

KC1 Cotransporter Gene Expression.

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HbS polymerization is the basis for sickle cell pathophysiology, but disease severity and clinical course are influenced by other factors, including cellular dehydration, which markedly accelerates HbS polymer formation. The KC1 cotransporter (KCC) normally regulates cation and water content in reticulocytes. However, in sickle red blood cells (SS RBC), high expression of KCC and abnormal response to cell volume may both contribute to SS reticulocyte dehydration. Thus, KCC plays a role in sickle cell pathology, and a potential therapeutic strategy is to reduce KCC activity to improve RBC hydration. One approach to this end would be the genetic manipulation of KCC expression. A comprehensive understanding of the regulated expression of the KCC gene in RBC is the focus of the three specific aims of this project. Recent data indicates that the KCC1 isoform and several splicing isoforms are present in erythroid cells. The goal of Specific Aim 1 is to characterize the temporal sequence and level of expression of KCC1 and its isoforms during erythroid differentiation, comparing AA and SS cells. Bone marrow samples from AA and SS patients will be analyzed using in situ hybridization techniques to assess KCC1 mRNA levels in various erythroid precursors. Parallel studies will examine KCC1 mRNA levels by RT-PCR and RNase protection assays in differentiating erythroid cells in culture. Protein levels will also be assessed by immunocytochemistry and Western blot analysis. Studies in Specific Aim 2 will determine the critical *cis* elements regulating transcription of the human gene encoding KCC isoform 1. We have identified at least one functional promoter for this gene, with preliminary evidence suggesting a second promoter as well. These studies will involve transient and stable transfections of erythroid and control cell lines with various reporter gene constructs derived from these promoter regions. Experiments in Specific Aim 3 will characterize the *trans*-acting factors that bind the genetic elements identified in aim 2. These studies will include electrophoretic mobility shift assays, antibody supershifts to confirm known factors, and protein purification of uncharacterized factors. Together, these experiments will produce a clearer understanding of the mechanisms of KCC gene regulation and its possible abnormalities in sickle cell disease, with the ultimate goal of developing new therapies for altering red cell hydration to augment the treatment of sickle cell disease.