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Expression and Role of Integrins in Invasive Activity of Oncotransformed Fibroblasts Differing in Spontaneous Metastasizing

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Abstract—Four closely related lines of RSV-transformed Syrian hamster fibroblasts differing drastically in their spontaneous metastatic capacity were investigated for the surface expression of integrins, *in vitro* invasion, and production of MMP-2 collagenase. The highly metastasizing HET-SR-2SC-LNM cells differ from the lowly metastasizing parental HET-SR cells in a high level of the surface expression of the collagen-specific α1β1, α2β1, and αvβ3 integrins, a high invasive activity, and an increased production of MMP-2. The same properties are characteristic for the actively metastasizing cells of the independent HET-SR-1 line. The lowly metastasizing fibroblasts that are derived from HET-SR-2SC-LNM retain a high level of the expression of the α1β1 and α2β1 integrins, but, unlike the parental line, they exhibit a decreased expression of the αvβ3 integrin, invasion in Matrigel, and MMP-2 production. Substrate stimulation of the signal function of the collagen-specific integrins increases the production of MMP-2 by the metastatically active fibroblasts. Inhibition of the signal activity of the integrins by RGD-containing pentapeptide or by genistein reduces markedly *in vitro* invasion in Matrigel and MMP-2 production. The role of specific properties of the extracellular matrix surrounding tumor cells and of specific surface integrins expressed in these cells in developing of the malignant phenotype is discussed.

Key words: integrins, extracellular matrix, metastasizing, invasion

Invasion and metastasizing of tumor cells are multistage processes in which the interaction of the cells with the extracellular matrix plays a significant role. The main mediators in this interaction are integrins, receptors of the cytoplasmic membrane of the cell possessing an affinity to certain proteins of the matrix. The molecule of any of these receptors is a heterodimer composed of α- and β-subunits linked by noncovalent bonds [1, 2]. Besides the receptor function in the matrix–cell interactions, another important function of integrins is transmission of signals from the matrix to the cytosol allowing regulation of such intracellular processes as cell motility, proliferation, differentiation, apoptosis, etc. [3, 4].

Both of the indicated functions of integrins suggest their involvement in the mechanisms of growth and progression of tumors [5, 6]. However, the data on the role of integrin receptors in oncogenesis are incomplete and often contradictory. Most of the large family of integrins (more than 20 receptors) is virtually unstudied. Data concerning the explicitly characterized integrins demonstrate that any receptor can exhibit different properties in different cells, and no one can serve as the absolute marker of tumor progression, since no modification of any of these receptors has been revealed in all of the investigated lines. For the same integrins, an increased expression of the receptors in one type of metastasizing cells (compared to the non-metastasizing ones) and their decreased expression in other type of metastasizing cells were demonstrated [6-9].

These contradictions can be explained by the distinctiveness of the expression and ligand properties of the integrins. All cell types usually express in the surface membrane not one, but a number (spectrum) of these receptors. At the same time, each matrix protein is a ligand for several integrins, and each receptor recognizes several proteins. Thus, each integrin is interchangeable in the interaction of a cell with the matrix. It has been also shown that many integrins induce the same cell responses during signal transmission (for example, phosphorylation of certain proteins, activation of certain genes, etc.) [4, 10].

It can be assumed that the progression of tumor cells is connected with modification of the whole surface spec-

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trum of integrins, rather than a single receptor, thus resulting in the appearance of the optimal conditions for the interaction of these cells with their environment while developing the aggressive, malignant phenotype. Quantitative changes in the expression of a single integrin can be insignificant for tumor progression.

The data presented in this work support this assumption. Four lines of Syrian hamster fibroblasts transformed by the Rous sarcoma virus (RSV) were investigated: two of a high level and two of a low level of spontaneous metastasizing while growing of the primary tumor in the same medium (subcutaneous matrix rich in interstitial collagen). Although the highly metastasizing lines significantly differ in the character of their selection from the original transformed cells, both of them are characterized by virtually the same (compared to the non-metastasizing lines) changes in the spectrum of the surface expression of the collagen-specific integrins, integrin-dependent secretion of the collagen-cleaving proteases, and the integrin-mediated *in vitro* invasion.

MATERIALS AND METHODS

Chemicals. Polyclonal antibodies to the cytoplasmic domains of human integrin α1 and α2 subunits were purchased from Chemicon (USA), antibodies to the α3 and α5 subunits were generously supplied by Dr. Ruoslahti (Cancer Research Center, La Jolla, USA); polyclonal antibodies to the αvβ3 dimer were supplied by Gibco/BRL (USA); Matrigel, genistein, oligopeptides (GRGDS and GRDES) were from Sigma (USA). Preparations of type I collagen, fibronectin, and laminin were isolated and purified using described methods.

Cell models. We used the following lines of Syrian hamster embryo fibroblasts transformed *in vitro* by RSV (Schmidt–Ruppin strain): 1) HET-SR, with a low level of spontaneous metastasizing; 2) HET-SR-1, with an originally (after the transformation) high level of spontaneous metastasizing; 3) HET-SR-2SC-LNM, with a high level of spontaneous metastasizing; derived from HET-SR by *in vivo* selection of the cells with increasing metastatic activity while serial subcutaneous transplantations of the metastatic cells; 4) 2SC/20-2, with a low level of spontaneous metastasizing; derived from HET-SR-2SC-LNM.

