8 Summary

Quantitative Expression Studies of CLCA-Homologues in Murine Models of Cystic Fibrosis

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Members of the family of calcium-activated chloride channels (CLCA) have been implicated as modulators of the phenotype in cystic fibrosis (CF). Results of several different studies have shown that CF disease severity is modulated by other genetic factors than CFTR. Electrophysiological, mRNA-expression and allelic variation studies seem to imply that, at least in part, members of the CLCA gene family could be responsible for this modulation by mediating an alternative calcium-activated chloride conductance. Here, the expression levels of the murine mCLCA1, mCLCA2, mCLCA3 and mCLCA4 were quantified by *real-time* RT-PCR in the small intestines of CF (*cftr*$^{TgH(neoim)1Hgu}$, *cftr*^{tm1Cam}) and wild type BALB/cJ, C57BL/6J, DBA/2J und NMRI mice. Markedly different expression levels of all four CLCA-homologs were observed between the different wild type strains. Expression of mCLCA1 and mCLCA4 was similar in CF versus wild type mice. In contrast, mCLCA3 mRNA copy numbers were increased up to three-fold in all CF models. Immunohistochemichal detection of mCLCA3- and PAS-positive cells on consecutive tissue sections identified a similar increase in mCLCA3-expressing goblet cells, suggesting that elevated mRNA copy numbers of mCLCA3 are due to goblet cell hyperplasia rather than transcriptional regulatory events. Increased mCLCA2 mRNA copy numbers, however, were considered more likely to be due to transcriptional upregulation. Changes in mRNA copy numbers were not associated with altered cell kinetics as determined by immunohistochemistry using antibodies to phospho-histone 3 and activated caspase 3. The results suggest that both mCLCA2 and mCLCA3 may act as modifiers of the intestinal phenotype in CF.

Sequence analysis of the *open reading frame* of mCLCA3 failed to identify allelic variations in the coding region between the four different mouse strains and thus variations in coding sequences were not associated with differences in mCLCA-expression levels.