

cDNA Cloning of Mouse VLA-3 α Subunit

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Abstract cDNA clones for mouse VLA (very late antigen)-3 α subunit ($\alpha 3$ integrin) were isolated and sequenced. The encoded mouse $\alpha 3$ integrin subunit was composed of 1,053 amino acid residues. The results of sequence analysis revealed similar structural characteristics to other VLA α subunits. For example, the presence of a large extracellular domain including three putative metal binding sequences, a transmembrane domain, and a short cytoplasmic domain. A higher level of its message was detected in thymus than in kidney, stomach, spleen, liver, brain, or lung by Northern blotting analysis. © 1995 Wiley-Liss, Inc.

Key words: integrin, VLA (very late antigen), adhesion molecule, cDNA cloning, tissue distribution

Members of the integrin family play important roles in mediating cell-matrix and cell-cell interactions [Hynes et al., 1987]. Each member is composed of two subunits, α and β . Both of these subunits span the plasma membrane. The $\alpha 3\beta 1$ Integrin (VLA-3), a member of the $\beta 1$ integrin family, is proposed to function as a receptor for fibronectin, laminin, and collagens [Takada et al., 1988; Elices et al., 1991]. Several recent reports including ours have suggested that $\alpha 3\beta 1$ integrin plays a critical role in the intercellular adhesion events [Symington et al., 1993; Sriramarao et al., 1993; Takeuchi et al., 1994]. We have previously cloned the human $\alpha 3$ integrin cDNA from a human bladder carcinoma cell line cDNA library [Tsuji et al., 1991]. This human homologue of galactoprotein b3 seems to express on fibroblastic cells after oncogenic transformation at higher levels than before [Tsuji et al., 1990]. Enhanced expression of $\alpha 3\beta 1$ on transformed cells may be related to their oncogenic phenotypes [Saga et al., 1988; Plantefaber and Hynes, 1989]. However, $\alpha 3\beta 1$ levels have no apparent correlation with the malignant behavior of cancer cells according to other studies [Dedhar and Saulnier, 1990; Pignatelli et al., 1990]. Dedhar et al. [1993] reported that an invasive subpopulation of PC-3 human prostate carcinoma cell line showed lower level of $\alpha 3\beta 1$ integrin expression than parental cells.

In contrast, Natali et al. [1993] demonstrated that the levels of $\alpha 3\beta 1$ integrin on cutaneous malignant melanoma cells were positively correlated with the degree of dermal invasiveness. These studies suggest that this integrin plays crucial roles in the abnormal behavior of cancer cells. However, the regulation of and the function of $\alpha 3\beta 1$ integrin have poorly been understood. In the present paper, we have cloned and sequenced the cDNA for the α subunit of murine VLA-3, and analyzed its mRNA level in murine tissues. This is the first step toward the understanding of VLA-3 integrin regulation in mice and the generation of VLA-3 deficient mice.

MATERIALS AND METHODS

Cell Culture

SV-T2 Cells (SV-40 transformants of BALB/3T3 fibroblastic cells, ATCC CCL163.1; supplied by Japanese Cancer Research Resources Bank, Tokyo, Japan) were cultured in Dulbecco's modified minimum essential medium supplemented with 10% fetal bovine serum at 37°C under 5% CO₂.

RNA Preparation

Total RNA was prepared from SV-T2 cells or various mouse organs by the guanidine thiocyanate/CsCl gradient centrifugation method [Chirgwin et al., 1979]. Poly A⁺ RNA was isolated by two cycles of oligo (dT)-cellulose column chromatography.

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Construction and Screening of cDNA Library

cDNA was synthesized with SV-T2 from the poly A⁺ RNA (5 µg) as described above by the method of Gubler and Hoffman [1983] using the Pharmacia (Uppsala, Sweden) cDNA synthesis kit. cDNA was size-selected (> 1.5 kb) by electrophoresis on a 1% agarose gel, recovered from the gel using GeneClean (Bio 101, La Jolla, CA), and ligated to the dephosphorylated *Eco*RI arms of the λ gt10 vector. Ligated DNA was in vitro-packaged with a packaging extract (Gigapack, Stratagene, San Diego, CA). Recombinant phages were screened by hybridization with hamster galactoprotein b3 cDNA [Tsuji et al., 1990] that was labeled with ³²P dCTP by the random priming method [Feinberg and Vogelstein, 1983]. Hybridization was performed at 50°C for 24 h in 6 × NET, 10 × Denhardt's solution, 0.5% SDS. When 2 × 10⁵ recombinant phages were screened with this probe, five positive clones were obtained.