The indicated lines were obtained and characterized in the All-Russian Oncological Research Center [11, 12]. The HET-SR, HET-SR-1, and HET-SR-2SC-LNM lines were kindly supplied by Prof. G. I. Deichman; the 2SC/20-2 line was kindly supplied by Prof. A. A. Stavrovskaya. The cells were cultivated at 37°C in the presence of 6% CO_2 in RPMI-1640 medium containing 5% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin.

Cell adhesion on matrix proteins was investigated as previously described [13].

Biotinylation of cell surface proteins, immunoprecipitation of integrins, electrophoresis, and Western-blotting of the immunoprecipitates were performed as described in [13].

***In vitro* invasion** was assayed in Costar (The Netherlands) transwells (membrane diameter 6.5 mm, pore size 8 μm). The upper well was filled with gel: 50 μl of a solution of Matrigel (3 mg/ml) in RPMI-1640 medium were applied to the well and incubated for 2 h at 37°C to let the gel form. Cells ($(2\text{-}3)\cdot10^4$) in 200-300 μl of the same medium containing 0.5% fetal serum were seeded on the gel, and the lower well was filled with 1 ml of the medium. The wells were incubated at 37°C for 24 h, and then the number of the cells migrated into the lower well was determined.

Enzymographic analysis of matrix metalloproteinases (MMP) was performed in 96-well microtiter plates with matrix proteins immobilized on the plastic. The cells ($25\cdot10^3$) in serum-free medium were added to the wells and incubated for 24 h at 37°C. The culture liquid (50 μl) was supplemented with 12.5 μl of a solution containing 2.5% SDS, 10% sucrose, and 4 μg/ml of Phenol red, and then electrophoresis was performed in 10% polyacrylamide gel containing 1.5 mg/ml of gelatin. Then the gel was washed with 0.05 M Tris-HCl, pH 7.5, containing 0.005 M $CaCl_2$ and 2.5% Triton X-100 and incubated in the same buffer containing 1% Triton X-100 at 37°C for 3-6 h. The gel was fixed, stained with Coomassie R-250, washed, and photographed.

Statistical analysis of the data was performed by Student's *t*-test using the Sigma-Plot computer program. Differences between cell lines were considered as significant when $p < 0.05$.

RESULTS

Affinity to matrix proteins and expression of integrins by RSV-transformed fibroblasts. To evaluate the effect of the metastatic activity of the transformed fibroblasts on their interaction with matrix proteins, we investigated adhesion of the original lowly metastasizing HET-SR cells and the derivative highly metastasizing HET-SR-2SC-LNM cells on substrates containing separate matrix proteins. It was found that the lowly metastasizing cells do not differ from the highly metastasizing cells in terms of their adhesion on the basic matrix proteins, such as native collagen, fibronectin, and laminin (data not shown). However, the HET-SR cells bind to the substrate from denatured collagen with significantly less efficiency than the highly metastasizing HET-SR-2SC-LNM cells.

It is known that in the integrin family the αvβ3 receptor exhibits a specific affinity to denatured collagen

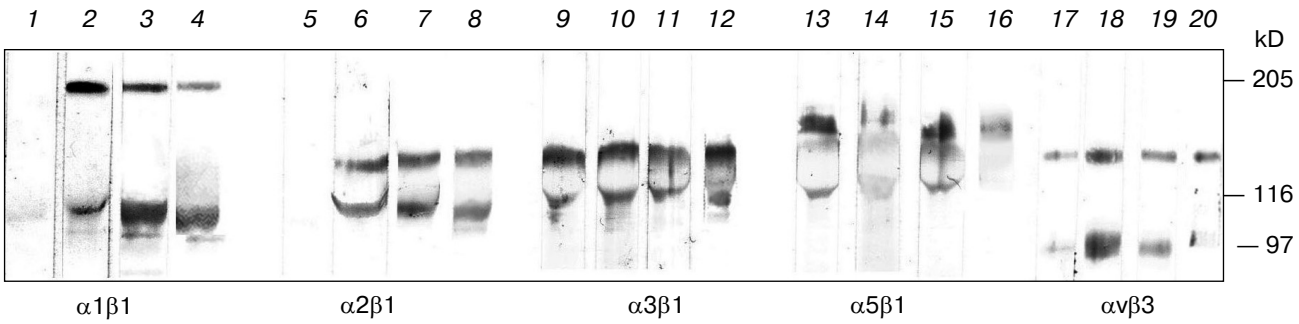

Fig. 1. Expression of integrins in the surface membrane of transformed fibroblasts differing in metastatic activity: PAGE and Western blotting of the immunoprecipitates of the biotinylated proteins from the cytoplasmic membrane of HET-SR (lanes *1*, *5*, *9*, *13*, *17*), HET-SR-2SC-LNM (lanes *2*, *6*, *10*, *14*, *18*), HET-SR-1 (*3*, *7*, *11*, *15*, *19*), and 2SC/20-2 (*4*, *8*, *12*, *16*, *20*). The proteins were precipitated by the antibodies to the α1 subunit (lanes *1-4*), to the α2 subunit (*5-8*), to the α3 subunit (*9-12*), to the α5 subunit (*13-16*), and to the αv subunit (*17-20*). The blots were developed with streptavidin phosphatase. Molecular weights of the protein markers are shown on the right in kD.

[1]. Consequently, it could be assumed that the highly metastasizing fibroblasts are characterized by an enhanced surface expression of the indicated integrin compared to the lowly metastasizing cells.

