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A Defect in Cell-to-cell Adhesion via Integrin-Fibronectin Interactions in a Highly Metastatic Tumor Cell Line

Yoshiko Abe,[1] Tateki Tsutsui,[1] Jie Mu,[1] Atsushi Kosugi,[3] Hideo Yagita,[4] Kenji Sobue,[2] Ohtsura Niwa,[5] Hiromi Fujiwara[1] and Toshiyuki Hamaoka[1]

[1]Department of Oncology, [2]Department of Neurochemistry, Biomedical Research Center, [3]School of Allied Health Sciences, Faculty of Medicine, Osaka University Medical School, 2-2 Yamada-oka, Suita, Osaka 565, [4]Department of Immunology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113 and [5]Research Institute for Nuclear Medicine and Biology, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734

We investigated the role of integrin-fibronectin (FN) interactions in tumor cell adhesion. Two cloned tumor cell lines designated OV-LM (low-metastatic) and OV-HM (high-metastatic) were isolated from a murine ovarian carcinoma, OV2944. OV-LM and OV-HM cells exhibited high and low RGDS-sequence-dependent adhesiveness to FN, respectively. Both lines expressed comparable levels of α5 and αv integrins, which are capable of reacting with RGDS on FN. To compare the functions of these integrins between the two tumor lines, the signaling mechanism following FN stimulation was examined. Significant levels of phosphorylation of focal adhesion kinase (FAK) were detected in both OV-LM and OV-HM cells before FN stimulation. Whereas the level of FAK phosphorylation was appreciably enhanced in OV-LM cells stimulated with FN, stimulation of OV-HM cells with FN induced a reduction in the FAK phosphorylation in association with a significant decrease in the amount of FAK protein in the soluble compartment of cell lysates. A difference in the deposition of FN on the cell surface was also observed between the two types of tumor lines; OV-HM cells had an appreciably smaller amount of FN than OV-LM. Consistent with the functional abnormality of the integrin-FAK system and the smaller amount of FN on OV-HM, this clone exhibited a reduced cell-cell adhesion in the *in vitro* cell aggregation assay. Namely, OV-LM cells displayed a time-dependent increase in the formation of cell aggregates, whereas most OV-HM cells remained single. The formation of aggregates by OV-LM cells was inhibited by addition of RGDS peptide. These results indicate that the highly metastatic clone, OV-HM, exhibits a decreased capacity of cell-cell adhesion mediated by integrin-FN interactions and suggest that this defect is mainly due to the dysfunction of integrins/FAK rather than a decrease in the amount of integrins expressed on tumor cells.

Key words: Tumor metastasis — Integrin — Fibronectin

The process of tumor metastasis is a complex multistep cascade.[1–3] Recent studies have shown that the adhesiveness of tumor cells either via cell-cell or cell-extracellular matrix (ECM) interactions is a critical factor in metastasis. Cell surface molecules involved in these interactions include a variety of integrins,[3,4] cadherins,[5] members of the Ig superfamily[6] and CD44.[7] Because each class of adhesion molecules could contribute to the net cellular and matrix adhesiveness of tumor cells, the roles of these molecules in tumor metastasis have been under active investigation.

Metastasis is initiated by the detachment of tumor cells from the primary site. Down-regulation of adhesion molecules has been shown to correlate with a higher propensity of tumor cells to detach from a primary tumor mass.[1,3] Most of the preceding studies have been focused on the role of cadherins in regulating tumor cell detachment.[8] Cadherins are a family of glycoproteins that mediate homotypic intercellular adhesion in various normal tissues, as well as in various tumors.[8,9] Subsequent studies suggested that decreased adhesiveness through the reduction of cadherin action may favor tumor cell detachment and contribute to early stages of the metastatic process.[5] However, the overall adhesiveness of tumor cells should not depend solely on the function of cadherins, but should also be influenced by other adhesive mechanisms, such as those mediated by cell-ECM interactions. Nevertheless, little is known regarding the role of integrins expressed on tumor cells in mediating tumor cell adhesion.

