

Construction of Sickle Cell Anemia Mice Carrying Chromosomes from the Human Sickle Cell Anemia Haplotypes

Y.W. Kan Principal Investigator

A. Specific Aims

The aim of this project is to make mouse models of sickle cell anemia that carries the different human sickle cell haplotypes. We plan to use bacterial artificial chromosomes (BAC) to isolate the β globin gene cluster in patients with sickle cell anemia carrying the different haplotypes and then make transgenic mice out of them. These mice will then be mated with mice that have knockouts of the endogenous α and β mouse globin genes already in our laboratory. Because the mice contain the whole β globin gene cluster including the control regions, they may be very useful for testing drugs that stimulate fetal hemoglobin production.

B. Studies and Results

Previously, we had made a sickle cell anemia mouse by incorporating a yeast artificial chromosome that contained the β^S gene. However, this mouse responded poorly to drugs that stimulated fetal hemoglobin because the DNA was not obtained from a sickle cell anemia patient. Therefore, we have used this mouse to begin to test another approach to treat sickle anemia using embryonic stem cells. We had reported making ES cells from these mice and corrected the mutation of β^S to β^A by homologous recombination. We differentiated these cells into erythroid cells that showed β^A as well as β^S globin production. We have now characterized these ES cells in order to see if they are suitable for transplantation. This report covers both the isolation of a BAC that contains the whole β globin gene cluster, and the characterization of the ES cells converted from β^S to β^A .

For cloning the β globin gene cluster with BAC, we previously used the *Sfi*1 site to isolate the β globin gene cluster. There are three *Sfi*1 sites in this region, 2 flanking the β globin gene cluster, and one cleaves the DNA at the upstream hypersensitive site. To block cleavage at this site, we used an oligonucleotide homologous to this region and RecA protein and oligonucleotide and were successful in obtaining 130 kb DNA that contained the β globin gene cluster. However, when we cloned this DNA into the BAC vector, we kept on obtaining incorrect inserts after many attempts. We think that "N" positions of the *Sfi*1 recognition sites allow the BAC pick up irrelevant sequences. Therefore, we went back to the method using partial digestion with *Eco*R1. We used both partial digestion with *Eco*R1 as well as *Eco*R1 methylase to achieve high molecular weight DNA. Pulsed Field gel was used to separate the fragments, and the region of the gel between 97kb to 145kb was excised and the DNA eluted. The DNA was ligated to the pBACe3.6 vector and propagated on bacteria. Screening with human γ globin probe identified several positive clones. We are very pleased to report that we have now isolated a BAC that contains the human β globin gene cluster extending from the 5'HS to include the β globin gene just before the 3'HS. Haplotype analysis shows that it is the CAR haplotype, from a sickle cell anemia patient who is homozygous for this haplotype. We plan to proceed to make transgenic mice out of the 110 kb. We plan to proceed to make transgenic mice out of this BAC clone.