Expression level of integrin α5 on tumour cells affects the rate of metastasis to the kidney

N Tani1,2, S Higashiyama2, N Kawaguchi2, J Madarame2, I Ota2, Y Ito2, Y Ohoka2, S Shiosaka1, Y Takada2 and N Matsuura2

1Division of Structural Cell Biology, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-0101, Japan; 2Department of Pathology, School of Allied Health Science, Faculty of Medicine, Osaka University, 1-7 Yamada-oka, Suita, Osaka 565-0817, Japan; 3Department of Vascular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Tumour metastasis is known clinically to have organ specificity. We hypothesised that integrins might be involved in determining the organ specificity of tumour metastasis. Here, we report the results of spontaneous metastasis tested in nude mice that were inoculated with Chinese hamster ovary (CHO) cells expressing integrin α5β1 at various levels. The growth of the primary tumour inversely correlated with the α5 expression level on CHO cells, which is consistent with a previous report (Scheiner et al., 1991). The rates of pulmonary, lymph node, and adrenal metastases that developed in nude mice were not related to changes of the α5 expression level on CHO cells. Kidney metastasis developed in 40% of nude mice inoculated with α5B2 cells (CHO cells overexpressing α5) and in 20% of mice with CHO-K1 cells (CHO cells expressing native α5), whereas inoculation with CHO-B2 cells (α5-defective mutants) and α5CHO cells with the highest expression of α5 did not lead to development of kidney metastasis. Furthermore, α5CHO, which shows the slowest growth of these cell types, did not lead to primary tumours in nude mice. These findings suggest that there is an appropriate level of α5 expression on tumour cells that leads to metastasis. Microscopic observations revealed that micrometastasis in the kidney was formed in glomeruli. An adhesion assay using frozen sections of the kidney demonstrated that α5B2 cells, but not CHO-B2 cells, effectively adhered to glomeruli. Kidney metastasis in vivo and the adhesion of α5B2 to glomeruli shown ex vivo were significantly suppressed by the administration of GRGDSP peptide. Finally, we conclude that the interaction of α5β1 on tumour cells with fibronectin in kidney glomeruli is involved in kidney metastasis and that the tumour has appropriate levels of integrins crucial for metastasis.

Keywords: tumour metastasis; α5β1 integrin; kidney; CHO-K1

INTRODUCTION

Clinically, it is well known that tumour metastasis has organ specificity. Selectivity of the organs to which the tumour metastasises is thought to be affected by interactions between the tumour and molecules surrounding it (seed and soil theory) (Paget, 1898), and the direction of blood flow (mechanical theory) (Ewing, 1928). The process of tumour metastasis consists of detachment of cells from the primary tumour, invasion of extracellular matrix (ECM), intra- and extravasation, and growth at the secondary site. Numerous data have been reported to support the seed and soil theory as well as the mechanical theory (Buck and Zetter, 1984). Tumour cells that metastasised selectively to a specific organ showed intense adhesion to the endothelial cells derived from this organ (Nicolson, 1988; Pauli and Lee, 1988). Furthermore, an adhesion molecule designated Lu-ECAM-1 is specifically expressed on endothelial cells derived from the lung but not from other organs (Zhu et al., 1991). These findings suggest that there might be specific molecules or proportions of proteins in endothelial cells or ECM proteins in organs, that determine the metastatic sites of tumour cells. Therefore, adhesion molecules appear to be crucial for mediating the tumour–endothelial or tumour–ECM interactions.

Integrins are transmembrane glycoproteins that consist of α and β subunits and mediate cell–matrix and cell–cell adhesions (Hynes, 1992). Varieties of α and β subunits produce ligand selectivity to ECM (Albelda and Buck, 1990). To elucidate the role of integrins in tumour progression leading to metastasis, integrin expression levels have been compared in normal and tumour cells, or in primary and secondary sites in cancer patients using polymerase chain reaction (PCR), in situ hybridization, or immunohistochemical techniques.

