ABSTRACT (EN)

Based on the promising results concerning the anti-cancer properties of redox-silent analogue vitamin E α-tocopheryl succinate (α-TOS), we prepared its mitochondrially targeted derivative MitoVES by attaching the positively charged triphenylphosphonium (TPP^+) tag to α-TOS molecule. We tested the hypothesis that 'sending' the drug directly to its cellular site of action, mitochondria, should enhance its anti-cancer properties, which would result in more effective anti-cancer agent while making it possible to reduce the effective concentration.

We provide evidence that, indeed, MitoVES is a highly effective anti-cancer compound, superior to untargeted α-TOS both *in vitro* and *in vivo*. We show that MitoVES exerts its anti-cancer effects by interfering with complex II (CII) activity specifically at the ubiquinone binding site (Q_p), where it blocks further electron transfer resulting in increased reactive oxygen species (ROS) production, which then leads to apoptosis induction via the intrinsic mitochondrial pathway, preferentially engaging the pro-apoptotic Bak protein causing mitochondrial membrane permeabilisation.

We further show that mitochondrial targeting on the basis of higher mitochondrial membrane potential (ΔΨ) is important for MitoVES pro-apoptotic activity. This feature endows the agent with selectivity for cancer cells, which have higher ΔΨ than normal cells, and as we found, high ΔΨ is also propensity of proliferating endothelial cells (ECs) in contrast to growth-arrested ECs. This provides MitoVES with yet another anti-cancer property, inhibition of angiogenesis in newly formed tumor.

Mutagenesis of CII Q_p site confirmed MitoVES interaction with this site and further corroborated the importance of ROS production for efficient cell death induction, as the Q_p site mutations conferred resistance to MitoVES treatment. This was reflected by less efficient inhibition of CII-derived respiration, lower ROS production and cell death. Interestingly, we found that another CII Q_p site inhibitor, TTFA, showed different efficiency in Q_p site inhibition than MitoVES, since the S68A mutant was more responsive to the drug than the wild-type cells and the other mutants. In this case, the potency of Q_p site inhibition again correlated with the extent of induced cell death. Surprisingly, we discovered that the high-affinity Q_p site inhibitor atpenin A5 did cause neither ROS generation nor cell death induction. We demonstrate that this is due to rapid accumulation of intracellular succinate, which is incompatible with ROS generation from CII.

Altogether, this work has established MitoVES as promising anti-cancer agent and defined mitochondrial CII as its target site. Moreover, CII Q_p site mutagenesis revealed a direct correlation between the efficacy of CII Q_p site inhibition, ensuing ROS production and the level of cell death induction, unless intracellular succinate is high.