A new alternative transcript encodes a 60 kDa truncated form of integrin β_3

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A cDNA for integrin β_3 isolated from a human erythroleukaemia (HEL) cell library contained a 340 bp insert at position 1281. This mRNA, termed β_{3C}, results from the use of a cryptic AG donor splice site in intron 8 of the β_3 gene, and is different from a previously described alternative β_3 mRNA. The predicted open reading frame of β_{3C} stops at a TAG stop codon 69 bp downstream from position 1281. It starts with the signal peptide and the 404 N-terminal extracellular residues of β_3, encompassing the ligand binding sites, followed by 23 C-terminal intron-derived residues, corresponding to a truncated form of β_3 lacking the cysteine-rich, transmembrane and cytoplasmic domains. Expression of β_{3C} mRNA was demonstrated in human platelets, megakaryocytes, endothelial cells and HEL cells by reverse transcriptase/PCR. The β_{3C} transcript was also demonstrated in the mouse, suggesting its conservation through evolution. Finally, a 60 kDa polypeptide corresponding to the β_{3C} alternative transcript was demonstrated in platelets by Western blotting using a polyclonal antibody raised against a synthetic peptide designed from the β_{3C} intronic sequence. Taken together, these results suggest a biological role for β_{3C}, the first alternative transcript showing an altered extracellular domain of a β integrin.

INTRODUCTION

The integrins are a superfamily of cell surface receptors that mediate cell–cell and cell–matrix adhesion [1–3]. They have become a subject of extensive study because of their profound biological importance in development, wound healing, metastasis, inflammation, immune responses, and thrombosis and haemostasis [1,2]. Eight subfamilies of integrins have been described to date. All members within each subfamily share a common β subunit, which is non-covalently associated with an α subunit. For example, in the β_3 subfamily, $\alpha_{IIb}\beta_3$ (also termed GPIIb–IIIa), the fibrinogen receptor on platelets, and $\alpha_V\beta_3$ (also termed VNR), a vitronectin receptor expressed by several cell types, share the same β_3 subunit. Some β subunits such as β_3 are more widely distributed than the associated α subunit (α_{IIb}), which is restricted to platelets and cells of megakaryocytic potential [4]. The β subunits of all integrins are remarkably similar in structure: a signal peptide at the N-terminus, a large extracellular domain containing ligand recognition sequence(s) and four cysteine-rich repeats, a transmembrane domain, and a short cytoplasmic tail at the C-terminus [1,5–18]. The amino acid sequences of all the β subunits are highly similar, with specific structural features being conserved over a wide variety of species [19].

$\alpha_{IIb}\beta_3$ mediates platelet aggregation via binding of adhesive proteins, mainly fibrinogen, fibronectin and von Willebrand factor [4]. Like other integrins, it provides a link between extracellular ligands and cytoskeletal components. The extracellular domain, particularly the N-terminal portion of the receptor, is involved in direct interactions with ligands. Residues 109–171 of β_3 are involved in the recognition of the tripeptide Arg-Gly-Asp [20,21], which is present in a number of matrix proteins and is of widespread importance in cell adhesion [2]. Residues 204–229 are also involved in fibrinogen binding [22]. Similarly, an α_{IIb} extracellular sequence is involved in the specific recognition of the C-terminal dodecapeptide of the fibrinogen γ chain [23]. Binding of soluble ligands to $\alpha_{IIb}\beta_3$ requires a conformational change of the receptor, which is probably induced by intracellular signalling, maybe via the cytoplasmic tail of β_3 [24]. Another identified function of the cytoplasmic tails of β integrins is incorporation into focal contact sites through interactions with cytoskeletal components, namely talin, vinculin and α-actinin for β_1 and β_3 [25], an essential step in the machinery linking the cytoskeleton and extracellular matrix.

