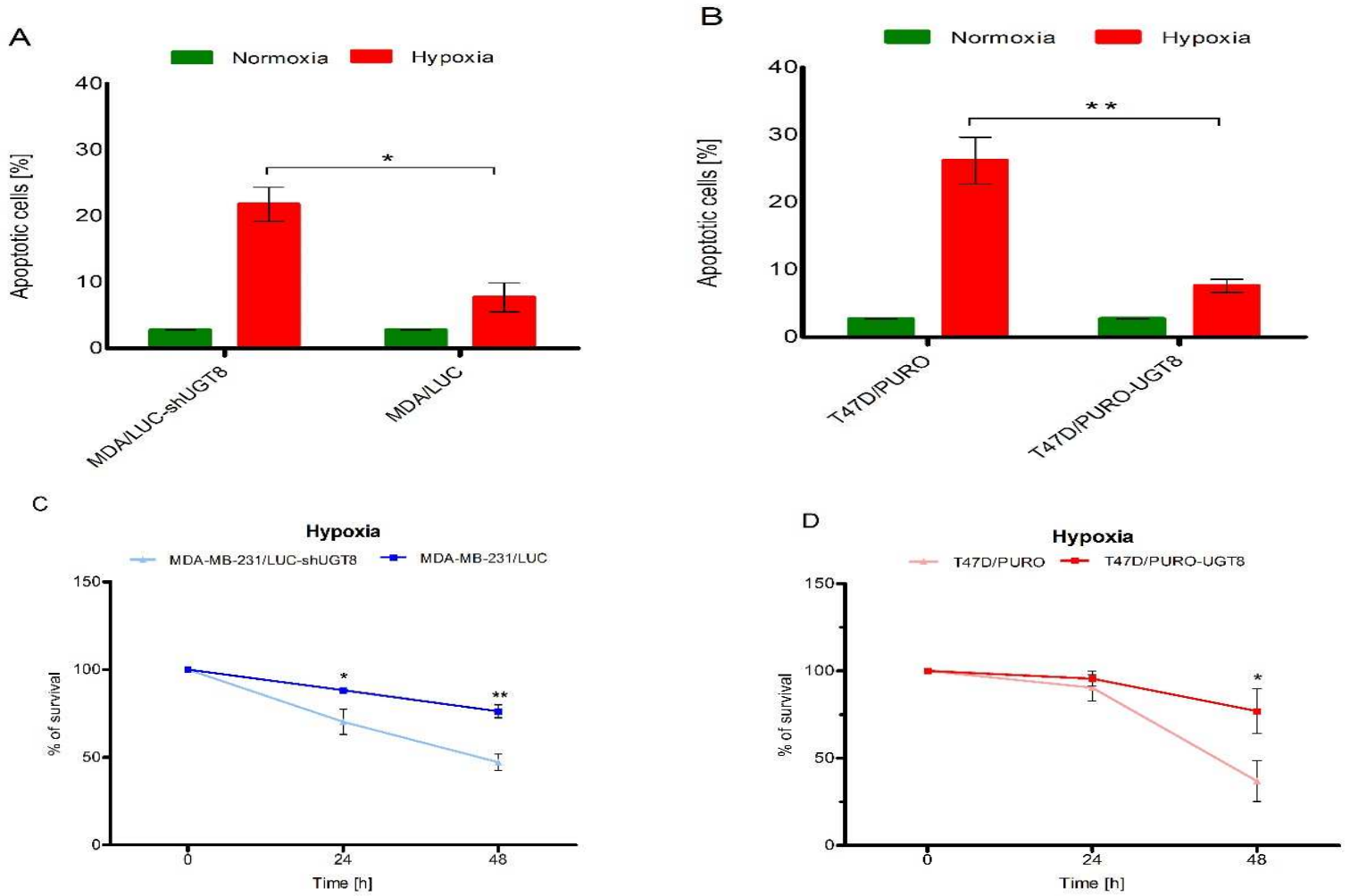
Hypothesis

Accumulation of GalCer in tumor cells protect them from apoptosis induced by microenvironmental stressors.