To test this assumption, the expression of integrins in the cell cytoplasmic membrane of the parental and the derivative lines (HET-SR and HET-SR-2SC-LNM, respectively) was investigated.

The data presented in Fig. 1 demonstrate that the HET-SR-2SC-LNM fibroblasts are much more active in the surface expression of the αvβ3 receptor compared to the HET-SR cells. However, these lines also differ in the expression of a number of other integrins. As seen from Fig. 1, the HET-SR fibroblasts are virtually inactive in the expression of the α1β1 and α2β1 receptors that specifically interact with native collagen, while the HET-SR-2SC-LNM cells are highly active in the expression of these receptors. The contrary situation is observed in the case of the α5β1 fibronectin-specific integrin, whose expression is much higher in the lowly metastasizing cells compared to the actively metastasizing fibroblasts. Integrins presented in Fig. 1 predominate in the typical integrin spectrum of fibroblasts. As seen from the figure, only the α3β1 receptor with an affinity to fibronectin, laminin, and collagen (in some types of cells) is expressed with the same intensity by the highly and lowly metastasizing cells.

Thus, the data on the cell adhesion on different substrates and on the expression of different integrins by the cells showed that the characteristic of the highly metastasizing HET-SR-2SC-LNM fibroblasts that distinguishes them from the lowly metastasizing parental HET-SR cells is a higher affinity to denatured collagen, a higher level of the expression of the collagen-specific α1β1, α2β1, and αvβ3 receptors, and a lower level of the expression of the fibronectin-specific α5β1 integrin.

It was important to determine whether these properties were characteristics for the metastatic phenotype or the differences observed were a random feature that was revealed only in one pair of the compared lines.

To clarify this point, the expression of integrins by the transformed HET-SR-1 fibroblasts was studied. The HET-SR-1 cells, as well as HET-SR-2SC-LNM, exhibit a high level of spontaneous metastasizing, but they are not derivatives from the HET-SR line. The results of this analysis presented in Fig. 1 show that the HET-SR-1 cells as well as HET-SR-2SC-LNM cells exhibit a high level of the expression of α1β1 and α2β1 integrins. The HET-SR-1 fibroblasts, unlike the HET-SR cells, express the αvβ3 integrin, although its expression level is lower than in the HET-SR-2SC-LNM cells.

Thus, the active expression of the collagen-specific receptors is observed in both independent lines of the transformed fibroblasts with a high metastatic potential. At the same time, the HET-SR-1 cells, unlike the HET-SR-2SC-LNM fibroblasts, also exhibit a high expression of the α5β1 fibronectin-specific receptor.

It was of special interest to compare these data with the results of the investigation of the 2SC/20-2 line that exhibits a lower metastatic activity compared to their parental HET-SR-2SC-LNM line. It was demonstrated that the decrease in the metastatic activity did not affect the expression of the α1β1 and α2β1 integrins, but resulted in the sharp down regulation of the surface expression of the αvβ3 receptor (Fig. 1).

Invasive activity and production of matrix-specific proteinases (MMP) by RSV-transformed fibroblasts. The initial stages of metastasizing involve destruction of the surrounding tissue by the tumor, migration (invasion) of the tumor cells from the primary point, and their invasion in the blood vessels and lymphatics [4, 14]. A model imitating these processes *in vitro* is penetration of the cells into gels formed by matrix proteins and destruction of the gels [15, 16].

Figure 2 presents the data of comparative analysis of the invasion of the fibroblasts with different metastatic

activity in Matrigel, a gel composed of the proteins of the basal membrane (mainly type IV collagen and laminin). It is seen that the HET-SR-2SC-LNM and HET-SR-1 cells exhibiting a high metastatic activity migrate through Matrigel much faster than the lowly metastasizing HET-SR cells. It is also seen that among two metastasizing lines, the HET-SR-2SC-LNM fibroblasts exhibiting a higher level of expression of the αvβ3 integrin possess a higher invasive potential.

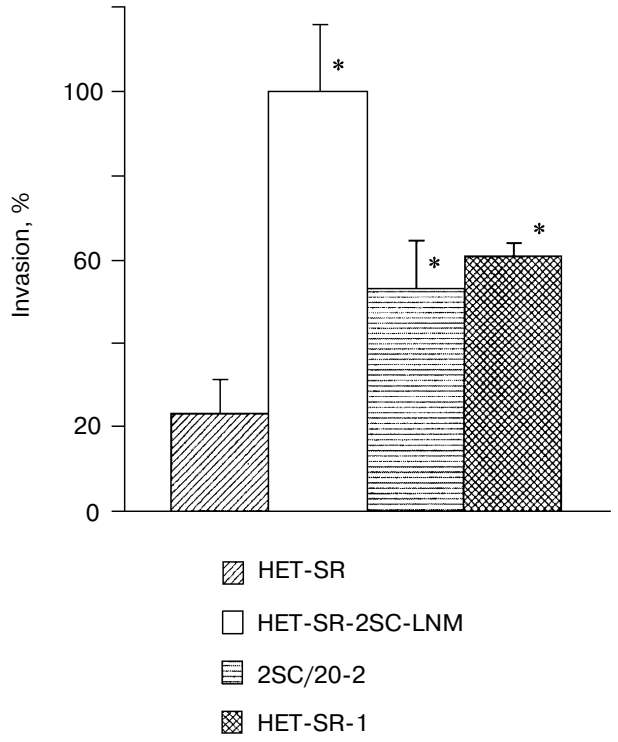

Fig. 2. *In vitro* invasion assay of oncotransformed fibroblasts with different metastatic activity. The cells ($5 \cdot 10^4$) were placed into the upper well of a transwell; after 48 h of incubation, the number of cells in the lower well was determined as described in "Materials and Methods". The invasive activity of the HET-SR-2SC-LNM cells was taken as 100%. Results of three experiments are presented as mean ± SE. * $p < 0.05$ relative to HET-SR.

