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Genomic structure, organization and promoter analysis of the human F11R/F11 receptor/junctional adhesion molecule-1/JAM-A

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Abstract

The F11-receptor (F11R) (a.k.a. JAM-1, JAM-A, CD321) is a cell adhesion molecule of the immunoglobulin superfamily involved in platelet adhesion, secretion and aggregation. In addition, the F11R plays a critical role in the function of endothelial cells and in platelet adhesion to inflamed endothelium. In the present study, we used partial sequences of the human F11R gene, F11R cDNAs, and information in unannotated human genome databases, to delineate the F11R gene. We found that the F11R gene is composed of 13 exons (E1a, 1b, 1c, E1–E10) encoding two groups of mRNAs differing in length and sequence at their 5′ UTRs, referred to as type 1 and type 2 messages. Type 1 cDNAs are shorter at the 5′ end and contain a region not found within type 2 messages. Type 1 mRNAs are present in endothelial cells (EC), platelets, white blood cells and in the cell lines CMK, HeLa, K562, HOG and A549, while type 2 messages are limited to EC. Type 1 messages contain exons E1–E10 whereas type 2 messages usually contain exons E1a, 1c, part of E1 and E2–E10. The translation start site is localized in the 3′ end of E1, common for both type 1 and type 2 messages. Expression of these messages is regulated by two alternative promoters, P1 and P2. P1 is a TATA-less promoter containing an initiator element, multiple transcription start sites, several GC and CCAAT boxes, and GATA, NF-κB and ets consensus sequences. The cloned P1 drives efficient expression of the luciferase reporter gene. A high level of similarity between human P1 and its rat and mouse counterparts was observed. Promoter P2, located upstream of P1, contains a TATA box, GC boxes, a CCAAT box and GATA and ets consensus sequences. 3′ RACE provided evidence for variability in the 3′ UTR due to the presence of two polyadenylation signals. The finding of multiple regulatory sites in the promoters supplements the biochemical evidence that the F11R has several different roles in the functional repertoire of endothelial cells, platelets and other cells. In particular, the presence of NF-κB provides additional evidence to the significance of the F11R function in the initiation of inflammatory thrombosis.

Keywords: F11 receptor; F11R; JAM; JAM-A; Cell adhesion molecule; Immunoglobulin superfamily; Platelets; Endothelium; CD321

Abbreviations: CAM, cell adhesion molecule; CASK, Ca2+/calmodulin-dependent membrane-associated kinase; CPBP, core promoter binding protein; CRE, cAMP response element; E1a, 1b, …, exon 1a, 1b, …; CIP, calf intestinal phosphatase; CNS, central nervous system; DPE, downstream core promoter element; EC, endothelial cells; EKLF, erythroid Krüpel-like factor; F11R, F11 receptor; FCS, fetal calf serum; FGF, fibroblast growth factor; gDNA, genomic DNA; HGP, Human Genome Project; HOG, human oligodendroglioma; HUVEC, human umbilical vein endothelial cells; I1, 2, …, intron 1, 2, …; Ig, immunoglobulin; Inr, initiator; IRF, interferon regulatory factor; JAM-1, junctional adhesion molecule-1; JAM-A, junctional adhesion molecule-A; M.Ab.F11, monoclonal antibody F11; NF1, nuclear factor 1; NF-AT, nuclear factor of activated T-cells; NF-Y, nuclear factor Y; Oct, octamer-binding factor; PKC, protein kinase C; RACE, rapid amplification of cDNA ends; SP1, stimulating protein 1; TAP, tobacco acid pyrophosphatase; TGFβ, transforming growth factor β; TNF-α, tumor necrosis factor α; WBC, white blood cells

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1. Introduction

The F11 receptor was discovered and characterized originally as the protein target of a stimulatory monoclonal antibody M.Ab.F11, which induces platelet activation and aggregation (Kornecki et al., 1990). Two forms of the F11R protein, 32 and 35 kDa, were identified, differing in degree of *N*-glycosylation (Naik et al., 1995). Subsequently, a cDNA clones providing the complete amino acid sequence of the human and mouse F11R proteins were obtained from platelets and epithelial cells (Martin-Padura et al., 1998; Ozaki et al., 1999; Sobocka et al., 2000; Naik et al., 2001). The F11R is a type I transmembrane protein containing two extracellular immunoglobulin folds and has been classified as an Ig-CAM. We have reported that human platelets express on their surface 8067±1307 F11R molecules/platelet (Kornecki et al., 1990).

