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# ANALYSIS OF INTEGRIN mRNA IN HUMAN AND RODENT TUMOR CELLS

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SUMMARY. Several human and rodent tumor cell lines were examined for the presence of integrin mRNAs by dot- and Northern- blot analysis. All tumor cells tested expressed mRNAs for $\alpha 5$, $\alpha IIb$, $\beta 1$ and $\beta 3$. The mRNA of $\beta 2$ integrin was not detectable and that of $\alpha_V$ integrin was found only in certain cells. Northern blotting was carried out in three selected tumor cell lines: Clone A, HEL and B16a. An apparent difference in the mRNA species coding for the $\alpha IIb\beta 3$ integrin, but not for $\alpha 5$ and $\beta 1$, was found. Our result suggests that alternative splicing of integrin genes may be one of the important mechanisms in regulating $\alpha IIb\beta 3$ expression and function in different tumor cells. 

Integrins are a family of $\alpha$ and $\beta$ subunit glycoprotein heterodimers serving as receptors for extracellular matrix proteins (1). More than 16 distinct noncovalent $\alpha/\beta$ heterodimers have been found (2). Although it has been shown recently that an $\alpha$ subunit may form dimers with different $\beta$ subunits, the integrin superfamily was originally divided into three subfamilies according to a common $\beta$ subunit ($\beta 1$, $\beta 2$ or $\beta 3$) in association with one of the $\alpha$ subunits: the VLA protein subfamily ($\alpha\beta 1$ heterodimers), the leu-CAM subfamily ($\alpha\beta 2$ heterodimers) and the cytoadhesin subfamily ($\alpha\beta 3$ heterodimers) (3-5). These cell surface receptors play an important role in cell-cell and cell-extracellular matrix interactions during processes such as embryogenesis, wound healing, thrombosis, immune reactions and tumor progression (1-6). Most of the cDNAs coding for integrins have been cloned, sequenced and genes have been localized to chromosomes (6). However, the integrin gene expression and regulation in different cells, especially in tumor cells, and the mechanism by which the integrins are involved in tumor cell metastasis is still poorly understood. In our earlier studies, we demonstrated the

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presence of $\alpha_{IIb}\beta_3$ or an immunologically related glycoprotein (originally termed IRGpIIb/IIIa) in tumor cells and its participation in stimulated tumor cell adhesion to subendothelial matrix or endothelial cells, in tumor cell induced platelet aggregation, and therefore its possible role in tumor cell metastasis (7-9). Recently, we showed that the authentic $\alpha_{IIb}\beta_3$ genes were functionally expressed in B16a cells by Northern blot and immunoprecipitation (10). Here, we extend our study to other integrins and different tumor cell lines. The expression of integrin mRNA for the VLA protein ($\alpha_5$ & $\beta_1$), the leu-CAM protein ($\beta_2$) and the cytoadhesin ($\alpha_v$, $\alpha_{IIb}$ & $\beta_3$) was examined in a group of tumor cells from different species (human & rodent) and origins (leukemia, adenocarcinoma, carcinosarcoma & melanoma). In particular, the expression of $\alpha_{IIb}\beta_3$ mRNA was analyzed in three selected tumor cells by Northern blot. The results are consistent with and extended our previous studies (7-10). In addition, a different mRNA splicing pattern of $\alpha_{IIb}\beta_3$ integrin in different tumor cells (leukemia, adenocarcinoma & melanoma) is described.

## MATERIALS AND METHODS

Cell culture Human erythroleukemia cell (HEL), human cervical carcinoma cell (MS751), human colon adenocarcinoma cell line (Clone A), murine amelanotic melanoma cell line (B16a) and rat Walker carcinosarcoma cell line (W256) were obtained and cultured as described before (7,8,10).
Total RNA and poly(A)+ RNA isolation Total RNA was isolated by the guanidium thiocyanate/cesium chloride method and poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (10).
RNA dot and Northern blotting analysis 1-5 $\mu$g of poly(A)$^+$ RNA was denatured by the glyoxal method and was either loaded onto a 1% agarose gel for Northern blot (10) or spotted directly onto membrane which has been prewashed with $H_2O$, 20x SSC (3 M NaCl, 0.3 M NaCitrate, pH 7). The blot was hybridized, washed and exposed as described in Ref.10.