Aside from the many combinations of α and β subunits, an additional mechanism that increases the diversity of the integrin superfamily is provided by alternative splicing. This has been described for both α and β subunits [26–30]. Interestingly, in the latter case, all alternative transcripts so far described have contained the β cytoplasmic tails. In the case of β_1, two alternative transcripts were found [28,29], one at least of which encoded a β_1 cytoplasmic variant that is functionally different from regular β_1, as indicated by its non-incorporation into adhesion plaques [30]. In the case of β_3 van Kuppevelt et al. [27] have reported an alternatively spliced mRNA which also generated an alternative cytoplasmic domain of β_3, but actual expression of a corresponding translation product was never demonstrated. We report here a new β_3 alternative transcript, the first β integrin alternative mRNA which truncates the extracellular domain and excludes the cytoplasmic domain. We analyse its tissue expression at the mRNA level and demonstrate expression of a corresponding protein product.

MATERIALS AND METHODS

Cloning and sequencing

A cDNA for β_{3C} was isolated from a human erythroleukaemia (HEL) cell cDNA library constructed in λgt10 [31]. It was subcloned either in M13mp18 phage vector or in pBluescript phagemid (Stratagene, San Diego, CA, U.S.A.) for sequencing of

Abbreviations used: HEL cells, human erythroleukaemia cells; RT/PCR, reverse transcription/PCR.

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single-stranded DNA and double-stranded DNA respectively. Sequencing was performed by the dideoxy chain-termination method using Sequenase version 2.0 (USB, Cleveland, OH, U.S.A.); primers used were a universal M13 primer, T3 and T7 primers, or oligonucleotides derived from the β_3 cDNA sequence.

Preparation of cells and tissues

Human platelets were isolated as described [24]. Megakaryocytes were isolated from human femoral bone marrow using the magnetic beads method [26] (informed consent was obtained from patients undergoing surgery). Briefly, sheep anti-mouse IgG conjugated to magnetic beads (Dynal A.S., Compiègne, France) was incubated with monoclonal antibody P_2 (Immunotech, Marseille, France), specific for the $\alpha_{IIb}\beta_3$ complex, at room temperature for 30 min and then washed three times in RPMI-1640. Human bone marrow was aspirated through a 18-gauge needle and collected in RPMI-1640 containing 200 units/ml heparin (Sigma) and 5% fetal calf serum. The marrow suspensions were washed once in RPMI-1640 and then incubated with the P_2/sheep anti-mouse IgG/magnetic beads complex at room temperature for 30 min. Cells with surface-bound P_2–beads were purified using a magnetic concentrator (Dynal A.S.) according to the manufacturer's instructions. About 30% of the nucleated cells were megakaryocytes, as determined morphologically using Wright–Giemsa stains. HEL cells were obtained from the American Type Cell Culture Collection, and were cultured as described [32]. Lungs and kidneys from adult 3T3 mice were immediately frozen in liquid nitrogen, and kept at −80 °C until RNA extraction.

RNA isolation

Total cellular RNA was isolated from human platelets, megakaryocytes, smooth muscle cells and HEL cells as described [33], and from mouse lung and kidney using the guanidine isothiocyanate method followed by ultracentrifugation [34]. RNA from human umbilical endothelial cells was generously provided by M. Nakache, Hôpital Lariboisière, Paris, France.

Reverse transcription (RT)/PCR

An initial single-strand cDNA was synthesized from 1 μg (0.1 μg for megakaryocyte RNA) of human total cellular RNA with 200 units of Moloney murine leukaemia virus reverse transcriptase (BRL) and 1 μM primer E_1 (5′-CACAGATGCTCCAGGACAAA-3′; complementary to nucleotides 1322–1303 of β_{3C} mRNA, i.e. nucleotides 41–60 of intron 8 of the β_3 gene [35,36]). PCR was performed essentially as previously described [24] in a final volume of 100 μl containing 200 nM each of primers E_1 and F_3 (5′-AACTATAGTGAGCTCATCCC-3′; corresponding to nucleotides 1056–1075 of β_3 mRNA, and exon 7 of the gene), 200 μM of each dNTP, 50 mM KCl, 10 mM Tris/HCl (pH 8.3), 2.0 mM $MgCl_2$, 0.01% gelatin and 2.5 units of *Taq* DNA polymerase (Amersham-France, Les Ulis, France). After 5 min at 95 °C enzyme was added, and then PCR was performed for 30 temperature cycles (each of 94 °C/1.5 min, 55 °C/1.5 min and 72 °C/3 min steps; the last cycle included a 10 min/72 °C step) in an IHB thermal reactor (Hybaid Ltd., Teddington, Middlesex, U.K.). Genomic DNA PCR was performed under the same conditions, except that 1 mM $MgCl_2$ was used.

