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REVIEW

Evolution of Cell Interactions with Extracellular Matrix during Carcinogenesis

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Abstract—Interaction of cells with extracellular matrix (ECM) largely defines migration capacity of cells and ways of their dissemination in normal tissue processes and during tumor progression. We review current knowledge about structure of cell adhesions with ECM and their alterations during carcinogenesis. We analyze how changes in structure of cell–matrix adhesions and ECM itself lead to acquisition of neoplastic properties by cells. Modern concepts of tumor cell motility and changes in the relationships of cells with ECM during tumor development are presented. Contemporary approaches for influencing the cell–ECM adhesion structures for inhibition of invasion and metastasis are briefly discussed.

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Normal functioning of an organism is possible when activities of all organs and cells are coordinated. Cells are in close interaction with each other and with the surrounding extracellular matrix. The cell environment is the source of different signals regulating the cell behavior, in particular, their reproduction state—transition to cell division, cell cycle arrest or to apoptosis, as well as migration during wound healing or embryonic development. An important role in these regulations belongs to specific structures providing for cell interactions with their environment, such as cell–cell contacts and adhesions to extracellular matrix [1-3]. These structures and their regulatory functions have been studied for many years, but recently great methodical advances have been made in studies of cell interactions with extracellular matrix. The combination of molecular biological methods with the most advanced methods of microscopy (the use of confocal or multiphoton microscopes and live cell videomicroscopy) made it possible to observe the motility of cells both *in vitro* and in living organisms *in vivo*, as well as to study the participation of individual structural and regulatory molecules in affording the process of cell interaction with its environment.

This review deals with present concepts of cell adhesion to extracellular matrix, changes in cell relationships with extracellular matrix during tumor progression, and new trends in investigations in this field associated with the possible effects on cell behavior in carcinogenesis.

CHANGE IN CHARACTER OF CELL MIGRATION DURING CARCINOGENESIS

A peculiarity of tumor cells is their enhanced ability to migrate and invade into adjacent tissues. In the course of invasion tumor cells leave normal structures by passing through basal membrane and migrate into the surrounding stroma. These events include significant changes in cell morphology as well as close relationships of cells with extracellular matrix (ECM) and structural rearrangement of the latter [4-7].

Recent research has shown that tumor cells can disseminate in different ways during carcinogenesis. This may be the so-called migration of individual cells (in such tumors as lymphomas, leukemias, and sarcomas) or collective migration characteristic for tumors of epithelial origin, when cells remain bound to each other and

Abbreviations: ECM) extracellular matrix; EMT) epithelial-mesenchymal transition; FAK) focal adhesion kinase; FC/FA) focal contacts/focal adhesion; MAT) mesenchymal-amoeboid transition; MMP) matrix metalloproteases.

migrate in a group [8]. In many tumors, both types of dissemination can exist simultaneously.

In the case of cell dedifferentiation during tumor progression, the so-called epithelial–mesenchymal transition (EMT) takes place when transformation results in rupture of cell–cell contacts and cells begin to move separately. Usually, the lower the extent of tumor differentiation the more often it spreads as individually migrating cells [8, 9]. In this case their movement resembles migration of fibroblasts and consists of several successive steps: protrusion of the active edge of the cell mainly due to actin polymerization, making adhesion contacts with the substrate, translocation of the cell body via contraction of actin–myosin fibrils, disassembling of adhesion structures in the back part of the cell, and retraction of the rear. The migration efficiency depends of the coordination of these steps [10]. Each step suggests significant rearrangements of the cell cytoskeleton, mainly of the actomyosin microfilament system that is in tight cooperation with other cytoskeleton systems, in particular with microtubules [11]. These rearrangements are under strict control of external factors (signals from ECM, growth factors, etc.) and intracellular regulators. The most important intracellular regulatory elements of this system for both normal and transformed cells are proteins of the family of small GTPases (Rac, Rho, Cdc42), each of which has its own function [12-14]. Thus, Rac is responsible for actin polymerization at the cell edge, regulating formation of lamellipodia (thin wide pseudopodia) and nascent focal complexes, while Rho is responsible for formation of actomyosin bundles (stress fibers), their tension, and maturation of focal contacts, whereas Cdc42 is responsible for generation of filopodia (narrow processes) [15-17]. The activation of Rac during fibroblast motility (mesenchymal migration) results in enhancement of the ability of cells to migrate, whereas an increase in Rho content results in formation of large focal contacts and retardation of cell motility.

