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Cloning and characterization of the human *FCRL2* gene☆

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Abstract

We report cloning and characterization of *FCRL2,* a novel human gene that belongs to the FcR family. The gene is closely linked and structurally similar to the recently identified *FCRL/FREB/FcRX* gene. The encoded protein is composed of three Ig-like domains and a C-terminal mucin-like domain containing a conserved α-helical motif with dileucine signals. Intraexonic splicing may generate two alternative transcripts, coding for isoforms with the third and fourth domains replaced by entirely different amino acid sequences. Like FCRL, the full-length isoform of FCRL2 is expressed intracellularly in transfected 293T cells. Expression analysis revealed FCRL2 mRNA only in placenta. The gene transcripts were not detected in lymphoid tissues or in the main leukocyte subsets isolated from peripheral blood. However, we found that *FCRL2* is differentially expressed by transformed B cell lines. Of interest is also the finding that the gene expression may be up-regulated in the progression of melanocytic tumors.

Keywords: FcR family; Placenta; Intraexonic splicing; B cell tumors; Melanoma

The Fc receptors play an important role in adaptive and innate immunity. The ability to bind the immunoglobulin constant regions involves Fc receptors in phagocytosis, antibody-dependent cell cytotoxicity, immediate hypersensitivity, and transcytosis of immunoglobulins [1–6]. In structural terms, Fc receptors comprise a heterogeneous group of molecules. Except for the low-affinity IgE receptor (CD23), known Fc receptors belong to different subsets of the immunoglobulin superfamily. Thus, FcRn, the receptor responsible for the catabolism and transcytosis of immunoglobulins, is a homolog of class I MHC molecules [6]. The extracellular regions of Fcα/μR and poly(Ig)R are composed of the V-type Ig-like domains [4,7]. The human receptor for IgA (CD89) and bovine receptor for IgG2 belong to the family of KIR receptors [5]. The largest group, sometimes called the leukocyte Fc receptors (FcRs), is divided into four major classes, FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcεRI. The Ig-like domains of FcRs are of the C2 type and may be divided into three structural subtypes further referred to as D1, D2, and D3. The extracellular region of FcγRI is composed of D1D2D3, the others have a D1D2 structure [1–3].

Recently, the FcR family has been extended. Several research groups have reported that the human and mouse genomes possess, respectively, eight and seven genes encoding proteins structurally related to leukocyte FcRs. Seven human and four mouse genes have been characterized at the cDNA and some at the protein level [8–17]. The functional properties of the FcR-like proteins remain unknown. None have been shown so far to bind to immunoglobulins. However, the specific expression in the cells of the human immune system suggests their involvement in the differentiation of lymphocytes ([8–16], Ershova et al., submitted for publication). A distinctive feature of the

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FcR-like proteins is the remarkable diversity of their domain architecture. Six of the human FcR-like molecules are surface transmembrane receptors with extracellular regions composed of three to nine extracellular Ig-like domains. In addition to D1–D3, two more domain subtypes (D4, D5) have been recognized in the FcR-like proteins. Each receptor has a unique domain composition. One of the newly defined proteins, designated FCRL/FREB/FcRX, is expressed intracellularly [12,13,16]. FCRL is made up of four domains. The N-terminal is a truncated and diverged variant of the D1 subtype, the second and third belong to the D2 and D3 subtypes, respectively. The C-terminal domain of FCRL shows no clear-cut similarity to the known protein sequences, yet based on the abundance of the proline, serine, and threonine residues, it may be assigned to the mucin-like. The *FCRL* gene is tightly linked to the *FcγRIIb* gene in the human and mouse genomes. Computer analysis of the neighboring genomic sequences demonstrated that, downstream of the *FCRL* gene, there is yet another tightly linked and *FCRL*-related gene we designated *FCRL2* [12]. Also based on computer predictions, Davis and colleagues called the gene *FcRY* [17]. The aim of the present study was to characterize the *FCRL2* gene at the cDNA and protein levels.