In the present study, we investigated whether either the expression or the function of integrins on tumor cells influences cell-cell adhesion via integrin-ECM interactions. The results show that OV-LM (low-metastatic line) and OV-HM (high-metastatic line) ovarian tumor cells exhibited high and low adhesiveness to fibronectin (FN), respectively, despite expressing comparable levels of α5 and αv integrins. An abnormal pattern of focal adhesion kinase (FAK) response and a reduction of FN deposition on tumor cells were seen for OV-HM. In

accordance with these findings, OV-HM showed apparently reduced cell-cell adhesiveness in a cell aggregation assay. Thus, the results obtained using high and low metastatic lines of ovarian tumor indicate that (i) cell-cell attachment involves integrin-FN interactions and (ii) the dysfunction of FAK rather than the amount of expressed integrins underlies the reduced cell-cell adhesiveness observed for the high-metastatic line.

MATERIALS AND METHODS

Tumors An ovarian tumor (OV2944) developed in a female (C57BL/6×C3H/He) F_1 ($B6C3F_1$) mouse given a single whole-body neutron irradiation of 2.7 Gy from a ^{252}Cf source.[10] Two lines with high or low metastatic property were isolated from the parental OV2944 tumor.[10] A single cloned line was established from each of the high and low metastatic lines and designated as OV-HM or OV-LM, respectively.

Mice Female (C57BL/6×C3H/He)F_1 mice were obtained from Shizuoka Experimental Animal Laboratory, Hamamatsu and used at 6–9 weeks of age.

Reagents The following protein and peptides were purchased: rat FN (Sigma Chemical Co., St. Louis, MO); peptides GRGDSP (RGDS sequence) and GRGESP (RGES sequence) (Bachem, Torrancel, CA). Anti-α4 (PS/2) (from Dr. K. Miyake, Saga Medical College, Saga), anti-αv (RMV-7)[11] and anti-α5 (HMα5-1)[12] monoclonal antibodies (mAbs) were purified from culture SNs of the respective hybridomas. Rabbit anti-mouse FN Ab was purchased from Bioproducts, Indianapolis, IN. This Ab exhibited only marginal cross-reactivity to bovine FN contained in fetal calf serum (FCS). Anti-$p125^{FAK}$ mAb (2A7)[13] and anti-phosphotyrosine mAb (PY20) were obtained from UBI, Lake Placid, NY and ICN, Costa Mesa, CA, respectively. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rat Ig and FITC-conjugated rabbit anti-hamster IgG were from Becton Dickinson, Mountain View, CA. FITC-conjugated goat anti-rabbit IgG was from Cappel Research Products, Durham, NC. Control normal rat Ig and normal hamster IgG were from BioMeda, Foster City, CA and Cappel Research Products, respectively.

Preparation of a single tumor cell suspension Tumor cells were harvested from monolayer cultures by treatment with ethylenediamine tetraacetate (EDTA) when they were required for cell binding assays, cell-cell aggregation assays and flow cytometry analyses. Tumor cells were also harvested by trypsinization when they were required for FAK immunoprecipitation and western blot analyses.

Assay for binding of cells to plate-coated FN Microculture plates (96-well, flat-bottomed: Corning No. 25860, Corning, NY) were coated with 10 μg/ml of FN in PBS overnight. The monolayer of tumor cells was ^{51}Cr-labeled. The labeled cells (5×10^4/well) were dispensed in a volume of 100 μl of RPMI 1640 medium into FN-coated wells and cultured at 37°C in a CO_2 incubator in the absence or presence of blocking peptides. After 60 min incubation, the plates were washed with RPMI 1640 three times, and radioactivity remaining in each well was determined. Results were obtained as the mean radioactivity of triplicate cultures. The data are presented as the relative % binding: [(mean radioactivity in the presence of blocker − mean radioactivity in BSA-coated wells)/(mean radioactivity in the absence of blocker − mean radioactivity in BSA-coated wells)]×100.