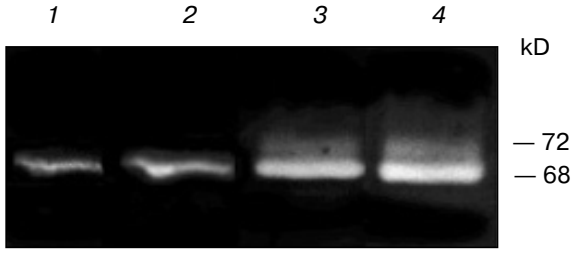

Fig. 3. Production of MMP-2 by the transformed fibroblasts differing in their metastatic activity. The cells were grown in medium containing 5% fetal bovine serum to reach subconfluence and then incubated in serum-free medium at 37°C for 24 h. Enzymographic analysis of the conditioned medium was performed as indicated in "Materials and Methods". *1*) HET-SR; *2*) 2SC/20-2; *3*) HET-SR-2SC-LMN; *4*) HET-SR-1. The molecular weights of the proenzyme (72 kD) and the active enzyme form (68 kD) are presented on the right in kD.

On comparing the invasion of the HET-SR-2SC-LNM fibroblasts and the derivative 2SC/20-2 cell line with a lower metastatic potential, it can be seen that the decrease in the metastatic activity is accompanied with a sharp decrease in the expression of the αvβ3 receptor and a significant decrease in the *in vitro* invasion, although its level remains higher than in the parental HET-SR cells.

The role of the enzymes cleaving the extracellular matrix proteins in the malignant progression of tumor cells is well known [14, 17]. In a number of works, a role of integrins in the transmission of the signals controlling the production of metalloproteinases (MMP), in particular the enzymes responsible for degradation of different types of collagen, was demonstrated [15, 18]. Previously we showed that the HET-SR-2SC-LNM fibroblasts are much more active than the HET-SR cells in the production of the MMP-1 collagenase cleaving the interstitial types of collagen (types I and III) [13]. We were interested in comparing the production of the MMP-2 and MMP-9 collagenases by the fibroblasts with different metastatic activity. These enzymes possess the same substrate specificity (cleave type IV collagen, the main component of the basal membrane, and denatured type I collagen), but differ in their molecular weights.

The activity of the collagenases MMP-2 and MMP-9 was assayed enzymographically in the conditioned medium obtained after cultivation of four lines of the transformed fibroblasts. The data presented in Fig. 3 demonstrate that the cells with a high metastatic potential are much more active in the production of MMP-2 than the lowly metastasizing fibroblasts. As in the case of MMP-1 [13], the enzyme is secreted in two forms: inactive form (proenzyme) with the molecular weight of 72 kD and the active one of 68 kD. The MMP-9 collagenase (92 kD) was not revealed in the investigated cell types.

Investigation of the role of integrin-mediated signals in production of MMP-2 by RSV-transformed fibroblasts and in their invasive activity. *1. Effect of inhibition of tyrosine protein kinases.* The results presented above demonstrate a strict correlation between the expression of the collagen-specific integrins by the metastatically active fibroblasts, the *in vitro* invasion of these cells, and their production of one of the collagenolytic enzymes. These properties could be a random coincidence of phenotypic features. But it can be assumed that the signals mediated by the collagen receptors play a significant role in metastatic evolution of the investigated cells. It is known that cellular signaling is a cascade of reactions of protein phosphorylation catalyzed by protein kinases [3, 19].

Inhibitors of these enzymes interrupt the signal and modify cell behavior. Tyrosine phosphokinases, especially FAK-kinase, are of special importance in the transmission of the integrin-mediated signals [20]. If the assumption concerning the role of integrins in the development of metastatic properties in the cell is correct, the inhibition of tyrosine phosphokinases must block the *in vitro* invasion and the production of collagenases.

To check this assumption, we investigated *in vitro* invasion of the cells in the presence of a strong inhibitor of tyrosine phosphokinases, genistein. In the presence of genistein, the migration of the HET-SR-2SC-LNM and HET-SR-1 fibroblasts into Matrigel was shown to reduce 4-5-fold (Fig. 4). The effect of the protein kinase inhibitor is not caused by its destructive influence, because virtually all the fibroblasts retain viability at the genistein concentrations employed.

At the same time, a strict correlation was found between the effect of genistein on the invasive activity and on the MMP-2 production (Fig. 5). It is seen that the inhibition of tyrosine protein kinases results in the virtually complete blocking of MMP-2 production by two independent lines of metastasizing fibroblasts.

2. Stimulation of signal activity of integrins by the substrate and its inhibition by RGD-containing peptide. The data concerning the effect of genistein can be considered as an indirect confirmation of the involvement of integrins into the transmission of intracellular signals controlling invasive activity of the transformed fibroblasts. To obtain direct confirmations, we used an approach based on the fact that the interaction of a cell with a substrate results in the activation of the receptor specific for this substrate and in the transmission of the signal affecting the gene activity and cell behavior. In the present work, this approach was used to reveal the role of the integrin-mediated signals generated by different matrix proteins in the production of MMP-2 by the transformed fibroblasts with different metastatic potential.

