[Frontiers in Bioscience 7, d219-227, January 1, 2002]

SPLICE VARIANTS OF HUMAN β1 INTEGRINS: ORIGIN, BIOSYNTHESIS AND FUNCTIONS

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1. ABSTRACT

The integrin β1 subfamily of adhesion receptors consists of 12 members and forms the biggest subfamily among integrins. Human integrin subunit β1 has five cytoplasmic splice variants (β1A, β1B, β1C-1, β1-C2, β1D). Even though cytoplasmic splice variants do not change the ligand-specificity of a β1 integrin, clustering of these different splice variants triggers signaling pathways that lead to a different cellular response. The main focus of this review is on the origin and specific functions of the less abundant human integrin β1 splice variants (B, C-1, C-2, D).

2. INTRODUCTION

Integrins constitute a major group of cell-surface receptors for extracellular matrix and cell-surface proteins. The term "integrin" was introduced by R. Hynes and co-workers in 1986 and designates a cell surface receptor that connects cell cytoskeleton to the extracellular matrix (ECM). Since the publication of the full-length cDNAs of *the fibronectin receptor* (current name $\alpha_5\beta_1$) in 1986 - 1987 (1, 2), 18 α and 8 β subunits are now described in mammals. An integrin is composed of non-covalently associated transmembrane α and β subunits. The known combinations of different α and β subunits give rise to 24 different heterodimers. The integrin family can conditionally be divided into subfamilies dependent on their subunit composition and ligand specificity. The main focus of this review - integrin subunit β1 can combine with 12 α subunits and forms the biggest integrin subfamily with broad ligand specificity.

Importantly, integrins do not act only as molecular bridges linking intracellular filament systems (actin filaments and intermediate filaments) with ECM but are also important for mediating signals from ECM that regulate growth, death, differentiation, and movement of cells. Since integrins do not possess any known intrinsic kinase activity they transduce signals by spatially compartmentalizing docking and adapter proteins that link integrins to cytoplasmic kinases. Furthermore, cells themselves can dynamically regulate integrin-mediated cell adhesion. Modulation of integrin affinity by intracellular factors results in extensive conformational changes in the receptor that affect the ligand-binding interface.

The short cytoplasmic tails of integrins are absolutely required for integrin activation and signaling. Great efforts have been made to identify residues in the cytoplasmic tail of β subunits required for these specific aspects of integrin function. In particular, the cytoplasmic domains of β1, β2, and β3 have been subjects for extensive studies regarding the effects of deletions and amino acid substitutions.

Integrin signaling is complex and far from being understood. One given receptor can often bind different ligands that in turn result in activation of different signaling pathways. One should keep in mind that integrin signaling

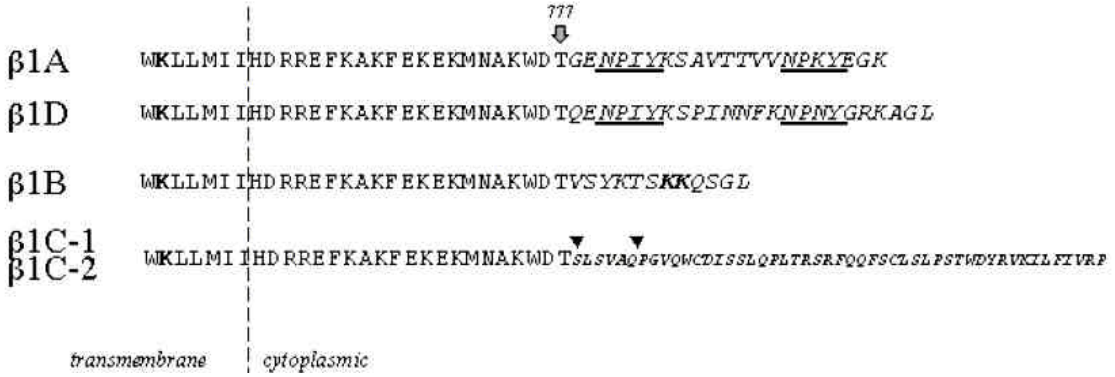

Figure 1. The amino acid sequences of the cytoplasmic splice-variants of human integrin β1. The variant specific regions are shown *in italics*. The NPXY motifs in β1A and β1D are underlined. A double lysine motif in β1B is *in bold*. Arrowheads indicate 6 amino acids lacking in β1C-2. The sequence up to T^{777} is encoded by exon 6.

