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Theories on the metastatic process and possible therapeutic options

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Abstract A sequence of steps are prerequisite for cancer cells before metastases are established. Metastasis has been shown to be an inefficient process limited by both random and selective events. By differentiating invasion from metastasis, sequential steps in the metastatic cascade have been defined and studied separately. This approach has yielded a variety of new potential therapeutic strategies. However, increasing knowledge of the mechanisms relating to metastasis has also revealed the complexity of each step. In spite of difficulties in translating results obtained in preclinical models into the clinical setting, continued development of such model systems and further research into the genetic control of metastatic dissemination will lead to improved strategies for prevention of metastasis formation and for treatment of metastatic tumor cells.

Key words Metastatic cascade · Invasion
Anti-metastatic therapy

Approximately 50% of all cancers have already metastasized by the time of diagnosis. In spite of extended surgical procedures and the availability of new chemotherapeutic agents, most patients will ultimately die because of metastatic dissemination [8]. The propensity for metastasis formation varies significantly within different tumor entities. The metastatic process involves a sequence of steps that are prerequisite for distant metastases to be established [15]. Of all the tumor cells that reach the circulation, only a small percentage (< 0.01%) will ultimately succeed in initiating metastatic colonies [39], the majority of tumor cells being eliminated by random events [78]. Both experimental and clinical evidence suggests that metastasis is a highly selective process. Continued cell heterogeneity in primary tumors as well as in metastases has been shown to be a critical factor favoring the survival of a subpopulation of tumor cells with metastatic potential [16]. The relative size of this subpopulation has been a matter of debate in the past. However, evidence from the use of genetic markers suggests that the metastatic subpopulation dominates the primary tumor early in its growth [32]. Studies with clinical tumor samples have also demonstrated that genetic alterations detected in the primary tumor can be correlated with clinical parameters of metastasis and recurrence [44, 68]. Continued selection of tumor cell subpopulations and increasing genetic instability probably account for progressive dissemination even in organ sites not characteristically involved with a given tumor system.

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The metastatic cascade

Some genetic changes lead to unrestrained growth, and additional genetic changes are required for invasion and metastasis. Therefore, tumorigenicity and metastatic potential are viewed as both separate and overlapping features [43]. Advances in understanding the molecular mechanisms involved in metastasis have been hindered by the complexity of the multistep tumor/host interactions. Investigators have therefore separated invasion and metastasis into a series of defined steps (Fig. 1).

Local invasion

An early event during invasion is the penetration of the epithelial basement membrane and penetration into the interstitial stroma. The continuous basement membrane is a dense matrix (collagen, glycoproteins and proteoglycans) not normally allowing for passive cell traversal. Invasion of the basement membrane is an active process requiring (a) attachment to the basement membrane, (b) matrix dissolution and (c) cell migration.