The data presented in Fig. 6 demonstrate that while using the major matrix proteins as the substrates, the production and secretion of MMP-2 are significantly higher in metastatically active fibroblasts compared to the lowly metastasizing cells. At the same time, the production of MMP-2 significantly depends on the substrate. As seen from the figure, type I collagen, both in the native and in the denatured forms, exhibits a higher ability to stimulate MMP-2 production by the metastatically active cells compared to fibronectin and laminin.

This observation is in agreement with the data presented above on the expression of integrins, according to which the HET-SR fibroblasts virtually do not express the collagen-specific $\alpha1\beta1$, $\alpha2\beta1$, and $\alpha v\beta3$ integrins, while the HET-SR-2SC-LNM cells are highly active in the expression of these receptors.

The second approach used for the investigation of the role of separate receptors consisted in the study of the

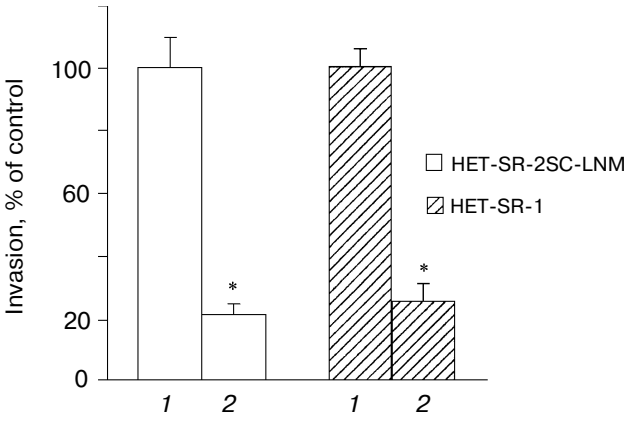

Fig. 4. Effect of genistein on *in vitro* invasion of the transformed fibroblasts with the high metastatic activity. The cells were incubated in medium containing genistein (20 μg/ml) for 30 min at 37°C, and then applied on Matrigel. The invasion was assayed as described in "Materials and Methods". *1*) Invasion of the cells not treated with genistein (control); *2*) invasion of the cells treated with genistein. Results of three experiments are presented (mean ± SE). * $p < 0.01$ relative to the control.

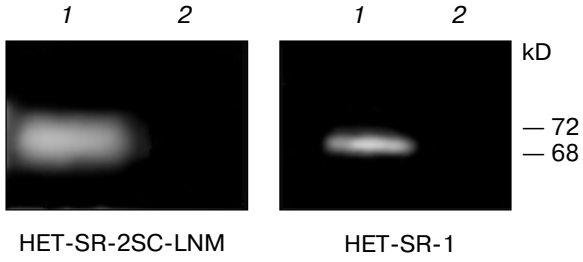

Fig. 5. Effect of genistein on production of MMP-2 by the fibroblasts with high metastatic activity. The cells were incubated in the presence of genistein as indicated in the legend to Fig. 4 and then incubated in a serum-free medium for 24 h. Enzymographic analysis of the conditioned medium was performed as described in "Materials and Methods". *1*) Cells not treated with genistein; *2*) cells treated with genistein.

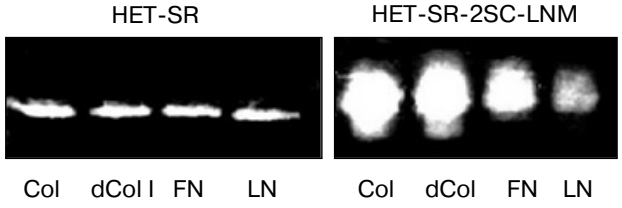

Fig. 6. Effect of substrates on the production of MMP-2 by fibroblasts with high and low metastatic activities. The cells were applied onto 24-well plates containing immobilized matrix proteins and incubated in serum-free medium for 24 h at 37°C. Then enzymographic analysis of the conditioned medium was performed as described in "Materials and Methods". Col) type I collagen; dCol) denatured type I collagen; FN) fibronectin; LN) laminin.