Microenvironmental stressors associated with tumor growth

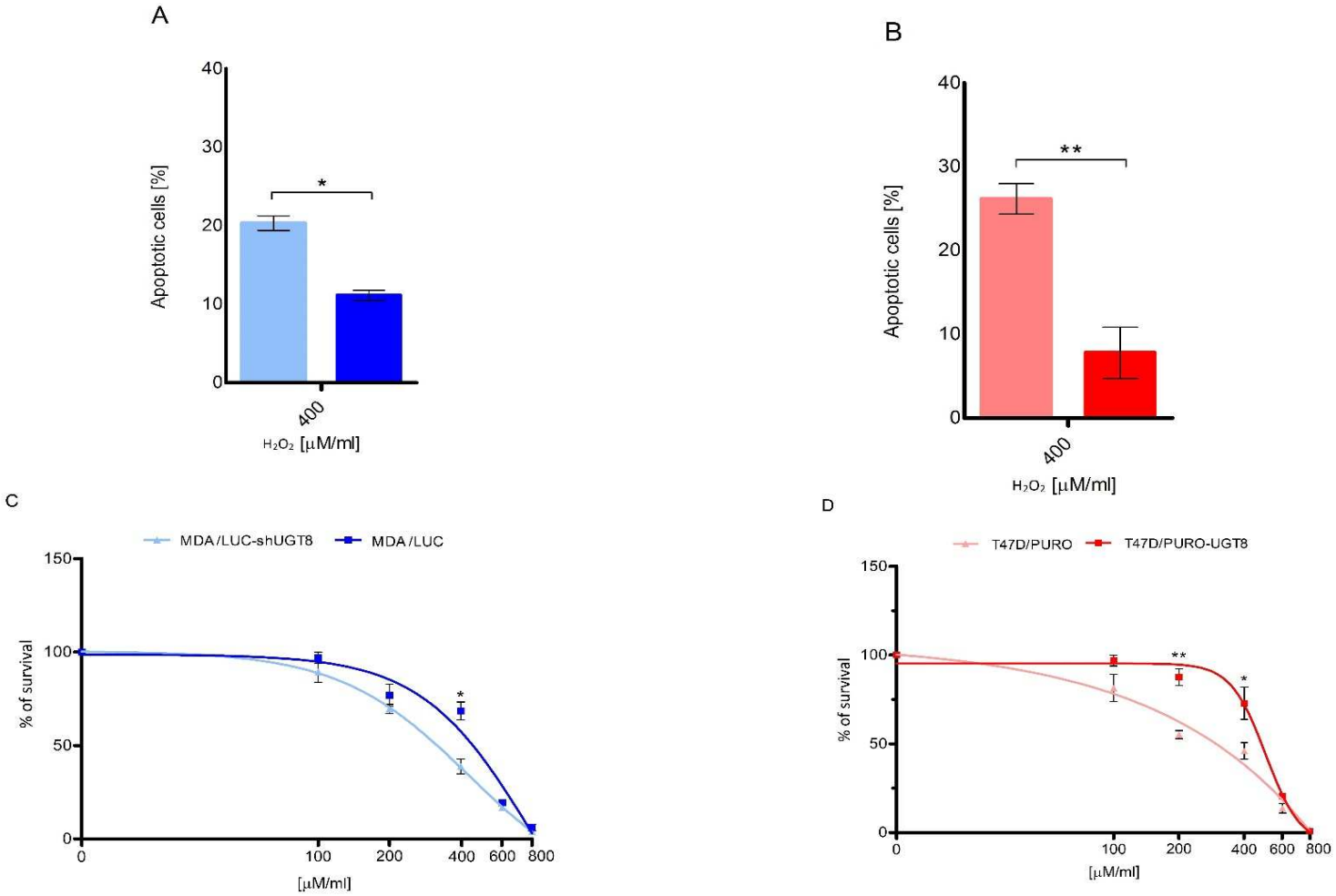


The resistance of breast cancer cells with different expression of UGT8/GalCer to apoptosis induced by hypoxia



A, B – cellular response measured by presence of active form of caspase-3; C, D – cell viability determined using WST-1 assay

The resistance of breast cancer cells with different expression of UGT8/GalCer to apoptosis induced by free radicals