Changes of integrin expression or its localisation in transformed cells appear to contribute either positively or negatively to the transformed cell phenotype. For instance, α5β1 integrin is a key molecule in the invasion at least of melanoma, osteosarcoma, and glioblastoma cells, while αVβ3 integrin also appears to be associated with increased invasiveness (Ruoslahti, 1992). The localisation of the fibronectin receptor on the cell surface is altered in the malignant transformation of cultured human cells in comparison with normal cells (Akiyama et al., 1990). In sarcoma
Kidney metastasis enhanced by α5 expression on tumour cells

N Tani et al

virus transformation of several rodent cell lines, α5β1 integrin disappears from the cell surface, whereas α3β1 levels remain constant (Heino, 1993). In melanoma, alterations of α2β1, α3β1, and α6β1 integrins appear to be associated with malignancy (Chen et al, 1991; Natali et al, 1991,1993; Moretti et al, 1993). In breast cancer, α2β1, α5β1, or α6β4 integrins seem to be crucial for malignant transformation (Pignatelli et al, 1991; Natali et al, 1992; Zutter et al, 1993; Weaver et al, 1997). In pancreatic carcinoma cell lines, expression in the bone marrow (Matsuura et al, 1996). Here, we constructed CHO transfectants expressing α5β1 at various levels and examined metastatic sites in nude mice. In this study, we especially focused on the relation between the change in α5β1 levels on tumour cells and rates of metastasis.

In another strategy, tumour cells or mice with genetic changes of integrin expression have been used for analysing the effects of integrin on tumourigenesis and organ-preferrential metastasis. Rhabdomyosarcoma (RD) cells transfected with cDNA encoding the α2 subunit enhanced metastasis in nude mice, demonstrating the specific effect of α2β1 integrin on metastasis (Chan et al, 1991). CHO cells transfected with cDNA encoding signal peptide integrin α2b, whereas α7 integrins are detected in adenocarcinomas and ampullary tumours, in the normal pancreas, reduced levels of expression occur, or in the case of some integrins there is no expression (Hall et al, 1991). These strategies are beneficial in that integrin expression is directly detected on tissues from patients or tumour cell lines.

MATERIALS AND METHODS

Cells and culture conditions

CHO-K1 cells (ATCC, VA, USA) were used as parent cells in this study. CHO-B2 cells are α5-defective mutants of CHO-K1 cells, that exclusively express α5β1 integrin (Schreiner et al, 1989), and their transfectants were designated as α5β2 and α5CHO cells, respectively. A pBJ-1 vector carrying α5 cDNA driven by the SV40 promoter (Takebe et al, 1988) was transfected into CHO-K1 or CHO-B2 cells. Cells expressing α5 were selected by flow cytometric sorting and by resistance to geneticin (G418). α5B2F187A cells are transfectants of CHO-B2 cells with mutant α5 cDNA containing an alanine substitution of phenylalanine 187 (Irie et al, 1995). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Nihonseiyaku, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS, Dainippon Pharmaceutical Co, Ltd, Tokyo, Japan) and 50 U ml−1 of penicillin plus 50 μg ml−1 of streptomycin (Life Technologies, Frederick, MD, USA) in 10% CO2.

Flow cytometric analysis

Cells in DMEM supplemented with 1% FBS and 0.03% sodium azide were incubated with a monoclonal antibody against human/hamster α5 (KH72) for 30 min at 4°C. After washing with DMEM as described above, the cells were incubated with FITC-conjugated mouse IgG (DAKO, Copenhagen, Denmark) for 30 min at 4°C. After washing, cells were resuspended with the same DMEM and analysed by FACS Calibur (BD Biosciences, CA, USA).

Spontaneous metastasis model in nude mice

Nude mice (BALB/cAnNCrj-nu/nu, 4 weeks, female) were subcutaneously injected with 1 × 106 cells of parental, mutant, or transfectant CHO cells. Subcutaneous tumours were measured with a dial-caliper twice a week and volumes were determined using the formula width3 × length × 0.52. Each volume value indicates the mean ± standard error (s.e.). Primary tumours were removed surgically at a size of 2500–3500 mm3 to prevent mice from dying. The mice were killed and metastatic sites were examined 7–8 weeks after inoculation. To investigate whether kidney metastasis was caused by adhesion of α5β1 on tumour cells to fibronectin, the effects of intravenous tail vein injection of GRGD or GRGSE peptide (20 mg kg−1 in 50 μl each) in PBS had been tested every other day starting from day 5 for 7 weeks.

