THE ROLE OF THE HOMEOBOX PROTEIN *HEX* IN HEMATOPOIETIC LINEAGE COMMITMENT AND DIFFERENTIATION

David L. Mack

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Stephen R. Dlouhy, Ph.D., Committee Chair

Doctoral Committee:

Yan Chen, Ph.D.

Robert Hromas, M.D.

April 18, 2003

Ann Roman, Ph.D.

ABSTRACT

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The orphan homeobox gene Hex was cloned in a yeast one-hybrid screen by its ability to bind regulatory sequences located in a downstream intron of the Breakpoint Cluster Region (BCR) gene. Luciferase reporter assays showed that Hex was able to transcriptionally regulate the BCR promoter by binding these intronic regulatory sequences. This finding is particularly interesting given the location of the BCR Intron Factor (BIF) suppressor site in the vicinity of the breakpoint for BCR during the BCR/ABL reciprocal translocation. Therefore, the BCR/ABL fusion gene that causes chronic myelongenous leukemia (CML) may be transcriptionally regulated through the BIF suppressor sites and, in turn, the chronic phase of CML might be prolonged through repression of the fusion oncogene. Previous studies in mice linked aberrant expression of the Hex protein to leukemogenesis independent of BCR/ABL. Hex protein is expressed in a range of multipotent hematopoietic progenitor cells and is generally downregulated during terminal differentiation, suggesting a role for this protein in early hematopoietic lineage decisions. To further investigate the role that Hex might play in myeloid and lymphoid differentiation, two transgenic mouse lines were constructed, each using a different promoter that directs protein expression in different hematopoietic tissues. Since Hex expression is normally down-

regulated as myeloid cells differentiate and is not expressed at any stage in the T-cell lineage, these murine models were designed to address the question of how T-cell differentiation is affected if Hex is not down-regulated but is continuously expressed as T-cells develop in the thymus. Flow cytometric analyses on mice from both lines showed a shift in the ratio of T-helper cells and cytotoxic/suppressor T-cells, and an accumulation of more primitive T-cell progenitors in the thymus. These data indicated that down-regulation of Hex plays an important role in T-cell differentiation. Also, the rate at which hematopoietic marrow progenitors proliferate was dramatically increased, which may explain how activating mutations in Hex cause leukemia. The oncogenic potential of Hex is supported by one additional observation. Fibroblasts expressing Hex showed dramatic changes in their cellular morphology, including a rearrangement of the actin cytoskeleton, membrane ruffling and cell surface protrusions.

Stephen R. Dlouhy, Ph.D., Committee Chair

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