Molecular Cloning and Expression of CD34: A Haemopoietic Progenitor-Associated Cell Surface Glycoprotein

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Introduction

The CD34 group of monoclonal antibodies recognize a 105–120 kDa cell surface glycoprotein which is selectively expressed by human myeloid and lymphoid progenitor cells probably including the haemopoietic stem cell [1–3] (for a recent review see [4]). CD34 antibodies therefore offer a prospect of using highly enriched human stem cells for analysis of their regulation [5–7], gene transfection and/or transplantation [8]. The CD34 antigen is also expressed on vascular endothelial cells [9].

The restricted pattern of expression of CD34 in haemopoiesis suggests that it may have a significant function in the earliest stages of blood cell differentiation in the bone marrow. However, neither biochemical characterization of the antigen nor functional studies using monoclonal antibodies have so far identified its function. The protein sequence of the antigen has been deduced from a complementary deoxyribonucleic acid (cDNA) clone (D. Simmons and B. Seed, personal communication) and is in agreement with N-terminal amino acid sequence derived from the purified protein [10]. The sequence predicts that the CD34 antigen is a type 1 transmembrane protein with a protein backbone molecular weight of 39 kDa and with no close homology to any other protein in the data bases. The protein is extensively glycosylated with both N- and O-linked carbohydrate, and the epitopes recognized by most of the monoclonal antibodies are dependent on the presence of the carbohydrate including sialic acid residues [10, 11]. The gene encoding CD34 has been mapped to the long arm (q) of human chromosome 1 [12, 13].

We report here characterization of the CD34 gene and its pattern of expression in murine and human cells.

Cloning of Murine CD34

We have shown previously that human CD34 cDNA hybridizes under stringent conditions to DNA from three different rodent species [12], indicating some degree of conservation of the gene. This has allowed the isolation of a murine CD34 cDNA and genomic coding regions containing the complete coding sequence. The strategy used for cloning murine CD34 is illustrated in Fig. 1 and details are given in Brown et al. [14].

The murine gene is organized in eight exons in 22 kb of DNA. The first exon lies in a GC and CpG rich island. The sequence of the gene and the cDNA predict a 382 amino acid long protein containing a N-terminal signal peptide and one transmembrane region 73 amino acids from the carboxyl terminus. The extracellular part of the protein contains a 140 amino acid long N-terminal region 40% of whose residues are serine or threonine – potential attachment sites for O-linked carbohydrate as well as five potential attachment sites for N-linked carbohy-