Histological analysis

Kidney and lung were excised and fixed with formalin. Horizontal sections (6 μm thick) were prepared from the centre of the tumour in paraffin-embedded tissue. The sections were stained with haematoxylin–eosin and metastatic sites were examined by microscopy.

Adhesion assay

A total of 96-well plastic plates were coated with 10 μg ml−1 of fibronectin in phosphate-buffered saline (PBS) for 2 h at 37°C and then treated with 3% bovine serum albumin (BSA) for 1 h at 37°C, or were coated with only BSA for negative control. The cells (5 × 104 cells well−1) in serum-free DMEM containing 0.1% BSA were added and incubated for 1 h at 37°C. After removal of the medium, a 0.04% crystal violet solution was added and incubation was conducted for 10 min at room temperature. The wells were washed three times with PBS and 20 μl of Triton X-100 was added for permeabilisation. After addition of distilled water to 100 μl, the optical density was measured at 550 nm. The value indicates the mean ± s.e. for triplicate data representing three independent experiments.

Spreading assay

Cells (5 × 104 cells well−1) in serum-free DMEM containing 0.1% BSA were added to plates coated with 10 μg ml−1 of fibronectin (96 wells), and incubated for 3 h at 37°C. The cells were photographed on slide film under microscopy and the images on the film were analysed by NIH Image (National Institutes of Health, Bethesda, MD, USA). The spreading area of each cell type was calculated. The value indicates the mean ± s.e. for 20 cells randomly sampled.

Migration assay

Cell migration to soluble fibronectin was assessed by multiwell Boyden chambers (Neuro Probe, Gaithersburg, MD, USA). Polycarbonate filters (pore size of 8 μm) were coated with 10 μg ml−1 of fibronectin (Asahi Techno Glass, Tokyo, Japan) for 30 min at room temperature and air-dried. The lower part of the chamber was filled with 10 μg ml−1 of fibronectin in serum-free DMEM containing 0.1% BSA or with a medium containing BSA only. Cells (2 × 105 well−1) in 50 μl of DMEM containing 0.1% BSA were applied to the upper part of the chamber and incubated for 3 h under 5% CO2 at 37°C. The upper side of the filter was scraped with a cotton swab. The filters were fixed with formalin and stained with haematoxylin. The number of cells on the lower side was counted. The value indicates the mean ± s.e. for triplicate data representing three independent experiments.

Adhesion assay on the tissue

An adhesion assay on kidney sections was performed according to the Stamper–Woodruff assay (Stamper and Woodruff, 1976). The kidneys were removed from nude mice and immediately frozen by liquid N2. Horizontal sections (10 μm thick) were cut for one assay were prepared from the same tissues and mounted on glass slides. Mock-transfected CHO-B2 and α5β2 cells (1 × 106 cells in 1 ml of DMEM supplemented with 10% FBS) were fluorescence-labelled by 3 μl of 1 μM BCECF-AM DMSO solution (Wako Pure Chemical

British Journal of Cancer (2003) 88(2), 327 – 333 © 2003 Cancer Research UK
Industries, Ltd, Hokkaido, Japan) and incubated for 30 min at room temperature. Excess B/EClF was washed off with PBS and the cells were resuspended in 10 ml of HEPES (pH 7.4). Cells (1 x 10^5 in 0.1 ml of HEPES) were preincubated with GRGDS or GRGES peptide (200 μg/ml) for 30 min at 4°C and applied onto tissue sections marked by a wax pen and incubated for 30 min at room temperature with moderate rotation (60 r.p.m.). Unbound cells were washed away with PBS and the sections were fixed with 3% glutaraldehyde in PBS. Cell nuclei were stained with propidium iodide. The sections were air-dried and the number of cells adhering to glomeruli were counted by confocal laser microscopy (LSM-GB200, OLYMPUS, Tokyo, Japan). The value indicates the mean ± s.e. for 20 glomeruli randomly selected and representing three independent experiments.

Statistical analysis

The χ² test was used to determine the significance of the in vivo metastasis experiments. The statistical analysis of the adhesion assay was performed by Kruskal–Wallis ANOVA. A P < 0.05 value was regarded as statistically significant.