is often cell-type specific. In addition, integrins exist in several splice variants (extracellular- and intracellular) that further increases the complexity. The highly recommendable review by de Melker and Sonnenberg gives a good overview about extra- and intracellular splice variants of different integrins (3). The aim of this review is to describe some of the integrin β1 cytoplasmic tail splice variants in more detail and underline some specific aspects of splice variants β1B, β1C-1 and β1C-2 that have been largely neglected.

3. SPLICE VARIANTS OF β1

Integrin subunit β1 is expressed in all mammalian cells, except for mature erythrocytes. Knockout experiments in mice have shown that integrin subunit β1 is absolutely required for embryonic development (4, 5). Specific deletion of β1 integrin subunit in the nervous system (in neurons and glia cells) showed that β1 integrins are required for anchorage of glial endfeet, the remodeling of basement membranes but not essential for neuron-glia interactions and neuronal migration during corticogenesis (6). Fetal and adult blood stem cells lacking β1 integrins cannot colonize hematopoetic organs (7). In skin, β1 integrins are required for hair follicle development and the maintainace of the epidermal-dermal junction (8).

For human β1, five different cytoplasmic splice-variants are characterized, namely β1A, β1B, β1C-1, β1C-2, β1D (Figure 1) (9-14). All splice-variants of human β1 share the common N-terminal part until the sequence WDT^{777} that corresponds to the 3' end of exon 6 in the β1 gene (Figure 1). We know for today that the cytoplasmic splice-variants of β1 do not change the ligand specificity for a given heterodimer, but they can modulate receptor affinity towards the ligand (15, 16). Essentially nothing is known about specific intracellular signals that these variants may generate.

4. SPLICE VARIANT β1A

The splice-variant A, mostly referred to as β1 only, is very conserved at the amino acid level amongst different species from sponge to human, particularly in the transmembrane and cytoplasmic domains (17). Most review articles dealing with integrin β1 signaling and affinity regulation are covering data obtained by studying β1A and therefore β1A will not be discussed in this review. For further information, the reader is referred to several review articles covering integrin activation and signaling (18-21).

5. SPLICE VARIANTβ1B

The β1B isoform was isolated from a human placenta library probed with a synthetic oligonucleotide corresponding to the cytoplasmic domain of β1A. The last 12 amino acids of β1B that are different from β1A are derived from the intronic sequence that follows immediately downstream of exon 6 (Figure 1 and 2) (9). Analysis of the nucleotide sequence of the mouse β1 gene has revealed that the mouse intronic sequence after exon 6 could potentially code for 15 amino acids (VSYETLLRAVGWFLK) that show no significant homology to human β1B, except for first three amino acids (10). Thus, the human β1B described in the literature has no orthologue in mouse; it is not known how is the situation in primates.

The β1B specific transcript has been detected at low levels in all human tissues and cell lines tested by RT-PCR, but the protein was reported to be detectable only in skin (keratinocytes) and liver (hepatocytes) (9, 22). Expression of human β1B in CHO cells showed that β1B can dimerize with α subunits and bind to a fibronectin affinity matrix in an RGD-dependent manner in the presence of Mn^{2+}. In contrast to β1A, the β1B integrins did not localize to focal contacts when cells were plated on fibronectin (22). However, β1B can accumulate to some extent to focal contacts in a ligand-independent manner (15). Further analysis revealed that the human β1B isoform does not mediate cell spreading and activation of focal adhesion kinase (FAK) in cells plated on anti-human β1 mAb (TS2/16) (23). Furthermore, induction of ligand occupied conformation by the small GRGDS peptide of β1B integrins does not trigger activation of FAK and