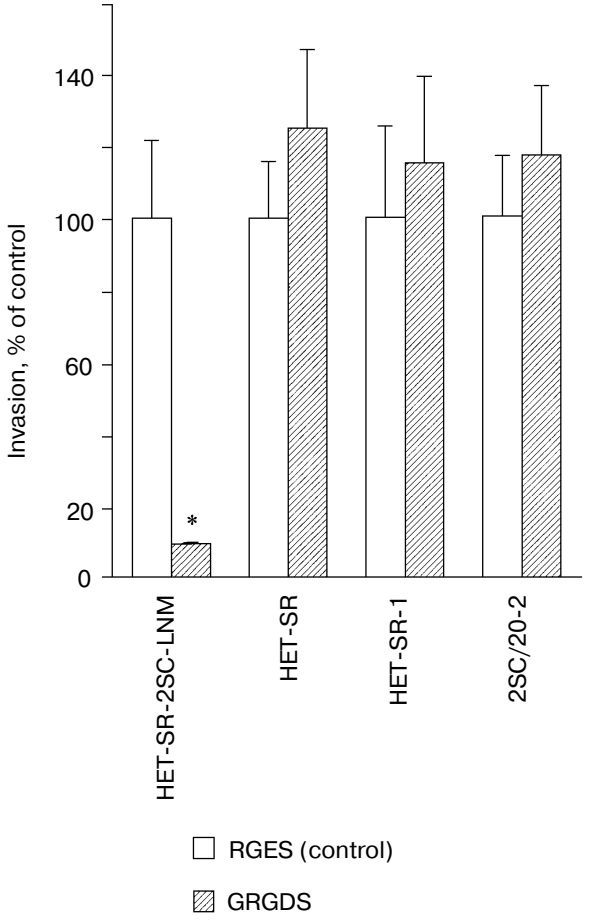

Fig. 7. Effect of RGD-peptide on the *in vitro* invasion of the transformed fibroblasts differing in their metastatic activity. The cells were incubated in medium containing 0.5% fetal bovine serum and 500 μg/ml of GRGDS pentapeptide or RGES peptide (control) for 30 min at 37°C and then placed on Matrigel. The invasion was assayed as described in "Materials and Methods". The results of three experiments are presented as mean ± SE. * $p < 0.01$ compared to the control.

effect of the integrin antagonists on the invasion and production of MMP. RGD pentapeptide (GRGDS) blocking the interaction of cells with the matrix that is mediated by the α5β1 and αvβ3 integrins was used as the antagonist.

The data presented in Fig. 7 show that in the presence of RGD peptide, the invasion of the HET-SR-2SC-LNM fibroblasts in Matrigel decreases 10-fold compared to the control (invasion in the presence of the inactive RGES peptide). RGD peptide did not affect significantly the invasion of cells of other lines. Comparing the effect of RGD peptide on the invasion of the fibroblasts of the investigated lines with the expression of the RGD-dependent integrins by these cells, it can be concluded that decreased invasion takes place only in the case of the cell line that is highly active in the expression of the αvβ3 receptor. The invasion of the cells with low expression of αvβ3, but active in the expression of other RGD-dependent integrin α5β1 (HET-SR cell line) or the cells with the low activity in the expression of the both indicated receptors (2SC/20-2 line) was shown to be insensitive towards RGD peptide. Consequently, in the investigated system of three relative lines of the RSV-transformed fibroblasts, it is the collagen- and vitronectin-specific αvβ3 integrin that is responsible for the *in vitro* invasive activity, while the fibronectin-specific α5β1 receptor is inert in this system. However, the absence of the effect of RGD peptide on the signal function of α5β1 can be accounted for by the fact that Matrigel does not contain a significant amount of fibronectin, the only substrate that this receptor is capable of interacting with.

However, the conclusion concerning the role of the αvβ3 integrin disagrees with the fact that RGD peptide does not inhibit the invasion of the HET-SR-1 cells expressing αvβ3, although the degree of its expression by these cells is lower than by the HET-SR-2SC-LNM fibroblasts. In our opinion, this contradiction also can be connected with the absence of fibronectin in Matrigel. It can be assumed that on inhibiting the invasion of the HET-SR-1 cells that are highly active in the surface expression of the α5β1 receptor compared to the HET-SR-2SC-LNM fibroblasts, α5β1 competes with αvβ3 for the RGD-containing peptide and decreases the efficiency of its action. If this assumption is correct, it can be expected that the RGD-containing peptide will be inefficient as the competitor while interacting of the HET-SR-2SC-LNM cells with fibronectin, but it will be an efficient competitor on the interaction of the HET-SR-1 fibroblasts with this substrate.

This assumption was tested in the investigation of the production of MMP-2 collagenase by the cells of the

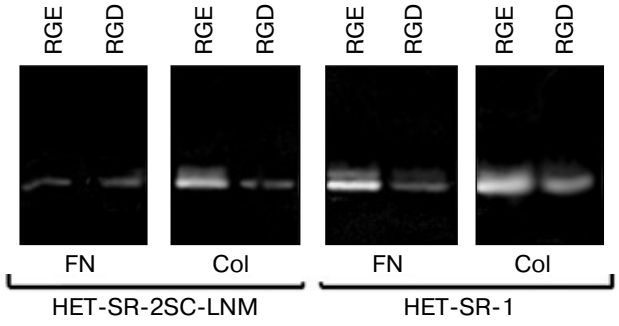

Fig. 8. Effect of RGD pentapeptide on the production of MMP-2 by the fibroblasts with high metastatic activity. The cells were incubated in medium containing 0.5% fetal bovine serum and 500 μg/ml of GRGDS pentapeptide or RGES-tetrapeptide (control) for 30 min at 37°C and then applied on the matrix proteins immobilized on 24-well plates. The cells were incubated in serum-free medium for 24 h at 37°C, and then enzymographic analysis of the conditioned medium was performed as described in "Materials and Methods". FN) fibronectin; Col) native type I collagen.

indicated lines under the conditions of their interaction with a fibronectin substrate and (for comparison) with a substrate from collagen (Fig. 8). It is seen that the HET-SR-2SC-LNM cells adhered to the fibronectin substrate are significantly less active in the production of MMP-2 than those adhered to the collagen. RGD-peptide inhibits production of the enzyme by the indicated cells adhered to the collagen substrate, but does not affect the MMP-2 production by these cells adhered to the fibronectin. Another situation takes place in the case of the HET-SR-1 cells. As seen, RGD-peptide inhibits the MMP-2 production by these fibroblasts adhered to fibronectin and does not effect the production of the enzyme when the cells are adhered to the substrate from collagen.