Reverse transcription and PCR amplification of β_{3C} mRNA from total RNA of mouse kidney and lung were performed as described above using primer E_1 (human intron 8) and primer F_{2S} (5′-CCCCACCACAGGCAATCAA-3′), which is derived from the sequence of mouse β_3 mRNA [34] and corresponds to nucleotides 599–617 (exon 3) of human β_3 mRNA. To prevent artefactual co-amplification of the human sequence, mouse primer F_{2S} was chosen because it exhibits four base differences from the corresponding human sequence. The annealing temperature in the PCR programme was elevated to 60 °C to prevent misannealing.

Production and characterization of anti-β_{3C} antibodies

To examine whether the β_{3C} mRNA was translated into an actual polypeptide, rabbit polyclonal antibodies were prepared against the synthetic peptide CPGASVGTGPPFFLL, corresponding to the C-terminal residues of the presumably translated intronic sequence of β_{3C}. The peptide was synthesized using a standard protocol with a Milligen 9050 apparatus (Waters). An immunogen was obtained by incubating 15 mg of the peptide and 15 mg of keyhole lympet haemocyanin overnight at 22 °C in the dark in the presence of 0.06% glutaraldehyde in 15 ml of phosphate buffer. No attempts were made to characterize the immunogen further. The conjugate (3 mg in 3 ml of saline) was emulsified with 3 ml of Freund's complete adjuvant (Difco, Detroit, MI, U.S.A.), and 2 ml was injected subcutaneously into each of two rabbits. The first injection booster was given 6 weeks later (200 μg per animal), and rabbits were bled weekly. Boosters were performed every month and the same follow-up protocol was used. Antisera were tested (titre and sensitivity) in a competitive e.l.i.s.a. using the β_{3C} synthetic peptide covalently linked to acetylcholinesterase using the heterobifunctional reagent succinimidyl 4-[*NN*-maleimidomethyl)cyclohexane-1-carboxylate as tracer, as previously described [37]. The antiserum with optimal sensitivity was selected by its ability to displace the tracer with the β_{3C} peptide.

Western blot analysis

Platelet proteins (30 μg per lane) were separated by SDS/PAGE and blotted on to nitrocellulose according to standard procedures. Membranes were stained with Ponceau Red (5%) and then blocked overnight in 20 mM Tris, pH 7.6, 400 mM NaCl, 0.15% Tween-20 and 5% fat-free dry milk (Gloria, Courbevoie, France) at 4 °C. Incubations with primary antibodies were carried out overnight under mild agitation at 4 °C in the same buffer (TBS/Tween/milk). Where required, presaturation of the antibodies (serum diluted 1000-fold) by the synthetic peptide (100 μg/ml) was achieved in the same buffer at 4 °C overnight. Washes were for 4 × 10 min in TBS/Tween/milk and 2 × 20 min in TBS/Tween. Bound antibodies were detected by the ECL technique (Amersham-France) according to the manufacturer's instructions. Briefly, the secondary affinity-purified anti-rabbit IgG coupled to horseradish peroxidase was incubated in TBS/Tween/milk at a 1:5000 dilution for 30 min at room temperature. Washes were for 2 × 15 min and 2 × 5 min in TBS/Tween. Peroxidase activity was revealed by H_2O_2 and Luminol followed by film exposure (autoluminogram) for between 30 s and 15 min.