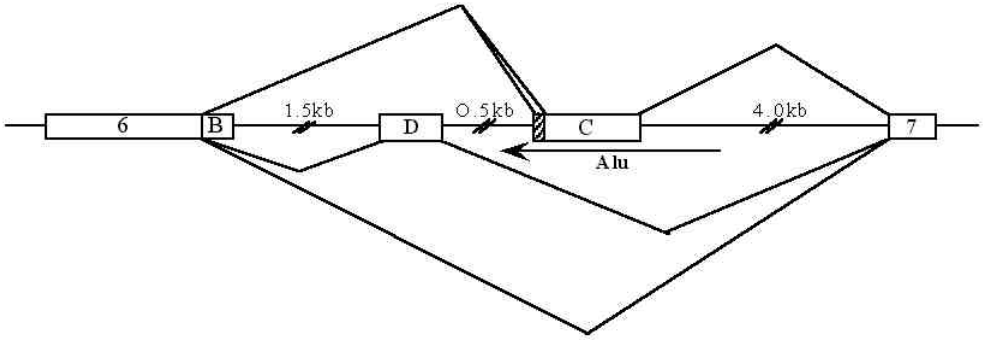

Figure. 2. Partial exon-intron organization of the human integrin β1 gene. The exons are depicted as boxes and the introns as horizontal lines connecting the boxes. The other lines are depicting the splice-patterns used to generate the various transcripts. The orientation and position of the Alu element that exon C is part of is indicated with a horizontal arrow. The exons and introns are not drawn to scale.

tyrosine phoshorylation of paxillin (15); A.Armulik, unpublished results). The cytoplasmic part common for all integrin β1 splice-variants is thus not sufficient for FAK activation. Interestingly, triggering of a ligand occupied conformation in β1B integrins results in modest tyrosine phosphorylation of CAS (A.Armulik, unpublished results).

Expression of β1B in CHO cells was reported to reduce cell spreading on fibronectin and laminin-1 but not on vitronectin (23). The attachment of cells to fibronectin and laminin-1 was only affected in clones expressing high levels of β1B (50% of that of endogenous β1A). The migration on gelatin of CHO cells expressing the β1B was similar to that of CHO cells expressing β1A when vitronectin, but not fibronectin, was used as a chemoattractant. Again, similarly to cell attachment data, higher β1B expression levels caused stronger inhibition. From these experiments it was concluded that β1B has a dominant negative effect on endogenous β1 integrins and it was suggested to be caused by the competition of β1B with endogenous β1A for available α subunits (23) and subsequently a failure of β1B to bind to extracellular ligands and activate intracellular signaling pathways.

β1B expressed in the β1-deficient cell line GD25, similar to β1A, dimerizes with α5, α3 and α6 subunits (24). GD25 cells do not adhere to laminin-1 but expression of β1A in GD25 cells restored the ability of these cells to attach to laminin-1 via α6β1A (25); however, the expression of β1B subunit did not promote cell adhesion unless Mn^{2+} was present in the medium (24). Analysis using antibodies recognizing epitopes exposed only in the ligand-competent/occupied integrins revealed that the extracellular domain of β1B integrins possesses an inactive conformation (15, 24). The inactive ectodomain conformation could be changed to active by addition of Mn^{2+} or the GRGDS peptide (15, 24). The spreading and organization of actin stress fibers of GD25-β1B cells on fibronectin was found to be impaired compared to GD25 cells (24). The authors concluded that β1B has a dominant negative effect not only on β1A integrins but also on αvβ3/β5 integrin, since the attachment of GD25 to fibronectin is mediated via these latter integrins (25). The vitronectin substrate was not tested in the former report. In contrast to this finding (24) we found that β1B does not have a dominant-negative effect over the αv integrins (15). GD-25 cells expressing human β1B were not impaired in cell attachment, spreading, and in organization of actin cytoskeleton neither on fibronectin nor on vitronectin (15). As mentioned above, expression of β1B in CHO cells also did not inhibit cell spreading on vitronectin (i.e. adhesion mediated via αv integrins) (23).

The β1B integrins were found not only to be unable to mediate the assembly of fibronectin matrix but were reported to inhibit this process in CHO, GD25 and FRT cells (24, 26). Overexpression of constitutively active RhoA in FRT cells abrogated the negative effect of β1B on matrix assembly (27).

Studies on human keratinocytes, one of the few cell types that was reported to express the β1B variant at a detectable protein level, showed that overexpression of β1B in keratinocytes results in intracellular accumulation of the protein, which could be overcome by deleting the KK sequence (Figure 1) (28).