RESULTS

Expression level of α5 subunit on each CHO cell

To confirm the expression level of the α5 subunit on each CHO cell type, we carried out a flow cytometric analysis (Figure 1). Since the counterpart of the α5 subunit is only the β1 subunit, the expression level of the α5 subunit indicates that of α5/β1 integrin. α5CHO cells showed the highest level of α5/β1 integrin. α5B2 cells had a higher expression of α5/β1 than CHO-K1 (mock) cells with the endogenous level. No α5 expression was observed in CHO-B2 (mock) cells, which are α5-defective mutants. α5 expression on α5B2F187A cells was almost the same level as α5B2 cells.

Growth of primary tumour in vivo and cell proliferation in vitro

After the cells were inoculated subcutaneously into nude mice, the size of the tumour was measured twice a week (Figure 2). The growth of the primary tumour was inversely correlated with α5 expression levels on CHO cells as previously reported (Schreiner et al, 1991). α5CHO cells expressing α5/β1 at the highest level did not produce a primary tumour. In a cell-proliferation assay, CHO cells with increased α5 expression on fibronectin showed reduced cell proliferation as well as reduced cell growth in vivo (data not shown).

Figure 1 Flow cytometric analysis of α5 expression on CHO. Human and Chinese hamster α5 was detected by mAb KH72 on each CHO cell. CHO-K1 cells expressed α5 at the endogenous level and CHO-B2 cells, α5-defective mutant, did not express α5. Among three transfectants of α5B2, α5B2F187A, and α5CHO cells, α5CHO showed the highest expression of α5.

Metastatic sites and rate of metastasis observed in nude mice subcutaneously inoculated with each CHO cell type

To identify the role of α5 expression on CHO cells in metastasis, we examined the organs with spontaneous metastasis in nude mice (Table 1). Lung metastasis was frequently seen in nude mice inoculated with parent CHO-K1 (mock, 100%), α5-defective mutants (CHO-B2, mock, 90.4%), or α5 transfectants (α5B2, 89.4%). This means that lung metastasis at least in this study appears to be primarily because of physical trapping of CHO cells by microvessels rather than interactions between α5/β1 on CHO cells and fibronectin. Metastases in adrenal glands and lymph nodes were observed in nude mice at a lower level of 0–30%. These metastases also seem to have no relation to a change of α5 expressions on CHO cells. On the other hand, the rate of kidney metastasis changed in association with the expression levels of α5 on CHO cells. Both CHO-B2 cells with no α5 expression and α5B2F187A cells expressing mutated α5 did not give rise to kidney metastasis in nude mice. CHO-K1 cells with a native level of α5 produced kidney metastasis in nude mice at levels of 22.7–27.7%. α5B2 cells with a higher level of α5 than CHO-K1 cells caused kidney metastasis at a higher level of 30–50%. α5CHO cells with the highest level of α5 did not give rise to kidney metastasis in nude mice, since α5CHO cells could not produce a primary tumour because of their very slow growth. These findings indicate the possibility that kidney metastasis might be particularly caused by interactions between α5/β1 on tumour cells and fibronectin in the kidney. Furthermore, kidney metastasis induced by α5B2 was significantly inhibited by intravenous administration of GRGDS peptide (10%) when compared to GRGES peptide (42.1%) (P < 0.05), whereas the administration of GRGDS peptide did not affect the metastatic rate of any other organs investigated. This suggests that the adhesion of α5B2 cells to fibronectin via integrin α5/β1 might have a crucial role in the induction of kidney metastasis.