Assay for *in vitro* cell-cell aggregation A single cell suspension of tumor cells (2×10^5/tube) in RPMI 1640 medium was centrifuged at 1200 rpm and cell pellets were incubated at 37°C in a CO_2 incubator in the absence or presence of blocking peptides. After 10–30 min incubation, cells were resuspended by gentle pipetting, and the number of cells remaining single, without forming cell aggregates, was evaluated by microscopy. The percentage of single cells following incubation was expressed as the ratio to the total cell number.

Immunofluorescence staining and flow cytometric analyses Tumor cells were stained at 4°C with anti-α4, anti-α5 or anti-αv mAb or normal IgG (rat or hamster) as a control, followed by FITC-conjugated anti-rat Ig or anti-hamster IgG. Tumor cells were also stained with anti-FN Ab followed by FITC-conjugated anti-rabbit IgG. Cells were washed with Hanks' balanced salt solution containing 0.5% BSA and 0.5% sodium azide, and analyzed on a FACScan (Becton Dickinson).

Preparation of cell extracts Cells were harvested by trypsinization, and an equal volume of ice-cold PBS containing 1.0 mg/ml soybean trypsin inhibitor (Wako Pure Chemicals, Tokyo) was added to the cell suspension. After having been washed twice with PBS, the cells were suspended in RPMI 1640 medium containing 1% BSA (10^5/ml) and incubated for 30 min at 37°C to permit them to regenerate integrins. Cells were washed with RPMI 1640 twice, and resuspended in RPMI 1640 without FCS. Cells (2×10^6/dish) were incubated on 10 μg/ml FN-coated dishes (ϕ 10 cm) for 60 min and the medium was removed. Cells were solubilized by adding ice-cold lysis buffer (1% Triton X-100, 50 m*M* Tris HCl pH 7.5, 100 m*M* NaCl, 2 m*M* $MgCl_2$, 5 m*M* EGTA, 1.8 mg/ml Na orthovanadate, 1 m*M* PMSF, 100 μg/ml aprotinin and 10 μg/ml leupeptin) to the dishes. The lysates were harvested, and the nuclear pellet was removed by centrifugation. Protein concentration of each lysate was determined using BCA Protein Assay (Pierce, Rockford, IL).

Immunoprecipitation and western blot analysis The lysates were immunoprecipitated with anti-$p125^{FAK}$ mAb

(2A7), prebound to protein G-Sepharose (Pharmacia, Piscataway, NJ) for 1 h at 4°C. The immunoprecipitates were washed with the lysis buffer, subjected to 7.5% SDS-PAGE under reducing conditions and electroblotted onto polyvinylidene fluoride membrane (Immobilon-P, Millipore Corp., Bedford, MA). Membranes were blocked in Tris-buffered saline (TBS) containing 5% BSA, and incubated either with PY20 in a 1 : 1000 dilution or with 2A7 in a 1 : 500 dilution. They were then washed with TBS containing 0.2% Tween20 and 0.5% BSA, incubated with horseradish peroxidase-conjugated $F(ab')_2$ fragment of sheep anti-mouse IgG (Amersham, Buckinghamshire, England), washed again and developed using the ECL system (Amersham).

RESULTS

Comparison of metastatic and invasive properties between OV-LM and OV-HM cloned tumor lines Previous studies showed that the OV-LM and OV-HM tumors derived from a parental ovarian carcinoma line are low- and high-metastatic lines, respectively.[10] We confirmed this (Table I). Different numbers of OV-LM and OV-HM cells were inoculated s.c. into syngeneic $B6C3F_1$ mice, and metastasis to lymph nodes and invasion into the peritoneal cavity were determined two months later.

Table I. Comparison of Metastasis and Invasion between Two Ovarian Carcinoma Cell Lines

Tumor cell line	No. of cells inoculated[a]	Incidence of[b]: metastasis	invasion
OV-LM	10^6	0/6	1/6
	10^7	1/6	1/6
OV-HM	10^5	5/6	5/6
	10^6	6/6	6/6

a) The indicated numbers of OV-LM or OV-HM tumor cells were inoculated s.c. into syngeneic $B6C3F_1$ mice.
b) Incidence of metastasis to axillary and inguinal lymph nodes, as well as peritoneal invasion, was determined 2 months after tumor implantation.