## RESULTS AND DISCUSSION

Distribution of integrins in tumor cells These cell surface receptors were originally studied in different areas, e.g. thrombosis, embryogenesis and lymphocyte immunology. Since the realization that this family of integral glycoproteins have a common structure, similar functions, homologous sequences and are highly conserved during evolution, new information concerning these glycoproteins is appearing at a rapid pace. The tissue and cell distribution of different integrins has been studied in several laboratories by immunostaining and immunoprecipitation techniques (3,6, 11-13). In our laboratory, we have investigated the presence of the $\alpha_{IIb}$ $\beta_3$ receptor, a prominent platelet integrin, in a series of tumor cells. A wide distribution of the $\alpha_{IIb}\beta_3$ complex was found (7-9, unpublished).

Table 1
DISTRIBUTION OF INTEGRINS IN TUMOR CELLS

| | Integrins | | | | | |
|---|---|---|---|---|---|---|
| Cells | $\alpha_5$ | $\alpha_V$ | $\alpha_{IIb}$ | $\beta_1$ | $\beta_2$ | $\beta_3$ |
| HEL | + | ± | + | + | − | + |
| MS751 | + | + | + | + | − | + |
| Clone A | + | + | + | + | − | + |
| B16 a | + | − | + | + | − | + |
| W256 | + | + | + | + | − | + |

1 μg mRNA from each cell line were analyzed by bot blotting. +: positive signal was obtained after hybridization and exposing to X-ray film for 24 hr with intensifying screen. ±: detectable in some HEL clones. -: no signal was obtained after 4 days exposure with intensifying screen.

However, it is known that there are highly homologous regions among the integrin α subunits (50-70%) and β subunits (50-75%) in amino acid sequence (14). Therefore crossreaction of the anti-$\alpha_{IIb}\beta_3$ antibodies, used in our previous studies, with other integrin proteins was a possibility. Thus, we previously named the protein "immunologically related $\alpha_{IIb}\beta_3$ glycoprotein", i.e. IRGpIIb/IIIa. In this study, we employed the Northern blot technique with integrin subunit-specific probes to determine the distribution of the various α and β subunits among tumor cells of different species and origins. The specificity of integrin probes was insured by dot blot integrin cDNA to membranes followed by hybridization with random primer labeled probes. No crosshybridization was found (data not shown). Under this condition, the expression of integrin mRNAs for $\alpha_V$, $\alpha_5$, $\alpha_{IIb}$, $\beta_1$, $\beta_2$ and $\beta_3$ was analyzed in human HEL, Clone A, MS751 and rodent B16a, W256 tumor cells by dot blot. The mRNAs for $\alpha_5$, $\alpha_{IIb}$, $\beta_1$ and $\beta_3$ were detectable in all tumor cell lines, but the $\beta_2$ transcript was absent (Table1). The result for $\alpha_5$, $\beta_1$ and $\beta_2$ was in agreement with published data (3,6,11-13), in that the $\alpha_5\beta_1$ integrin is widely distributed in normal cells and tumor cell lines, and that the $\beta_2$ integrin was confined to leukocytes. The existence of the authentic $\alpha_{IIb}\beta_3$ mRNAs in these tumor cells confirmed our earlier results using immunofluorescent staining with $\alpha_{IIb}\beta_3$ specific antibodies. The $\alpha_V$ mRNA was detected in Clone A, MS751 and W256 tumor cells but not in murine B16a cells (Table1). It is reported that the $\alpha_V$ protein was widespread among different tumor cells (11) and absent in lymphocytes (6, 11). Albeda et al (3,12) have shown the presence of $\alpha_V$ protein in human melanoma cells by immunoprecipitation. However, in our case

using the murine B16a cell, the $\alpha_V$ mRNA was not detectable (Table1), although genomic DNA Southern blot revealed the presence of the $\alpha_V$ gene (unpublished). Suzuki et al (15) have detected a trace level of $\alpha_V$ mRNA in HEL cells. Yet, under our conditions, the transcript of $\alpha_V$ was not consistently detectable in different HEL clones. Since synthesis and cell surface expression of integrins are subject to cellular regulations, it is not surprising that different cells or even the same cell under different physiological conditions may express this receptor differently. For example, when T cells were cultured from 3 days to 6 months, a change in VLA protein expression was observed (6).