Macroscopic and microscopic findings of kidney metastasis in nude mice

The macroscopic appearance of kidney metastasis is shown in Figure 3A. The arrowhead indicates a metastatic site formed in the right kidney of a nude mouse inoculated with α5B2 cells, this mouse also has an adrenal metastasis. To identify where α5B2 cells were first trapped in the kidney, we observed micrometastatic sites in kidney sections stained with haematoxylin–eosin. In all nude
mice with kidney metastasis, metastatic sites were formed in the kidney cortex region (Figure 3B, arrowhead). Furthermore, all micrometastases were shown in the kidney glomeruli (Figure 3C, arrowhead). These findings demonstrate that kidney metastasis develops first by the adhesion of α5β1 on CHO cells to fibronectin in the kidney glomerulus.
Adhesion and migration of CHO cells to fibronectin in vitro

To estimate whether it is cell adhesion or migration that is supposed to be crucial for kidney metastasis formation, the behaviour of each CHO cell type relative to fibronectin was analysed in vitro. In the adhesion assay, CHO cells with greater α5 expression effectively adhered to fibronectin, whereas α5B2F187A cells with the alanine mutation and CHO-B2 cells did not adhere to fibronectin (Figure 4A). Likewise, CHO cells showed an increase in cell spreading that correlated with the overexpression of α5 (Figure 4B). Since tumour cells in intravasation penetrate into blood vessels containing abundant soluble fibronectin (300 µg ml⁻¹) in serum (Mosesson and Umfleet, 1970), the cell migration of each CHO cell type was estimated by a cell migration assay. In contrast to the results of adhesion and spreading assay, of all the CHO cells investigated, CHO-K1 cells with the native level of α5 migrated most effectively to fibronectin (Figure 4C). These data are consistent with the theory that cell migration is controlled by dynamic interactions between cell receptors and substratum ligands in a manner representing events at the front and rear of the migrating cells (Palecek et al., 1996, 1997; Regen and Horwitz, 1992). The rate of kidney metastasis appears to shift in parallel with the adhesion of CHO cells to fibronectin, except for α5CHO cells that do not develop a primary site.

Adhesion of α5B2 cells to kidney tissue sections and inhibition by GRGDS peptide

To identify whether kidney metastasis is primarily caused by interactions between α5β1 on CHO cells and fibronectin in glomeruli, we performed an adhesion assay with frozen sections. In this assay, the artificial binding of CHO cells to cortex regions such as uriniferous tubules other than glomeruli was seen because of adhesion of tumour cells in the absence of extravasation. Therefore, adhesion of α5B2 cells to glomeruli was estimated by focusing on the binding of cells (yellow spots) to glomeruli (accumulating red spots) (Figure 5A). The number of cells on glomeruli for the different CHO cell types investigated is shown in

![Figure 4](image_url)

**Figure 4** Adhesion and migration of CHO cells to fibronectin. (A) Adhesion assay demonstrating that adhesion activities to fibronectin in each CHO cell depend on α5 expression level. Cells (5 × 10⁴ well⁻¹) in serum-free DMEM containing 0.1% BSA were plated on coated fibronectin (10 µg ml⁻¹) and incubated for 1 h at 37°C. Cells binding to fibronectin that were stained with crystal violet were assessed by measuring optical density at 550 nm. Results are expressed as the percentage compared with CHO-K1 cells (mock). Each value is the mean±s.e. of triplicate data representative for three independent experiments. (B) Spreading assay quantifying increased binding area in cell adhesion to fibronectin compared with cell spreading on BSA. Each cell in serum-free DMEM containing 0.1% BSA was plated on coated fibronectin (10 µg ml⁻¹) and incubated for 3 h at 37°C. Each value is the mean±s.e. of 20 cells randomly selected. The same results were obtained in two independent experiments. (C) Boyden chamber assay evaluating migration activity of each CHO to soluble fibronectin. A chamber with 10⁵ cells well⁻¹ in the upper chamber and 10 µg ml⁻¹ fibronectin in the lower chamber was incubated for 3 h at 37°C in 10% CO₂. The number of cells migrating to the lower side of the filter was assessed as a percentage compared with CHO-K1 cells (mock). Each value is the mean±s.e. of triplicate data, representative for three independent experiments.

