CLCN7 promoter regulation and disruption of ClC-7 function: potential for osteoporosis therapy

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Chloride channels are essential for a wide range of physiological functions in the plasma membrane and intracellular organelles of all eukaryotic cells. Mouse gene knockout models have demonstrated that the disruption of the ClC-7 chloride channel prevents the production of acid required for the resorption of bone, resulting in a severe osteopetrotic phenotype. In addition to this, CLCN7 mutations have been found in human osteopetrotic patients. While osteopetrosis is a rare disease in humans, it is possible that down-regulation of CLCN7 expression by promoter manipulation could be used to treat the far more common disorder, osteoporosis. We have isolated the human CLCN7 promoter and characterised it with respect to promoter activity and transcription factor binding. Truncation of this promoter region has revealed the presence of two small approximately 30 bp regions of significant activity. Site-directed mutagenesis has identified one potential E-box transcription factor binding site that is responsible for the majority of this promoter activity and, as has been shown by EMSA, it may bind the myc protein. As an alternative to down-regulation, deliberate disruption of ClC-7 function could be therapeutic. In osteoclasts, endogenous ClC-7 is restricted to endosomes and the ruffled border membrane of the resorption lacuna. Possibly because of similar trafficking to endosomes during heterologous over-expression in other cells, ClC-7 has been found to be difficult or impossible to characterise by patch-clamp. We have, therefore, inserted a FLAG-tag epitope into either the extracellular BC or IJ loop of a CLCN7 cDNA construct to allow us to follow and optimise trafficking in HEK 293 cells. By western blot, we have then confirmed expression of ClC-7 and demonstrated its presence in the surface membrane by flow cytometry. As yet, however, we have been unable to show functional activity, or to disrupt it, in the patch-clamp. Our work on ClC-7 could lead towards complementary approaches to current osteoporosis therapy.