Alternative splicing of $\alpha_{IIb}$ and $\beta_3$ integrins in tumor cells Integrins are cell surface receptor glycoproteins containing extracellular, transmembrane and cytoplasmic domains (1). The extracellular domain binds to adhesive proteins such as fibronectin and vitronectin, in many cases via the RGD motif in the ligand (16). The cytoplasmic domain is connected to the cytoskeleton and mediates the extracellular matrix-cytoskeleton interactions (17). Recently, van Kuppevelt et al (18) demonstrated the presence of an alternative spliced $\beta_3$ mRNA in osteogenic sarcoma MG63, placental cells and HEL cells resulting in a variant $\beta_3$ with a different cytoplasmic domain. In our previous study (10) and this study, a band of 6.6 kb corresponds to the $\beta_3$ mRNA and a band of 3.3 kb corresponds to the variant $\beta_3$ mRNA were found in HEL cells. Moreover, a band of 4.8 kb was detected simultaneously, and may represent another variant $\beta_3$ subunit. In the experiments of van Kuppevelt et al (18) and ours (10, this study) both $\beta_3$ and variant $\beta_3$ were found in HEL cells. Interestingly, only the 6.6 kb $\beta_3$ was found in B16a cells and only the variant $\beta_3$ (3.3 kb & 4.8 kb) were detectable in clone A cells. Similarly, alternative splicing of $\alpha_{IIb}$ integrin was also observed among different tumor cells. The HEL cells expressed a major 3.9 kb transcript and a minor 4.4 kb transcript, the B16a cells expressed both messengers (3.9 & 4.4 kb) at a similar level, and the clone A cells expressed only the 4.4 kb mRNA (Fig.1). This is the first evidence showing the possibility of an alternative splicing of $\alpha_{IIb}$ integrin. Further experiments in this direction are underway.

Study of the distribution and expression of integrins in cells and tissues is important not only because of the pivotal role these receptors play in normal cell-cell and cell-extracellular matrix interactions, but also their critical role in tumor cell dissemination (2). It has been shown that the variant $\beta_3$ (probably translated from 3.3 kb mRNA) has a different cytoplasmic domain lacking the tyrosine phosphorylation sequence and losing three threonine residues within that fragment (18). Although unlike the $\beta_1$ subunit which can be phosphorylated at a tyrosine residue (19), the $\beta_3$ subunit was phosphorylated predominantly on threonine

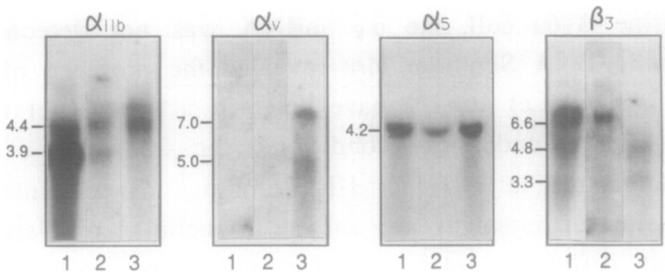


Fig. 1. Northern blot analysis of integrin mRNAs. 5 μg mRNA of each cell line were denatured with glyoxal, migrated through a 1% agarose gel, transferred and hybridized with $\alpha IIb$, $\alpha 5$, $\alpha v$ or $\beta 3$ cDNA probe respectively. The numbers are estimated molecular weight. 1: HEL cell, 2: B16a cell, 3: Clone A cell.

residues in the human platelet and this phosphorylation was linked to $\alpha IIb\beta 3$ functions (20). The finding of differential splicing of $\alpha IIb\beta 3$ integrin mRNAs in tumor cells is intriguing. This might result in a variant $\beta 3$ in an activated form not subject to phosphorylation regulation. Furthermore, not only the function of the $\alpha IIb\beta 3$ protein itself is subject to the phosphorylation regulation, but also the $\alpha IIb\beta 3$ integrin can regulate the phosphorylation of other cellular proteins (21). The preferential expression of the large mRNA (4.4 kb) of $\alpha IIb$ subunit in solid tumors in addition to the variant expression of the $\beta 3$ subunit may enable tumor cells to form unique $\alpha\beta$ heterodimer(s) which may participate in the process of malignant transformation and metastasis.

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