![Figure 5](image_url)

**Figure 5** Effects of GRGDS peptide on adhesion of α5B2 to the glomerulus in the kidney section. (A) Laser microscopic appearance of adhesion assay on the kidney section. 10⁶ cells/section were incubated for 30 min with gentle rotation. a, CHO-B2 cells (yellow spots), α5-defective mutant, slightly adhere to the glomerulus (red spots). b, α5B2 cells which were α5 transfectants effectively adhered to the glomerulus. c, Adhesion of α5B2 cells to the glomerulus was not changed by administration of 200 µg ml⁻¹ GRGES peptide. d, Adhesion of α5B2 cells to the glomerulus was inhibited by administration of 200 µg ml⁻¹ GRGDS peptide. (B) Cell number per glomerulus (n = 20). Note that adhesion of α5B2 cells with GRGES treatment to the glomerulus was significantly inhibited as compared with GRGES treatment (**P < 0.0001**).
Kidney metastasis enhanced by $\alpha_5$ expression on tumour cells

N Tani et al

Objectives

In this study, we constructed CHO cell types with various levels of integrin $\alpha_5 \beta_1$ to investigate the relation between $\alpha_5 \beta_1$ expression on tumour cells and organ-preferential metastasis. Furthermore, we focused on alterations of the metastatic rate associated with different levels of $\alpha_5 \beta_1$. Previous studies have reported that the expression of $\alpha_5 \beta_1$ on tumour cells is inversely correlated with tumourigenicity (Giancotti and Ruoslahti, 1990; Schreiner et al., 1991). Moreover, the loss of fibronectin or $\alpha_5 \beta_1$ on tumour cells had no effect on tumourigenesis or metastasis in chimeric mice containing many $\alpha_5$-null cells (Taverna et al., 1998). However, it remains unknown how metastatic rates change in the case of abundant $\alpha_5$ expression on tumour cells. Furthermore, since the expression of $\alpha_5 \beta_1$ on tumour cells has been thought to make the phenotype of tumour cells normal, $\alpha_5 \beta_1$ expression might play a minor role in the tumour metastatic process. Here, we report that the expression of $\alpha_5 \beta_1$ on tumour cells is involved in the development of kidney metastasis. The inhibition of kidney metastasis in vivo and the adhesion of $\alpha_5 \beta_2$ cells to kidney sections ex vivo by administration of GRGDS peptide, both suggest that kidney metastasis might be induced by adhesion of tumour cells via $\alpha_5 \beta_1$ to fibronectin in the kidney glomerulus. Since RGD peptide is also found in laminin, collagen, and vitronectin, in addition to fibronectin, GRGDS peptide does not necessarily specifically inhibit the adhesion of $\alpha_5 \beta_1$ to fibronectin. CHO cells, however, predominantly express $\alpha_5 \beta_1$, and no ligand molecule other than fibronectin has been found in previous reports. Therefore, GRGDS peptide specifically inhibits the adhesion between $\alpha_5 \beta_1$ on CHO cells and fibronectin. Since a quarter of the total blood volume effused from the heart flows into the kidney (Chan et al., 1991), many tumour cells seem to interact with capillaries in the glomeruli, suggesting the possibility that kidney metastasis could develop from tumour cells physically trapped by vessels. In this study, however, CHO-B2 cells with no $\alpha_5 \beta_1$ hardly adhered at all. The adhesion of $\alpha_5 \beta_2$ cells to glomeruli was significantly inhibited by the administration of GRGDS peptide, but not by RGDES peptide, when compared with the adhesion of $\alpha_5 \beta_2$ cells with no treatment ($P<0.0001$). These findings demonstrate that micrometastasis in glomeruli appears to be brought about primarily by the adhesive interaction of $\alpha_5 \beta_1$ on tumour cells with fibronectin in glomeruli.

In a spontaneous metastasis test, both CHO-B2 (mock) cells with no $\alpha_5 \beta_1$ and $\alpha_5 \beta_2$ CHO cells with the highest level of $\alpha_5 \beta_1$ did not give rise to kidney metastasis in nude mice, whereas kidney metastases were seen in about 40% and 25% of mice inoculated with $\alpha_5 \beta_2$ and CHO-K1 cells, respectively (Table 1). This demonstrates that tumour cells have intrinsic expression levels appropriate for developing metastatic sites. At a primary site, tumour cells with lower levels of $\alpha_5 \beta_1$ show rapid growth adequate for developing metastatic sites. However, once tumour cells intravasate, cells with higher levels of $\alpha_5 \beta_1$ appear to adhere easily to fibronectin to form metastatic sites.