RESULTS

Nucleotide and corresponding amino acid sequence of β_{3C}

The nucleotide sequence of β_{3C} cDNA is identical to that of β_3 cDNA [7,31,38], with the exception of a 340 bp insertion at nucleotide position 1281 (Figure 1a). The open reading frame is

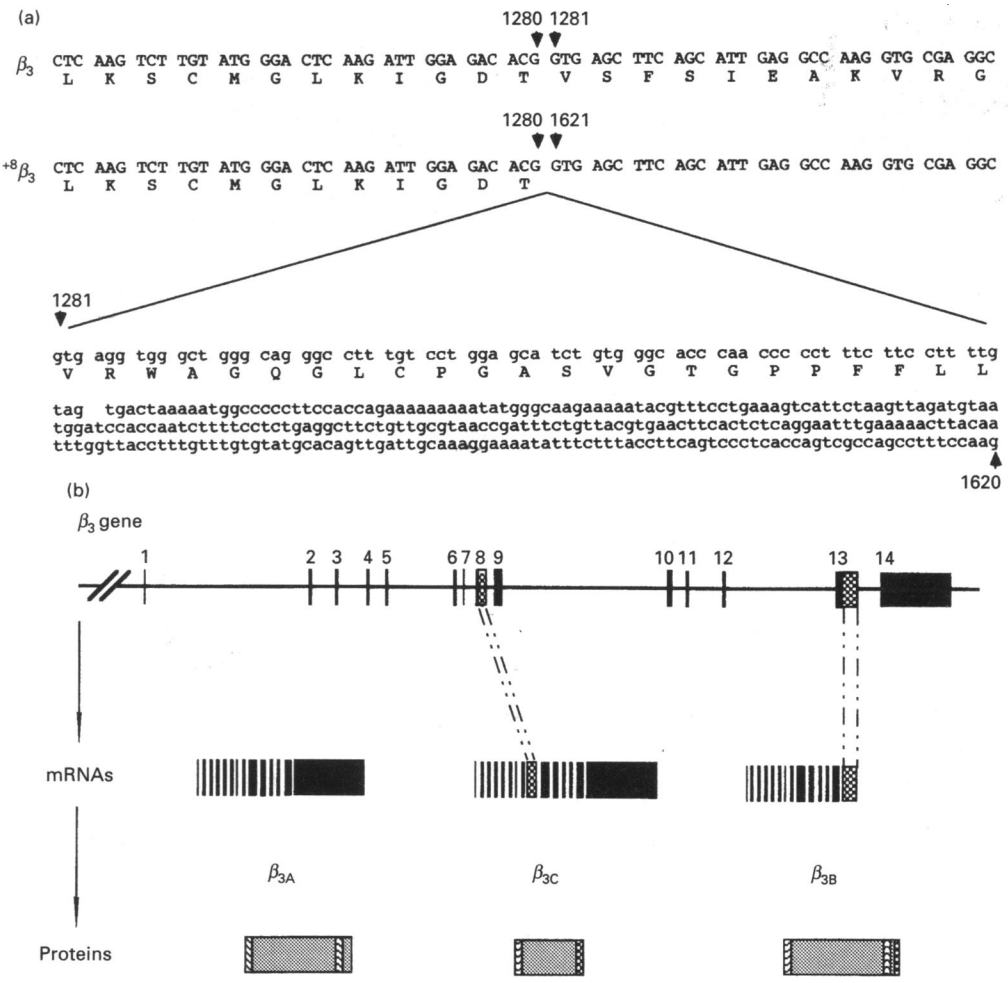

Figure 1 (a) Partial cDNA and deduced amino acid sequence of β_{3C}, and (b) splicing patterns of β_{3A}, β_{3C} and β_{3B}

