Abstract in English

Mitochondrial electron transport chain (ETC) targeting shows a great promise in cancer therapy. However, why modern ETC-targeted compounds are tolerated on the organismal level and what are the molecular reasons for this tolerance remains unclear. Most somatic cells are in a non-proliferative state, and features associated with the ETC in quiescence might therefore contribute to specificity. Thus, we investigated the ETC status and the role of two major consequences of ETC blockade, reactive oxygen species (ROS) generation and inhibition of ATP production, in cell death induction in breast cancer cells and in proliferating and quiescent non-transformed cells.

First, we characterised the effect of a newly developed ETC inhibitor mitochondria-targeted tamoxifen (MitoTam) in *in vitro* and *in vivo* tumour models of breast cancer with varying status of the Her2 oncogene. We document that $Her2^{high}$ cells and tumours have increased assembly of respiratory supercomplexes (SCs) and increased complex I-driven respiration *in vitro* and *in vivo*. They are also highly sensitive to MitoTam. Unlike the parental compound tamoxifen, MitoTam efficiently suppressed experimental $Her2^{high}$ tumours without systemic toxicity. Mechanistically, MitoTam inhibits complex I-driven respiration and disrupts respiratory SCs in $Her2^{high}$ background *in vitro* and *in vivo*, leading to elevated reactive oxygen species production and cell death. This suggests that in cancer settings increased respiration sensitizes to ETC inhibition by stimulating ROS generation.

Next, we investigated if differences in mitochondrial respiration between proliferating and quiescent cells, representing cancerous and normal tissue, could explain the specificity of ETC targeting. Therefore, endothelium-derived (EA.hy926) and epithelium-derived (MCF10A) non-transformed cells capable of contact inhibition were cultured in low (1 g/L) and high glucose (4.5 g/L) media, representing conditions of limited and ample glucose supply, respectively. Both, proliferating and quiescent (contact-inhibited) cells were exposed to direct ROS inducers that do not interact with the ETC, specific inhibitors of the ETC or inhibitors of mitochondrial ATP production. We also performed similar experiments in EA.hy926 cells with depleted mitochondrial DNA featuring defective ETC to clarify ETC's role in the selectivity phenomenon. Cell death and ROS production, as well as various metabolic parameters, particularly mitochondrial respiration, antioxidant defence expression and activity, glycolysis rate and ATP levels

were assessed. The role of several components of the antioxidant defence systems (SOD2- and GPX1-, TRX-reductase- and GSR) was interrogated by shRNA-mediated knockdown. In the end of study, we confirmed the results *in vivo* using FVBn/c-neu mouse model of spontaneous Her2^{high} mammary carcinoma.

Even though quiescent cells, compared to proliferating cells, featured increased SC assembly and a higher rate of respiration, proliferating cells were more susceptible to cell death induced by direct ROS inducers, ETC and ATP synthase inhibitors when cultured in high glucose conditions. All these agents also induced more ROS in proliferating cells. Interestingly, in low glucose conditions the pattern of cell death sensitivity was reversed for ETC and ATP synthase inhibitors, but not for direct ROS inducers. The amount of cell death in this situation correlated with significant ATP depletion in quiescent cells, rather than with ROS production. This was related to the inability of quiescent cells to compensate for the loss of mitochondrial ATP production by the upregulation of glucose uptake. Regardless of glucose availability, quiescent cells featured increased protein levels and activity of antioxidant enzymes (SOD2, TRX2, PRX3 and GPX). However, silencing of SOD2 or TRX-reductase 2 by RNA interference increased ETC inhibition-induced cell death and ROS production exclusively in quiescent cells under high glucose conditions. Experiments with ETC-deficient cells then revealed that functional ETC paradoxically complements the protective role of antioxidant defence in quiescent cells. This interesting aspect will be investigated in follow-up research.

In conclusion, our data suggest that lack of ATP is the major factor for cell death induction when ETC is blocked in a situation of glucose limitation, while the amplitude of ROS generation becomes dominant when glucose is not limiting. The combination of antioxidant defence and glucose availability therefore co-determines the specificity of ETC-inhibition-induced cell death and explains why ETC inhibition can effectively suppress cancer without considerable collateral damage to normal tissue.