DE GRUYTER OPEN

Bull Vet Inst Pulawy 58, 1-9, 2014
DOI: 10.2478/bvip-2014-0001

Extracellular matrix in tumours as a source of additional neoplastic lesions - a review

Janusz A. Madej

Department of Pathomorphology and Forensic Veterinary Sciences, Department of Pathology, Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw, Poland
janusz.madej@up.wroc.pl

Received: July 2, 2013 *Accepted: October 9, 2013*

Abstract

The review describes the role of cells of extracellular matrix (ECM) as a source of neoplastic outgrowths additional to the original tumour. The cells undergo a spontaneous transformation or stimulation by the original tumour through intercellular signals, *e.g.* through Shh protein (sonic hedgehog). Additionally, cells of an inflammatory infiltrate, which frequently accompany malignant tumours and particularly carcinomas, may regulate tumour cell behaviour. This is either by restricting tumour proliferation or, inversely, by induction and stimulation of the proliferation of another tumour cell type, *e.g.* mesenchymal cells. The latter type of tumour may involve formation of histologically differentiated stromal tumours (GIST), which probably originate from interstitial cells of Cajal in the alimentary tract. Occasionally, *e.g.* in gastric carcinoma, proliferation involves lymphoid follicles and lymphocytes of GALT (gut-associated lymphoid tissue), which gives rise to lymphoma. The process is preceded by the earlier stage of intestinal metaplasia, or is induced by gastritis alone. This is an example of primary involvement of inflammatory infiltrate cells in neoplastic progression. Despite the numerous histogenetic classifications of tumours (*zygotoma benignum et zygotoma malignum,* or *mesenchymomata maligna et mesenchymomata benigna*), currently in oncological diagnosis the view prevails that the direction of tumour differentiation and its degree of histologic malignancy (grading) are more important factors than the histogenesis of the tumour.

Key words: neoplasia, extracellular matrix (ECM), tumour cell-tumour cell communication, tumour cell-EM communication.

Introduction

Apart from the physiological role, extracellular matrix (ECM) also hosts several pathological processes, such as inflammatory processes and proliferative diseases of non-neoplastic type (*metamorphoses progressivae non neoplasmaticae*), or neoplastic type (*metamorphoses progressivae neoplasmaticae*) (18).

The tumour represents a cell cycle disease, evoked by several possible processes. They are processes such as passing the phase G_1 restriction point, which is often not sufficiently controlled, genomic alterations, disturbed cell differentiation, and disturbed intracellular, intercellular, and extracellular signalling (3, 7). The genomic alterations are in protooncogenes, genes controlling apoptosis and/or suppressor genes, *e.g. TP53, Rb*, and genes controlling repair of DNA damage. The disturbance in signalling develops in ECM and involves either intercellular matrix, and/or basement membrane. ECM provides a sublayer for a tumour or its stroma, and it controls the biology of neoplastic tissue, since it unites and nourishes the tumour through its vascular network. The network, in both spontaneous and experimental tumours, originates mainly from the tumour-surrounding tissue. After grafting of a tumour, venous circulation develops first and it prevails in development of the entire vascular supply. This indicates that blood outflow is more efficient than blood inflow. Tumours house at least eight microangiographic systems, the histological type of the tumour mediating which of them develop (28). The structure of the blood vessels remains under the influence of mechanical factors, such as compression of the tumour tissue (the sandwich tumour), it is modified by lytic properties of the tumour, and by disturbances in chemical composition of the tumour-infiltered tissue. Compared to normal blood vessels, *vasa vasorum* manifest a tortuous course, they are devoid of nerves, and their contractile elements are infrequent or absent (27).

Moreover, the blood vessels manifest leakiness and enhanced vascular permeability (20). In parallel with the blood vessels, the stroma contains also lymphatic vessels, participating in drainage of metabo-lic and tissue destruction products.

The stroma may provide a criterion for morphological classification of tumours, which yields non-epithelial malignant tumours or sarcomas, *i.e.* stroma-producing tumours, and epithelial malignant tumours or carcinomas taking advantage of the local tissue stroma.