It has been shown in experiments on cell migration in a 3D matrix that proteolytic degradation is the essential component of individual cell movement in ECM. An elongated cell with protrusions at the leading edge moves with formation of integrin-dependent contacts with ECM. Metalloproteases are accumulated at the leading edge and this process, in turn, depends on establishment of integrin contacts. Cell migration following the mesenchymal mechanism through 3D matrix is facilitated by degradation of ECM by secreted proteases, i.e. it depends on the presence and activity of metalloproteases [18, 19].

STRUCTURE AND FUNCTIONS OF FOCAL ADHESIONS, DIFFERENT ADHESION TYPES, ASSOCIATION WITH CYTOSKELETON

As noted above, interaction of a cell with a substrate is necessary to provide for cell movement along the substrate surface. This interaction is performed by specialized structures – focal adhesions. The first data on structure and function of focal adhesions were obtained more than 30 years ago during *in vitro* studies of cells on a 2D substrate [20-22]. It was shown that the cell–matrix interaction are effected through small regions associated with actin bundles and tightly adjacent to the substrate. At the present time it is known that adhesion or focal contacts (FC) are highly specialized structures responsible for cytoskeleton binding to extracellular matrix and playing an essential role in cell morphogenesis, thus providing for cell migration along the substrate [10]. The FC formation, size, and amount are closely associated with the arrangement of actin cytoskeleton. Disintegration of microfilament bundles (stress fibers) using inhibitors of actin polymerization or actin–myosin contractility results in destruction of FC [23-25]. Inhibition of FC formation (like that caused by inhibition of integrin interaction), in turn, blocks formation of stress fibers [26, 27]. Moreover, the enhanced contractility or additional cell tension caused by a force applied from the outside results in the enlargement of FC and growth of actin stress fibers [28, 29].

More than fifty proteins associated with focal contacts and adhesions with ECM are now known [30]. These proteins can directly interact with each other or be involved in complex regulatory relationships [30]. They can be separated into several groups in accordance with their functions. The first group includes transmembrane proteins integrins, the main receptors binding the cytoskeleton to ECM [31]. The second group consists of proteins incorporated in the focal adhesion structure from the cytoplasmic face of the adhesion site and associated with the cytoskeleton (tensin, vinculin, paxillin, α-actinin, etc.). Most of them are able to carry out both structural and regulatory functions. The third group includes a large number of regulatory proteins (tyrosine kinases, serine-threonine kinases, protein regulators of small GTPase activities, tyrosine phosphatases, and other enzymes) also associated with adhesion structures. Interaction of these proteins with each other and their activation are under strict control of the cell signals and those from the outside. Different types of adhesion structures can be formed in response to these outside signals, which, in turn, defines cell behavior, in particular, its ability for directed migration and the rate of this migration.

According to the current classification, several types of cell adhesion contacts with ECM are distinguished, such as focal complexes, focal contacts, and fibrillar adhesions [25, 30]. Focal complexes are small (0.5-1.0 μm), punctate, short-lived adhesions with the substrate [15, 16, 32]. They usually emerge at the active edge of a cell in the region of lamellipodia or filipodia [33-35] and their formation depends on activity of the small GTPases Rac and Cdc42 [15, 16]. Focal complexes exist

for several minutes and then either dissociate or undergo maturation to larger focal contacts. It was shown that despite small dimensions and rapid exchange of molecules comprising focal complexes could support stronger traction force transmitted to substratum than mature focal contacts [36]. As a rule, focal complexes are characteristic of highly motile cells like keratocytes and melanoma B16. Mature focal contacts are oval structures of 3-10 μm in length associated with the ends of microfilament bundles. Their formation is regulated by the activation of GTPase Rho [15, 16] and depends on tension developed due to contraction of actomyosin stress fibers [29, 37] or is activated by outside applied force [28]. The FC assembly–disassembly takes 10-120 min, and these structures are typical for relatively slowly moving cells. Thus, the focal contact forming cells move in the 3D matrix with the rate of 0.1-2 μm/min [38]. In stationary cells, the tensile stress that can be developed by focal contacts relative the substrate is proportional to the size of the FC [39].