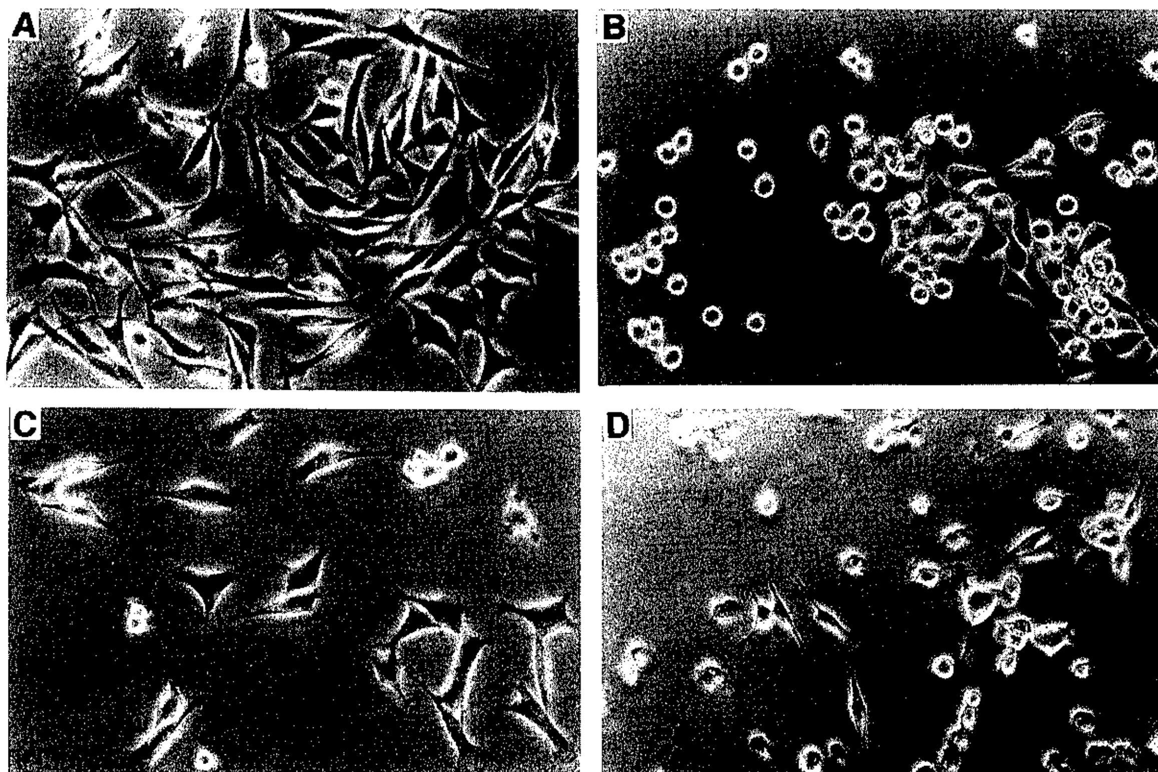

Fig. 1. Differential cell spreading between OV-LM and OV-HM tumor lines. Panels A and B, OV-LM (A) and OV-HM (B) tumor cells (5×10^4/well) were cultured overnight in 24-well culture plates in medium containing 10% FCS. Panels C and D, Wells of culture plates were coated with 20 μg/ml of FN. OV-LM (C) or OV-HM (D) tumor cells (5×10^4/well) suspended in FCS-free medium were incubated in the FN-coated wells for 60 min.

Fig. 2. Inhibition of FN-binding of the two tumor lines by addition of RGDS peptide. Microculture plates (96-well, flat-bottomed) were coated with 10 μg/ml of FN. ^{51}Cr-Labeled OV-LM (open symbols) or OV-HM (closed symbols) cells (5×10^4/well) were cultured in the FN-coated wells at 37°C for 60 min in the presence of RGDS (□ and ■) or control RGES (○ and ●) peptide. The plates were washed with PBS, and the radioactivity (remaining cells) in each well was determined.