(**a**) Nucleotide positions are indicated by numbers and arrows. Amino acids are indicated in the single-letter code. Part of the published β_3 sequence is shown for comparison [7,31,38]. The 340 bp internal sequence in β_{3C} indicated in lower case letters is not found in β_3. The open reading frame of β_{3C} extends within this sequence for 69 nucleotides before reaching a TAG stop codon. (**b**) ■, exons and corresponding sequences in mRNA; ⊠, alternatively spliced-in parts of the introns; ▨, signal peptides and transmembrane domains. The splicing at the usual sites results in the longest open reading frame (788 amino acids) of β_3 with the transmembrane and cytoplasmic domains. The splicing in of the 5′ part of intron 8 results in an alternative form, β_{3C}, with a frame shift leading to premature termination. The intron 13-in and exon 14-out form of splicing previously reported [27] results in β_{3B}, with an alternative cytoplasmic domain.

not interrupted by the inserted sequence, but extends within the intervening sequence until a premature TAG stop codon, 69 nucleotides downstream from position 1280. Therefore β_{3C} cDNA encodes a putative protein composed of the 26-amino-acid signal peptide, the first 378 N-terminal amino acids of mature β_3, and an additional C-terminal 23 amino acids encoded by the inserted sequence. However, it does not contain the cysteine-rich repeats, or the transmembrane and cytoplasmic domains of β_3. Thus the putative β_{3C} polypeptide could correspond to a secretable subspecies of β_3. No sequence identity could be found between the C-terminal 23 amino acids of β_{3C} and sequences in the GenBank, NBRF and SwissProt data bases. Within this protein there are 14 cysteine residues, one of which lies within the C-terminal 23 intron-derived amino acids; the remaining 13 are in the upstream sequence. There are also three potential N-linked glycosylation sites.

Comparison of β_{3C} with β_3 and β_{3B}

Comparison with the genomic sequence of β_3 showed that the 340 bp insert of the β_{3C} cDNA corresponds to the first 340 bases of intron 8 of the β_3 gene [35] (Figure 1b). Hence this cDNA corresponds to a β_3 mRNA containing intron 8 which is alternatively spliced at a cryptic GT donor site, located 340 bp downstream from the regular donor site of β_3 at the exon 8/intron 8 boundary [35,36] (Figure 1b). The AG acceptor site at the intron 8/exon 9 junction of β_{3C} is the same as for the regular splicing of β_3 [36]. An additional nucleotide A at position 1388 in the non-coding portion of the β_{3C} mRNA was not found in the published sequence of the β_3 intron 8 [35]. Whether this represents a polymorphism remains to be investigated.

β_{3C} differs from a previously reported alternative transcript that we term β_{3B} [27]. The latter has the same sequence as β_3 prior to nucleotide position 2322, after which it diverges. In contrast to β_{3C}, β_{3B} contains neither the sequence from intron 8 nor the sequence from exon 14, but instead contains the 5′ part of intron 13 as a result of the non-splicing of intron 13, within which there is a AATAAA polyadenylation site. The putative protein encoded by β_{3B} would be a subspecies of β_{3A} with an alternative cytoplasmic domain, contrary to the β_{3C} subspecies

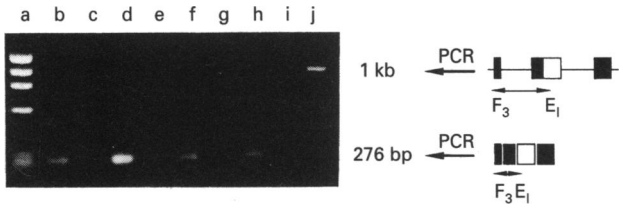

Figure 2 Detection of β_{3C} mRNA by the RT/PCR method

Total RNA prepared from human platelets, megakaryocytes, HEL cells and endothelial cells was reverse-transcribed. The resulting cDNAs were amplified by PCR. Primer E_I is derived from the spliced-in intronic sequence so as to amplify β_{3C} cDNA rather than β_3 cDNA. The PCR products were analysed by ethidium bromide staining of a 1.5% agarose gel. Lane a, ϕX174/*Hae*III-cut standard (Pharmacia, Uppsala, Sweden); lanes b, d, f and h are PCR amplifications of human platelets, megakaryocyte-enriched bone marrow cells, HEL cells and endothelial cells respectively; lanes c, e, g and i are control amplifications of the same cells, but without reverse transcriptase; lane j is the amplification of the genomic DNA from human peripheral leucocytes.