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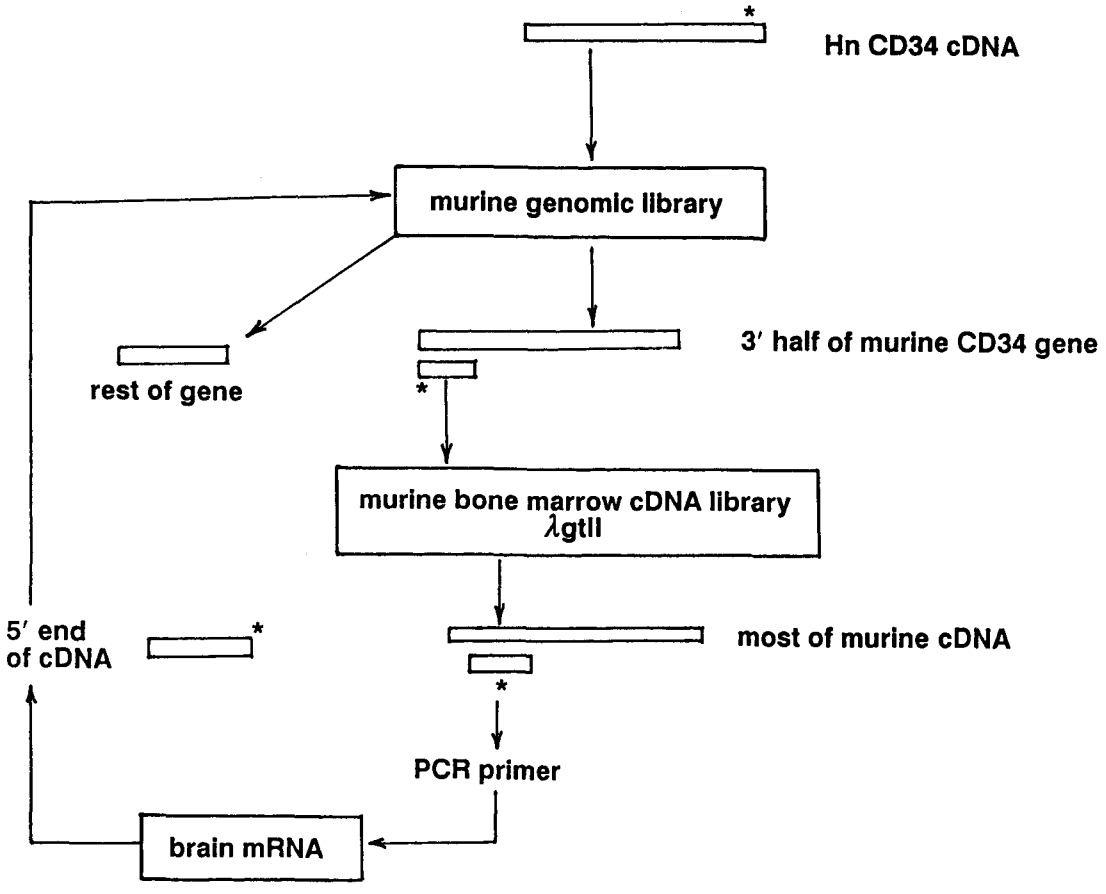

Fig. 1. Strategy used to isolate murine genomic and cDNA clones. The mouse gene and cDNA homologous to human CD34 were isolated by a combination of genomic DNA and cDNA library screening and the PCR. Initially a murine genomic DNA library was screened at low stringency with the human CD34 cDNA. This allowed the isolation of the 3′ half of the murine gene. The most 5′ coding region of this murine genomic DNA was used to screen a murine bone marrow cDNA library and lead to the isolation of cDNA clones which encoded most of the coding region of the murine CD34. The coding region at the 5′ end of the cDNA was isolated by the PCR technique using a primer complementary to a sequence near the 5′ end of the cDNA and a primer complementary to a polyA tail added to the first strand cDNA. The murine cDNA clones were then used to complete the isolation of the murine gene

drate. Proximal to the extracellular membrane, there is a 79 amino acid long cysteine rich region. The homology with the human sequence is highest in the intracellular domain (90% amino acid identity) and lowest in the N-terminal region (43% amino acid identity) (Fig. 2). The protein is not homologous with any other proteins currently in the data bases.

Expression of CD34

Since CD34 antibodies may recognize carbohydrate-dependent epitopes [4, 10], it is not clear whether the pattern of CD34 antigen expression in haemopoietic cells reflects activity of the CD34 gene itself or whether alterations in glycosylation determine the expression and availability of antigenic epitopes. The protein product encoded by the CD34 gene might, therefore, be more widespread in its expression than anticipated from antibody studies. A comparison of CD34 mRNA and antigen expression of a panel of different haemopoietic cell types (Table 1) indicates that there is, in fact, a good correlation between presence of mRNA and accessible epitopes, confirming therefore that the CD34 gene

