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Functional analysis of novel pro-apoptotic factor CLTAP

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Renal cell carcinoma (RCC) accounts for about 90% of kidney cancer, one of the top ten most common human cancers. The subtype RCC has been shown to be caused by dysregulation of VHL, mTOR and other angiogenic pathways, however these mechanisms do not explain the complexity of RCC pathogenesis. A differential diagnosis of RCC is insufficiently developed, and 30% of RCC cases are metastatic at the time point of diagnosis, decreasing the efficiency of therapy. The current treatment options are limited, making the discovery of other possible therapeutic strategies crucial. CLTAP is a newly discovered TGFbeta-inducible gene in human macrophages. Bioinformatics analysis revealed that CLTAP is almost exclusively expressed in normal kidney, but is lost in RCC. Preliminary data from our laboratory indicated that CLTAP is absent in kidney carcinoma cell lines and interacts with CAMLG, a regulator of apoptosis, in a yeast two-hybrid assay. However, the biological function of CLTAP and its role in the regulation of apoptosis remained unknown. The aims of the current study were to establish a model system for studying the function of CLTAP, to investigate the role of CLTAP in the regulation of apoptosis, to analyze the interaction of CLTAP with CAMLG by *in vitro* binding assays, and to investigate their co-localization during the course of apoptosis. In order to establish a model system for gain-of-function of CLTAP, the kidney carcinoma cell line A498, negative for endogenous CLTAP, was selected. To introduce recombinant CLTAP, the lentiviral-based expression construct, TWEEN-CLTAP, was generated. The use of a lentiviral vector provided efficient overexpression of recombinant CLTAP in almost 100% of cells and the possibility to use a precise negative control. Using the newly created lentiviral construct TWEEN-CLTAP, analysis of the effect of CLTAP on the apoptosis of A498 cells was performed. Apoptosis was induced by staurosporine which works via a caspase-independent mechanism, for 12-72 hours. Overexpression of CLTAP increased the proportion of apoptotic cells at all time points. A major effect on apoptosis was observed 18 hours post stimulation, with a 2-fold higher percentage of apoptotic cells in the TWEEN-CLTAP transduced cells compared with the control. Overexpression of CLTAP also stimulated apoptosis in the absence of the stimulating agent staurosporine. A 3-fold higher percentage of apoptotic cells in the CLTAP transduced cell population was detected 48 hours post infection in comparison with the control, indicating that CLTAP is a pro-apoptotic factor. To examine the mechanism of CLTAP-mediated induction of apoptosis, the interaction of CLTAP and CAMLG was analyzed using the pull-down assay. GST-fused recombinant CLTAP protein fragments, corresponding to amino acids 70-183, 91-183 and 116-183, were purified out of a bacterial system. Full length recombinant CAMLG, corresponding to amino acids 1-296, and its fragments corresponding to amino acids 1-199 and 29-93 were produced by *in vitro* translation. The interaction assay demonstrated that CLTAP amino acids 91-116 are responsible for the interaction with CAMLG. In CAMLG, amino acids 29-93 were important for the interaction with amino acids 91-116 of CLTAP. To examine whether CLTAP and CAMLG interact in A498 cells, immunofluorescence analysis and confocal microscopy were used. In the non-apoptotic A498 cells, CLTAP and CAMLG were localized separately in vesicular structures. Induction of apoptosis using staurosporine resulted in the co-localization of CLTAP and CAMLG after 12 hours. The data indicates that CLTAP and CAMLG form a transient complex prior to the highest effect of CLTAP on the rate of apoptosis which was identified by flow cytometry. The data suggests, that the interaction of CLTAP with CAMLG is needed for the pro-apoptotic effect of CLTAP. The results of the study suggest that the pro-apoptotic activity of CLTAP can be used in the future to develop novel therapeutic approaches in RCC.