Fig. 3. Expression of various integrins on the two tumor cell lines. OV-LM (upper panels) and OV-HM (lower panels) cells were stained with anti-α4, anti-α5 or anti-αv mAb followed by FITC-conjugated anti-rat or anti-hamster Ig. Dotted lines indicate the staining of cells with normal (control) IgG.

When the same number of tumor cells (10^6/mouse) was inoculated, there was a significant difference in tumor size between the two tumor lines. Therefore, comparisons were made between OV-LM-bearing and OV-HM-bearing mice which had been inoculated with the same number (10^6/mouse) and a different number (10^7 vs. 10^5/mouse) of tumor cells. Much higher incidences of metastasis to lymph nodes were observed with the OV-HM tumor, in agreement with the previous results.[10] The present results also show that tumor invasion from the primary s.c. site to the peritoneal cavity occurred in most OV-HM-bearing mice along with metastasis to lymph nodes. Thus, compared to OV-LM, the OV-HM tumor line exhibits high-metastatic and invasive properties.

Differential adhesiveness to FN of OV-LM and OV-HM lines We observed that OV-LM and OV-HM cells grew in culture medium containing 10% FCS in different shapes. Namely, OV-LM cells spread like untransformed fibroblasts (Fig. 1A), whereas most OV-HM cells did not (Fig. 1B). The difference in cell spreading was also observed in FCS-free culture medium in the culture plates that had been coated with FN (Fig. 1C vs. D), although most of the cells in both lines appeared to adhere to FN-coated wells. To determine whether the differential spreading is related to a difference in the strength of adhesion to FN between the two tumor lines, we examined their capacity to bind to FN (Fig. 2). Both lines of tumor cells were incubated in FN-coated microculture plates in the presence of serially diluted RGDS or control RGES peptide. After 60 min incubation, cells that did not adhere or only loosely adhered were removed by washing with PBS. In the absence of blocking peptides, OV-LM cells exhibited a higher binding capacity to FN than did OV-HM cells (% cell binding: 41.8 vs. 29.5). When extensive washing was performed, both types of tumor cells were removed, depending on the presence of RGDS peptide during incubation (data not shown). However, the adhesion of OV-LM cells to FN was only slightly inhibited by RGDS peptide. In contrast, addition of RGDS peptide to OV-HM cultures resulted in more potent and peptide dose-dependent inhibition of FN binding. Thus, OV-HM cells exhibit apparently weaker RGDS-dependent binding to FN than OV-LM cells.

Expression of integrins with FN-binding capacities on two tumor lines We examined which types of integrins interacting with FN are expressed on OV-LM and OV-HM tumor cells and whether there exists a difference in the levels of their expression between these two tumor lines. Both tumor cell lines expressed α5 and αv integrins, but did not express α4 integrin (Fig. 3). The levels of α5 and αv expression on OV-LM and OV-HM were comparable. These results suggest that the differential capacity of FN-binding between OV-LM and OV-HM lines may not be ascribed simply to a difference in the expression levels of integrins that react with FN.

Abnormality in integrin-mediated signaling in the OV-HM tumor cell line Earlier studies[14–16] have demonstrated that adhesion of cells to FN via integrins such as $\alpha5\beta1$ and $\alpha v\beta3$ results in tyrosine phosphorylation of focal adhesion kinase (p125^{FAK}) and that the activation of this kinase is an important early step in the intracellular signal transduction pathway triggered in re-

