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Adhesiveness of β5 integrin variant lacking FNK$^{767-769}$ is similar to that of the prototype containing FNKFNK$^{764-769}$

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Abstract

Little is known about the functions of two different β5 integrins: repeated-FNK (FNKFNK$^{764-769}$) and single-FNK (FNK$^{764-766}$) amino acid sequences in the cytoplasmic domain. We examined whether they occurred as germ line mutations or somatic mutations associated with neoplastic transformation, and whether there were functional alterations. Out of six cultured cell lines, only KATO-III cells had the single-FNK β5 sequence. The single-FNK β5 was found in 9 out of 79 patients with colon carcinoma, but no somatic mutations were detected in cancerous tissues. CHO cells were transformed with expression vectors containing single-FNK or repeated-FNK β5 cDNA, which were derived from KATO-III cells. CHO cells transfected with single-FNK and repeated-FNK showed similar adhesiveness to, and proliferative activity on, vitronectin substrates.
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Keywords: β5 Integrin; Adhesion; Vitronectin

1. Introduction

Members of the integrin family are characterised by their ability to dynamically regulate their ligand binding affinity; integrin-mediated cell anchorage to the extracellular matrix has also been shown to induce signal transduction pathways (Hynes, 1992; Guadagno et al., 1993; Juliano and Haskill, 1993). The cytoplasmic sequences of the integrins appear to be involved in signalling events (O'Toole et al., 1994), and experimental studies have revealed a crucial role of the cytoplasmic domains in maintaining the proteins' functions (Stephens et al., 1993). In addition, it was reported that alternative splicing variants of β subunits in α6, β1, β3 and αIIb usually occur in cytoplasmic domains, with many of the variants being functionally inactive.

The αvβ5 integrin is expressed by most epithelial cells (Pasqualini et al., 1993). Its function differs from that of the major vitronectin receptor, αvβ3, and the cytoplasmic domain of β5 can transduce adhesion information to subsequent signalling pathways (Leavesly et al., 1992; Pasqualini and Hemler, 1994). The cDNA sequence of the β5 subunit from the UCLA-P3 human lung carcinoma cell line (McLean et al., 1990), and that obtained from a thymic epithelial cDNA library (Ramaswamy and Hemler, 1990) have a repeated-FNK sequence (FNKFNK$^{764-769}$) in the cytoplasmic

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domain. Moreover, a different β5 sequence has been reported by Suzuki et al. (1990) in an uncharacterised cell line in which $FNK^{767-769}$ was deleted, resulting in a single FNK sequence. Although Ramaswamy and Hemler (1990) suggested that the repeated FNK sequence was the prototype of β5, they identified five out of eight clones in a λgt11 thymic epithelial cDNA library that lacked the second FNK. In addition, other types of heterogeneity were found at positions 336–338, 379–381 and 708. These findings indicate molecular polymorphism of the β5 integrin subunit, whereas the cytoplasmic domains of other integrin subunits are highly conserved, except for alternative splicing. However, nothing is known about the significance of the polymorphism of β5.

In this report, we examine the incidence of the variant type β5 integrin containing a single FNK sequence in colon tissue, and examine whether it occurs as a somatic mutation in carcinoma tissue. Furthermore, we have tried to clarify whether it has functional alterations. Here, we report that adhesiveness and proliferative activity of the variant type, which appears in 10% of the population, are similar to those of the prototype β5 integrin.

2. Materials and methods

2.1. Carcinoma cell lines

A variety of cell lines, including the oral squamous carcinoma lines OSC-19 and HSC-3 (Kawahara et al., 1995), the lung carcinoma lines Calu-1, VMRC-LCP, ACC-LC-73 (Suzuki et al., 1993) and LU65, and the gastric carcinoma cell line KATO-III (Sekiguchi et al., 1978), were used. Sub-confluent cultures were used to examine mRNA expression. All cell lines were maintained in Eagle's minimum essential medium containing 10% foetal bovine serum (Gibco BRL, Rockville, MD, USA). HSC-3, VMRC-LCP, LU65 and KATO-III were obtained from the Japanese Cancer Resource Bank, and ACC-LC-73 and Calu-1 were provided by Dr. T. Takahashi (Aichi Cancer Institute, Japan).

2.2. Reverse transcription–polymerase chain reaction (RT–PCR)

Total cellular RNA was extracted by the guanidinium isothiocyanate method (Chomozynski and Sacchi, 1987) using a Total RNA Separator Kit (Clontech Laboratories, Inc., Palo Alto, CA, USA). Poly (A^+) mRNA was obtained from the cultured carcinoma cells using an oligo-dT column (Poly (A) Quick Kit, Stratagene, La Jolla, CA, USA). First-strand cDNA was synthesised using AMV reverse transcriptase (Takara Bio, Ohtsu, Japan). The resulting cDNA was used for PCR amplification of an intracytoplasmic domain of β5 integrins, using primer pairs that amplify a 219-bp fragment encoding the cytoplasmic region of β5, i.e., B5VNR5, 5′-^{2514}TGTGGTCGGTAGCATCCTCC^{2533}-3′ and B5VNR3, 5′-^{2765}CCCGCTCCAGCCCCTCG GAG^{2746}-3′, according to the sequence of Hemler et al. (EMBL/GenBank/DDBJ, accession no. X53002). The PCR conditions were as follows: 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were electrophoresed on agarose gels, which were stained with ethidium bromide before or after electrophoresis. *Hae*III fragments of ϕX174 replicative form DNA were used as molecular size markers (Toyobo, Osaka, Japan).

2.3. PCR/single-strand conformation polymorphism (SSCP) analysis

SSCP analysis was performed as described previously (Akasofu and Oda, 1995). DNA was subjected to PCR using a set of primers, B5VNR5 and B5VNR3, labelled with [γ-^{32}P]ATP (7000 Ci/mmol, 160 mCi/ml, ICN, Costa Mesa, CA, USA) by the polynucleotide kinase reaction (Takara). The PCR products in formamide were heated to 80 °C for 5 min, and then loaded onto gels. Polyacrylamide gel electrophoresis was performed at 40 W for 3 h at 4 °C. The gels were dried on filter paper and exposed to X-ray film at −80 °C for 1–12 h with an intensifying screen.

2.4. S1 nuclease digestion

After electrophoresis, bands were cut out of the agarose gels and the DNA was extracted using filtration tubes (Suprec-01, Takara). The purified samples were incubated for 30 min at 37 °C with 750 U of S1 nuclease (Takara) in 200 μl of S1 nuclease solution (30 mM sodium acetate, pH 4.6, 280 mM NaCl and 1 mM $ZnSO_4$). After ethanol precipitation, the samples were electrophoresed in 2% agarose gels, and then stained with ethidium bromide.

2.5. Direct sequencing

After PCR and electrophoresis on agarose gels, the bands were cut out and extracted using filtration tubes. The amplified strands were labelled with fluorescein by the dideoxynucleotide chain termination method using a DyeDeoxy Terminator Cycling Kit (Perkin–Elmer, Foster City, CA, USA). DNAs were analysed with an autosequencer and a 373 DNA sequence analyser (Perkin–Elmer).

2.6. TA cloning

The PCR products were subcloned into vectors by the TA cloning method (Clark, 1988) using a TA cloning kit

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