How do tumour cells interact with fibronectin in kidney glomeruli? Since basement membranes of glomerular vessels contain a small amount of fibronectin compared with collagen and laminin (Yurchenco and Schüttner, 1990) as well as general basement membranes, CHO cells with $\alpha_5 \beta_1$ might have difficulty in adhering directly to fibronectin in basement membranes of glomeruli during metastasis. The glomerular capillary is known to have a characteristic structure in relation to its surrounding basement membranes. In glomeruli, several blood vessels are surrounded by a basement membrane along the lateral side of endothelial cells, whereas a common blood vessel is covered with a basement membrane. Furthermore, at the medial side of the glomerular capillary, endothelial cells and mesangial cells without basement membranes are present (Ramzi et al., 1999). Since mesangial cells surrounded by glomerular blood vessels are known to produce ECM proteins containing abundant fibronectin (Couchman et al., 1994), tumour cells seem to interact easily with fibronectin by invading only the endothelial cells but not the basement membranes. On the other hand, lung, adrenal, and lymph node metastatic rates appear to be independent of $\alpha_5$ expression level on CHO cells. In these organs, integrins other than $\alpha_5 \beta_1$ or other molecules on tumour cells might be involved in the development of metastatic sites in addition to physical trapping of cells by microvessels.

In conclusion, we demonstrated that $\alpha_5$ expression levels on tumour cells might affect kidney metastasis rates. Therefore, the inhibition of kidney metastasis by targeting cell adhesion might be essential for metastasis therapy.