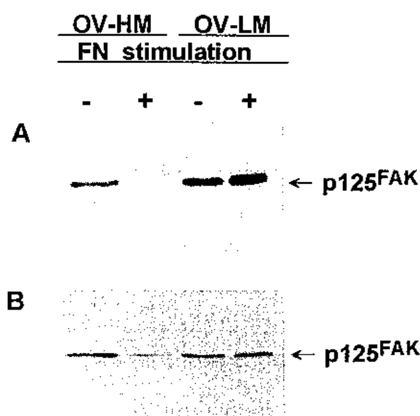

Fig. 4. Different patterns of p125^{FAK} phosphorylation in OV-LM and OV-HM cells following stimulation with FN. OV-LM and OV-HM cells stimulated with FN were solubilized with 1% Triton X-100 lysis buffer, and the nuclear pellet was removed by centrifugation. Cell lysates were immunoprecipitated with anti-p125^{FAK} mAb, and the anti-p125^{FAK} immunoprecipitates were electrophoresed in 7.5% SDS-PAGE under reducing conditions and immunoblotted either with anti-phosphotyrosine mAb, PY20 (Panel A) or with anti-p125^{FAK} mAb, 2A7 (Panel B).

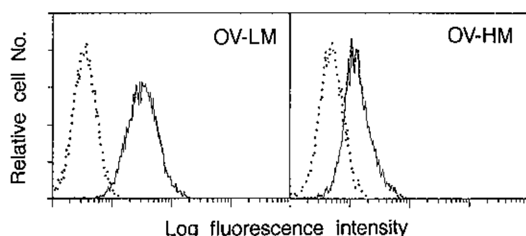

Fig. 5. Differential deposition of FN on OV-LM and OV-HM cell surfaces. OV-LM and OV-HM cells were stained with anti-FN Ab followed by FITC-conjugated anti-rabbit IgG.

sponse to cell attachment to FN. We investigated the phosphorylation of p125^{FAK} in OV-LM and OV-HM cells following attachment to FN. OV-LM and OV-HM cells were allowed to attach to FN-coated plates. Sixty minutes later, cells were harvested and cell lysates prepared. These lysates were subjected to immunoprecipitation with anti-p125^{FAK} mAb. The resultant anti-p125^{FAK} immunoprecipitates were electrophoresed and then immunoblotted with anti-phosphotyrosine (Fig. 4A) or anti-p125^{FAK} mAb (Fig. 4B). Fig. 4A shows that FAK from both OV-LM and OV-HM cell lysates exhibited considerable levels of phosphorylation before FN stimulation. Stimulation of OV-LM cells with FN resulted in

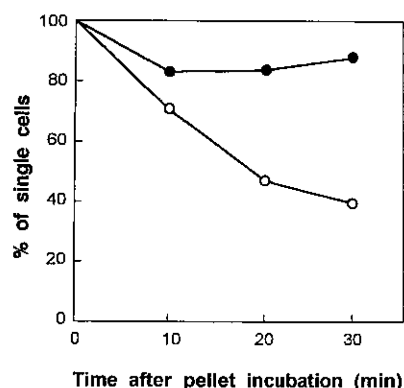

Fig. 6. Time-dependent cell aggregation in OV-LM, but not in OV-HM pellet. An OV-LM (○) or OV-HM (●) single cell suspension was centrifuged and the cell pellets were incubated at 37°C for 10–30 min. Cell were harvested at the indicated time points and the incidence of cells remaining single was determined.

an appreciable enhancement of FAK phosphorylation. In contrast, the level of FAK phosphorylation was markedly reduced in the OV-HM cell lysate following FN stimulation. Another important finding was that the amount of FAK immunoprecipitated from the OV-LM lysate did not change before and after FN stimulation, whereas that from the OV-HM lysate was apparently decreased following FN stimulation (Fig. 4B). Thus, these results suggest that, as in fibroblasts[14] or OV-LM cells, stimulation of OV-HM cells with FN induces downregulation of p125^{FAK} phosphorylation, along with a decrease in the amount of FAK protein in the soluble compartment.

Comparison of the deposition of FN on the surface of tumor cells We next examined whether there exists a difference in the amount of FN between the two tumor lines. OV-LM and OV-HM tumor cells during cultures in FCS-positive medium were harvested, and FN was stained with anti-FN Ab. As shown in Fig. 5, the amount of FN deposited on OV-HM cells was found to be significantly less than that on OV-LM cells.

