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## **ER<sup>+</sup>-derived breast cancer stem cells reveal a high expression of the serpin protease inhibitor PI-9.**

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**Introduction:** Breast cancers (BC) are the major cause of death in women. More than 70% of BCs express high levels of estrogen receptor- $\alpha$  (ER $\alpha$ ) and are sustained for their growth by the hormone. Estrogens seem to protect BC cells from apoptosis mediated by immunosurveillance associated with cytotoxic T lymphocytes and NK cells granzyme B release. However, the production of granzyme B inhibitor PI-9 by tumor cells causes a short-circuit in immunosurveillance's signalling. Although it has been shown the role of PI-9 in BC cells, its presence has not been investigated in tumor stem cells so far.

**Methods:** Cell viability was evaluated by MTT, cell cycle by propidium iodide staining; mRNA and protein levels by qPCR and western blotting. Tumorspheres from ER $\alpha$ +BC MCF7 cells were isolated in ultra-low attachment conditions. The higher expression of stemness markers (Nanog, Oct3/4 and Sox2) was found in tertiary tumorspheres which were used in our study.

**Results:** Low doses (10 nM-10  $\mu$ M) of 17- $\beta$  estradiol consistently increased the number of MCF7 cells more than tumorspheres, while higher doses (50-100  $\mu$ M) reduced cell number as a consequence of G2/M cell cycle arrest. The analysis of ER $\alpha$  disclosed the presence of three different isoforms (66, 46 and 36 kDa) in MCF7 cells. In contrast, tumorspheres exhibited an increase in ER $\alpha$ 36, which lacks transcriptional activity, while the level of ER $\alpha$ 66 was undetectable. Then, we analyzed the level of PI-9, which is transcriptionally regulated by ER $\alpha$ 66. Surprisingly, we found that tertiary tumorspheres, express higher levels of both PI-9 protein and mRNA than MCF7 cells.

**Conclusions:** Our data provided evidence that the high level of PI-9 in ER<sup>+</sup> tertiary tumorspheres could supply a selective advantage to BC stem cells by interfering with immune-surveillance systems. Ongoing studies aim to elucidate the relationship between the levels of different ER $\alpha$  isoforms and PI-9 high expression in BC-stem cells.