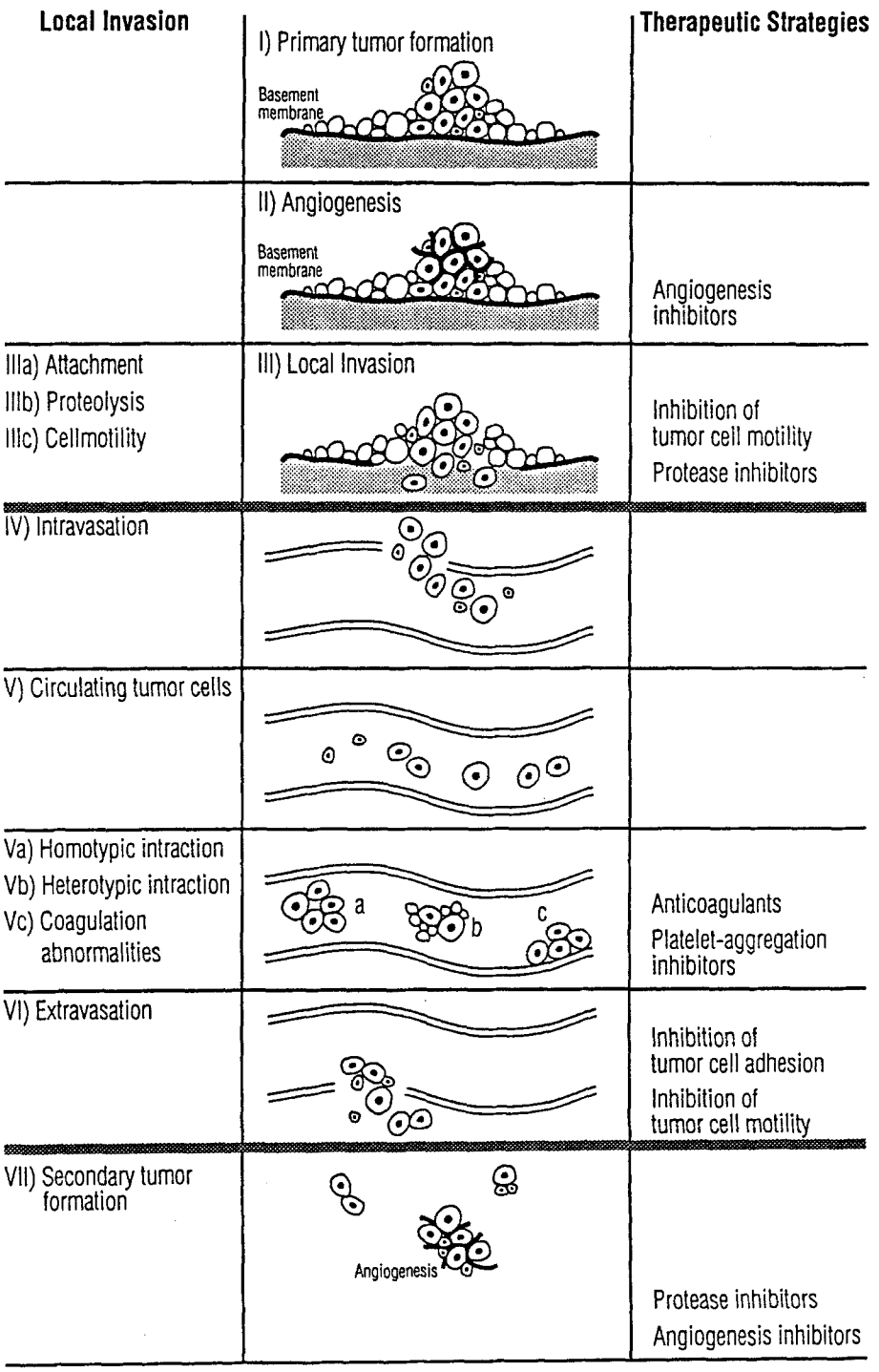

Fig. 1 The metastatic cascade

a) Attachment is mediated by cell surface receptors of the integrin and non-integrin types [3, 30], which recognize basement membrane glycoproteins (e.g., laminin, type IV collagen and fibronectin).

b) Degradative enzymes are secreted by the tumor cells producing a localized zone of lysis immediately adjacent to the cell surface [10]. In this region, the amount of active enzyme outweighs natural proteinase inhibitors originating from the matrix, serum or surrounding cells. Proteolysis plays a crucial role not only during invasion at the primary site but also during intravasation, extravasation and successful invasion and establishment of metastases in distant organs (see below).

c) Cell migration is the third step required for invasion. In the area of matrix proteolysis the tumor cell must migrate through basement membrane and stroma. This cell move-

ment has been shown to be directional, mediated by ligands binding to the cell surface and inducing a coordinated mobilization of cytoskeletal elements. Cytokines which regulate random tumor cell motility have been identified, e.g., "autocrine motility factor (AMF)" [42] and "scatter factor" [22]. Increased random cell motility causes dispersion at the primary site. The level of AMF in the urine of bladder cancer patients has been shown to be associated with invasion levels, stage and grade of disease [23]. Melanoma cells produce AMF which stimulates their own motility and invasiveness [42]. AMF is suspected to mediate its effect through G proteins, which transmit signals received at the cell surface membrane. Recently, another human motility-stimulating protein [termed "autotaxin" (ATX)] has been isolated from culture medium of A2058 human melanoma cells [71]. This autocrine motility factor stimulates both random and directed motility. Its activity appears to be receptor mediated since pretreatment of the melanoma cells with pertussis toxin abolishes the response of tumor cells to ATX.