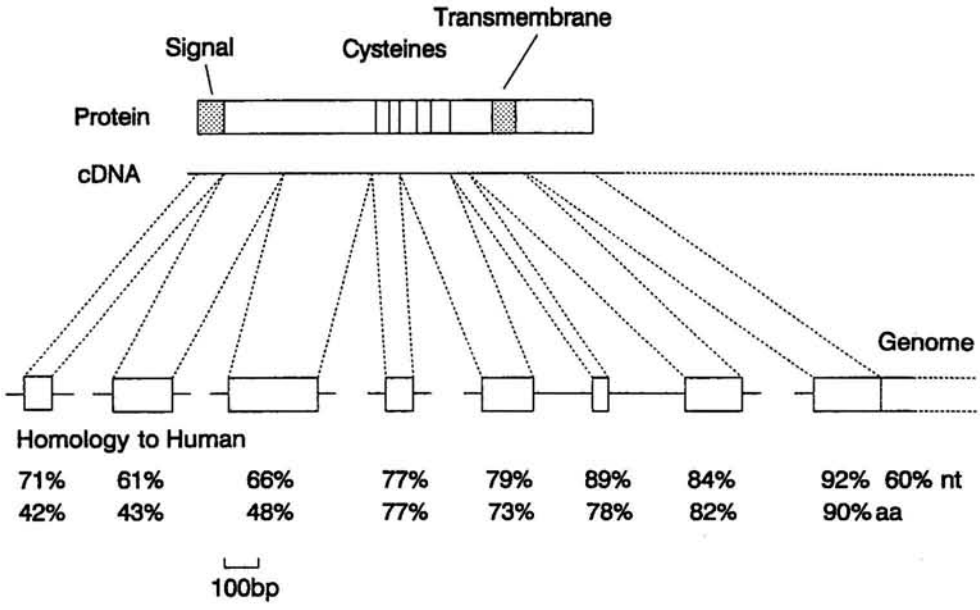

Fig. 2. Schematic diagram of murine CD34 protein, cDNA and exon organization. DNA and protein sequence homology with the human CD34 sequence (D. Simmons and B. Seed, personal communication) for each murine exon is shown in relation to a schematic diagram of the murine protein. The isolated and sequenced cDNA is represented by a *solid line*. The *broken line* represents the 3′ end of the cDNA deduced from the genomic sequence. (From [14])