No significant differences were revealed in molecular composition between focal complexes and focal contacts, although there are quantitative distinctions in the ratio of various proteins or in the level of their phosphorylation and hence in their level of activation. Thus, it was shown that the content of phosphorylated focal adhesion kinase (FAK), an important protein involved in regulation of adhesion structure formation, is lower in focal complexes compared to that in focal contacts, and protein zixin appears in focal complexes somewhat later than other proteins, and usually at the moment of the contraction of the cell edge, i.e. upon maturation of focal complexes to focal contacts ([34] and our unpublished results). Fibrillar adhesions are a different type of adhesion structures formed by fibroblasts. They differ in molecular composition from focal contacts. Fibrillar adhesions are enriched with integrin $\alpha_5\beta_1$ and they also include tensin and parvin [30, 40, 41]. Fibrillar adhesions bind to fibronectin fibers and, owing to tension, form fibronectin bundles in the surrounding extracellular matrix [42], but their formation and existence is independent of tension of actomyosin stress fibers [25, 41].

All these adhesion structures interact with ECM and thus, in turn, affect its structure. Formation of ECM structures depends on the tension developed by the cell and hence on its cytoskeleton structure and on the type of adhesion structures formed by the cell. The ECM structure can also be changed in response to the action of different proteases secreted by cells. There is still practically no data showing that any active degradation of extracellular matrix can be carried out with involvement of FC, focal complexes, or fibrillar adhesions. Only Takino et al. [43] showed on transformed HeLa and fibrosarcoma HT1080 cells that expression of matrix membrane type 1 metalloprotease (MT1-MMP) and activation of MMP2 take place in the region of focal adhesions, while fibronectin (a protein of extracellular matrix) disappears from these regions. No biochemical proof of real ECM degradation is given in this work.

In addition to the above-described adhesions, characteristic of all normal cells, specific integrin-containing adhesion structures, podosomes, are also distinguished; they were first described in fibroblasts transformed by Rous sarcoma virus [44] and are typical adhesion structures of tumor cells [45, 46]. Podosomes also occur in some normal cells, such as monocytes and macrophages, which are characterized by rapid migration [47]. In osteoclasts where podosomes are located at the cell periphery, they take part in resorption of bony tissue [48-50]. Since the presence of podosomes is characteristic of the most aggressive invading cells, the current interest in investigation of these structures is very high [45, 51-54]. The podosome has complex functions: it provides for cell adhesion to ECM, migration along extracellular matrix or inside it, and simultaneously degrades this matrix. The podosome adheres to adjacent substrate via a circular structure containing integrin and integrin-interacting proteins practically identical to those present in adhesion contacts. The main difference between podosomes and the above-considered adhesion structures is the presence of matrix metalloproteases, which are responsible for matrix degradation. Podosomes are short-lived structures; their assembly–disassembly takes only minutes [51].

STRUCTURAL ALTERATIONS OF CELL–MATRIX ADHESIONS DURING CARCINOGENESIS

Cell morphology, cytoskeleton, and structure of focal contacts undergo significant changes during transformation. Investigations *in vitro* have shown that the extent of cell spreading on the substrate significantly decreases, actin stress fibers practically disappear, and the number of mature focal contacts also becomes significantly lower [55]. Instead, a great number of small punctate focal complexes are formed in transformed cells [56]. The induction of this phenotype depends on activation of Rac, intensifying the dynamics of pseudopodia, and on lowering the Rho activity, which results in suppression of ROCK-dependent formation of stress fibers and reduction of adhesion contacts [8, 57, 58]. It was also shown that transformed cells developed lower tension and exhibited lower ability to deform a flexible substrate (silicon film) compared to normal cells [59], i.e. they exert lower pressure on the surrounding ECM. Many works are now published concerning changes, caused by neoplastic transformation, in the content or activity of individual proteins incorporated in focal contacts or regulating its functions.

Tumor cell invasiveness correlates with integrin expression and composition. Integrins (the main receptors involved in cell contacts with ECM) are heterodimers

consisting of transmembrane subunits α and β and having a large extracellular domain, an intramembrane domain, and an intracellular domain interacting with cytoskeleton proteins [60, 61]. In mammals 18 α- and eight β-subunits are known, which associate in different combinations and form 24 integrins [62, 63]. Different integrins usually provide for interaction with different substrates, but sometimes the same integrin can interact with different substrates, and vice versa, different integrins can participate in formation of adhesion structures with the same substrate [64]. Integrins provide for transmission of chemical and mechanical signals, which in turn, results in rearrangement of the cell cytoskeleton and activation of pathways that control cell survival and motility [65, 66].