Proteolysis

Proteolysis of tissue barriers is not restricted to malignant processes but also occurs during trophoblast implantation, embryo morphogenesis, tissue remodeling and angiogenesis. However, tumor cells couple proteolysis with motility at times and places inappropriate for normal cells. For a variety of classes of degradative enzymes (e.g., heparinases; serine-, thiol-, metal-dependent enzymes) a positive correlation with tumor aggressiveness was found [38, 54, 61]. The emerging picture is that probably all of these enzymes are involved in invasion and their interaction appears to be comparable to the proteolytic cascades involved in blood coagulation. This view has been corroborated by recent findings showing that inhibitors of any one of these enzymes can each block tumor cell invasion in vitro [47, 77].

Within the metalloproteinase family (interstitial collagenases, type IV collagenases, stromelysins), type IV collagenase has been under intense investigation and its association with invasion and metastasis has been documented both in vitro [41] and in vivo: almost all invasive colonic and gastric adenocarcinomas stained positive for this antigen [37]. Type IV collagenase activity has been successfully downregulated by retinoic acid treatment of human melanoma cells, resulting in loss of the invasive phenotype [25].

The action of proteinases is counterbalanced by natural proteinase inhibitors produced either by the tumor cell itself or by the host. Natural inhibitors such as "tissue inhibitors of metalloproteinases" (TIMPs) or "plasminogen activator inhibitors" (PAIs) may therefore function as metastasis suppressors as demonstrated in animal models [1]. TIMP levels correlated inversely with the invasive potential of intracranial tumors and purified TIMP inhibited invasion of the human amnion by sarcoma cells. Inhibition of TIMP by transfection of antisense-TIMP into 3T3 cells yielded highly invasive variants with increased type IV collagenase activity [12].

Intravasation

The access of tumor cells to the lumen of blood vessels is facilitated within the primary tumor. Because of structural vascular defects the newly formed vessels are abnormally "leaky" [13] and tumor cells either pass through junctions between endothelial cells or directly traverse the endothelial cells themselves (intracellular passage). This process is quantitatively related to the surface area of tumor vessels [40].

During their passage through the circulation tumor cells use interactions with themselves (homotypic) as well as interactions with blood cells (heterotypic) in order to increase metastatic capacity: homotypic interaction positively affects implantation in the microcirculation at the metastatic site [50]. By interacting with platelets and fibrin, tumor cells presumably become more insensitive to shear forces and immune effector cells. Inhibitors of platelet aggregation have been shown to inhibit metastasis in experimental animal tumors [29].

Heterotypic interaction also leads to coagulation abnormalities with increased turnover of constituents of the clotting system. Hemostatic abnormalities indicative of disseminated intravascular coagulation (DIC) are commonly detected in patients with various metastatic malignancies [64]. Furthermore, tumor cells have been shown to activate platelets. These are known to store lytic enzymes, serotonin and growth factors as well as arachidonic acid metabolites known to increase metastasis [18].

Numerous experiments with animal models as well as clinical observations have shown a correlation between a tumor's ability to activate the clotting system and interact with platelets and its ability to grow and metastasize [60]. The ability to generate proteolytic and adhesive properties is probably essential for invasion, implantation and angiogenesis. Various studies, however, revealed that different experimental models behaved differently and sometimes contradictory results were obtained. Different tumor types display a striking heterogeneity with regard to their interaction with the coagulation and fibrinolytic pathways: Immunohistochemical studies in small-cell carcinoma of the lung (SCLC) revealed the existence of an initiator of coagulation (termed "tissue factor") as well as of coagulation factor intermediates in the immediate environment of the tumor cells [82]. Conversely, colon cancer tissue does not display an intact thrombin-forming pathway. Instead, urokinase-type plasminogen activator (u-PA) was detected within colonic tumor cells [34] and was associated with the degree of invasion. The involvement of u-PA in colon cancer progression is also supported by a correlation of u-PA content with invasive properties of colon cancer explants in nude mice [11]. In breast cancer, u-PA was also found to be a marker associated with tumor aggressiveness and prognosis [31].