Subcloning

DNA from the phage clones was digested with *Eco*RI, and the inserts were subcloned into a pT7T3 18U plasmid (Pharmacia LKB Biotechnology) or a pBluescript SK(+) vector (Stratagene, San Diego, CA). After DNA-transformation of XL-1 Blue strain bacteria (Stratagene, San Diego, CA), the clones with inserts were screened by color selection with isopropyl-1-thio- β -D-galactopyranoside and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranosid.

DNA Sequencing

Dideoxynucleotide termination sequencing reactions [Sanger et al., 1977] were performed with single-stranded DNA produced from pT7T3 clones by superinfection with the helper phage M13KO7 [Vieira and Messing, 1987]. A set of deletions was prepared as previously described [Henikoff, 1984], and by the use of restriction sites. DNA sequencing was carried out with a Model 373A DNA sequencer (Applied Biosystems, Foster City, CA) using a Taq polymerase (Applied Biosystems, Foster City, CA) and an M13 universal primer.

Northern Hybridization

For Northern blotting analysis, 50 µg of total RNA were electrophoretically separated on 1% formaldehyde-agarose gels and transferred onto nylon membranes (Hybond-N, Amersham). The membrane filters were prehybridized in 50%

formamide, 5 × SSPE, 5 × Denhardt's solution, and 0.1% SDS at 42°C with a ³²P dCTP-random priming-labeled 600-bp DNA fragment obtained by digestion of mouse α 3 integrin cDNA clone 1 with *Eco*RI. The membrane filter was washed in 2 × SSC, 0.1% SDS at room temperature for 5 min twice, and then in 1 × SSC, 0.05% SDS at 68°C for 1 h. Autoradiography was performed with Kodak X-OMAT films (Rochester, NY).

RESULTS

cDNA Cloning of Mouse VLA-3 α Subunit

After screening of murine SV-T2 cells cDNA library (2 × 10⁵ independent phages) with a hamster galactoprotein b3 cDNA probe, it resulted that five positive clones were isolated. From the results of restriction mapping, these five clones were strongly suggested to align to each other as shown in Figure 1.

Characterization of cDNA Clones for Mouse VLA-3 α Subunit

The cDNA sequence and the deduced amino acid sequence in the coding region of mouse α 3 integrin are shown in Figure 2. The cDNA sequence was constructed from the five overlapping partial cDNA clones. We concluded that these clones represent murine α 3 integrin because i) these clones have a high sequence homology to the hamster galactoprotein b3 (93% of amino acids were identical) and the human α 3 integrin (88% of amino acids were identical), ii) all 19 Cys residues were located at matched positions to hamster and human α 3 integrins (Fig. 3), iii) Southern blotting analysis indicated that mouse α 3 integrin gene was a single copy gene [Tsuji et al., 1990], and iv) the length of the constructed cDNA sequence was in good agreement with that of a transcript of α 3 integrin (~4.8 kb) detected in Northern blotting analy-

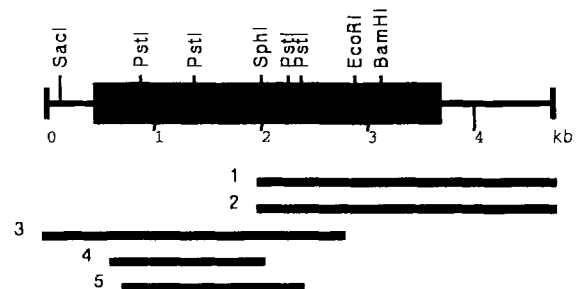


Fig. 1. Restriction map for five overlapping clones encoding mouse α 3 integrin. cDNA was cloned in five overlapping clones (1 to 5) to cover approximately 5 kb. Protein coding and untranslated regions are shown by a box and lines, respectively.