β1B has been suggested to have a regulatory role of adhesion-mediated signaling. However, the modulating effects of β1B over β1A have only been observed at expression levels many-fold higher than what apparently occurs *in vivo*. Thus, the physiological relevance (if any) of human β1B remains to be established. The β1B specific part is generated by intron retention. Similar splice variants are found for human integrin subunit β3 (β3B) and β4 (β4E) (29, 30). So far no specific function for these splice variants has been described. Intron retention resulting from aberrant pre mRNA splicing has been described for quite a number of other proteins (e.g. CD44, periaxin, rhodopsin kinase) (31-34). In most cases, intron retention results in a truncated non-functional protein with an intron-encoded C-terminus, similar to β1B. It is most likely that human β1B represents just a splicing error occurring at low frequency rather than an obscure way to regulate cell adhesion.

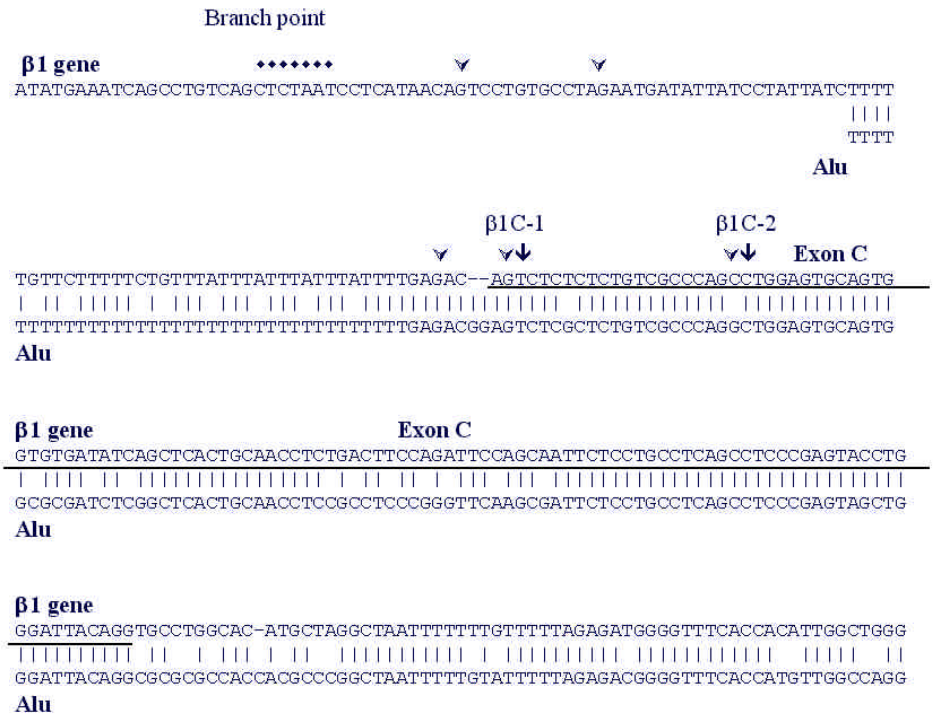

Figure 3. Alignment of the genomic sequence of exon C and its surrounding intron sequence with the complementary Alu sequence. Exon C is underlined and identical nucleotides between the β1 genomic sequence and Alu sequence are indicated with vertical bars. A consensus branch point is indicated with rombs, and potential splice-acceptor sites (AG di-nucleotides) are indicated with arrowheads. The splice acceptor sites used to generate β1C-1 and β1C-2 are indicated with vertical arrows.

5. SPLICE VARIANTS β1C-1 AND β1C-2

The transcript for the splice variant β1C-1 was first isolated from a human erythroleukemia (HEL) cell λ-cDNA library (11), and the transcript for β1C-2 was identified using RT-PCR with total RNA from the human HL60 cell line as template (12). The β1C-1 differs from β1C-2 by six amino acids (Figure 1) that in β1C-2 are missing as a result of the utilization of a more distal 3' splice acceptor site (Figure 2) (12). Similar to β1B, the splice-variants β1C-1 and β1C-2 are only found in human and not in mouse (11, 12). Exon C is part of an Alu element and Alu elements are primate specific retrotransposable elements (12). Alignment of the genomic nucleotide sequence around exon C with the reverse complement of a consensus Alu sequence clearly demonstrates the homology (Figure 3). The calculated homology at the nucleotide level between the exon C-Alu element and a consensus Alu element is 81% and within the 116 bp exon C-region, it is as high as 91% (12).