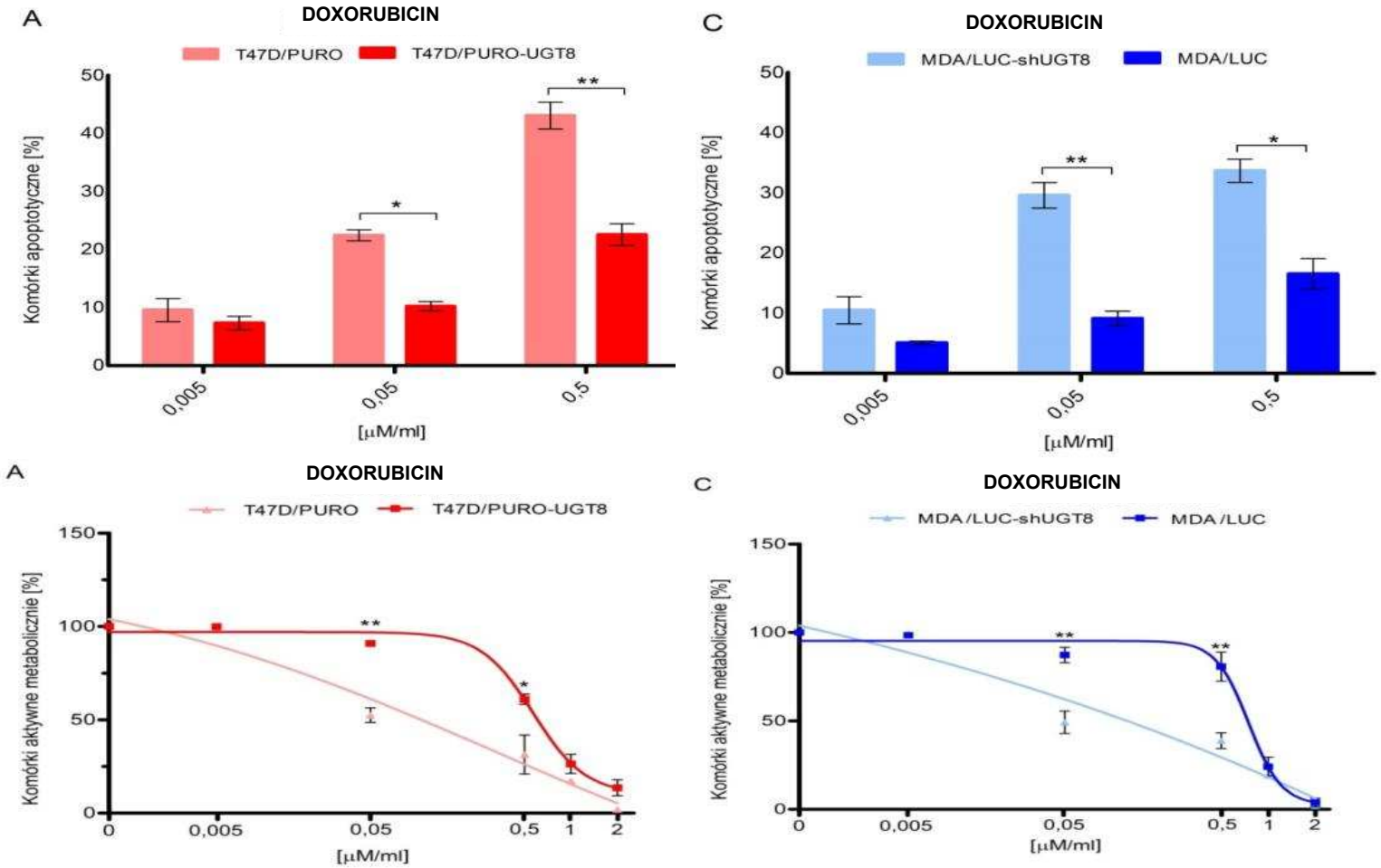


A, B – cellular response measured by presence of active form of caspase-3; C, D – cell viability determined using WST-1 assay

These studies confirmed our hypothesis that the accumulation of GalCer in tumor cells protect them from apoptosis induced by microenvironmental stressors.

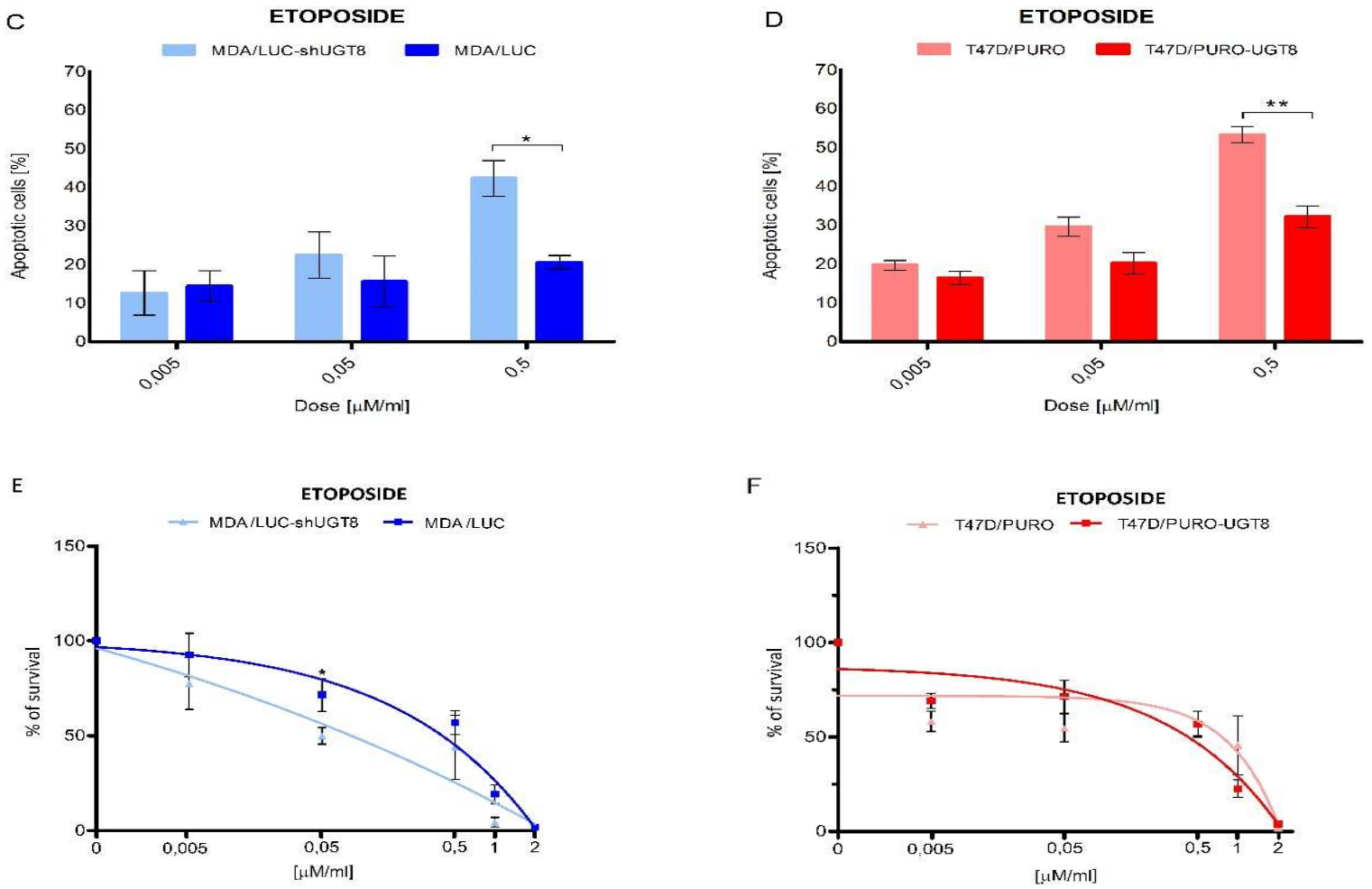
Hypothesis: UGT8 expression and presence of GalCer increase the resistance of breast cancer cells to apoptosis induced by anticancer agents/drugs.

