Ubiquinone-binding site mutagenesis reveals the role of mitochondrial complex II in cell death initiation

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Respiratory complex II (CII, succinate dehydrogenase, SDH) inhibition can induce cell death, but the mechanistic details need clarification. To elucidate the role of reactive oxygen species (ROS) formation upon the ubiquinone binding (Q_p) site blockade, we substituted CII subunit C (SDHC) residues lining the Q_p site by site-directed mutagenesis. Cell lines carrying these mutations were characterized on the bases of CII activity and exposed to Q_p site inhibitors MitoVES, TTFA and Atpenin A5. We found that I56F and S68A SDHC variants, which support succinate-mediated respiration and maintain low intracellular succinate, were less efficiently inhibited by MitoVES than the wild-type variant. Importantly, associated ROS generation and cell death induction was also impaired, and cell death in the wild-type cells was malonate- and catalase-sensitive. In contrast, the S68A variant was much more susceptible to TTFA inhibition than the I56F variant or the wild-type CII, which was again reflected by enhanced ROS formation and increased malonate- and catalase-sensitive cell death induction. The R72C variant that accumulates intracellular succinate due to compromised CII activity was resistant to MitoVES and TTFA treatment and did not increase ROS, even though TTFA efficiently generated ROS at low succinate in mitochondria isolated from R72C cells. Similarly, the high affinity Q_p site inhibitor Atpenin A5 rapidly increased intracellular succinate in wild-type cells but did not induce ROS or cell death, unlike MitoVES and TTFA that upregulated succinate only moderately. These results demonstrate that cell death initiation upon CII inhibition depends on ROS and that the extent of cell death correlates with the potency of inhibition at the Q_p site unless intracellular succinate is high. In addition, this validates the Q_p site of CII as a target for cell death induction with relevance to cancer therapy.