As mentioned, Alu elements are primate specific repetitious genomic DNA sequences that belong to a group of sequences called short interspersed nucleotide elements (SINEs) (35, 36), and they are present at a copy number of approximately 500 000 per haploid human genome, making up around 5% of all human DNA. Alu elements are occasionally found to be part of coding regions of mRNA (for a review see: (37). In most of these cases, the Alu-derived sequence has been included into the transcript by a splice-mediated insertion of intronic Alu sequence. Investigation of the consensus Alu element in more detail has revealed that it contains several nucleotide regions that are similar to eukaryotic splice acceptor and donor sites. The requirement for a polypyrimidine tract is met by the presence of the reverse complement of the polyadenyl tail and the adenine-rich linker located at the end, and in the middle, of the Alu element, respectively. Both sense and antisense Alu elements can be spliced into mRNA (for review see: (37). A comparison of the Alu-exon encoded amino acid sequences demonstrates that sequences translated in the same reading frames can give rise to amino acid regions of high homology (Figure 4). Thus, to some extent, inclusion of Alu-exons can provide proteins with specific Alu-derived-domains. However, the functional significance of these domains is still unclear and most of these alternative transcripts have been identified to occur at a very low frequency compared with the normal protein variants and they are all expressed simultaneously as the normal variant, similarly to β1C-1 and β1C-2.

Analysis of the genomic region around exon C has identified an upstream branch-point sequence (CTCTAAT) and several potential AG dinucleotides (Figure 3) (12). Downstream of the polypyrimidine tract, which consists mostly of thymidines, the sequence GAGACAG follows and then the β1C-1 exon starts. Within the next 18 nucleotides, another stretch of mostly pyrimidines follows before a CCAG-sequence followed by the start of the β1C-2 exon. The splice-donor site used by

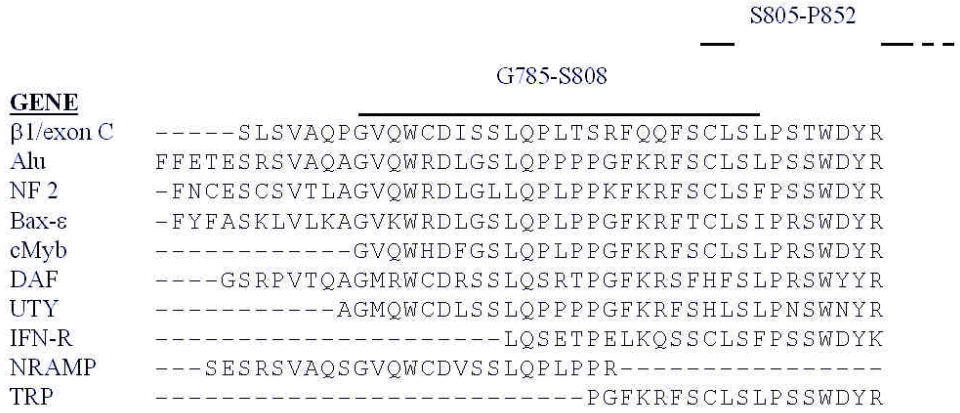

Figure. 4. Alignment of translated Alu-derived exons. Amino acid sequences of various cDNAs containing regions derived from Alu elements. The regions corresponding to the β1C peptides used to generate anti-β1C-specific antibodies are indicated with horizontal lines and labeled G785-S808 and S805-P825, respectively. The abbreviations used are: NF2, neurofibromatosis type 2; DAF, decay-accelerating factor; UTY, ubiquitously translated tetratricopeptide repeat gene of the Y chromosome: IFN-R, interferon receptor; NRAMP, natural resistance.associated macrophage protein; TRP, transformation-related protein.

both β1C-1 and β1C-2 is CAG|GTCCT and contains the important AG|GT combination at the exon-intron border. Comparing the β1C-1 and β1C-2 splice sites with the consensus splice sequence shows that they are not optimal, suggesting that this might be part of the reason why exon C is included in the mature β1-mRNA at such low frequency (12).