The resistance of breast cancer cells with different expression of UGT8/GalCer to apoptosis induced by doxorubicin



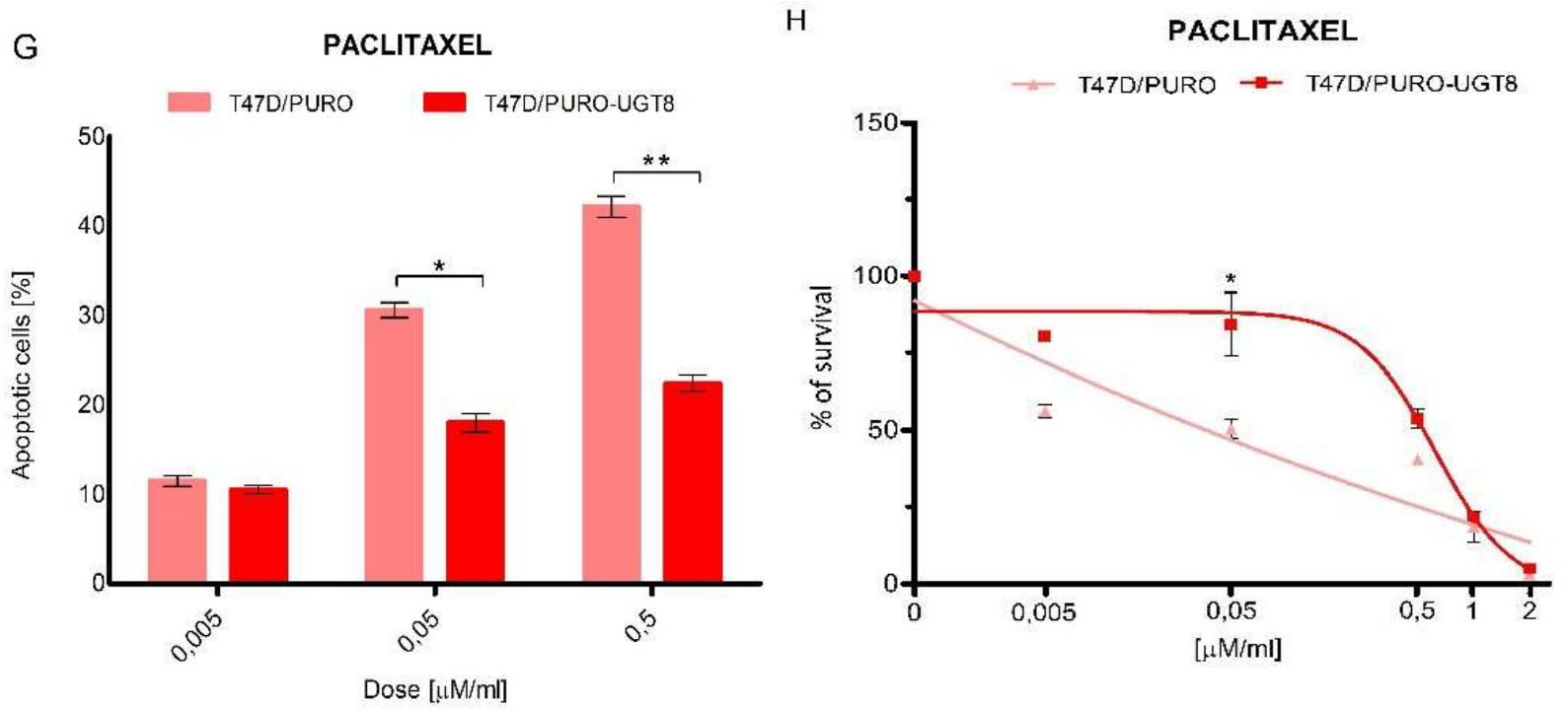
A, B – cellular response measured by presence of active form of caspase-3; C, D – cell viability determined using WST-1 assay.