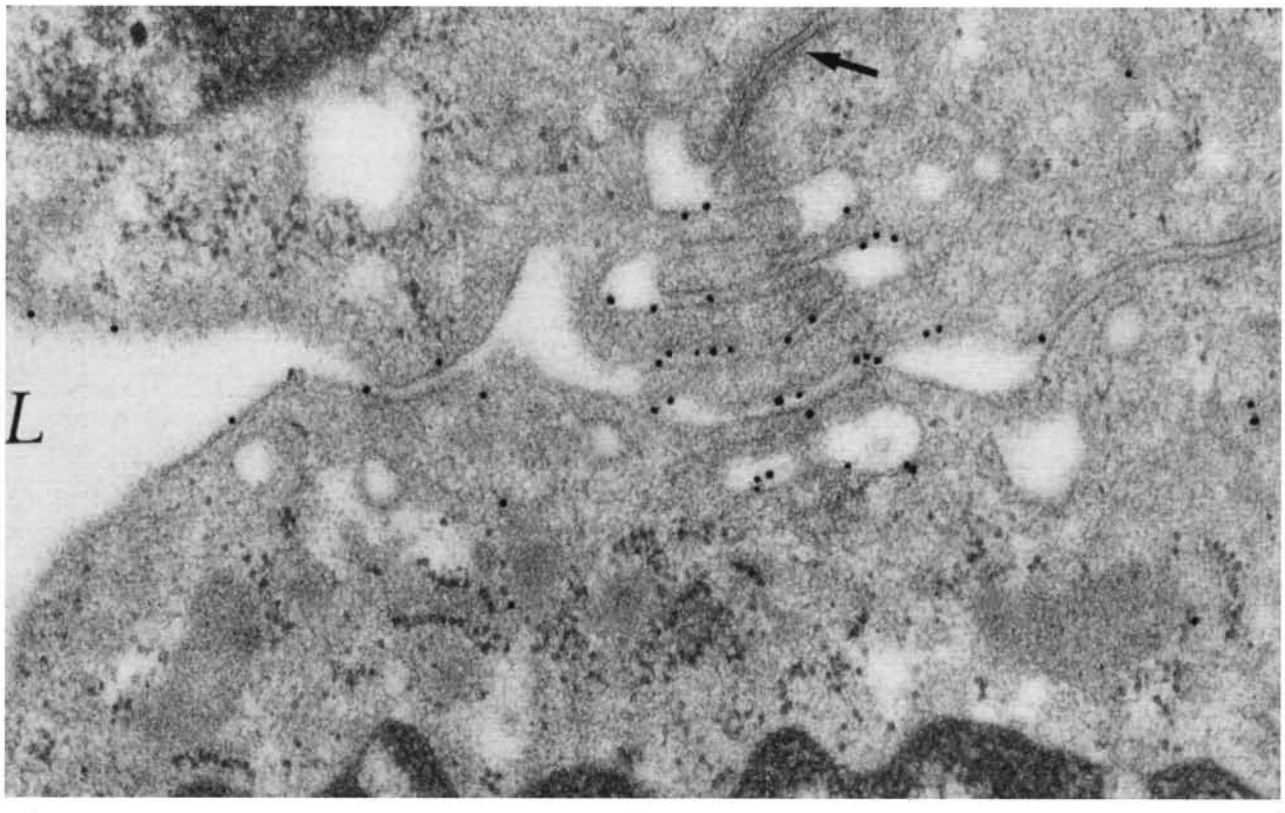

Fig. 3. Ultrastructural localization of CD34 antigen. Electron micrographs of endothelial cells. The sections have been reacted with a CD34 monoclonal antibody (QBEND-10) localized with colloidal gold markers. The figure shows a region of opposing membranes of two endothelial cells from human umbilical artery. Some colloidal gold is present on the lumenal membrane (*L*) of the cells, but the majority of the gold markers are located on the lateral membranes of the cells, where they form complex interdigitations. Colloidal gold is not present on the membrane in areas of cell junctions, such as tight junctions (*arrow*). Original magnification, ×77 500. (From [9])

Table 1. Co-expression of CD34 mRNA and antigen in human heamopoietic cell lines

Cell type	Line[a]	Antigen[b] (%)	mRNA
Multi-lineage progenitors	KG1	>90	+++
	KG1a/b[c]	>90	+++
	K562	–	–
Myeloid precursors	HL-60	–	–
	EM1/EM2	–	NT
	U-937	–	–
B precursors	REH	–	–
	NALM-6	–	–
	RS4:11	–	–
	TOM-1	~10–15	+
	KM3	–	–
	P3	–	–
	NALM-1	–	–
Mature B	B85	–	–
T precursors	JM	~10–15	+
	CEM	–	–
	PEER	–	–
	MOLT13	~10–15	NT
	T-ALL (FE)	~10–15	NT

[a] All derived from leukaemic cells except B85, which is an EBV transformed normal B cell.
[b] % cells stained by immunofluorescence labelling with monoclonal CD34 antibodies and flow cytometry assessment, or stained by alkaline phosphatase labelled antibodies and assessed by light microscopy.
[c] Sub-lines of KG1 co-expressing some early lymphoid markers [17] (S. Watt and M. F. Greaves, unpublished observations).
NT: not tested.

itself, at least at the level of stable mRNA, has a highly restricted pattern of expression in early haemopoiesis. Expression of the CD34 gene is not, however, limited to haemopoietic cells.