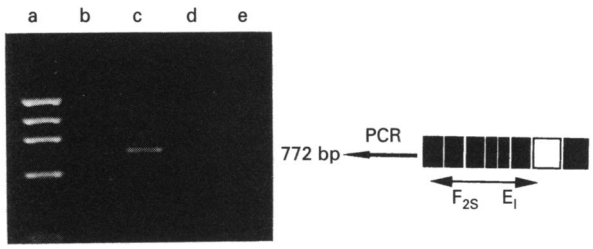

Figure 3 Detection of mouse β_{3C} mRNA by RT/PCR

Total RNA prepared from mouse kidney and lung was reverse-transcribed. The resulting cDNA was amplified by PCR. Primer E_I was described in the legend to Figure 2. Primer F_{2S} is derived from mouse β_3 cDNA [34]. The combination of the two primers specifically amplified cDNA from mouse β_{3C} mRNA. The PCR products were analysed by ethidium bromide staining of 1.5% agarose gel. Lane a, ϕX174/*Hae*III-cut standard; lanes c and e, amplifications of mouse kidney and lung respectively; lane b and d, control amplifications of the same tissues, but without reverse transcriptase. The drawing on the right represents relative positions of the primers relative to exons (■) and intron 8 in β_{3C} mRNA.

which does not contain the transmembrane and cytoplasmic domains (Figure 1b).

Detection of β_{3C} mRNA by PCR

We did not detect the β_{3C} transcript by the conventional Northern blotting method, either because of a low level of expression or because it is of a size too close to that of the regular transcript. We thus decided to use RT/PCR. An initial single-stranded cDNA for β_{3C} was reverse-transcribed from total cellular RNA using primer E_I, which is specific for intron 8 and therefore cannot prime regular β_{3A} mRNA. This cDNA was then amplified by PCR after addition of primer F_3, which was designed from exon 7. Amplification of β_3 transcripts could therefore be distinguished from amplification of potentially contaminating genomic β_3 sequence, since primer F_3 was separated from E_I by intron 7. β_{3C} mRNA was detected as a 276 bp PCR product in platelets (Figure 2, lane b), in megakaryocyte-enriched bone marrow cells (lane d), in HEL cells (lane f) and in endothelial cells (lane h). This 276 bp PCR product hybridized with a β_3 cDNA after Southern transfer, confirming its identity (results not shown). β_{3A} mRNA was expressed in all cells examined (results not shown). Thus β_{3C} RNA message is present in these cells and does not represent a cloning artefact from the HEL cDNA library.

Detection of mouse β_{3C} mRNA

To investigate whether β_{3C} mRNA is conserved in evolution, we amplified β_{3C} mRNA using the human intron 8 primer E_I and the mouse primer F_{2S} (corresponding to human exon 3) from the total RNA of mouse kidney and lung (Figure 3). A 772 bp product was obtained, indicating the presence of a mouse β_{3C} mRNA. Amplification was restricted to mouse β_{3C} mRNA, since primer F_{2S} was derived from the mouse β_3 sequence and contained four mismatches with the human β_3 mRNA [34]. Primer E_I, which is derived from the human intronic coding sequence of β_{3C} mRNA, was used for direct testing of the presence of related intronic mouse sequence. The size of the product obtained was identical to the predicted distance between the two primers on the human β_{3C} cDNA, and further suggests that mouse β_{3C} mRNA is very similar to its human counterpart.