The resistance of breast cancer cells with different expression of UGT8/GalCer to apoptosis induced by etoposide



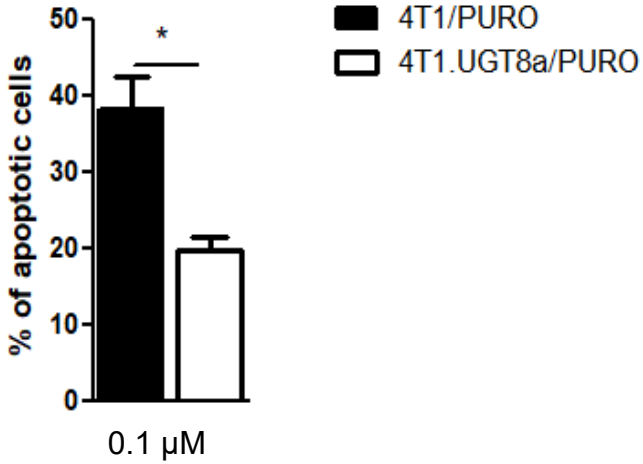
C, D – cellular response measured by presence of active form of caspase-3; E, F – cell viability determined using WST-1 assay.

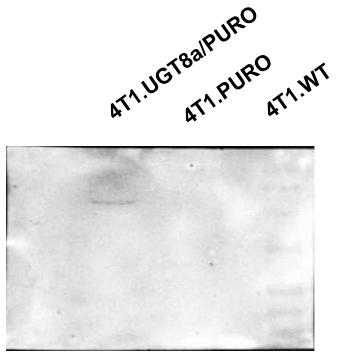
The resistance of breast cancer cells with different expression of UGT8/GalCer to apoptosis induced by paclitaxel



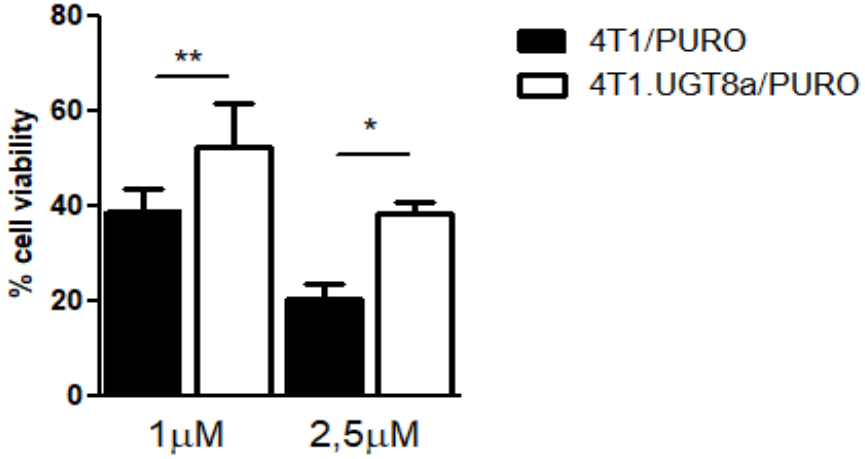
G – cellular response measured by presence of active form of caspase-3; H – cell viability determined using WST-1 assay.

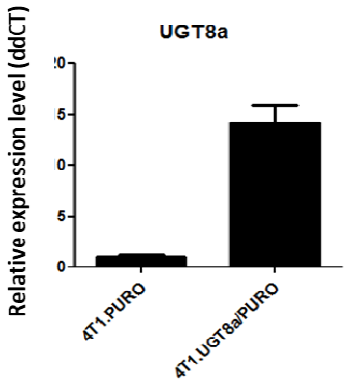
The resistance of murine mammary carcinoma 4T1 cells with expression of murine UGT8/GalCer to apoptosis induced by doxorubicin





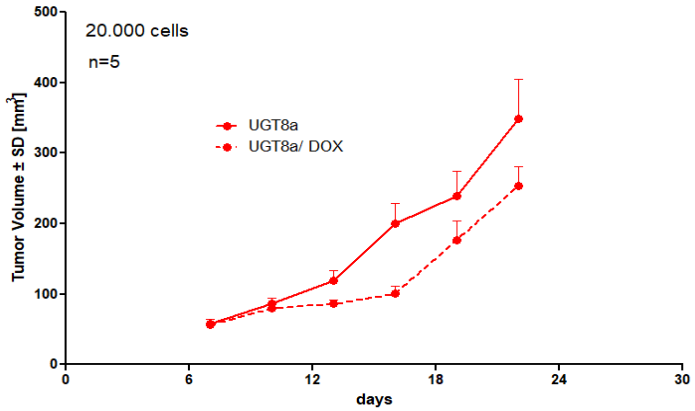
Western blot analysis of anti-UGT8 rabbit polyclonal antibodies binding to cellular proteins of 4T1 cell sublines