The F11R is involved in the regulation of platelet aggregation and adhesion to the inflamed endothelium. The N-terminus of F11R and first Ig domain of F11R play critical roles in these processes (Babinska et al., 2002a,b). The F11R becomes phosphorylated in platelets activated by the physiological agonists thrombin and collagen, and M.Ab.F11 stimulatory antibody itself (Sobocka et al., 2000, 2004). Platelet activation by M.Ab.F11 involves cross-linking of F11R to FcγRII, followed by platelet shape changes as a result of cytoskeletal reorganization, filopodia formation, secretion of the granular contents and platelet aggregation (Kornecki et al., 1990; Naik et al., 1995; Sobocka et al., 2000). The biochemical events associated with these changes include activation and translocation of specific PKC isozymes (Wang et al., 1995), an increase in intracellular free-ionized calcium levels, activation of phosphatidylinositide 3-kinase, and phosphorylation of pleckstrin and myosin light chain (Kornecki et al., 1990; Sobocka et al., 2000, 2004).

In addition to its role in platelets, the same protein was also studied extensively in the endothelium. Immunofluorescence studies revealed F11R as a component of tight junctions in different types of epithelial cells (Martin-Padura et al., 1998; Williams et al., 1999; Gupta et al., 2000; Liang et al., 2000; Liu et al., 2000). In epithelium, F11R colocalizes with the tight junction components ZO-1 (Bazzoni et al., 2000; Ebnet et al., 2000) and calcium/calmodulin-dependent serine protein kinase, CASK (Martinez-Estrada et al., 2001). Association with the tight junction components is mediated by the PDZ type II-binding motif found to be present in the C-terminal region of F11R. Expression of F11R in HUVEC cells is regulated by the proinflammatory cytokines IFN-γ and TNF-α. Treatment of HUVEC with these cytokines caused a decrease of F11R in intercellular junctions with concomitant increase on the cell surface without change in the total number of expressed protein (Ozaki et al., 1999). The F11R has been identified as a potential target receptor for the T3 reovirus, which spreads to the CNS and causes lethal encephalitis (Barton et al., 2001). In cultured endothelial cells, reovirus infection induced apoptosis by activation of the nuclear factor NF-κB. Both NF-κB activation and apoptosis were found to be F11R-dependent.

Although all reported F11R cDNA sequences appear to encode an identical protein (except *AF154005.1*, Liu et al., 2000, which encodes a shorter protein of 259 amino acids), there are variations in lengths and nucleotide sequences of their 5′ untranslated regions (5′ UTRs). The longest 5′ UTRs are present in four cDNAs: *AF154005.1*, *AF111713* (Ozaki et al., 1999), *AF191495* (Gupta et al., 2000) and *AF172398.1* (Naik et al., 2001). Northern blot analysis of F11R, using as a probe the full coding region of the message (Williams et al., 1999; Naik et al., 2001), provided evidence for two F11R mRNAs of 2.0–2.4 and 4.4 kb. It suggests that the 4.4 kb message contains a 5′ and/or a 3′ UTR sequence that is longer than cDNA clones identified thus far.

The study presented here addresses the issue of variability among the F11R mRNAs, proposes the organization of the F11R gene with localization of the F11R promoters and presents detailed analysis of the 3′ UTR demonstrating the presence of two major F11R species.

2. Materials and methods

2.1. Reagents

The GeneRacer™ Kit for full-length, RNA ligase-mediated rapid amplification of 5′ and 3′ cDNA ends (RLM-RACE), the TOPO-TA™ Cloning Kit and Thermozyme™ thermostable DNA polymerase, the THERMOSCRIPT™ RT-System, the Platinum® Taq High Fidelity thermostable DNA polymerase, Agarose 1000™ and a dNTPs mixture were purchased from Invitrogen Corporation (Carlsbad, CA). The QIAprep Miniprep plasmid DNA isolation kit, QIAquick PCR purification kit, QIAquick gel extraction kit, the Effectene™ Transfection Reagent, RNeasy Mini kit including QIAShredder™ columns and on-column DNase digestion kit were purchased from QIAGEN (Valencia, CA). The pGL3-Basic (no promoter) and pGL3-Promoter (SV40 promoter) firefly luciferase reporter plasmids, pRL-TK vector, Luciferase Assay System and the Wizard® Genomic DNA Purification Kit were purchased from Promega (Madison, WI). Restriction endonuclease *EcoRI* was purchased from New England Biolab (Beverly, MA) and *KpnI* and *NheI* from Promega. All other reagents were obtained from Sigma (St. Louis, MO).

2.2. PCR primer design

Primer design for PCR of genomic DNA or cDNA was conducted by use of the rawprimer program (http://alces.med.umn.edu/rawprimer.html). All custom primers were synthesized by Invitrogen and their sequences are listed in Fig. 1B. Primers (oligo dT_{20} and random hexamers) used for reverse transcription were included in the THERMOSCRIPT™ RT-PCR System. Primers used for amplification of the 5′ and 3′ cDNA ends: GeneRacer™ 5′ [forward] Primer (GeneRacer 5′F), GeneRacer™ 5′ Nested [forward] Primer (GeneRacer 5′NF) and GeneRacer™ 3′ reverse were included in the GeneRacer™ kit.