Thus, integrins not only provide for interaction with ECM, but besides they are involved in regulation of biological processes like proliferation, differentiation, apoptosis, and angiogenesis. The disturbance of integrin-dependent contacts with ECM in normal cells results in their death caused by a specific type of apoptosis – anoikis [67]. The ability to survive without contacts with a substrate is a feature of tumor cells. However, in this case tumor cells still depend on signals from integrins both at the beginning of carcinogenesis and in the course of tumor progression [68, 69]. It was shown that expression of different integrins changed significantly during carcinogenesis [70]. In particular, it was shown that expression of integrin $\alpha_6\beta_4$, involved in cooperation with the epidermal growth factor receptor in intracellular transmission of mitogenetic signals is enhanced in many carcinomas [71, 72], whereas expression of integrin $\alpha_v\beta_3$ cooperating with platelet-derived and epidermal growth factors was enhanced in glioblastomas and melanomas [73, 74]. The regulatory pathway initiated by these integrins results in increased cell proliferative activity [75, 76]. Besides, some integrins were able to stimulate tumor invasion due to regulation of expression, activity, or maturation of matrix metalloproteases degrading components of basal membrane and extracellular matrix [77-79]. Activation of some integrins ($\alpha_v\beta_3$, $\alpha_6\beta_4$) or their regulatory pathways stimulates expression of blood vessel endothelium growth factor (VEGF) and activates its receptor, which contributes to tumor vascularization [80, 81].

Change in focal adhesion kinase (FAK) during carcinogenesis. Focal adhesion kinase is another component of cell adhesion contacts that is often changed during carcinogenesis. In many human tumors either the level of FAK expression or the level of its phosphorylation increase, i.e. the enzyme undergoes activation [82-85]. These changes correlate with acquiring of invasive cell phenotype and with increased number of metastases [86-88]. FAK is a regulatory protein associated with focal contacts and playing an essential role in their dynamics and cytoskeleton modeling during normal cell motility [8, 89-91]. FAK causes disassembly of focal contacts by an unknown mechanism and thus stimulates more rapid exchange of contact structures, which in turn increases the rate of cell migration. FAK stimulates the activity of Rac (a small GTPase responsible for formation of cell protrusions), and this process in turn results in activation of cell motility [92, 93]. In addition, FAK activation results in increased MMP synthesis [84] and hence in ECM degradation and enhancement of cell invasion. It was also shown that FAK can be involved in extracellular matrix remodeling not only by degrading the latter by metalloproteases, but also due to enhanced cytoskeleton contractility. In this case regulatory cellular pathways, leading to increased cell proliferation and inhibition of anoikis, are switched on [93], i.e. the enhancement of FAK activity or expression causes not only structural rearrangement of adhesion structures, but rather a shift of the regulatory pathways toward manifestation of neoplastic behavior of the cell.

Change in other focal adhesion proteins during carcinogenesis. Paxillin was shown to play a special role among structural proteins of FC. Its phosphorylation level is of significant importance for regulation of cell migration [94, 95]. Recently it was directly shown that paxillin overphosphorylation is associated with metastatic potential of human osteosarcoma [96]. It is supposed that paxillin phosphorylation mediates activation of small GTPase Rac1 and thus enhances cell migration.

It was also shown that the development of breast cancer metastases is accompanied by a decrease in the amount of tensin 3, which is responsible for integrin binding to actin cytoskeleton. Instead, expression of cten, a protein also belonging to the tensin family, but unlike tensin 3, deprived of actin-binding domain, increases. In this case the interaction of integrin receptors with actin cytoskeleton is disturbed, which results in disintegration of actin stress fibers and acquiring by cells of a mobile fibroblast-like phenotype [97], i.e. this not only (and not so much as) regulates the FC morphology, but causes total cytoskeleton rearrangement.

Increase in cell invasiveness is often caused by accelerated FC assembly–disassembly. Thus, there are numerous data showing that enhanced expression of the cytoplasmic protease calpain is directly associated with *in vivo* invasion [98]. This protease cleaves focal contact components (talin, cytoplasmic tails of integrin subunits β_1 and β_3), which contributes to the more active rotation of adhesion structures and participates in actin cytoskeleton rearrangement [99, 100].

Thus, in all cases of changes in the cell–ECM adhesion structure or in activity of any protein incorporated in these structures, the acquiring by cells of invasive phenotype is connected with complicated regulations and rearrangements concerning the whole actin cytoskeleton and often with alteration of the cell proliferative status or its ability to survive.