Results

cDNA cloning

In the human genome, the *FCRL2* gene is closely linked to the *FCRL* and *FcγRIIb* genes. NCBI annotated this gene as FLJ31052. However, both mRNA and the protein product of the annotated FLJ31052 appeared to be predicted incorrectly. From our gene structure analysis we inferred that the *FCRL2* gene may consist of six exons, two coding for the leader peptide and four for distinct domains. A search of the EST databases enabled us to identify 19 human cDNAs corresponding to this gene. All the cDNAs were truncated at the 5′ end, most were from tumor cell libraries, including melanotic melanoma and B cell lymphoma RAMOS. Using RT-PCR, we tested the human melanoma cell line G-361 and several cell lines of hemopoietic origin, including B cell lines Raji, BL-2, IM-9, CBMI, RPMI 1788, and BJAB, for the presence of the FCRL2 transcripts. The forward primer corresponded to the genomic sequence immediately adjacent to the predicted exon for the leader peptide; the reverse primer corresponded to the 3′ untranslated region, and it was chosen on the basis of the cDNAs from the EST database. RT-PCR for 30 cycles demonstrated the presence of a fragment of the expected length in the melanoma and a fuzzy band presumably containing several fragments in BJAB cells. Forty cycles gave a positive reaction in Raji and BL-2, too (Table 1 and Fig. 5). The other cell lines were negative under all conditions.

Cloning of the fragments yielded 2 melanoma and 11 BJAB cDNAs. The cDNAs derived from G-361 were identical to each other and their sequences were identical to the predicted. The cDNAs encoded a protein similar to FCRL with respect to domain architecture (Fig. 1). The deduced amino acid sequence may be subdivided into a hydrophobic leader peptide, three Ig-like domains, and the C-end domain enriched with proline, serine, and threonine residues. In contrast to FCRL, the N-terminal domain of FCRL2 is typical Ig-like. It shares 30–46% identical residues with the D1-type domains of the known FcR and FcR-like proteins. The D2- and D3-type domains of FCRL2 are 42 and 37% identical to the respective domains of human FCRL (Fig. 2). The C-terminal domains of the two proteins share 20% identical residues. The unique conserved feature of the FCRL2 and FCRL C-terminal domains is the presence of a motif comprising charged amino acids interspersed with dileucine repeats (Fig. 3). The motif was predicted to adopt an α-helical structure. The deduced amino acid sequence contains two potential N-glycosylation sites. Of 11 cDNA clones derived from BJAB, 10 fell into four additional sequence variants. Two presumably resulted from the use of alternative acceptor splicing sites located in the middle of the fifth exon normally encoding the D3-type domain (Figs. 1 and 2). In both instances, the reading frame was shifted and two new proteins arose, in which new amino acid sequences different from each other and from the full-length FCRL2 C-end followed the second Ig-like domain. These alternative C-end sequences showed no significant homology with known protein sequences. Each one of the two alternative cDNA variants, designated FCRL2b and FCRL2c, was subdivided into two variants either containing the second exon for the leader peptide or not.

Comparison of the human FCRL2 amino acid sequence with that of the mouse protein predicted from genomic analysis demonstrated strong conservation of the gene

Table 1
RT PCR analysis of the FCRL2 gene expression in subpopulations of peripheral blood leukocytes and hemopoietic cell lines

Cell line	Expression
B cell lymphoma IM9	–
B cell lymphoblastoma CBMI	–
B cell lymphoma BJAB	++[a]
B cell lymphoma BL2	+
B cell lymphoma RPMI 1788	–
B cell lymphoma Raji	+
T cell lymphoma Jurkat	–
T cell lymphoma MOLT4	–
Melanoma G-361	++
Erythroleukemia K562	–
Peripheral blood leukocyte subsets	
$CD14^+$ cells	–
$CD8^+$ cells	–
$CD19^+$ cells	–
$CD4^+$cells	–
$CD4^+$ mitogen-activated cells	–
$CD8^+$ mitogen-activated cells	–

++ indicates that a positive reaction was detected at 30 cycles.
[a] Forty cycles of amplification were generally applied.