Extravasation/adhesion

While lymphatic and vascular drainage at the primary site initially determine tumor cell access to secondary sites (e.g., mechanical entrapment of tumor cell emboli in the capillary bed), both experimental and clinical evidence strongly support an active interaction between tumor cells and host endothelial cells, as well as the extracellular matrix and the stromal and parenchymal cells thus resulting in site-specific metastasis [9, 45, 56]. This was already proposed in 1889 by Paget as the "seed and soil" hypothesis [55]. Tumor cells adhere to junctional regions between endothelial cells (basal lamina) and cause endothelial cell retraction. "Cell adhesion molecules" (CAMs) are thought to mediate organ-specific tumor cell adhesion [52]. Several classes of adhesion receptors have been described. Best characterized are the integrin family of adhesion receptors [21, 30], adhesion receptors belonging to the IgG superfamily [51], also implicated in blood cell interactions, the Ca^{2+}-dependent cadherins, which mediate homophilic adhesion [74], and the LEC CAMs, which are expressed on a variety of cell types and mediate lectin-like adhesive cell-cell interactions [9].

Protein kinase C (PKC) is a Ca^{2+}- and phospholipid-dependent enzyme that plays an important role in cell-surface signal transduction and controls a wide number of physiological processes including cellular growth and differentiation as well as tumor promotion [53]. PKC is activated by diacylglycerol formed in response to extracellular signals by turnover of phosphoinositides. Certain growth factors such as platelet-derived growth factor (PDGF) and interleukin-2 (IL-2) mediate their mitogenic effects through phosphatidyl inositol hydrolysis [5]. Phorbol esters and cellular regulators that elevate intracellular diacylglycerol induce a PKC association to the plasma membrane and thereby influence the cell-surface properties of the cells. Membrane-bound PKC is believed to influence both Ca^{2+}-regulated cell attachment and release of proteolytic enzymes. In addition, increased association of PKC with nuclear-cytoskeletal components has been described in response to phorbol esters, indicating an involvement of PKC in cell motility [73]. Recently, a correlation between levels of membrane-bound PKC activity and hematogenous metastasizing abilities of melanoma sublines was demonstrated [20].

Angiogenesis

Formation of new blood vessels is a fundamental requirement for tumor expansion since avascular tumors are restricted by the limits of oxygen and nutrient diffusion [17]. The ability of tumor cells to stimulate angiogenesis through various soluble factors such as basic fibroblast growth factor has been demonstrated [57]. Angiogenesis is a complex event starting with the stimulation of resting endothelial cells in the parent vessel to degrade the basement membrane. This is followed by endothelial cell migration into the perivascular stroma and by formation of a capillary sprout [2]. Through further proliferation of the endothelial cells a functioning circulatory network is developed. During the exit from the parent vessel endothelial cell migration occurs in a manner that is functionally very similar to cancer cell invasion. In experimental systems proteinase inhibitors block both endothelial cell invasion and tumor cell invasion in the same assay [46]. In angiogenesis, the balance between proteinases and proteinase-inhibitors regulates vascular morphogenesis [48].

During the final steps of dissemination the release of proteolytic enzymes (hydrolases, collagenases, cathepsins, plasminogen activators) and active tumor cell motility complete metastasis formation, again resembling those mechanisms which occur during invasion at the primary site.

Genetic basis of tumorigenicity and metastasis

It has been demonstrated that transfection of oncogenes into appropriate recipient cells could induce the complete phenotype of tumorigenicity, invasion and metastasis. This was initially shown for the *ras* family of oncogenes [76] and later also for the serine-threonine kinases *mos* and *raf*, tyrosine-kinases *src*, *fes*, *fms* and mutant *p53* tumor suppressor gene [43]. Nevertheless, subsequent experiments suggested that *ras*-induced tumorigenicity and metastasis were dependent on different downstream pathways: firstly, adenovirus 2 E1A can abolish *ras*-induced metastatic potential without affecting *ras*-induced tumorigenicity [59]; secondly, *ras* transfection can induce tumorigenicity without necessarily conferring metastatic capacity [49]. It is therefore assumed that invasion and metastasis are driven by effector genes over and above those required for tumorigenicity. If *ras* only induces tumorigenicity without metastasis then the effector genes are missing or suppressed. The list of possible effector proteins includes proteinases such as type IV collagenase, cathepsin L and motility-associated cytokines [42].