FORMATION OF A NEW TYPE OF CELL–MATRIX ADHESIONS DURING CARCINOGENESIS. PODOSOMES

Further changes in focal adhesions during transformation include the appearance of a different type of adhesion structures – podosomes. As indicated above, a podosome is a highly dynamic point adhesion structure. Besides integrins and proteins typical of other adhesion structures, the podosomes also harbor the actin polymerization machinery components Arp2/3, leading to the actin cytoskeleton rearrangement. In addition, the podosome is characterized by the presence of metalloproteases involved in ECM rearrangement [51, 101]. Formation of podosomes is described in many transformed cells of epithelial origin, including HeLa and MCF-7 [102]. Podosomes can also be induced in endothelial and smooth muscle cells after treatment with the tumor promoter PMA (phorbol 12-myristate 13-acetate) [103] or growth factors. This process is connected with local actin rearrangement [104, 105]. Signal pathways regulated by small GTPases and serine-threonine kinases, FAR- and Src-kinases, are very important for podosome formation [51]. Single cells degrade ECM with the involvement of podosomes, thus providing for cell penetration through basal membrane and invasion into adjacent tissues.

FURTHER DEDIFFERENTIATION LEADS TO ATTENUATION OF CELL–MATRIX INTERACTION

Further cell dedifferentiation can result in emergence of a different type of cell migration, independent of or slightly dependent on formation of adhesion structures with extracellular matrix [8, 18]. In this case, cell morphology changes from the extended fibroblast-like to the rounded or ellipsoid, and integrin receptors on the cell surface undergo redistribution. The level of metalloprotease expression significantly decreases and becomes less important compared to that for movement of fibroblast-like cells. The cells become very mobile and begin to move in a way resembling that of an amoeba, by squeezing through ECM [8], i.e. cells change their movement from mesenchymal to amoeboid mode. This means that the mesenchymal–amoeboid transition (MAT) takes place in this case [18]. Such type of movement was shown to be characteristic of lymphomas and small-cell cancers of lungs and prostate. When assembly–disassembly of focal adhesions is not necessary, cells can move at rates almost 30 times higher than that of cells forming integrin adhesions.

In vitro on a 2D substrate, the cell transition to amoeboid type of mobility due to reduced adhesion to the substrate results in the cell detachment from the substrate surface. Owing to this, the description of such type movement, evidently widespread *in vivo*, became possible only recently during investigation of cell mobility in a 3D matrix or in an organism. The amoeboid mode of movement also requires significant rearrangement of actin cytoskeleton. Practically no stress fibers are detected in such cells. It is assumed that motility of these cells is associated with cortical actin.

It was shown that tumor cells can change the type of migration depending on the environmental conditions. For example, inhibition of matrix metalloprotease (MMP) activities or inhibition of Rho-dependent regulatory pathways results in MAT. In *in vitro* experiments, the highly invasive fibrosarcoma HT1080 cells treated with inhibitors of proteolytic enzymes (MMP, cathepsins, and serine proteases) changed their type of motility from mesenchymal to amoeboid, i.e. from the movement dependent on the proteolytic enzyme activities to independent. Thus, the MAT model was obtained in experimental conditions [8].

CHANGE IN EXTRACELLULAR MATRIX COMPOSITION DURING CARCINOGENESIS

In addition to alteration of their own morphology and character of interaction with ECM, tumor cells also change ECM composition. A great number of proteolytic enzymes, including MMP and serine and cysteine proteases, are activated during tumor progression [106-108]. Their activity results in degradation of matrix structures. However, in addition to ECM degradation the level of expression of different matrix proteins also changes during carcinogenesis. In particular, a high level of fibronectin expression positively correlates with invasion into lymph nodes and with bad prognosis for patients with breast cancer [109]. There are data showing that the composition of laminins can change during tumor progression. In particular, the increased expression of laminin-8 was shown in gliomas and in highly invasive mammary gland duct cancers [6]. The rearrangement of ECM by tumor cells due to enhanced expression of individual ECM components significantly increases survival of tumor cells in response to chemotherapy [110].

Thus, practically all changes essential for transformation, even if they concern just individual proteins of adhesion structures or matrix, result in complex changes of regulatory pathways involving not only adhesion structures but leading to global changes of the cell cytoskeleton, proliferative status, and relationships with ECM. In this case, a general tendency for development of the cell–ECM relationships during tumor progression is the acquiring by the cell of higher independence from the environment and more pronounced aggression towards the environment.

