TRPV2 as a Potential Therapeutic Target of Granulosa Cell Tumors

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We previously identified the ion channel transient receptor potential vanilloid 2 (TRPV2) in cells of the human ovary (Eubler et al., *Mol Hum Reprod* 2023). In isolated human granulosa cells (GCs), derived from patients undergoing medical reproductive procedures, activation of TRPV2 by cannabidiol (CBD) resulted acutely in increased levels of intracellular calcium and stimulated the secretion of inflammatory factors, including interleukin (IL) 6 and IL 8. We concluded that it is involved in the regulation of inflammatory processes. Granulosa cell tumors (GCTs) are derived from granulosa cells. They amount to 2-5% of all ovarian cancers and are malignant stromal tumor types. If diagnosed at an early stage, treatment includes surgical removal of the tumor tissue followed by chemotherapy, but further treatment options are limited. By employing immunohistochemistry, we found that TRPV2 is expressed in a set of human GCT (in 52% of 29 investigated primary GCT samples). TRPV2 was also found in the well-established GCT-derived cell line, KGN (Nishi et al., *Endocrinology* 2001), which was therefore used for further studies. Acutely, the addition of CBD elicited increased intracellular calcium levels (5-30 µM CBD; n = 250 cells from 10 independent experiments), implying channel opening. Within hours (4-6 h), and in striking contrast to primary human GCs, CBD caused cell death in KGN cells, which occurred in a concentration and time-dependent manner. While being a preferred ligand of TRPV2, CBD is known to also interact with other proteins and channels. Therefore, we generated TRPV2-deficient KGN clones using the CRISPR/Cas9 technique and two (clones #4 & #13) were examined more intensively e.g. by cell counting and live cell imaging. The loss of TRPV2 led to several changes, including increased cell size (mean diameter: 16.7 µm in normal KGN vs. 19.5 µm (#4) and 19.8 µm (#13) in TRPV2-

deficient KGN clones; n = 10, each), higher proliferation (mean cell counts after 24 h: 142.8% in normal KGN vs. 206.8% (#4) and 203.8% (#13); n = 9, each) and a higher migration speed (0.32 µm/min in normal KGN vs. 0.46 µm/min (#4) and 0.43 µm/min (#13); n = 28 cells, each). Mass spectrometry (n = 5, each) further supported and complemented the results. Importantly, the absence of TRPV2 made these clones less vulnerable to CBD-induced cell death, as demonstrated e.g. by means of ATP assays, making this ion channel a new therapeutic target for GCTs.

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