All seven of a set of CD34 monoclonal antibodies also recognized human vascular endothelium (details in [9]). Capillaries of most tissues were CD34 positive, as were umbilical artery and, to a lesser extent, vein, but not the endothelium of most large vessels and the endothelium of placental sinuses. Angioblastoma cells and parafollicular mesenchymal cells in fetal skin were also CD34 positive, as were some stromal elements. An ~ 110 kDa protein can be identified by western blot analysis with CD34 antibodies in detergent extracts of freshly isolated umbilical vessel endothelial cells, and CD34 mRNA is present in cultured umbilical vein cells as well as other tissues rich in vascular endothelium (breast, placenta) [9]. These data indicate that the binding of CD34 antibodies to vascular endothelium is to the CD34 gene product, and not to cross-reactive epitopes. Electron microscopy of umbilical artery, breast, and kidney capillary vessels revealed that in all three sites CD34 molecules are concentrated on membrane processes, many of which interdigitate between adjacent endothelial cells. However, well-established endothelial cell contacts with tight junctions are CD34 negative [9].

Although no antibodies to murine CD34 are as yet available, the availability of murine CD34 cDNA allowed us to investigate the pattern of gene expression

in mouse cell types and tissues in comparison with the data available from studies on human cells (details in ref [14]).

The results of probing northern blots of mouse tissue mRNAs show that the murine homologue of the CD34 gene is expressed as a 2.5–2.7 kb mRNA, identical in mobility to the human mRNA [9], both in haemopoietic tissues (liver, spleen, bone marrow, and thymus) and in two non-haemopoietic tissues (brain and testis). Comparison of the intensities of bands suggests that CD34 is expressed at about a 100-fold lower level than actin in the mouse tissues analyzed, except in brain where the level of CD34 mRNA is only about tenfold lower than actin and where a minor higher molecular weight (3.7 kb) RNA is also seen. The presence of a variety of cell types in these tissues prevents identification of the actual cell lineages expressing CD34. An analysis of the expression of CD34 in haemopoietic cell lines revealed a selective pattern of expression similar to that in human cells. Among the haemopoietic cell lines tested so far, CD34 expression was detected only in progenitor cells lines [15]: A4, Clone 17, and 416B. Two other progenitor cell lines, Clone 15 and LyD9 did not have detectable levels of CD34. CD34 mRNA could also not be detected in the B lineage precursor cell line 18–8 and was not detectably expressed in cell lines with a more mature phenotype, including EL4 (a T cell), WEHI 3B (myelomonocytic cell), WEHI 274 (monocyte/macrophage) and NS1 (plasma cell/myeloma). Of the non-haemopoietic cell lines tested, CD34 expression was not detected in C3H 10T1/2 an embryonic cell line, but very high levels of CD34, approximately equal to actin, were detected in three other embryonic fibroblast lines: NIH 3T3, Swiss 3T3 and Swiss 3T6 but not in a fibroblast line derived from lung tissue (line CMT 64/61).

The high level expression of CD34 in certain murine fibroblasts accords with recent ultra-thin section electron microscopic evidence for CD34 antibody binding to stromal fibroblasts in vivo; established cell lines of human fibroblasts are, however, CD34 negative (M. F. Greaves, D. Robertson and S. Pegram, unpublished observations).

Conclusions

These studies have indicated that the CD34 gene and protein are so far unique with no strong homologies to other cloned genes. The gene itself is relatively highly conserved, though asymmetrically, between murine and human DNA, and although its expression may be restricted to stem cells and progenitor cells in haemopoiesis, it is expressed in other cell types, including vascular endothelial cells and certain fibroblasts. This then implies a function that is not restricted to haemopoiesis, and a clue as to what this might be comes from the ultra-structural studies revealing that CD34 protein may be concentrated on the surface of interdigitating membranes of adherent vascular endothelial cells. Additional studies have shown that CD34 molecules are localised on abluminal endothelial processes occurring at the tips of vascular sprouts during angiogenesis [16]. These observations clearly imply a role for CD34 in cell-cell and/or cell-matrix interaction. This possibility is now being pursued by attempting to isolate a ligand from cells or stromal matrices that will bind to purified CD34 molecules.

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