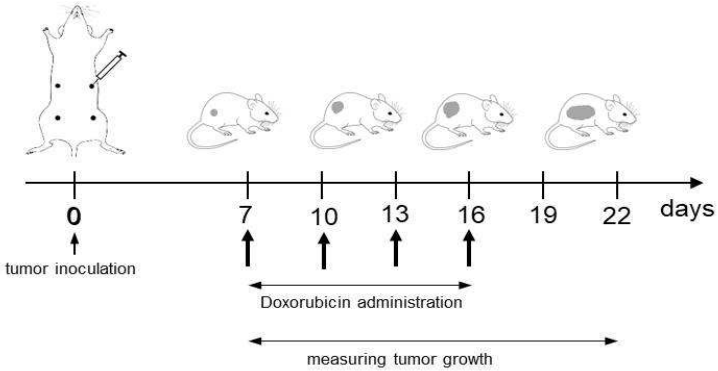


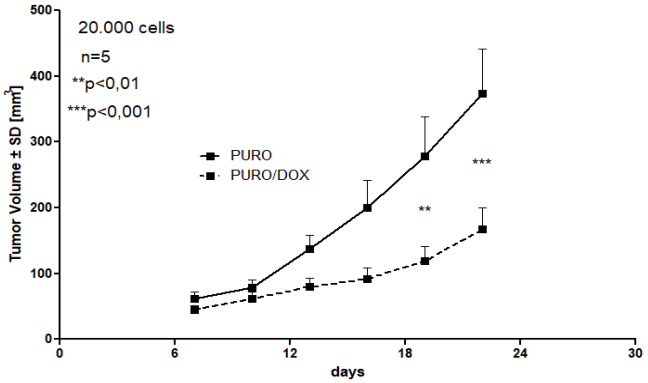


Expression of UGT8 mRNA in breast cancer cell lines. Real-time RT-PCR was used to analyze UGT8 mRNA

Therapeutic effect of i.v. administration of doxorubicin on 4T1.UGT8a/PURO and 4T1/PURO tumor growth



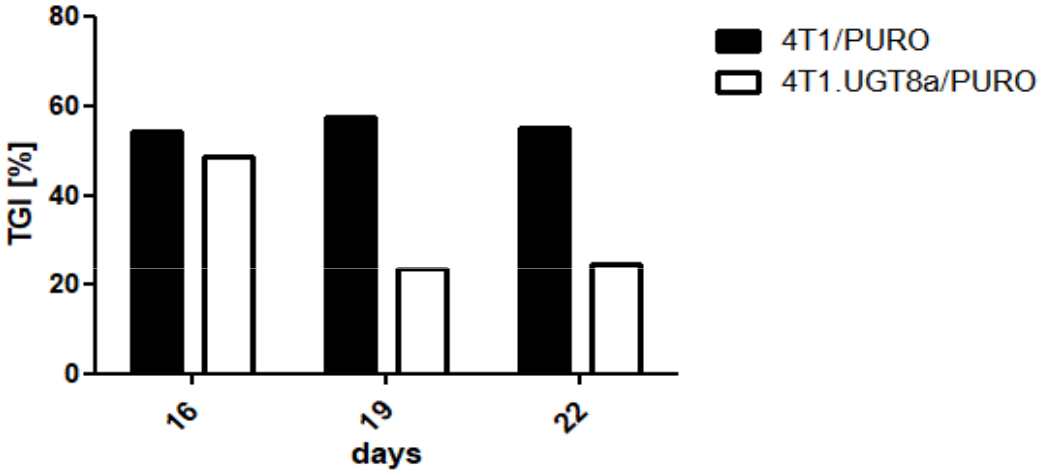




4T1/PURO tumors responded better to doxorubicin treatment then 4T1.UGT8a/PURO tumors overexpressing UGT8.

Data are shown as the mean tumor volume for each group of mice (n=5) ±SE at each indicated time point.

Therapeutic effect of i.v. administration of doxorubicin on 4T1.UGT8a/PURO and 4T1/PURO tumor growth

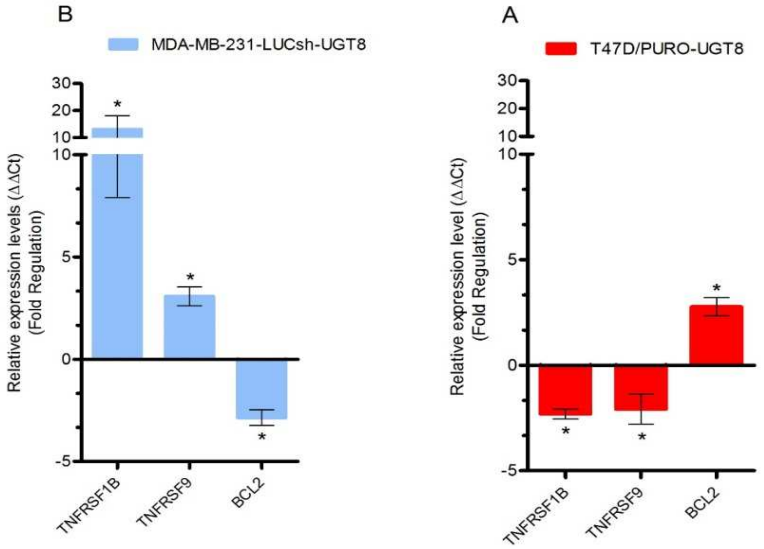


Tumor Growth Inhibition was calculated using the formula:

$$\mathbf{TGI\ (\%) = 100 - TV_T/TV_C \times 100\%}$$

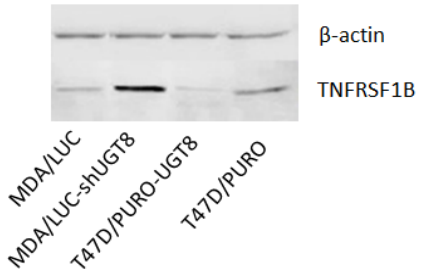
where TV_T is the tumor volume in mice treated with doxorubicin, TV_C – the tumor volume in un-treated mice.