INVOLVEMENT OF SMALL GTPases IN INVASION AND CARCINOGENESIS

As mentioned above, GTPases of the Rho family play an important role in regulation of cytoskeleton rearrangement [14]. It has been shown that proteins of the Rho family are also able to influence expression of individual genes and thereby effect cell proliferation and survival [111]. All these functions are of crucial significance in carcinogenesis. The change in proliferation level or activation of Rho proteins is shown for many tumors [111]. It has been shown that enhanced expression of Rac or Rho and activation of their regulatory pathways involving ROCK or MLCK result in enhanced migration of tumor cells *in vitro* and correlate with increased invasiveness *in vivo* [58, 112-114]. Changes in expression level or activation of Rho proteins are significant for revealing different stages of tumor progression, beginning from primary features (attenuation of cell–cell contacts and acquiring by cells of mesenchymal mobile phenotype) and for both the enhanced cell motility and for ECM degradation. Rho and Rac can regulate cell mobility at the expense of activation of cytoskeleton proteins ezrin, moesin, and radixin, which bind actin cytoskeleton to plasma membrane and thus determine the character and rate of cell migration [115, 116].

RhoA and Rac1 can model ECM degradation and rearrangement by regulation of MMP expression level or, in contrast, of expression level of tissue inhibitors of metalloproteases [117, 118].

It is understandable that Rho proteins play an important role in tumor development, but data on changes in expression level of these proteins in different tumors are quite controversial. This can probably be explained by the multicomponent action of Rho proteins at different stages of carcinogenesis. Tumor cells following mesenchymal and amoeboid types of dissemination move using different mechanisms. Accordingly, Rho proteins can play different roles depending on the type of cell mobility. For cells moving by the mesenchymal mechanism, the Rac-dependent protrusion formation at the leading edge is essential, whereas Rho signaling is not so important [119]. Since cortical actin, rearrangement of which is defined by the Rho/ROCK-dependent regulations, plays an important role in amoeboid movement [120], this means that suppression of the Rho/ROCK pathway inhibits the amoeboid-type migration. At least sometimes tumor cells can switch from one type of movement to the other depending on the activity of small GTPases. Thus, inhibition of Rac in HT1080 fibrosarcoma cells leads to switching to the amoeboid-type mobility, and vice versa, inhibition of the Rho/ROCK pathway results in exhibition of mesenchymal features [19]. Thus, the balance between Rac and Rho activities is able to define the character of tumor cell migration.

The ability of tumor cells to change migration mechanisms and, respectively, the ability for invasion due to morphological or functional dedifferentiation or depending on surrounding conditions is called plasticity [8].

SOME MODERN APPROACHES TO SUPPRESS INVASION AND MIGRATION ABILITIES OF TUMOR CELLS

Recent success in studying mechanisms of tumor cell migration and invasion made it possible to outline approaches for therapeutic treatment on these processes. They are mainly connected with attempts to regulate protein activities that change during carcinogenesis. One such approach concerns attempts to influence FAK in tumor cells [93, 121]. Thus, several groups of researchers have synthesized compounds (PF-573,228, PF-562,271, and NVP-TAE226) influencing FAK catalytic activity. These compounds interact with FAK by ATP binding site and effectively inhibit FAK kinase activity [122-125]. These agents inhibit cell migration. One of them (PF-562,271 or PF-00562271) passed preclinical tests. Regression of human tumors transplanted into nude mice or tumor growth arrest were shown for cancers of prostate, mammary gland, large intestine, lung, glioblastoma, and cancer of the pancreas. Phase 1 of clinical trials has been carried out on patients with solid tumors of different localization, and encouraging results were obtained [93, 126].

Much attention is given to the search for agents influencing regulatory pathways of small Rho GTPases as key regulatory proteins involved in carcinogenesis. But so far there is no great success in this direction, probably because, as already noted above, different Rho proteins are required at different stages of tumor progression, and migration of tumor cells may follow different mechanisms [8]. Inhibition of functions of one member of the Rho family does not produce a desired result, because transformed cells are able to switch from one type of movement to another. Evidently, just due to tumor cell plasticity, attempts to influence invasion and migration abilities of tumor cells by direct effect on receptor molecules or on proteolytic enzymes have still not given any significant results [8].

There are numerous attempts to obtain molecular antagonists of integrins inhibiting signal pathways from these proteins which lead to MMP or FAK activation, or stimulate vessel development in tumors [127]. In particular, monoclonal antibodies to integrin $\alpha_v\beta_3$ blocking angiogenesis have been developed. Derivatives of these preparations are now at the stage of clinical trials [127].

Thus, investigations of cell and ECM interactions during carcinogenesis are at the stage of intensive development, and owing to modern approaches they produce remarkable material for understanding carcinogenesis and development of approaches to combat carcinogenesis.

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