STEM CELL ANTIGEN CD34 IN NATIVE AND ENGINEERED FORM ALTER ITS BINDING ABILITY TO STROMAL CELLS AND LIGANDS: A CLASSICAL EXAMPLE OF CLINICAL BENEFITS OF THERAPEUTIC GENETIC ENGINEERING OF STEM CELLS IN TRANSPLANTATION

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The CD34 antigen is a 115 KD surface glycoprotein present on primitive stem cells that has been widely employed as a marker for hematopoietic progenitors that are capable of reconstituting bone marrow (1). CD34-specific antibodies have recently become available and are an important tool in the study of CD34 function. The choice of cells that are $CD34^{+\,(positive)}$ is important for transplantation; however, the subsequent identification of cells with no CD34 marker (negative) has contradicted the only sole potential of CD34+ (positive) cells in successful reconstitution of bone marrow (2). Although recent studies have documented the ability of CD34- (negative) cells to confer rapid engraftment with fewer risks of malignancy relapses, a large number of clinicians still prefer to use CD34+ (positive) cells for transplantation (3).

Although the utility of CD34 as a selection marker has been well established, little effort has been made to determine its precise role in the hematopoietic microenvironment. We have engineered CD34 expression on the cell surface of human HSCs and studied its effects in modulating homing and engraftment behaviors. We have systematically performed a structure/function analysis of the CD34 protein using computational techniques to identify critical amino acids necessary for CD34 function and created site-directed mutations in these critical residues in order to determine their functional significance in vivo.

The human CD34 protein sequence we used was from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) Entrez database. Using various in silico tools, we identified possible functional/ structural homologs that we used to identify structural features indicative of protein binding and interacting sites, as well as motifs that have implied biological functions. The most significant among them was a Src homology 2/3 (SH2/3) domain, which enables binding to the cytoplasmic adapter protein Crk-L, a protein that mediates downstream signaling relevant to adhesion (4). Several other potential sites for critical interactions in downstream signaling were identified. These studies motivated us to generate alanine substitution mutations via PCR-based site-directed mutagenesis at Ser306 and Tyr318 and to analyze the effects of these mutations.

Native and mutated forms of CD34 transfected into human hematopoietic cells were expressed ectopically by doxycycline-mediated induction using the Tet-on system **(Fig.1a).** Proliferation, the surface expression levels of adhesion molecules, and the adhesion affinity of transfected cells to fibronectin and stromal cells (both irradiated and non-irradiated to mimic damaged marrow) were evaluated in transfected cell lines.

The native form of CD34 displayed a two-fold enhancement in adherence to fibronectin relative to the control **(Fig.1b).** Similarly, cells expressing CD34 displayed a two-fold enhancement in cellular adhesion, whereas cells expressing CD34Ser306 showed no enhancement in adhesion compared to the control. Cellular adhesion is dependent upon surface adhesion molecules such as ICAM-1, integrin- β_1, integrin-αL, integrin-α5 and integrin-β_2; however, there were no changes in the expression levels of these molecules as assayed by flow cytometry. There was also no effect on cellular proliferation as assayed by MTT assay. These observations suggest an essential role for CD34Ser306. Enhanced adhesion by native CD34 and mutant CD34Tyr318 appears to involve high affinity/avidity binding states in the surface adhesion molecules.

In conclusion, the change in binding affinity of hematopoietic cells to both fibronectin and bone marrow stromal cells monolayer in culture were significant **(Fig.1c).** In addition, their binding, even to deteriorated

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stroma exposed to ionizing radiation, seems to be additive. These observations suggest that HSC CD34 induces signaling and that its overexpression increases binding, overcoming the rate limiting binding affinity necessary for HSC retention in the bone marrow extra-vascular space. These studies suggest that $CD34^{+}$ cells have efficient homing/engraftment capabilities that may dramatically reduce the number of cells required for clinical hematopoietic stem cell transplantation.

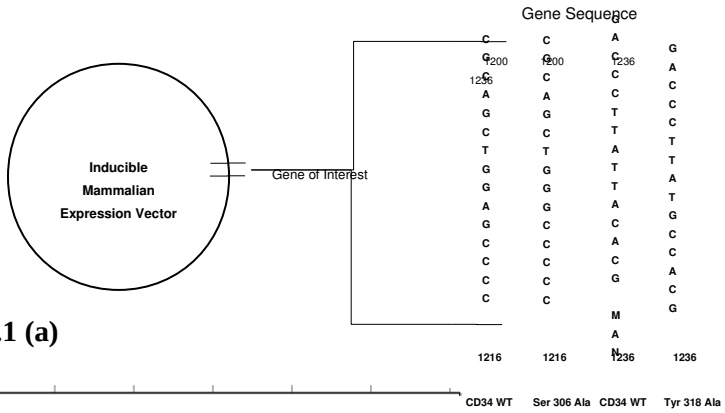

Figure.1 (a)

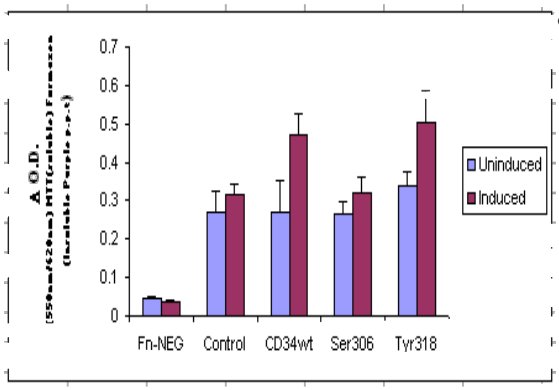

Figure1(b)

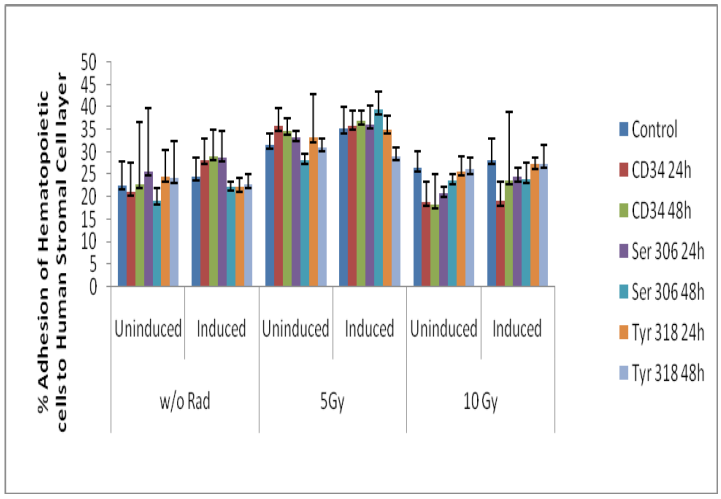

Figure.1(c)

Figure 1 (a-c). Modulation of hematopoietic cells adhesion by CD34 expression (A) The human CD34 gene was cloned into a tightly regulated inducible mammalian expression vector. In addition, two substitution mutants corresponding to Ser306Ala and Tyr318Ala located in the CD34 cytoplasmic tail region were generated. Expression of these mutants in hematopoietic cells showed significant enhancement of HSC binding to both fibronectin-coated surfaces (B) and human bone marrow stromal cell layer (C).

References

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