In view of the above, a tumour stroma may represent a proper, tumour-induced stroma, or it may consist of stroma of the tissue in which the tumour grows. In particular, the latter pertains to tumours which grow in the infiltrative manner. As a rule, stroma is very rare in mesenchymal tumours, in contrast to carcinomas, where it is abundant. In contrast to benign tumours, in cells and stroma of malignant tumours several retrogressive lesions develop, *i.e.* hyaline degeneration, mucoid degeneration, calcification, ossification, development of cysts, dilations of blood vessels, precipitation of melanin granules, and oedematous lesions (26). In oedematous regions, the stroma becomes loose and it contains blood vessel-resembling clefts lined with endothelial cells and individual capillaries, which all resemble a tissue of foetal type.

Factors controlling ECM metabolism in tumours

The surface of several cells, including neoplastic ones, carries around 20 types of ADAM proteins (disintegrins and metalloproteinases – EC 3.4.24.46), which contain cytoplasmic, transmembrane, and extracellular domains (4, 8) (Fig. 1).

The latter carries a fragment of disintegrin, joining the neighbouring cells and preventing binding of integrin with ECM, *i.e.* glycoproteins of fibronectin or laminin type. Fibronectin is described as a large extremal transformation sensitive protein (LETS) and, therefore, it undergoes reduction upon neoplastic transformation. This results in inhibition of intercellular contacts (22). In turn, the fragment with activity of zinc (Zn^{++})-dependent matrix metalloproteinase (MMP) allows for decomposition of ECM components and for motility of the cells in the extracellular matrix. This fragment does so with cooperation of other proteases located on the cell surface. Moreover, the fragment excises proteins anchored in the cell membrane, such as tumour necrosis factor-α (TNF-α) and EGF, and releases pro-inflammatory cytokines (IL-1, IL-6, and IL-12) to extracellular fluid (15). Among the metalloproteinases are strome-lysins (decomposing collagen type IV and elastin), collagenases 1, 2, 3 (decomposing collagen type I, II, III, and IV), and gelatinases A and B (decomposing collagen type I). MMPs, destroying basement membranes and ECM, promote metastatic potential and growth of tumour cells. These proteins also participate in angiogenesis of small capillaries (13). Independently, both neoplastic cells and ECM cells contain inhibitors of metalloproteinases (TIMP or tissue inhibitor of metalloproteinase), which inhibit the degradation of basement membrane and prevent the development of metastases. On the other hand, their low activity promotes the development of tumour metastases (8).