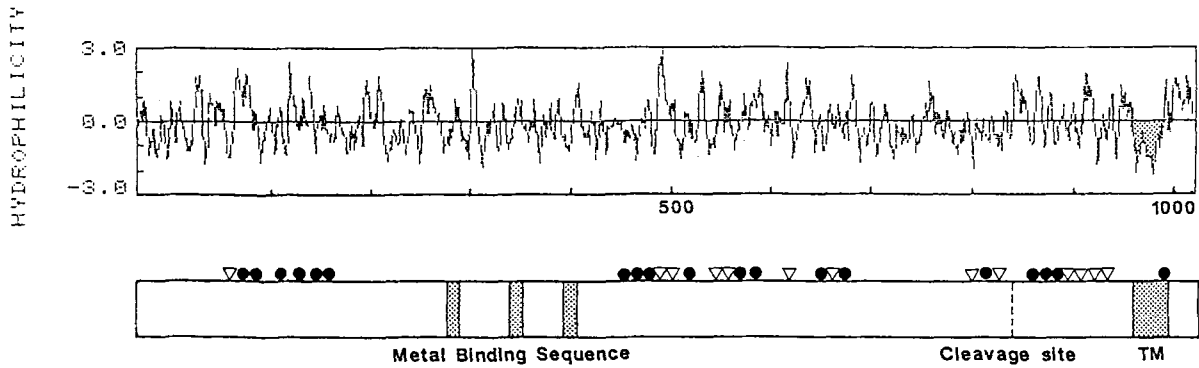


Fig. 4. Hydropathy profile and schematic representation of mouse VLA-3 α subunit. The hydropathy plot (upper panel) was derived from the predicted amino acid sequence of mouse VLA-3 α subunit by the program of Kyte and Doolittle [1982] with a window size of seven. The schematic representation of

mouse VLA-3 α subunit structure (lower panel) includes the positions of Cys residues (\bullet), potential N-linked glycosylation sites (∇), the putative transmembrane (TM), the possible cleavage site (broken line), and putative divalent cation-binding sites (stippled boxes).

near the C-terminus. This hydrophobic amino acid-cluster was likely to be a transmembrane domain. Since all 13 potential carbohydrate attachment sites were exclusively located on the N-terminal side of this putative transmembrane domain, the N-terminal 961 amino acid segment was probably located an extracellular domain and a short C-terminal 32 amino acid segment was probably a cytoplasmic domain. At approximately one third of the distance from the N-terminus, three homologous sequence motifs with the consensus sequence of D-D/N-D/NG-D were found. This sequence was known to be present in a variety of Ca^{2+} and Mg^{2+} binding proteins [Hynes, 1992]. This sequence also had a putative cleavage site, which might generate a heavy chain and a light chain. These structural characteristics of mouse $\alpha 3$ integrin were similar to hamster and human counterparts. Therefore, we concluded that the amino acid sequence of $\alpha 3$ integrin was highly conserved in the three species.

Distribution of $\alpha 3$ Integrin mRNA Among Murine Organs

The distribution of $\alpha 3$ integrin mRNA among various murine organs was examined by Northern blot hybridization using a mouse $\alpha 3$ integrin cDNA probe. As shown in Figure 5, hybridization at the position corresponding to that of $\alpha 3$ chain mRNA was seen. Higher intensity of $\alpha 3$ integrin cDNA binding was detected to total RNA from thymus than that from kidney, stomach, spleen, liver, brain, or lung. The same blotted membrane was tested for hybridization of a cDNA probe for β -actin. Very similar levels of β -actin signals were detected in these seven

lanes (data not shown). An additional component, which was smaller in size than the major component, was identified in thymus RNA.

DISCUSSION

We cloned cDNA for $\alpha 3$ integrin from a cDNA library derived from SV-40 transformed murine fibroblasts. The structure of murine $\alpha 3$ integrin deduced from nucleotide sequence of cDNA was similar to hamster and human counterparts, i.e., the number of amino acid residues (1,053 for mouse and 1,052 for hamster and human), the presence of a putative transmembrane domain near the C-terminus, a large extracellular domain with potential N-glycosylation sites and a short cytoplasmic domain, the presence of three tandem repeats of metal binding sequence motif, and a putative cleavage site to form a heavy and a light chains, which were thought to be specifically found in integrins $\alpha 3$, $\alpha 5$, $\alpha 6$, and $\alpha \text{II}\beta$ [Hemler, 1990]. The over all nucleotide and amino acid sequence was highly conserved among three species (hamster, human, and mouse).

The Northern blotting analysis revealed that thymus contained a higher level of the $\alpha 3$ transcript than other organs tested in this study. The mRNA expression was also detected in kidney and lung at lower levels, but that in other organs such as stomach, spleen, liver, and brain was undetectable. Fradet et al. [1984] investigated the localization of $\alpha 3$ integrin (mAb J143 epitope) by the immunofluorescence technique and found that its distribution in normal tissues in humans was restricted to kidney, thyroid, urothelium, skin, and esophagus. They did not detected the binding of this mAb to the sections

of thymus. However, our Northern analysis showed that mRNA for $\alpha 3$ integrin in thymus was more abundant than in kidney in mice. The apparent contradiction might be due to the different methods used in these experiments. Alternatively, this molecule in these organs may be localized in a very specific location. The presence of $\alpha 3$ integrin mRNA in a large quantity in thymus suggested a possible function of this integrin in the immune system. It should also be noted that Giunta et al. [1991] have suggested that VLA-3 was involved in thymocyte-thymic epithelial cell interactions. Fradet et al. [1984] also pointed out that $\alpha 3$ integrin was strongly expressed on a variety of cultured tumor cells, though its distribution in normal tissues was restricted.