Using RT-PCR the amount of both β1C-1 and β1C-2 transcripts have been found to be low compared to the β1A-transcript. Nevertheless, the β1C-1 and β1C-2 transcripts have been identified by RT-PCR in a whole range of human cell lines and tissues (11, 12). At the protein level, β1C has been detected from surface biotinylated HEL cells, TNFα-stimulated HUVEC, prostate carcinomas and from benign prostate tissue (11, 38-41). In these experiments, the antibody used was a peptide antibody generated against the 23 amino acid long peptide (S805-P825) deduced from the C-terminal end of the β1C-1 sequence (Figure 4) (11). This antibody would not distinguish between the β1C-1 and β1C-2 variants, thus, when considering the equal amounts of transcripts for the two β1C-variants in many cell types, the bands seen by this antibody most probably contain both β1C-1 and β1C-2 protein. Peptide antibodies raised against two regions of the β1C cytoplasmic region have been used in immunohistochemical studies. The anti-β1C (G785-S808) would in principle be able to recognize all proteins containing a similar region encoded by an Alu-derived exon, thus the specificity of this antibody is questionable (Figure 4). Twelve of the 21 amino acids constituting the peptide used to generate the anti-β1C (S805-P825) antibody are encoded by the Alu-derived exon, thus this antibody may also recognize other proteins than β1C. Regardless of this, both antibodies have been used to demonstrate a proposed specific expression of β1C in various tissues. In particular, several studies have been conducted in order to compare the expression-pattern of β1C in normal and carcinogenic human tissues (39-41), since overexpression of β1C-1 has been shown to inhibit cell proliferation in several cell lines. Using the anti-β1C peptide antibodies, a correlation between downregulated β1C-expression and neoplasia was identified. The proposed hypothesis is that normal cells express low levels of β1C in order to not become neoplastic, while reduced β1C expression would induce rapid cell proliferation. Interestingly, the anti-β1C (G785-S808) gave better immunohistochemical staining than the antibody anti-β1C (S805-P825), the latter being generated against the more C-terminal part of β1C (Figure 3) (39). This might indicate that the epitope (or epitopes) recognized by the former antibody is part of one or several proteins containing Alu-encoded sequences.

Expression of the β1C-1 cDNA in several cell lines has demonstrated that part of the β1C-specific cytoplasmic domain has inhibitory effects on cell proliferation (38, 40-43). Using deletion-mutants expressed in CHO and mouse 10T1/2 fibroblasts the growth inhibitory effect was located within the 18 amino acid long region from β1C-1 (Q_{795}-T_{812}) (43). Importantly, it was also shown that expression of the β1C-1 specific-domain β1C-1 (S_{778}-P_{825}) when fused to the green fluorescent protein (GFP) was equally potent as the intact β1C-1 protein. Hence, the growth inhibitory effect of β1C-1 is not dependent on the localization at the plasma membrane or any other feature provided by the whole β1C-protein. Information about the direct downstream effector molecules from β1C is lacking, but a cell-cycle inhibitor $p27^{kip1}$ has been identified as one of the nuclear effector molecules (40). Expression of β1C-1 in CHO cells was found to have an inhibitory effect on activation of ERK2 by fibronectin but not on activation of focal adhesion kinase (FAK) or Akt. Moreover, clustering of β1C integrins was shown to lead to the activation of the Akt pathway (44). Interestingly, even though β1C has been reported to

associate with α subunits (11) it has never been shown that β1C integrins can bind a ligand. So, even if a clustering of β1C-1 by mAbs could lead to a cellular response, it is unclear whether under physiological conditions β1C-1 has any signaling properties evoked by integrin ligand binding.

Furthermore, studies on β1C splice-variants expressed in the β1-deficient GD25 cells showed that these subunits are retained in the cell and degraded rather than localized to the cell surface (45).