Differential cell-cell adhesiveness via integrin-FN interactions between the two tumor lines The fact that OV-HM cells exhibit weaker FN adhesiveness and deposit a smaller amount of FN than OV-LM cells suggests that cell aggregation via integrin-FN interactions may be weaker in OV-HM than in OV-LM. This was directly investigated by an *in vitro* cell aggregation assay. A single tumor cell suspension of OV-LM or OV-HM cells was centrifuged, and each cell pellet was incubated for 10–30 min, then the incidence of cells remaining single, without forming cell aggregates, was compared between OV-LM

Table II. Effect of RGDS Peptide on the Formation of Cell Aggregates in an OV-LM Tumor Cell Pellet

Exp.	Tumor cell line[a]	Incidence of single cells in the presence of:				
		none	RGDS (mg/ml)		RGES (mg/ml)	
			0.02	0.1	0.02	0.1
1	OV-HM	88.1±3.1	88.7±3.3	84.2±4.1	88.3±2.8	91.2±4.3
	OV-LM	60.8±2.3	81.3±3.1	79.5±4.2	57.3±6.3	59.4±8.7
2	OV-HM	77.1±4.2	N.D.	76.6±4.0	N.D.	81.2±4.2
	OV-LM	42.4±6.3	N.D.	63.0±3.6	N.D.	43.9±5.7

a) An OV-HM or OV-LM cell pellet was incubated at 37°C for 30 min in the absence or presence of RGDS or RGES (control) peptide at the indicated concentrations.

and OV-HM pellets (Fig. 6). The results show that the incidence of single cells from the OV-LM pellet decreased in a time-dependent way, whereas that from the OV-HM cell pellet remained unchanged during 10–30 min incubation. To determine whether cell aggregation in the OV-LM pellet is mediated by the FN-integrin ($\alpha 5$ and αv) interaction, we examined the effect of RGDS peptide on the formation of cell aggregates (Table II). Higher incidences of single cells were again observed in the OV-HM cell pellet, irrespective of whether RGDS peptide was present. Addition of RGDS peptide to the OV-LM pellet significantly increased the incidence of cells remaining single. Control RGES peptide did not induce such an effect. Thus, these results indicate that there is a substantial difference in cell-cell adhesiveness via FN-integrin interactions between OV-LM and OV-HM.

DISCUSSION

The results presented here show that two cloned ovarian carcinoma cell lines (OV-HM and OV-LM) that induce high and low incidences of invasion/metastasis exhibit different capacities for cell-ECM (FN) interaction. Namely, the OV-HM line with a high-metastatic property adhered to FN weakly and the OV-LM line with low metastatic property did so strongly. However, both lines expressed comparable levels of integrins able to react with FN ($\alpha 5$ and αv). As for the mechanisms underlying the reduced FN-adhesiveness in OV-HM, our results suggested an abnormality in the activation of FAK in the integrin-mediated signaling pathway. Moreover, the amount of FN on cell surfaces was found to be significantly lower on OV-HM than on OV-LM cells. In accordance with this, OV-HM cells exhibited much weaker cell-cell adhesiveness via integrin-FN interactions.

Recent studies have shown that cell adhesion plays a critical role in various stages of the metastatic process and that modulation of adhesion mechanisms leads to the facilitation of metastasis.[1–4] However, the expression and function of adhesion molecules are, in general, controlled by a variety of factors, and therefore the relationship between metastasis and regulation of adhesion mechanisms appears to be complex. For example, down-regulation of certain adhesion molecules has been shown to correlate with a higher propensity of tumor cells to detatch from the primary tumor on the one hand. On the other, up-regulation of other adhesion molecules has also been correlated with a higher potential of tumor cells to metastasize. This seems reasonable, because tumor cells must show both decreased and enhanced adhesiveness, depending on the stage of the metastatic process. Release of tumor cells from the primary tumor, which represents the initial event of the metastatic process, would require a decrease in their adhesiveness to the adjacent tumor cells or surrounding ECM.

