SUMMARY

This report provides evidence for the important role of estrogens in the mechanism of PRODH/POX-dependent apoptosis in breast cancer cells.

PRODH/POX is a mitochondrial enzyme catalyzing the conversion of proline to Δ1-pyrroline-5-carboxylic acid (P5C). During the conversion of proline to P5C, electrons are transported to the respiratory chain, producing ATP or they are directly accepted by oxygen, generating reactive oxygen species (ROS). In the first case, activation of PRODH/POX leads to the production of ATP for survival, in the second one, ROS induce apoptosis. Although the mechanism for the switch between apoptosis/survival is not well understood, it has been postulated that the PRODH/POX-induced apoptosis or survival is a metabolic context-dependent process and proline availability for PRODH/POX-dependent functions may play a key role.

In this report it has been hypothesized that estrogens could play an important role in the mechanism of PRODH/POX-dependent apoptosis/survival as stimulators of collagen biosynthesis, that utilize a large amount of free proline, limiting substrate (proline) availability for PRODH/POX-dependent functions. Estrogens are implicated in collagen metabolism as stimulators of collagen biosynthesis. This process is accompanied by collagen degradation finalized by cytoplasmic imidodipeptidase, prolidase.

To explore the hypothesis four breast cancer cell models were used: ER-positive MCF-7 breast cancer cell line (expressing ERα and ERβ) and ER-negative MDA-MB-231 breast cancer cell line (expressing only ERβ) and respective shRNA PRODH/POX silenced clones. To up-regulate PRODH/POX, troglitazone (TGZ), the ligand of peroxisome proliferator-activated receptor-γ (PPAR-γ), known to stimulate PRODH/POX, was used.

It has been found that estrogens stimulate collagen biosynthesis by utilizing free proline and limiting its availability for PRODH/POX-dependent apoptosis. Interestingly, TGZ was found not only as a strong PRODH/POX activator but also an inhibitor of collagen biosynthesis. It has been documented that apoptosis (activated caspase-3, -9, and PARP) was highly pronounced in wild-type MDA-MB-231 cells cultured in the medium without estradiol or in the cells cultured in the medium with estradiol but deprived of ERβ (by ICI-dependent degradation), while in PRODH/POX-silenced cells the process was not found. PRODH/POX-induced apoptosis in these cells was reactive oxygen species (ROS) dependent. The effect was not found also in MCF-7 cells independently of the absence or presence of estradiol and in MDA-MB-231 cells cultured in the medium with estradiol. The mechanism for the process was

found at the level of collagen biosynthesis, the most effective process of proline utilization, that is up-regulated by estrogens. The data suggest that TGZ-induced apoptosis in MDA-MB-231 cells cultured in the medium without estradiol or deprived of ERβ is mediated by PRODH/POX and the process is facilitated by proline availability for PRODH/POX by TGZ-dependent inhibition of collagen biosynthesis. It suggests that combined PPAR-γ agonist and antiestrogen treatment could be considered in experimental therapy of estrogen receptor-negative breast cancers.