In conclusion: altogether there are several lines of evidence that overexpression of β1C or the β1C-specific cytoplasmic domain alone can inhibit cell proliferation but the actual mechanism for this and the molecules involved have not been identified. In addition, the immunohistochemical data ought to be considered with caution until the protein(s) recognized by the anti-β1C peptide antibodies have been identified. Like the β1B variant, the physiological role of the β1C variants is questionable.

6. SPLICE VARIANT β1D

The muscle specific integrin β1 isoform, β1D, is the only splice-variant that shares significant homology with β1A throughout the cytoplasmic tail (Figure 1). The β1D specific part (the C-terminal 24 amino acids) is encoded by exon D, which is localized between exons 6 and 7 in mouse β1 gene and between exons C and 7 in human β1 gene (Figure 1B) (13, 14). The β1D mRNA from human, mouse, rat and chicken have been sequenced and demonstrated a 100% homology at the amino acid level between the species in the β1D specific part (10, 14). *In vivo*, the β1D splice variant is only expressed in skeletal and cardiac muscles (not in smooth muscle), and it completely displaces β1A in terminally differentiated muscle, where it associates with α7 (46). In cardiac muscle, β1D is localized to the sarcolemma, costameres and intercalated discs, and in skeletal muscle, β1D was detected in sarcolemmas, costameres, myotendinous and neuromuscular junctions (46).

Expression of β1D in CHO cells and in GD25 cells demonstrated that β1D can localize to focal adhesions when plated on fibronectin, and clustering of β1D triggers activation of FAK and MAPK pathways (16, 46). Cells expressing the β1D isoform showed reduced spreading and migration and this occurred irrespective of the type of ECM. However, expression in C2C12, REF52 or NIH3T3 cells did not have any effect on adhesion and spreading, indicating that this might be cell type specific (47). A larger fraction of β1D than β1A was found to be in constitutively active conformation when expressed in both CHO and GD25 cells. Antibodies specific for epitopes for active ligand-binding conformation on β1 integrins recognized approximately 77-88% of β1D and only 27-44% of β1A subunit containing integrin receptors. The β1D subunit was shown to be more efficient than β1A in fibronectin matrix assembly, while β1A mediated cell migration to a greater extent than β1D. The effect on cell migration might be due to the stronger ligand binding and increased association with the actin cytoskeleton of β1D integrins compared with that of β1A and αV integrins, as shown by β1D displacement of both β1A and αV from focal contacts when cells (CHO, GD25) were plated on fibronectin (16). However, expression of β1D in NIH3T3 cells did not show any displacement of β1A from focal contacts - again this might be a cell type specific event (16).

It is interesting that despite the high degree of homology between β1A and β1D, β1D integrins display an increased affinity for fibronectin and enhanced association with the actin cytoskeleton (16). *In vitro* binding studies have shown that the cytoplasmic domain of β1D binds the cytoskeletal proteins talin and filamin with higher affinity than β1A (16, 48). On the other hand, more α-actinin was found to be associated with β1A than with β1D (16). The talin binding-site in the β1A cytoplasmic domain has been suggested to include the N_{780}PXY motif (49, 50). Apparently, β1D contains additional residues that strengthen the talin binding; however, the amino acids involved are unknown.

Recently a novel intracellular protein, melusin, was reported to interact with the common region of the β1 cytoplasmic tail (51). Although *in vitro* binding assays showed that melusin is able to bind all tested β1 variants (A, B, D), its restricted expression to skeletal and cardiac muscle suggests that the physiological function of melusin would be through binding to integrin β1D.

Of the potential phosphorylation sites in the β1 cytoplasmic tail, the two threonines (amino acid T788 and T789) are the only residues that are not conserved between β1A and β1D. Interestingly, these threonines have been shown to be essential for β1A mediated cell attachment and fibronectin fibril formation (52). The mutant β1ATT$^{788-9}$AA was shown to be in a conformation that is inactive for ligand binding. However, clustering by anti-β1 antibodies could induce phosphorylation of FAK, suggesting that the two threonines are not required for FAK activation, consistent wih the fact that β1D can activate FAK.