Regarding the adhesion molecules that influence the detachment of tumor cells from the primary mass, a critical involvement of cadherins has been shown in experimental and human tumors.[8, 17] Frixen *et al.*,[18] using a variety of human tumor cell lines, reported that invasive cell lines were often negative for expression of E-cadherins, whereas most of the non-invasive cell lines they examined were E-cadherin-positive. Furthermore, they demonstrated that invasiveness of malignant cell lines can be anulled by transfection of E-cadherin cDNA.[18] Differential expression of E-cadherin was also observed in the high and low metastatic sublines of the ovarian tumor used here.[10] Thus, it is likely that homotypic cell-cell adhesion via E-cadherin molecules plays a significant role in controlling tumor cell attachment/detachment.

Because cells can adhere to other cells via cell-ECM interactions,[17] it seems reasonable to consider that cell-ECM interactions may also contribute to promoting tumor cell-tumor cell adhesion or tumor cell aggregation.[19] In the course of our studies using the OV-HM and

OV-LM cloned tumor lines, we found that, compared to OV-LM cells, most OV-HM cells exhibited a weaker capacity to spread and adhere to culture plates in FCS-containing medium. Further, OV-HM cells show apparently reduced levels of adhesiveness to immobilized FN and deposition of FN on their surface. In the cell aggregation experiments, OV-LM cells formed more cell aggregates and this aggregation was inhibited by addition of RGDS peptide. Thus, our present study indicated a contribution of cell-ECM (FN) interactions to the promotion of cell-cell aggregation.

Despite the apparent difference in FN adhesiveness, OV-HM and OV-LM lines did not show a substantial difference in the expression levels of integrins able to react with FN ($\alpha 5$ and αv). Instead, our results revealed a critical difference in the activation of FAK following integrin-FN interactions. While a part of FAK in OV-LM cells was tyrosine-phosphorylated before FN stimulation, the phosphorylation level appreciably increased upon attachment to FN without a change in the amount of FAK protein in the soluble compartment, as has been observed for fibroblasts.[20] In contrast, attachment of OV-HM cells to FN resulted in a decrease in the amount of FAK protein, as well as a reduction in the phosphorylation level of FAK remaining in the soluble compartment. Thus, OV-HM cells exhibited a different pattern of FAK response from that observed in fibroblasts and OV-LM cells. The cause-effect relationship between these unusual patterns of the FAK response and the reduced FN-adhesiveness in OV-HM cells remains to be examined. A similar discrepancy between the cell surface expression and function of an adhesion molecule has been observed for cadherin molecules: metastatic carcinoma cells often express normal levels of E-cadherin and yet lack the E-cadherin-mediated adhesive function because of defects in catenins that regulate the action of cadherins inside the cell.[21] Recently, Owens *et al.* reported the overexpression of FAK protein in some primary human tumors, and this abnormality correlates with the invasive potential of tumor cells.[22, 23] Because the phosphorylation status of FAK was not examined in their study, the relevance to our results remains unclear. Nevertheless, these results underscore the importance for investigating the relation of the quantitative and/or qualitative (functional) status of FAK to the degree of malignancy in each tumor line.

After the tumor cells escape from the primary tumor, they must interact with basement membranes in the tumor vasculature to proceed toward the next step of the metastatic cascade. Cell surface receptors for laminin may mediate adhesion of tumor cells to the basement membranes prior to invasion.[2] Recent studies revealed the correlation of malignancy[24] and high incidence of metastasis[25] with high levels of expression of receptors to ECM other than FN, i.e., laminin receptors. Thus, tumor metastasis/invasion is positively and negatively regulated by interactions between a variety of integrins and the relevant ECM. Moreover, attachment of integrins with ECM appears to result in the expression of multiple genes, including those coding matrix metalloproteinases,[26] which are also critical for the promotion of metastasis.[1] Thus, further studies to investigate the overall function of integrin-ECM interactions at each stage of the metastatic process will be required for a better understanding of tumor metastasis.

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