The role of Prolactin Induced Protein in breast cancer cells apoptosis induced by doxorubicin

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Studies by Jabłońska et al. (2016 – team of prof. Dzięgiel) shown that Prolactin Induced Protein (PIP) can be a positive prognostic factor in breast cancer (BC) and plays role in breast cancer cell response to chemotherapy. It was shown that tumors from patients with low level or lack of PIP expression are significantly more resistant to doxorubicin and cyclophosphamide therapy than tumors from patients with high level of PIP (Jabłońska et al., 2016). Additionally, it was shown that the levels of PIP mRNA and protein decrease along with increasing tumor malignancy grade G and that its expression is the lowest in triple-negative (ER-, PR-, HER-2-) patients with poor prognosis (Jabłońska et al., 2016). From clinical point of view, it is also important that cases with high PIP expression were characterized by longer disease-free and overall survival (Hähnel et al., 1996; Jabłońska et al., 2016). Taken together, this data suggest that PIP may directly affect the sensitivity of breast cancers to adjuvant chemotherapy. Therefore, the present studies were undertaken to verify this hypothesis and define the role of PIP in breast cancer cell survival and apoptosis induced by cytostatics: doxorubicin, 4-hydroxycyclophosphamide and paclitaxel. These studies were conducted using breast cancer MDA-MB-231 cells with neoexpression of PIP, representing *gain of function phenotype*, and breast cancer T47D cells with suppressed expression of PIP, representing *loss of function phenotype*. Our *in vitro* results showed that breast cancer MDA-MB-231 cells with high PIP expression (MDA-231/PIP) were characterized by increased sensitivity to these cytotoxic drugs than control MDA-MB-231 cells with no expression of this protein (MDA-231/PURO). On the other hand, breast cancer T47D cells with suppressed expression of PIP (T47D puro/shPIP) were less sensitive to cytostatics than control T47D with high expression of PIP (T47D puro/ctrlsh).

This data was confirmed by *in vivo* experiments using nude mice model. Mice with orthotopically tumors formed by transplanted MDA-231/PIP cells with high expression of PIP responded better to doxorubicin treatment in comparison to mice bearing tumors formed by transplanted MDA-231/PURO with no expression of this protein. In summary, these experimental studies confirmed the clinical data, and revealed that PIP is directly responsible for increased sensitivity of breast cancer cells to apoptosis induced by cytotoxic drug.

The role of the PIP protein in the progression of breast cancer has not been fully explained. The majority of studies, so far, concentrated on the involvement of PIP in proliferation pathway of breast cancer cells, however, these studies brought inconsistent results. Therefore, using both cellular models cell cycle analysis was performed, and no differences in proliferation between breast cancer cells expressing high level of PIP (MDA-231/PIP and T47Dpuro/ctrlsh) and breast cancer cells without expression of PIP (MDA-231/PURO and and T47Dpuro/shPIP) were found. This data did not confirmed the previous proposal on the key role of PIP in the proliferation of breast cancer cells and are in agreement with our proposal on the involvement of PIP in apoptosis of these cells.

It is generally accepted that PIP is a secretory protein and act as a extracellular protein. Therefore, that's why using the recombinant PIP protein (PIP-LEXSY), to demonstrate the binding of PIP to the surface of breast cancer cells. It was found using flow cytometry that PIP binds specifically to the surface of MDA-MB-231 cells and, using western blotting, it was also shown that PIP binds to membrane protein of supposed molecular mass about 55 kD, which probably represent its specific receptor. The functional role of PIP as secretory protein was also confirmed by experiment showing that soluble PIP protein incresed the sensitivity of MDA-MB-231 cells to doxorubicin.

In summary, we have shown for the first time that PIP plays direct role in the induction of apoptosis in breast cancer cells by cytotoxic drugs. According to our data, PIP role in breast cancer progression seems more related to its pro-apoptotic than pro-proliferative properties. We have also confirmed that PIP acts as secretory protein and showed for the first time its binding to specific cell membrane protein. However, understanding the exact molecular mechanism of PIP action await further studies.