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**Abstract of invited talk at the International Biolron Society (IBIS) World Congress in Porto, Portugal, June 11, 2009. The title of the session was “Novel Therapies.”**

#### **GENE REGULATION THERAPY UTILIZING FERRITIN HEAVY CHAIN**

**R.H. Broyles** (Oklahoma), V. Belegu (Johns Hopkins), M. Trudel (IRCM, Montreal), S. Levi (Milan) and P. Arosio (Brescia, Italy).

Ferritin heavy chain (FtH) is an embryonically-expressed protein, prominently expressed in the erythroid lineage and in developing brain. In adults, FtH is highly expressed in CNS neurons, in heart and in kidney. In the adult erythroid lineage, FtH expression is low and disappears as erythroid differentiation and hemoglobin (Hb) synthesis begins. High FtH expression in embryonic erythroid cells versus low-to-nil expression in adult erythroid cells is a key developmental difference in the mechanism of developmental Hb switching (Broyles et al., *PNAS* **98**: 9145, 2001). FtH represses adult  $\beta$ -globin expression and activates  $\gamma$  (fetal)-globin gene expression in embryonic/K562 erythroid cells. These findings have led us to propose utilizing FtH as a therapeutic agent in sickle cell disease (SCD) and  $\beta$ -thalassemias, since it is well known that high expression of  $\gamma$  (fetal)-globin markedly alleviates both disorders. Our results with K562 cells that express only embryonic and fetal Hbs have supported the theoretical basis for FtH as an Hb switching factor. FtH localizes to the nucleus in K562 cells and represses the human adult  $\beta$ -globin promoter in transient expression assays. Chromatin immunoprecipitation (ChIP) assays using anti-FtH polyclonal antisera show that FtH is bound to the -150 promoter repression site *in vivo* in K562 cells in which the  $\beta$ -globin gene is repressed. An Alexa488-tagged antisense oligonucleotide to FtH transfected into K562 cells enters the nucleus and derepresses the  $\beta$ -globin gene. Strikingly, the anti-FtH oligo knocks down both FtH and  $\gamma$  (fetal)-globin gene expression by over 90 percent, confirming FtH's role as an activator of fetal Hb. Competitive EMSA assays have revealed that the mouse  $\beta$ Major-globin promoter has an analogous CAGTGC motif at -160 bp from the cap site that competes specifically with the human CAGTGC site for K562 nuclear FtH binding. However, the mouse  $\beta$ Minor-globin promoter lacks the -150/-160 CAGTGC motif and, therefore, the FtH binding site. Thus, a human FtH transgenic mouse, in which the FtH gene is driven by a truncated  $\beta$ -promoter lacking the CAGTGC motif, expresses human FtH in definitive erythroid cells which results in repression of  $\beta$ Major-globin but not  $\beta$ Minor-globin. Thus, these TgFtH mice are born with a reduced ratio of  $\beta$ Major/ $\beta$ Minor globins, resulting in  $\alpha$ -globin chain excess and a mild  $\beta$ -thalassemia. FtH-tg mice with high copy numbers of the transgene have greater numbers of target cells, due to inclusions of excess  $\alpha$ -globin. In applying this therapy to humans, thalassemia would not be expected since we have found that FtH represses  $\beta^S$ -globin and activates  $\gamma$  (fetal)-globin gene expression in maturing human erythroid precursor cells from pediatric sickle cell patients. FITC-labeled FtH enters these human erythroid precursor cells, presumably via a FtH-specific receptor that has been found on the surface of these cells. FtH receptors have also been found on liver cells and in the blood brain barrier, suggesting that FtH might be used as a therapeutic in a number of highly prevalent human diseases.