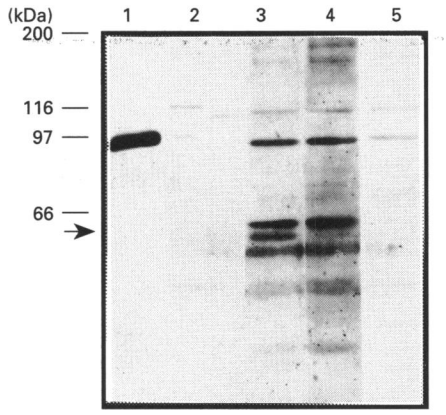

Figure 4 Detection of the β_{3C} polypeptide product by Western blotting

Platelet proteins (30 μg per lane) were separated by SDS/PAGE and blotted on to nitrocellulose membranes. Transferred proteins were reacted with specific antibodies, and bound IgG was detected with a secondary anti-rabbit IgG antibody coupled to horseradish peroxidase by the Luminol method and autoluminography. The exposure time was 30 s. Lane 1, polyclonal anti-β_{3A} (anti-GPIIIa) antiserum (1:20000 dilution); lane 2, non-immune serum (1:1000 dilution); lane 3, anti-(β_{3C} synthetic peptide) (1:1000 dilution); lane 4, anti-β_{3C} antibody (1:1000) preincubated with an excess of β_{3C} synthetic peptide (100 μg); lane 5, non-immune serum. The arrow on the left indicates the 60 kDa band detected by anti-(β_{3C} synthetic peptide) in lane 3, which disappears in the presence of excess free peptide in lane 4. Bands above and below correspond to background signal, since they are not sensitive to excess synthetic peptide.

Demonstration of a 60 kDa protein corresponding to the translation product of β_{3C}

Both its expression in normal tissues and its presence in at least one other species suggested biological significance for the β_{3C} transcript. We therefore decided to examine whether a protein product corresponding to β_{3C} was synthesized in normal tissues. Figure 4 shows a Western blot of whole platelet proteins treated with an anti-β_{3C} specific antibody raised against a synthetic peptide designed from the last 15 residues of the β_{3C} intronic sequence (lane 3) or the same antibody saturated with an excess of β_{3C} synthetic peptide (lane 4). A single band of 60 kDa was detected with the anti-β_{3C} antibody, which disappeared when the antibody was preincubated with an excess of β_{3C} synthetic peptide. This result demonstrated that the β_{3C} transcript was

translated into a polypeptide, further supporting its functional relevance.

DISCUSSION

We have identified a new alternative transcript for integrin β_3. Alternative mRNA splicing is a mechanism known for providing diversity in protein function and in the regulation of numerous genes [41]. It is therefore of potential interest to examine the exact nature of the alternative transcript of a given mRNA, its protein product and in some cases its regulation. The alternative β_{3C} mRNA which we identified in this study is due to the differential selection of a cryptic GT splice donor site 341 bp downstream from exon 8, resulting in the splicing in of 340 bp of the 5′ part of intron 8 of the β_3 gene. The corresponding AG acceptor splice site is the same as that for regular β_3 mRNA (β_{3A}) proposed by Lanza et al. [36], but is different from that proposed by Zimrin et al. [35]. In fact, the difference between the two propositions is a 5 bp shift for both the AG acceptor and GT donor sites, which does not affect the corresponding amino acids.

Alternative transcripts for integrin β subunits have been reported in the past, including two β_1 isoforms [28–30], one β_3 [27] and one β_4 [39] mRNA, leading to different cytoplasmic domains. All of these β integrin alternative mRNAs were generated by skipping of the last exon by non-splicing of the last intron or premature termination of transcription within the intron. This intron was in turn transcribed up to an alternative polyadenylation site. The mechanism described here for β_{3C} mRNA is very different, because there is no exon skipping or premature transcription termination, but partial splicing of intron 8 by use of a cryptic GT donor splice site, leaving the 5′ one-third of the intron unspliced. Moreover, the major difference in the present β_{3C} alternative sequence, in addition to the difference in terms of mechanisms, is that it is the first alternative transcript of a β integrin that differs in the extracellular region, and should lead to a truncated form of β_{3A} with no cytoplasmic or transmembrane domains.