Expression of β1D in several different cell lines has provided contradictory findings regarding the effect of β1D expression on cell proliferation (43, 47). Belkin and Retta (47) demonstrated reduced BrdU incorporation in β1D expressing C2C12, REF52 and NIH3T3 cells, while Meredith et. al. (43) did not see any such negative effects after β1D expression in mouse 10T1/2 fibroblasts. Similar results were obtained by expression of the complete β1D molecule, or with only the cytoplasmic domain as fusion-protein with the extracellular and the transmembrane domain of IL2. In addition, expression of β1D in CHO cells, but not in C2C12 cells, gave increased MAP kinase activation (46, 47). Using the NIH3T3 cells, the β1D mediated growth arrest was identified to the late G1 phase before the beginning of the S phase, and overexpression of a constitutively active form of Ha-Ras (but not Raf-1)

could abolish the growth arrest. In most pathways, Raf-1 is located downstream of Ras. However, in this case, alternative pathways must be involved. In contrast to the results from β1C, no short growth-inhibitory motif could be identified in β1D (43, 47). Instead, the only deletion mutant that did not affect the growth inhibition was a deletion of the C-terminal 6 amino acids.

Growth-arrest by expression of integrin β1D fits well with the observed onset of expression in muscle-tissues during embryogenesis where myoblasts fuse to form myotubes and stop proliferating (53, 54). In mouse embryos, β1A is the only β1 isoform expressed in skeletal muscle until embryonic day 17.5 (E17.5). After this, β1D is co-expressed with β1A until birth when β1A expression declines in skeletal muscle tissue and is restricted to the capillary walls, while β1D expression is located to the sarcolemma of the muscle cells. In cardiac muscle the situation is different. Brancaccio et al. (53) has reported that β1D is expressed already at embryonic day 11, while van der Flier et al. (54) claim that the onset of β1D expression is around the time of birth. In another report it was shown that expression of β1D can be detected at embryonic day 12 (in rat) in heart and prenatal expression of β1D was found to be <20% of that in adult ventricle (55). In any case, the β1A expression in cardiac muscle is negligible a few days after birth. The switch from expression of β1A to β1D also involves a change in the associated α-subunits. Undifferentiated C2C12 cells β1A in association with α3A, α5, α7B, and αV, while differentiated cells expressed β1D in association with α5, α7A (some α7B), and αV (54). In both skeletal and cardiac muscle, the onset of β1D parallels the start of α7 expression (53, 54, 56). Both α7A and α7B are expressed in skeletal muscle, while in cardiac muscle only α7B is expressed.

It has been suggested that replacement of the β1A isoform in muscles with β1D might be necessary to strengthen the cytoskeletal-matrix link in muscle cells (16). However, the lack of β1D isoform in transgenic mice (due to the exon D knockout) did not affect muscle formation and did not cause muscular degeneration. However, these mice showed some indications a mild ventricular dysfunction (57), and indeed, the requirement of β1D in the hyperthrophic growth of the cardiomyocytes was recently demonstrated (58). Little is known about the signaling pathways involved but there are indications that FAK is involved (55). In the converse situation, mice which express only the β1D variant (knock-in) were not viable and died in uteri because of a wide range of developmental defects (57). Embryonic β1D knock-in stem cells displayed reduced migratory activity. Expression levels of the β1D subunit were reduced when compared to β1A in wt embryonic stem cells; this could indicate that, when associated with other subunits than muscle-specific α7, the β1D protein is less stable (57).

Thus, even though β1A and β1D only differ by 13 amino acids, this difference is enough to have drastic effects on the function of the integrin, and further studies are needed to determine the critical residues and the signaling pathways involved.

7. CONCLUSIONS

Integrin signaling is complex and far from being understood. Cytoplasmic splice variants of integrin subunits add an additional level of complexity in integrin signaling. Even though five splice variants for human integrin subunit β1 have been described, most likely only two of them (A and D) are proteins with physiological functions. For three other splice variants (β1B, β1C-1 and β1C-2) the available data do not convincingly support the proposed view of their roles as physiological negative regulator of cell adhesion (β1B) or tumour-suppressor protein (β1C). Instead, these variants share characteristics typical for products resulting of abberant pre-mRNA splicing.

8. ACKNOWLEDGEMENTS

I would like to thank Dr. Svineng for rendered assistance.

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Key Words: Integrin, integrin subunit beta1, splice variant, signaling, Alu element, Review

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