Particular attention has recently been paid to a candidate metastasis suppressor gene called *nm23*. mRNA levels of *nm23* were dramatically reduced in several melanoma cell lines of high metastatic capacity compared with melanoma lines of low metastatic potential [70]. Later, the clinical significance of these findings was demonstrated in breast cancer patients: loss of *nm23* RNA was strongly associated with poor survival [26]. It is assumed that *nm23* is involved in signal transduction of cell-cell communication and thereby plays a critical role in normal tissue development. This is supported by its homology with the drosophila *awd* gene, which regulates drosophila morphology [43] and was shown to belong to the family of nucleoside diphosphate (NDP) kinases known to be critically involved in cellular processes relevant to both cancerous and normal development: By affecting microtubule assembly, *nm23*-like NDP kinases may regulate cellular functions such as mitotic spindle formation and cell locomotion [7]. This would also explain the high degree of aneuploidy (genetic instability) ob-

served in metastatic tumors due to aberrant mitosis. Furthermore, by interacting with a variety of G proteins, NDP-kinases may be involved in cell signal processes regulating development, oncogenesis and metastasis [43]. However, in other tumor systems (colon, neuroblastoma), metastasis was not linked to simply reduced *nm23* expression and an involvement of NDP kinase activity in metastasis suppression has recently been questioned [19].

Another candidate metastasis-suppressor gene, located on chromosome 18q, has recently been cloned and termed DCC ("deleted in colorectal carcinomas" [14]). It is expressed in many tissues including normal colonic mucosa but is absent or minimally expressed in colorectal carcinomas. Inactivation of DCC appears to occur at a late stage of tumorigenesis, suggesting that it acts as a suppressor of progression and metastasis formation. The gene sequence shows homology with the neural cell-adhesion molecule *N*-CAM, a surface glycoprotein of the Ig superfamily. Consequently, DCC is suspected to be involved in cell surface interactions such as adhesion properties.

Therapeutic approaches

Cancer-screening programs claim to result in a reduction of patient mortality of up to 30% [36]. However, this is true only for certain types of malignancy (e.g., colon cancer) where primary tumors grow to a relatively large size before metastatic dissemination occurs. In other tumor systems, 90% of metastases appear to be present when the age of the primary tumor is approximately 43 doubling times equaling an average size of *6 mm* [4]. Clearly, there are a majority of cancer patients who will eventually require systemic treatment in addition to local tumor control. Any such approach, however, is complicated significantly by the known biological and biochemical heterogeneity of tumor cells. Tumor cells differ with regard to their radiosensitivity, drug sensitivity, receptor status (e.g., hormone receptors) and antigenic properties.

Conventional cytotoxic drugs, applied with the intention of destroying metastases and/or primary tumors, can be defined as "antimetastasis" drugs. Considering the serious limitations and considerable side effects associated with such therapy, there clearly is a need for "antimetastatic" drugs which interfere with one or several steps of the metastatic cascade. An antimetastatic drug whose activity is limited to the prevention of new metastases would appear to be relatively ineffective since the majority of clinical cancers have already metastasized at the time of diagnosis. Rather, the desired drug should interfere with several steps of the metastatic cascade, thereby controlling growth of both primary and secondary tumors as well as preventing new metastases:

Treatment strategies:

1. Inhibition of tumor cell invasiveness
2. Suppression of angiogenesis
3. Enhancement of local stromal reaction
4. Enhancement of tumor cell immunogenicity/activation of the immune system
5. Inhibition of tumor cell attachment
6. Antibody-mediated selective tumor cell killing

The result would then be a "freezing" of the malignant state. Since several physiological processes are part of the metastatic cascade, such treatment will inevitably result in significant side effects. Ideally, an anticancer agent would allow for a selective killing of tumor cells at primary and secondary sites.

Anticoagulants

To date, most clinical trials with substances targeting the metastatic cascade have concentrated on anticoagulants. Coumarin and coumarin derivatives such as warfarin and phenprocoumon as well as heparin were shown to reduce the number of metastases after i.v. injection in some animal models [27]. Clinical trials performed in the early 1970s included several common tumor types for which existing therapy was unsatisfactory [79, 80]. Warfarin was chosen as the experimental drug in the initial trials since it had been in common use in humans for treatment of thromboembolic disorders and its mechanism of action was known. Of 431 patients with advanced SCLC and NSCLC (non-small-cell lung cancer) as well as colon, head/neck and prostate cancer, only patients with SCLC randomized to receive warfarin demonstrated a statistically significant increase in the interval to disease progression [79]. A possible explanation for the sensitivity of SCLC to warfarin therapy was provided later by immunohistochemical studies (see also above): SCLC tissues stained for tissue factor and several coagulation factors [81]. Furthermore, thrombin cleavage sites on fibrinogen were demonstrable in the connective tissue interface with the tumor cells [82]. This suggested the presence of enzymatically active thrombin which might have a direct tumor-promoting effect or contribute to formation of tumor stroma. The beneficial effect of warfarin in SCLC may therefore be due to its inhibitory effect on local thrombin production. In prostate cancer, NSCLC and colon carcinoma no evidence for local thrombin formation has been obtained and, as mentioned before, these tumor types did not respond to warfarin [83].

