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Analysis of the pro-apoptotic effects of CLTAP in renal cell carcinoma

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TMEM52B encoding CLTAP was identified in our laboratory as TGF-β induced gene in human macrophages. According to the BioGPS.org and GEO Profiles database, TMEM52B is expressed in normal kidney including the kidney cortex and medulla, and missing in clear cell renal cell carcinoma (RCC). Previous data from our laboratory demonstrated that CLTAP has a pro-apoptotic effect on kidney carcinoma A498 cells, and interacts with signaling integral membrane protein CAMLG via a special binding region between amino acids 91 and 116 within the CLTAP protein. However, the role of CLTAP in the regulation of apoptosis in other RCC cells, and the role of the CAMLG binding region in the pro-apoptotic function of CLTAP were unknown. The aims of the current study were to analyze the expression of CLTAP in human primary macrophages, to analyze the expression of CLTAP in the collecting duct renal cell carcinoma (CDC) patient samples; to analyze the pro-apoptotic effect of CLTAP in RCC cells using a lentivirus-based vector expressing CLTAP, to generate lentivirus-based vector expressing CLTAP-D1 with deletion of the CAMLG binding site, and to analyze the pro-apoptotic effect of CLTAP-D1 in RCC cells. CLTAP was found to be expressed on mRNA level but not on the protein levels in primary human macrophages. TGF-β was a critical cytokine that induced CLTAP gene expression in combination with M1 and M2 polarizing stimuli. CLTAP was found to be expressed in the collecting duct but neither in the proximal tubule nor in the CD68 positive macrophages in normal kidney tissue. Immunohistological analysis demonstrated that CLTAP was completely absent in CDC tissue but present in the adjacent normal tissue. Expression of the recombinant CLTAP introduced by a lentiviral vector had pro-apoptotic effects in RCC cell lines A498, Caki-1 and Caki-2 cells. The CAMLG binding region within CLTAP was deleted using gene splicing techniques to create a CLTAP mutant (CLTAP-D1). Comparison of full length CLTAP and CLTAP-D1 introduced by a lentiviral vector into A498, Caki-1 and Caki-2 cells demonstrated that deletion of the CAMLG binding site abrogated the pro-apoptotic effect of CLTAP in the RCC cells. In summary, the results of the study suggest that CLTAP is involved in the normal apoptotic process required for the termination of epithelial cell life span, whereas the pro-apoptotic effect of CLTAP requires the direct interaction with the CAMLG protein. The pro-apoptotic activity of CLTAP can be used in the future to develop novel therapeutic approaches in RCC and CDC.