DISCUSSION

Analysis of the expression of integrins in four related lines of oncotransformed fibroblasts showed that the integrin spectrum of the lowly metastasizing cells is rather poor in the collagen-specific receptors $\alpha1\beta1$, $\alpha2\beta1$, and $\alpha v\beta3$ compared to the cells exhibiting high metastatic activity (Fig. 1). A high correlation between the metastatic activity of the transformed fibroblasts and their *in vitro* invasion was also demonstrated.

Among three collagen-specific integrins, the most obvious accordance between the expression level and the metastatic and invasive activities was found for the $\alpha v\beta3$ receptor. This is concluded from Figs. 1 and 2 demonstrating that although the HET-SR-1 and HET-SR-2SC-LNM lines do not differ in the expression of the $\alpha1\beta1$ and $\alpha2\beta1$ receptors, the HET-SR-1 cells have significantly lower *in vitro* invasive activity. At the same time, differences in the invasive activity of these lines agree well with the differences in the expression of the $\alpha v\beta3$ integrin. This relation is revealed on comparing the integrin spectrum and the invasive activity of the HET-SR-2SC-LNM and the derivative 2SC/20-2 lines.

It should be noted that the results obtained do not exclude the involvement of the $\alpha1\beta1$ and $\alpha2\beta1$ integrins in the mechanisms of metastasizing and invasion of the investigated cells. This conclusion is supported by not only a higher level of the expression of these receptors in the metastasizing cells, but also by the data on the substrate dependence of the invasion demonstrating that both native collagen (the substrate of the $\alpha1\beta1$ and $\alpha2\beta1$ integrins) and denatured collagen (the substrate of $\alpha v\beta3$) stimulate the production of MMP-2 collagenase by the cells of the metastasizing line more effectively than fibronectin and laminin (Fig. 6).

Literature data on the role of integrins in the mechanisms of invasion and metastasizing are numerous and, at the same time, contradictory.

Investigations of the cells of osteosarcoma, mammary gland carcinoma, and stomach carcinoma demonstrate that the invasive and metastatic phenotype of the tumor cells significantly depends on the expression of the $\alpha1\beta1$ and $\alpha2\beta1$ integrins [8, 21-23]. The effect of these receptors is mediated by their signal-transducing activity resulting in the stimulation of the expression of the metalloproteinase stromalysin [21]. Transfection of cDNA of the $\alpha2$ subunit into the osteosarcoma cells resulted in hyperexpression of $\alpha2\beta1$ and increased their invasive activity [24].

These observations disagree with the data on the expression of the $\alpha2\beta1$ integrin in the normal mammary glands, fibroadenoma, invasive tumor of mammary glands, and metastases [25], and also with the observation that stimulation of the expression of $\alpha2\beta1$ in the lowly differentiated actively invading cells of the mammary gland carcinoma resulted in reconstitution of the differentiated, less invasive phenotype [26].

Data concerning the role of the $\alpha v\beta3$ integrin in the mechanisms of metastasizing are also ambiguous. Investigations of the expression of this receptor in the lines of different origin (melanoma [27], cervical cancer [28], hepatocellular carcinoma [29], etc.) revealed its important role in the development of the invasive and metastatic phenotype of tumor cells. Antagonists of the $\alpha v\beta3$ integrin decrease the level of metastasizing of tumor cells and increase the lifespan of animals [30, 31].

However, no expression of the $\alpha v\beta3$ receptor was revealed in two lines of melanoma cells exhibiting a high level of the tumorigenic and metastatic activities [32] and in the line of actively metastasizing cells of stomach carcinoma [33]. In other line of the $\alpha v\beta3$-deficient melanoma cells, re-expression of this integrin inhibited the experimental metastasizing [34].

Analogous contradictions were observed in investigations of the expression of the $\alpha5\beta1$ and $\alpha6\beta4$ integrins by tumor cells [6, 9, 34-36].

In our opinion, a disadvantage of the cited works is the absence of complex analysis of the expression of integrins exhibiting multiple ligand specificity and the analysis of the matrix microenvironment, which is involved in the realization of the invasive and metastatic potentials of tumor cells in each special case. It can be assumed that on progressing of each cell population, the cells are selected not by a single receptor, but rather by a spectrum of surface integrins that is optimal for the interaction of the given population with its microenvironment and for development of the aggressive, malignant phenotype. Quantitative changes in the surface expression of a single integrin might be insignificant for the neoplastic growth and tumor progression.

The correlation revealed in the present work between the metastatic activity of the oncotransformed fibroblasts, their invasive properties, and the level of the expression of the collagen-specific receptors indicates a strict coordination between the cell microenvironment (especially the surrounding matrix), adhesive properties of the receptors