Platelet aggregation inhibitors

Heterotypic aggregation between platelets and intravasal tumor cells is suspected of promoting metastatic spread through various mechanisms, e.g., protection of tumor cells against the external milieu, release of platelet components stored in granula and known to facilitate metastatic behavior of tumor cells [18]. A variety of compounds acting through different mechanisms as platelet inhibitors were tested as potential antimetastatic agents. Aspirin (cyclooxygenase inhibitor, inhibiting prostaglandin synthesis

and thromboxane synthesis) was tested in clinical trials in colorectal and small-cell lung cancer. However, no effect was demonstrated. Indomethacin (another prostaglandin synthesis inhibitor) was found to be ineffective in preclinical models. Conflicting results were obtained with thromboxane A_2 inhibitors [66].

Pyrimido-pyrimidine derivatives (dipyridamol, mopidamol) inhibit platelet aggregation through phosphodiesterase inhibition. Although preclinical results obtained with these compounds were again conflicting, mopidamol has been tested in some clinical trials. In combination with chemotherapy, this drug caused a prolongation of survival in non-small-cell lung cancer limited to one hemithorax [58]. In other tumors, it was ineffective.

Prostacyclin (PGI_2), an endothelial cell cyclooxygenase metabolite (a prostaglandin) of arachidonic acid, was identified in 1976 and turned out to be a potent natural inhibitor of platelet as well as tumor cell-platelet aggregation. It binds to a platelet receptor, thereby preventing aggregation. PGI_2 also interferes with tumor cell adhesion to endothelial cells and the subendothelial matrix [66]. Antimetastatic activity of PGI_2 was first reported by Honn et al. [28]. Due to its short half-life, however, it is not very suitable for clinical use. Prostacyclin analogs are nowadays available that have a significantly prolonged half-life and similar pharmacological properties [72]. PGI_2 analogs were tested in various animal systems (both after i.v. injection and in spontaneously metastasizing murine tumors) and most investigators were able to demonstrate an inhibitory effect on metastasis formation. Results strongly depend on experimental conditions (tumor system, route of drug administration, timing of drug administration, etc.). From a clinical point of view PGI_2 analogs would seem most beneficial for patients at high risk of metastasis but without evidence of metastatic dissemination. Selection of such patients, however, as well as the necessity for long-term treatment greatly complicates the design of clinical trials.

Inhibition of tumor cell motility

A novel carboxyamide-amino-imidazole compound, which blocks the activity of autocrine motility factor (AMF), has been described [35]. Little effect on primary tumor growth was observed in murine systems. However, a dramatic reduction in the number and size of pulmonary metastases was found. The compound, not having any significant side effects in animal models, is being evaluated in phase I protocols for ovarian, breast, colorectal, lymphoma and bladder tumors coordinated by the National Cancer Institute of the United States.

Suramin strongly inhibits invasion in vitro and is known to dissociate ligands (such as AMF and other cytokines stimulating invasion, e.g., tumor necrosis factor) from receptors. Apart from dissociation of ligands, suramin also inhibits many enzymes. Its activity might therefore relate to inhibition of cell motility and, possibly, to interference with degradative enzymes [62].

Inhibition of tumor cell adhesion

Interference with the function of CAMs could critically affect the metastatic process. In melanoma several up- and downregulated CAMs have been implicated. Expression of one of these, the vitronectin receptor (VnR), a member of the integrin superfamily, correlates with tumorigenicity of melanoma cell lines and represents a possible target for antibody-mediated therapy. Alternatively, injection of small peptides containing the VnR-binding site might abrogate adhesion as has been successfully performed in animal models [65]. A similar strategy appears to be feasible for other members of the integrin family. Currently, however, this approach is limited by the short half-life of the peptides, the high serum concentrations required and the lack of activity in patients whose cancers have already metastasized.