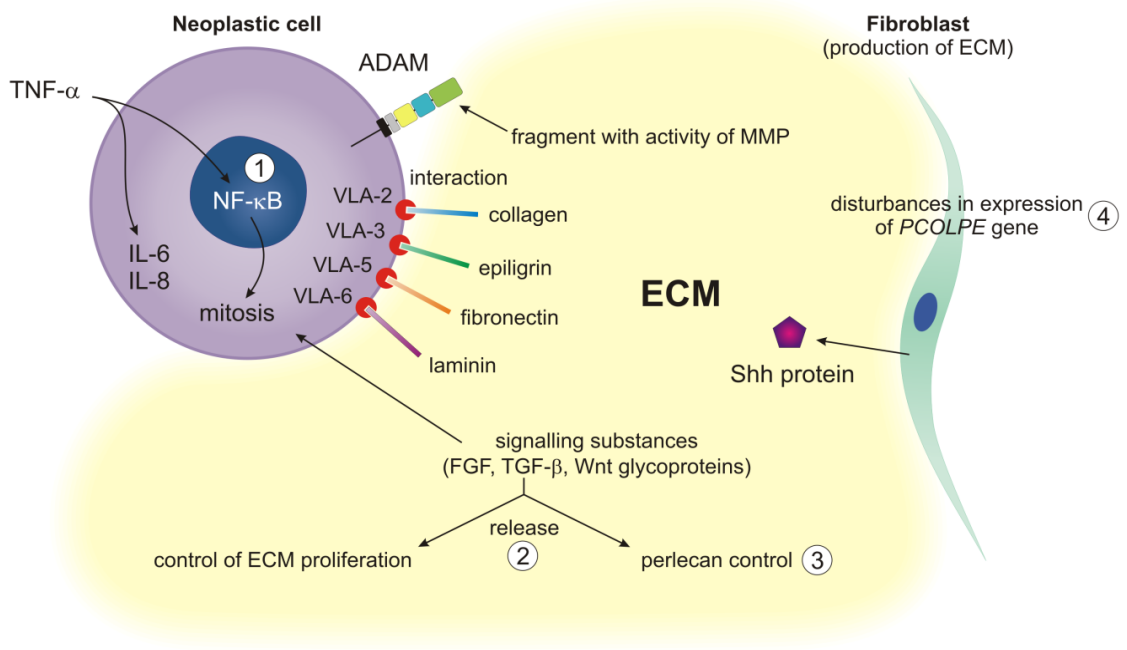

Fig. 1. Selected problems of extra-cellular (ECM) metabolism control in the neoplastic process. ADAM proteins (disintegrins and metalloproteinases) –containing disintegrin and metalloproteinase domain; MMP - metalloproteinase; VLA - very late antigens; Shh (sonic hedgehog) – transcription of genes in *Hox a* and *Hox d* group; 1 – NF-κB (nuclear factor κB), 2 – activity of MMP group proteinases and procollagen C-endopeptidase (BMP-1/mTLD - *bmp1* gene), 3 – anti-angiogenic factor, 4 – gene responsible for proliferation of smooth muscle cells in ECM (detailed description in the text)

Intracellular substance is produced locally by fibroblasts or their homologues, *e.g.* osteoblasts or less frequently smooth myoblast cells, and it produces basement membranes (non-fibrous collagen type IV, laminin, and proteoglycan) and ECM (fibrous collagen in the form of a triple helix, bound by transverse bonds, proteoglycan, and hyaluronate). Thus, despite epithelial cells and mesenchymatic cells (fibroblasts) having common components they differ in structure and in composition; nevertheless both bind to ECM with involvement of integrins, classified as cell adhesion molecules (CAM). For example, ubiquitinated fibronectin protein, belonging to the group of integrins VLA 5 (very late antigens), promotes adhesion of cells to ECM by binding them to integrin receptors (15). Domains of fibronectin may bind to cellular integrins through the tri-peptide motif of arginin-glycin-aspartic acid (RGD), the recognition of which is of key significance for cell adhesion to ECM (33). Adhesion to fibronectin may trigger the MAPK/ERK (mitogen-activated protein kinases, belonging to extracellular signal-regulated kinases pathway), phosphoinositide-3 kinase and protein kinase C. It also provides a mediating role in migration of cells containing a decreased level of fibronectin, *e.g.* neoplastic cells which profoundly augments motility of the cells and may promote development of metastases. In *in vitro* culture of some tumours, phosphorylation of tyrosin residues was noted in the cytoplasmic tail, which reduces affinity in integrin-tallin interactions, and is followed by a decreased cellular adhesion to fibronectin (33). The other integrins of VLA group, providing a receptor for ECM include the receptor for collagen (VLA 2), receptor for laminin (VLA 6), and receptor for epiligrin (VLA 3) (Fig. 1) (35). Therefore, elements of ECM manifest lower reactivity in contact with neoplastic cells compared to contact with normal cells.

Such a reaction determines the quality of the stroma of connective tissue and blood vessels, which can supply the tumour appropriately. For example, in ECM of uterine myomas, a disturbance is observed in the spatial orientation of collagen, as is an increased content of heparane, chondroitin, keratin, and heparin sulphates and a decreased number of disulphide bridges in dermatan disulphide in junctions with glycosaminoglycans (GAG) (34). It also should be mentioned that an accelerated proliferation and subsequent proliferation of ECM are noted in tissue anoxia. This is accompanied by a reduction in numbers of fibroblast mitochondria accounting for as much as 20% of their volume, and the cells switch to anaerobic glycolysis, with the resulting excess of pyruvates, subsequently decomposed to acetyl-coenzyme A. The latter, unoxidised in mitochondria, undergoes accumulation in the cells and stimulates their divisions (13). In a similar manner, neoplastic megakaryocytes, arising in myeloproliferative disorders (CMDs or Chronic Myeloid Disorders), produce factors which stimulate fibroblast proliferation: platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β), which exert a mitogenic effect leading to a progressive fibrosis of bone marrow (21). At present, CMDs in humans are categorised as anticipatory diseases, in 70% of cases associated with a loss or partial deletion of long arms in chromosomes 5 (del 5) or 7 (del 7), and with mutation of the *JAK 2 (V617F)* gene (21).