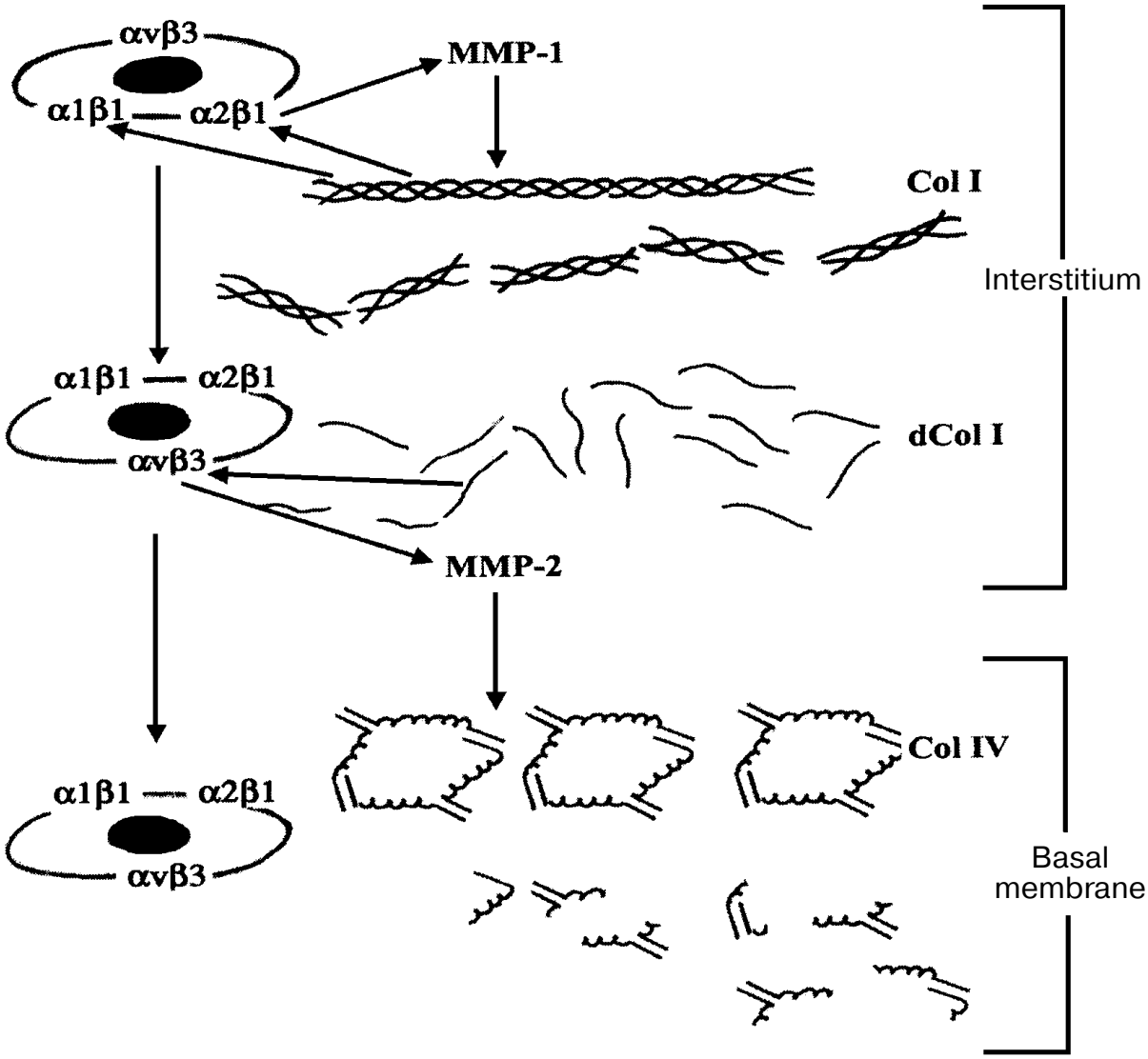

Fig. 9. Role of the integrin-mediated adhesion and the signal function of the collagen-specific integrins in the invasion of oncotransformed fibroblasts (see details in the text).

that are specific for the given environment, and signal functions of these receptors. We assume that evolution and selection of a metastasizing line results in elaboration of such a combination of properties that is optimal for separate stages of the metastatic process (invasion, intravasation, etc.). It is of importance that the specifying factor in this combination is the matrix surrounding the cells of the primary tumor. The cells adjust their integrin spectrum to the matrix while progressing of the malignant phenotype. This can explain numerous contradictions concerning the role of different integrins in invasion and metastasizing. Evidently, metastasizing tumors developing in different microenvironment expose to the cell surface different spectra of receptors.

Figure 9 demonstrates the relation between matrix and integrins during the invasion by an example of the investigated lines of fibroblasts.

All the investigated lines of transformed fibroblasts form tumors in the environment of interstitial collagen (mainly type I collagen). However, highly metastasizing lines exhibit a higher affinity to this type of collagen compared to the lowly metastasizing cells, since they express the $\alpha1\beta1$ and $\alpha2\beta1$ integrins that are specific for this type of collagen. On this interaction, the signal function of $\alpha2\beta1$ and possibly $\alpha1\beta1$ is activated, resulting in activation of the gene and production of MMP-1 collagenase [37], capable of cleaving interstitial collagen. Previously we found an increased level of the secretion of MMP-1 in

HET-SR-2SC-LNM cells compared to that of HET-SR [13]. Destruction of native collagen (collagen fibers) creates conditions for invasion. The destroyed collagen is easily subjected to denaturation, providing a substrate for αvβ3 integrin that is expressed by the highly metastasizing cells more actively (as for α1β1 and α2β1) compared to the lowly metastasizing cells. The interaction with the denatured collagen increases the signal activity of αvβ3 and up-regulates the synthesis and secretion of MMP-2 collagenase. This enzyme exhibits gelatinase activity and cleaves type I denatured collagen, thus facilitating further migration of the invading cells. Another substrate of MMP-2 is type IV collagen, one of the major components of the basal membrane. Due to this activity, the invading cells can destroy the basal membranes of blood vessels, providing conditions for their intravasation.

The suggested scheme is hypothetical and requires further experimental confirmation.

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