REFERENCES

Akiyama SK, Larjava H, Yamada KM (1990) Differences in the biosyntheses and localization of the fibronectin receptor in normal and transformed cultured human cells. Cancer Res 50: 1601–1607
Albelda SM, Buck CA (1990) Integrins and other cell adhesion molecules. PASEB J 4: 2868–2880
Chan BM, Matsuura N, Takada Y, Zetter BR, Hemler ME (1991) In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. Science 251: 1601–1602
Chen FA, Repasky EA, Bankert RB (1991) Human lung tumour-associated antigen identified as an extracellular matrix adhesion molecule. J Exp Med 173: 1111–1116
Churg J, Sobin LH (1982) Renal Disease: classification and Atlas of Glomerular Diseases, pp 23–32. Igaku-Shoin Ltd.: Tokyo
Couchman RG, Beavan LA, McCarthy KJ (1994) Glomerular matrix synthesis, turnover and role in mesangioproliferative expansion. Kidney Int 45: 328–335
Ewing J (1928) A Treatise on Tumours, 3rd edn. Philadelphia: W.B. Saunders
Giancotti FG, Ruoslahti E (1990) Elevated levels of the $\alpha_5 \beta_1$ fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell 60: 849–859
Hall PA, Coates P, Lemoine NR, Horton MA (1991) Characterization of integrin chains in normal and neoplastic human pancreas. J Pathol 165: 33–41
Heino J (1993) Integrin-type extracellular matrix receptors in cancer and inflammation. Ann Med 25: 335–342
Hynes RO (1992) Integrins: versatility, modulation and signaling in cell adhesion. Cell 69: 11–25
Irie A, Kamata T, Puzon-McLaughlin W, Takada Y (1995) Critical amino acid residues for ligand binding are clustered in a predicted beta-turn of the third N-terminal repeat in the integrin alpha 4 and alpha 5. EMBO J 14: 5550–5556
Matsuura N, Puzon-McLaughlin W, Irie A, Morikawa Y, Kakudo K, Takada Y (1996) Induction of experimental bone metastasis in mice by transfection of integrin $\alpha_5 \beta_1$ into tumour cells. Am J Pathol 148: 55–61
Moretti S, Martini L, Berti E, Pinzi C, Giannotti B (1993) Adhesion molecule profile and malignancy of melanocytic lesions. Melanoma Res 4: 235–239
Moessner MW, Umlleet RA (1970) The cold-insoluble globulin of human plasma. 1. Purification, primary characterization, and relationship to fibronectin and other cold-insoluble fraction components. J Biol Chem 245: 5728–5736
Natali PG, Nicotra MR, Bartolazzi A, Cavaliere R, Bigotti A (1993) Integrin expression in cutaneous malignant melanoma: association of the α3β1 heterodimer with tumour progression. *Int J Cancer* 54: 68 – 72
Natali PG, Nicotra MR, Botti C, Mottolese M, Bigotti A, Segatto O (1992) Change in expression of αβ4 integrin heterodimer in primary and metastatic breast cancer. *Br J Cancer* 66: 218 – 322
Natali PG, Nicotra MR, Cavaliere R, Giannarelli D, Bigotti A (1991) Tumour progression in human malignant melanoma is associated with changes in αβ1 laminin receptor. *Int J Cancer* 49: 168 – 172
Netland PA, Zetter BR (1984) Organ-specific adhesion of metastatic tumour cells *in vitro*. *Science* 224: 1113 – 1115
Nicolson GL (1988) Organ specificity of tumour metastasis: role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. *Cancer Metastasis Rev* 7: 143 – 188
Nicolson GL, Winkelhake JL (1975) Organ specificity of blood-borne tumour metastasis determined by cell adhesion? *Nature* 255: 230 – 232
Paget S (1898) The distribution of secondary growths in cancer of the breast. *Lancet* 1: 571 – 573
Palecek SP, Loftus JC, Glusberg MH, Lauffenburger DA, Horwitz AF (1997) Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* 385: 537 – 540
Palecek SP, Schmidt CE, Lauffenburger DA, Horwitz AF (1996) Integrin dynamics on the tail region of migrating fibroblasts. *J Cell Sci* 109: 941 – 952
Pauli BU, Lee CL (1988) Organ preference of metastasis: the role of organ-specifically modulated endothelial cells. *Lab Invest* 58: 379 – 387
Pignatelli M, Handy AM, Stamp GW (1991) Low expression of β1, α2 and α3 subunits of VLA integrins in malignant mammary tumours. *J Pathol* 165: 25 – 32
Rami SC, Vinay K, Tucker C (1999) Pathologic Basis of Disease, 6th edn, pp 930 – 996. PA, USA: W.B. Saunders Company
Regen CM, Horwitz AF (1992) Dynamics of beta 1 integrin-mediated adhesive contacts in motile fibroblasts. *J Cell Biol* 119: 1347 – 1359
Ruoslathi E. (1992) Control of cell motility and tumour invasion by extracellular matrix interactions. *Br J Cancer* 66: 239 – 242
Schreiner CL, Bauer JS, Danilov YN, Hussein S, Szekan MM, Juliano RL (1989) Isolation and characterization of Chinese hamster ovary cell variants deficient in the expression of fibronectin receptor. *J Cell Biol* 109: 3157 – 3167
Schreiner CL, Fisher M, Hussein S, Juliano RL (1991) Increased tumourigenicity of fibronectin receptor deficient Chinese hamster ovary cell variants. *Cancer Res* 51: 1738 – 1740
Stamper Jr HB, Woodruff JJ (1976) Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J Exp Med* 144: 828 – 833
Takebe Y, Seiki M, Fujisawa J-I, Hoy P, Yokota K, Arai K-I, Yoshida M, Arai N (1988) SRa promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol Cell Biol* 8: 466 – 472
Taverna D, Ullman-Cullere M, Rayburn H, Bronson RT, Hynes RO (1998) A test of the role of α5 integrin/fibronectin interactions in tumourigenesis. *Cancer Res* 58: 848 – 853
Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ (1997) Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. *J Cell Biol* 137: 231 – 245
Yurchenco PD, Schittny JC (1990) Molecular architecture of basement membranes. *FASEB J* 4: 1577 – 1590
Zhu D, Cheng CF, Pauli BU (1991) Mediation of lung metastasis of murine melanomas by a lung-specific endothelial cell adhesion molecule. *Proc Natl Acad Sci USA* 88: 9568 – 9572
Zutter MM, Krigman HR, Santoro SS (1993) Altered integrin expression in adenocarcinoma of the breast: analysis *in situ* hybridization. *Am J Pathol* 142: 1439 – 1448

Kidney metastasis enhanced by α5 expression on tumour cells
N Tani et al

© 2003 Cancer Research UK

British Journal of Cancer (2003) 88(2), 327 – 333

Experimental Therapeutics