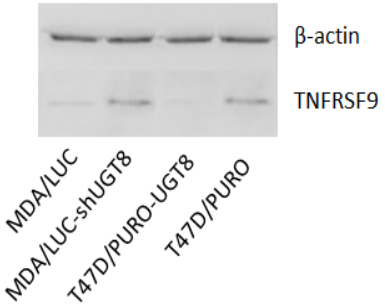
Expression of apoptotic genes in breast cancer MDA-MB-231 and T47D cells cells with high and low expression of UGT8 and GalCer (RT2 Profiler PCR Array Human Apoptosis Assay, real-time PCR, Western blotting)



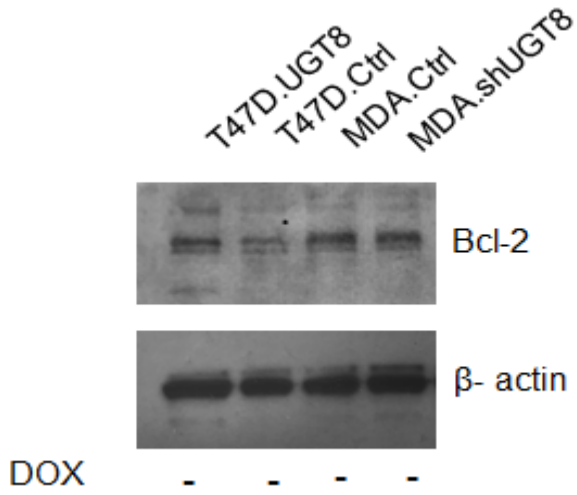
The relative expression of apoptotic genes in MDA-MB-231/LUC-shUGT8 cells *vs.* control MDA-MB-231/LUC cells and T47D/PURO-UGT8 vs. control T47D/PURO cells determined by real-time PCR.

ACTB as a reference gene. Data are presented as mean ± SD values of three measurements in three independent experiments (n = 9). **P* < 0.05 using the Student t-test.



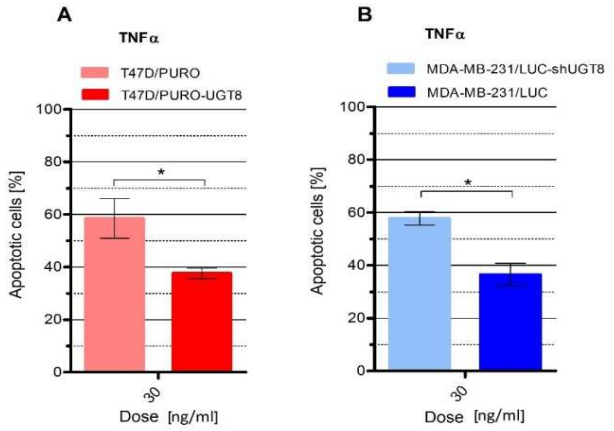


Expression of TNFRSF1B and TNFRSF9 determined by Western blotting.

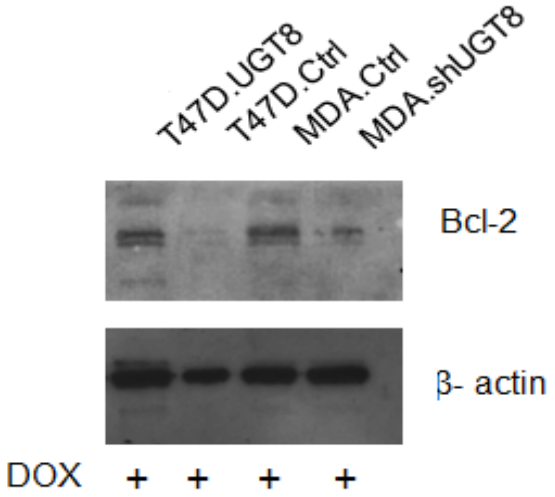


Expression of Bcl-2 determined by Western blotting.