We previously reported that a cell surface glycoprotein (galactoprotein b3) showing enhanced expression upon oncogenic transformation of human, murine, and hamster fibroblasts by SV-40 or polyoma virus was identified as $\alpha 3$ integrin through cDNA cloning [Tsuji et al., 1990, 1991]. Recently Natali et al. [1993] investigated the integrin expression in cutaneous malignant melanoma. They demonstrated that, while only low levels of $\alpha 3\beta 1$ integrin were detectable in benign lesions, this integrin was highly expressed in primary tumors and the levels correlated with the degree of dermal invasiveness. They also found that high levels of $\alpha 3$ integrin were expressed in metastatic lesions. Thus, the tumor progression in malignant melanoma seemed to be associated with changes in integrin phenotypes including $\alpha 3\beta 1$ integrin.

Based on the inhibition experiments of cell adhesion by $\alpha 3$ subunit-specific monoclonal antibodies (mAbs), $\alpha 3\beta 1$ integrin was claimed to function as a promiscuous adhesion receptor for multiple ECM proteins, e.g., fibronectin, laminin, collagen type I and IV, epiligrin, and entactin [Elices et al., 1991; Wayner and Carter, 1987; Carter et al., 1991; Dedhar et al., 1992]. In addition, it was recently shown that $\alpha 3\beta 1$ integrin was involved in the intercellular adhesion of keratinocytes mediated by its interaction with $\alpha 2\beta 1$ integrin [Symington et al., 1993], although their conclusions have been the subject of dispute. Other experiments also showed that $\alpha 3\beta 1$ integrin interacted homotypically with other $\alpha 3\beta 1$ molecule [Sriramarao et al., 1993]. Recently, Weitzman et al. [1993] and Takeuchi et al. [1994] demonstrated that anti- $\alpha 3$ integrin monoclonal antibodies induced homotypic cell

aggregation of $\alpha 3$ integrin-expressing cells in a cation-independent manner. In our observation, $\alpha 3\beta 1$ integrin molecules were found to be localized at the cell contact sites on these cells, suggesting direct involvement of this molecule in the adhesion. Furthermore, $\alpha 3$ integrin-expressing cells treated with the antibodies also adhered to $\alpha 3$ integrin-nonexpressing cells. These results suggested additional roles of $\alpha 3\beta 1$ integrin in intercellular adhesion events, besides its interaction with a basement membrane component called epiligrin [Carter et al., 1991] or kalinin [Rousselle and Aumailley, 1994]. Thus, the nature of counter ligands for VLA-3 integrin remains to be addressed. The cDNA clones for mouse $\alpha 3$ integrin would be a useful tool to elucidate the function of VLA-3 in tumor cells and normal tissues.

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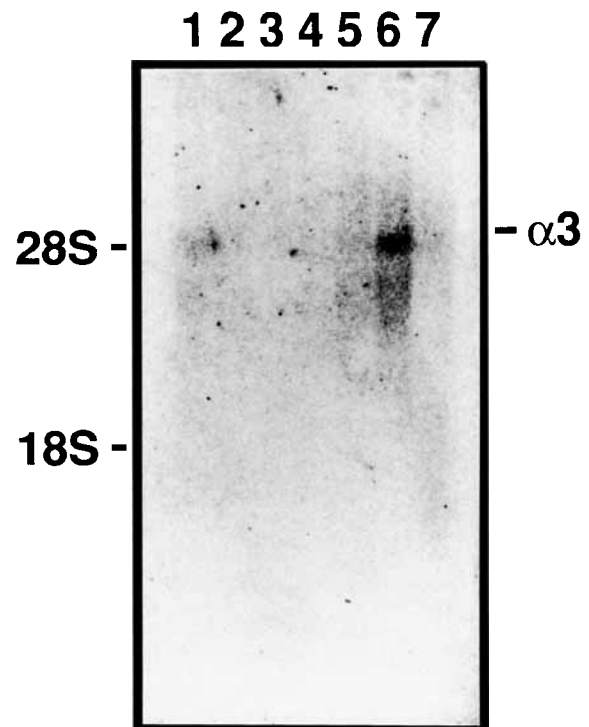


Fig. 5. Northern hybridization analysis for VLA-3 α subunit mRNA in various tissues. Total RNAs (50 μ g) from several murine tissues were electrophoretically separated on a 1% formaldehyde-agarose gel and transferred onto a nylon membrane. The membrane filter was probed with a 32 P-random priming-labeled 600-bp DNA fragment obtained by the digestion of mouse $\alpha 3$ integrin cDNA clone 1 with *Eco*RI. Lane 1, kidney; lane 2, spleen; lane 3, liver; lane 4, stomach; lane 5, lung; lane 6, thymus; lane 7, brain.

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