The important role of adhesion molecules in metastatic dissemination has again recently been demonstrated in lymphoma cells where injection of tumor cells deficient in LFA-1 (lymphocyte function-associated antigen), a CAM, failed to yield any metastatic deposits. Upon restoration of LFA expression, tumor-cell invasiveness was restored [63].

Inhibition of proteinases

Various studies have implicated TIMP (tissue inhibitor of metalloproteinases) in invasion and metastasis. Purified TIMP inhibited tumor-cell invasion in vitro, and periodic TIMP infusions inhibited lung colonization in animal models [33]. Clinical trials are currently underway. Similarly, high expression of plasminogen activator correlating with invasion and metastasis (see above) provides evidence for a role of proteolytic activities in invasive and metastatic properties. Anti-u-PA antibodies block cell invasion in vitro and metastasis of murine tumors [66]. Therefore, protease inhibitors may be considered as potentially useful drugs preventing tumor-cell dissemination.

Inhibition of angiogenesis

Heparin is able to induce angiogenesis. Early in vivo studies demonstrated that the heparin antagonist, protamine, inhibited angiogenesis and strongly reduced primary and secondary tumor growth in various animal systems [75]. Inhibition of angiogenesis provides an attractive concept because it interferes with several steps of the metastatic cascade. This approach is complicated by the variety of known and unknown angiogenic factors and the fact that none of those identified to date is tumor specific [6].

Growth factors with angiogenic properties appear to be appropriate targets for therapy, e.g., by using antibody-toxin conjugates against their receptors. Some receptors might be upregulated in proliferating epithelium, providing more specific targets for toxin conjugates. In animal

systems, beneficial results have been obtained with TGF-*Pseudomonas* exotoxin fusion proteins [24].

Anti-angiogenic peptides of two different categories have been described: those inhibiting production of angiogenic factors by tumor cells and those which inhibit endothelial cell proliferation. Interferons (IFN), alpha and beta, inhibit angiogenic stimuli in some systems [67].

Angiogenesis inhibitors are currently being evaluated in initial clinical trials and preliminary results are promising [69].

Conclusions

In recent years, knowledge of critical events of metastatic dissemination has increased significantly. Investigators have elucidated in detail many of the mechanisms involved at each step of the metastatic cascade. This has created new opportunities for therapeutic intervention. Many different approaches to inhibiting metastatic spread have been tested in various animal systems and promising results have been obtained. Concomitantly, however, researchers have become aware of the difficulties in translating results obtained in murine systems into the clinical setting. Growth of human tumors in murine hosts, albeit immunocompromized ones, is an artificial interaction which does not necessarily mimic autochthonous cases of neoplasia. Timing of drug administration and route of administration significantly influence results and in many cases cannot be translated into the clinical situation. Many of the compounds tested as antimetastatic drugs would appear to be effective primarily in patients whose cancers have not yet metastasized. The majority of cancer patients therefore would not benefit from such treatment. Design of clinical trials is further complicated by difficulties in patient selection. Most metastases are already present but undetectable at a time when the size of the primary tumor barely allows for its detection. Furthermore, simulation models as well as epidemiological studies of the natural history of breast cancer indicate that the median metastasis growth duration is approximately 18 doubling times or 3.7 years before metastases become clinically detectable. Therefore, identification of patients with identical stages of disease represents a major problem in clinical trials and adjuvant prophylactic clinical trials become inevitably lengthy. New diagnostic approaches, with increased sensitivity of metastasis detection, could help to alleviate such problems. Among these are new tumor markers and detection of micrometastases in bone marrow reported for patients with genitourinary as well as breast cancer. The application of the polymerase chain reaction (PCR) technique of DNA/RNA amplification should further increase the sensitivity of such approaches.

In patients with tumors that have already metastasized an antimetastatic drug could be used in order to prevent tertiary formation, thereby stabilizing the clinical situation. However, such patients with advanced stages of disease are likely to require the use of cytotoxic therapy in addition to antimetastatic drugs. In this area, further development of cytotoxic agents with increased specificity for tumor cells is certainly warranted.

For the future, identification of selective events in metastatic dissemination and comparison of metastatic and nonmetastatic variants of the same tumor should yield further insight into mechanisms relevant to the acquisition of metastatic capacity. Powerful new molecular biological techniques such as differential screening of cDNA libraries or subtractive genomic hybridization will allow identification of genes relevant to tumor progression. Subsequent elucidation of gene function will lead the way for new therapeutic strategies.

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