The β_{3C} mRNA was detected by RNA PCR in platelets, megakaryocytes, endothelial cells and HEL cells. Because of the intronic nature of primer E_I, which restricted the amplification to the mRNA containing the corresponding sequence, the amplification was specific for β_{3C}. Attempts to detect the β_{3C} transcript by Northern blotting failed (results not shown). The simplest explanation is that β_{3C} is a minor transcript, as suggested by its weak PCR signal compared with β_3, and is therefore difficult to detect by the less sensitive Northern blotting method. Interestingly, a recent report using the B16a mouse cell line showed the presence of several β_3 bands (9, 7 and 6 kb) [40]. It is tempting to speculate that the 7 kb band represents β_{3C}, since mouse β_{3A} has a mobility close to 6 kb [32].

Intron-containing mRNA precursors that are slowly processed [41] can be easily detected by PCR. However, β_{3C} mRNA is not merely an unmatured precursor, since it is the product of the processing of intron 8 through use of a cryptic GT donor site, leaving only the 5′ third of the intron unspliced. In addition, the fact that an identical alternative transcript exists in mouse further indicates that β_{3C} is an actual transcript and not a partially processed β_3 mRNA precursor.

Our finding that β_{3C} alternative mRNA was also expressed in the mouse is highly significant, and further suggests that β_{3C} is biologically relevant. None of the several alternative β integrin transcripts previously reported in the literature were shown to be conserved in evolution. This emphasizes the potential importance of the β_{3C} transcript and prompted us to examine the possible expression of a corresponding protein product. Because of the splicing in of the 340 bp intronic sequence, the open reading frame of β_{3C} mRNA is shifted, and stops at a premature TAG stop codon 69 bp downstream from exon 8, thus encoding 23 new C-terminal amino acids. We used this predicted sequence to produce a synthetic peptide and raise an antibody specific for the putative β_{3C} polypeptide. We found that β_{3C} resulted in expression of a 60 kDa polypeptide in platelets. The size of this polypeptide is slightly higher than the 50 kDa predicted from the amino acid sequence, suggesting that it is glycosylated, in agreement with the three N-glycosylation sites present in the β_{3C} sequence.

Both the conservation of β_{3C} and, most importantly, its translation into a polypeptide, argue strongly in favour of its potential biological significance. The function(s) of this protein may be inferred from its structural features. (1) It lacks a transmembrane domain; since it contains the β_3 signal peptide, it could be either secreted constitutively or targeted to the α-granules as a secretory protein. Although β_{3C} encompasses residues 110–350, which seem to be involved in the association with α_{IIb} [42], the substantial structural differences from β_3 and the absence of the cysteine-rich domain render this association with α_{IIb} questionable. (2) β_{3C} lacks the cytoplasmic domain and therefore cannot interact with cytoskeletal proteins; as a consequence, even if it associated with α_{IIb} or α_v, it would probably not be incorporated into focal adhesions, a phenomenon independent from integrin α subunits [25]. (3) It must lack the tight structure of β integrins conferred by the cysteine-rich domain that is absent from β_{3C}. Though no particular function has been attached to the integrin cysteine-rich domain, it is likely to play a fundamental structural role, since it is conserved throughout evolution and across the whole superfamily [1]. (4) β_{3C} may bind RGD-containing adhesive ligands, since it encompasses the ligand-binding domains of β_3 represented by residues 91–171 and 211–222 [20–22].

β_{3C} shows considerable overall structural differences from β_{3A} which probably result in distinct ligand-binding characteristics. In β_{3C} the capacity to bind fibrinogen should be preserved, but its ability to be activated through conformational change may be lost. Speculation on a potential role for this β_{3C} polypeptide is tempting; for example, secreted β_{3C} could act as a competitive inhibitor of fibrinogen (or other adhesive ligand) binding to $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$, and thus contribute to the regulation of platelet aggregation, or of adhesion to the extracellular matrix, or else to endothelial cell migration. Experiments are now under way to test this hypothesis.

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