TNFRs and Bcl-2 are directly involved in apoptosis of breast cancer cells

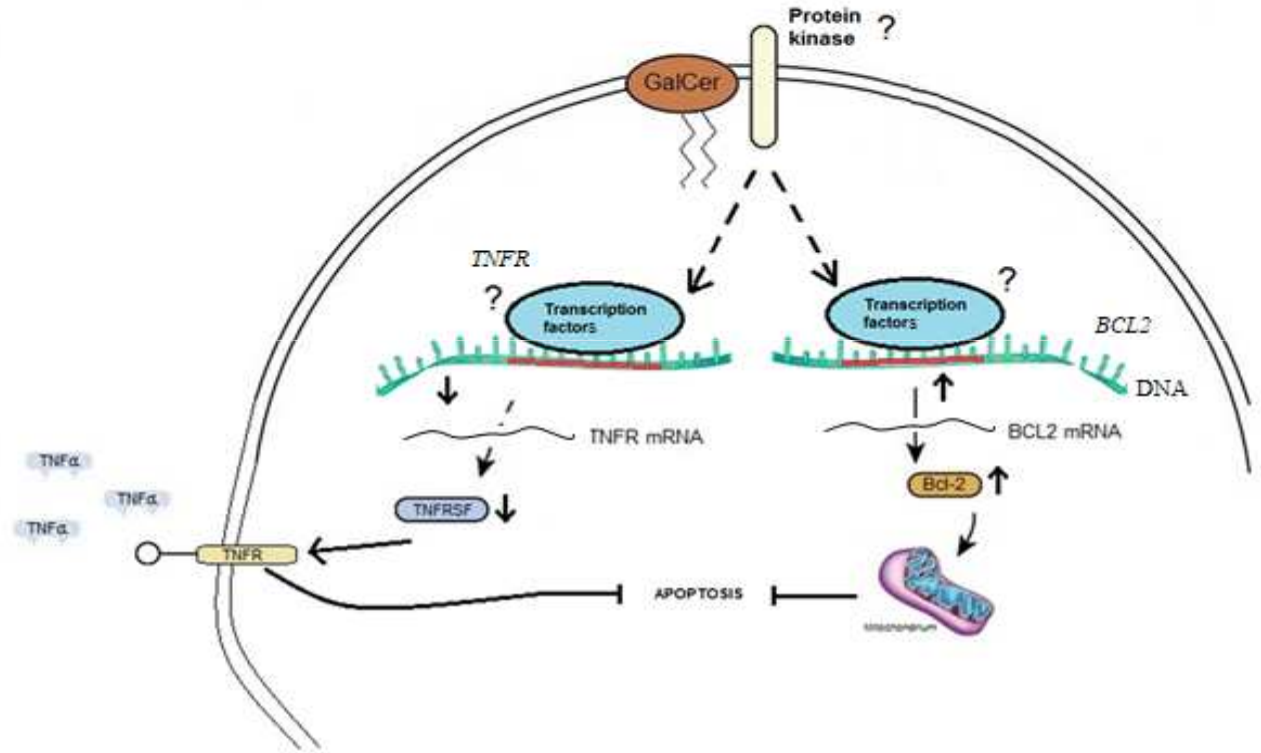


The T47D and MDA-MB-231 cells with high expression of UGT8/GalCer are more resistant to apoptosis induced by TNFα.

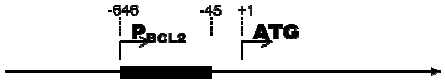


Bcl-2 protein levels are decreased in breast cancer cells with low expression of UGT8/GalCer during doxorubicin-induced apoptosis.

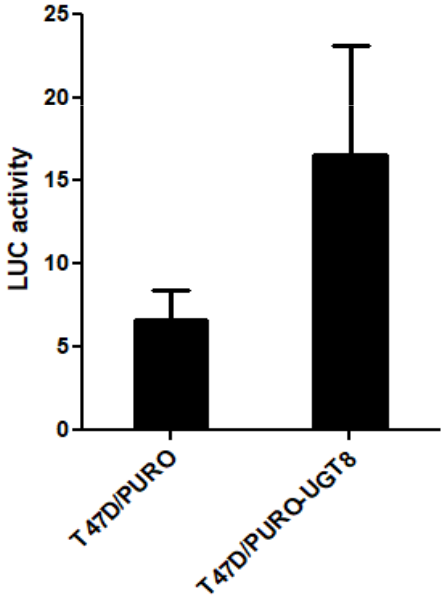
Proposed mechanism of GalCer involvement in the transcriptional regulation of apoptotic gene expression

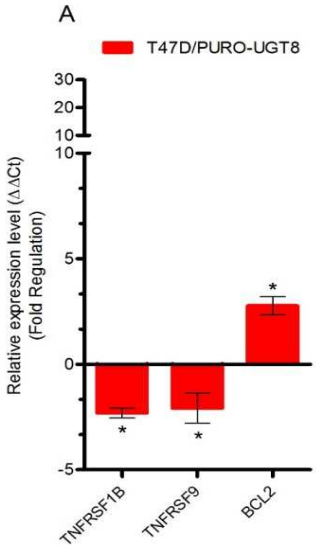


Activity of the *BCL2* promotor in T47D cells with different expression of UGT8



Schematic representation of cloned *BCL2* promoter





Activities of BCL2 promoter in T47D/PURO and T47D/PURO-UGT8 cells

The promoter activities were measured using the Dual-Luciferase Reporter Assay System (Promega) after transfection into T47D cells. The bars represent average luciferase activities compared with the control (not-transfected cells).



Tomasz Owczarek, **Jarosław Suchański**, **Safoura Reza** (Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science)

Bartłomiej Kocbach, **Alicja Kmiecik, Marek Chadalski Ewa Plażuk** (Laboratory of Glycobiology and Cell Interactions, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland)

P giel, **Bartosz Puła, Sandra Jethon, Agnieszka Gomułkiewicz, Marzena Podhorska-Okołów** (Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland).

Paweł Pasikowski (Laboratory of Mass Spectrometry, EIT+)

Keltouma Driouch, Rosette Lidereau (Oncogenetics laboratory/INSERM U 735, Saint-Cloud, France).