The secretory protein of Shh (sonic hedgehog) plays the role of a morphogene in the body, even if the molecular mechanisms of its action remain to be fully clarified (15). Both the hedgehog protein and its gene were described for the first time in *Drosophila melanogaster*. Most probably, the protein activates transcription of genes in the *Hox a* and *Hox d* group in mesenchymal cells, controlling morphogenesis in embryonal development. The expression of genes containing domains which reciprocally overlap each other provides the detailed structural pattern of tissues. The phenomenon was best recognised in the process of morphogenesis of extremities in birds. *In vitro* cultured chicken fibroblasts were enforced by genetic engineering to express the *shh* gene, and subsequently implanted into the primordium of an extremity in bird embryos, causing its deformation (15). Independently, disturbances in the intercellular signal transfer dependent on the action of Shh protein lead, among other outcomes, to development of dermal basal cell carcinoma and also to cyclopia (14). This type of reaction also fits the range of inductive interactions or an intercellular dialogue, but it has a restricted scope since the signalling substances, the inducers (ligands), occur in low concentrations (14). The signalling substances include, among others, fibroblast growth factor (FGF) stored in the basement membrane, from which it is rapidly engaged for stimulation of cell growth, for example; TGF-β; peptides of the mentioned above hedgehog family; and Wnt glycoproteins. The inducers may stimulate receptors in the cell membrane of a target cell, and activate a transcription factor, which changes gene expression in the cell nucleus of the induced cell, providing it with a new direction for its differentiation. This way, the ligand may remain linked to the cell membrane of the inducing cell, may remain anchored in ECM, or may diffuse to ECM (a paracrine secretion) (15).

TGF-β is one of the most important factors of neoplastic proliferation, the factor of an ambivalent activity, which stimulates ECM and, in parallel, inhibits synthesis of enzymes which degrade ECM (9). In its high concentration it blocks expression of PDGF receptors and, thus, represents a growth inhibitor; but in its low concentration it stimulates synthesis and secretion of PDGF and, therefore, exhibits an indirect mitogenic effect (30). The effects include an increased accumulation of ECM, accompanied by its decreased decomposition. In turn, the release of TGF-β requires proteolysis of ECM by proteinases of the MMP family

and procollagen C-proteinases (– PCP -EC 3.4.24.19) (27). The enzyme involves a protein of the BMP-1/mTLD subfamily (bone morphogenic protein/ mammalian Tolloid protein, discovered in *Drosophila melanogaster*, or the longer product of *bmp1* gene) (27). The subfamily may control proliferation of cells rich in ECM, for example of cells in uterine myomas. Moreover, oscillations in concentration of BMP-1/mTLD may lead to a decreased concentration of perlekan, the anti-angiogenic factor in ECM and close to basement membranes in particular, which results in an increased number of blood vessels in the neoplastic tumour (26). It was demonstrated that the number of mRNA copies encoding BMP-1/m TLD in endothelium of tumour blood vessels is increased compared to the copy number seen in normal endothelium. This points to the involvement of the protein in regulation of angiogenesis as appropriate to the altering environmental conditions (27). Similarly to *bmp1* gene, *PCOLPE* gene undergoes expression in embryonal tissues, which encodes PCPE 1 protein (procollagen C-proteinase enhancer 1), and acts in a similar manner to proteins of the TIMP family (36). Disturbances in normal expression of the gene are frequently encountered in smooth muscles, *e.g.* in uterine myoma cells, which are supposed to indicate loss of ability to inhibit growth and/or loss of tumour suppression ability in ECM (36). Moreover, it was discovered that TNF-α, through activation of the nuclear factor-kB (NF-kB) increases mitotic activity, expression of protein, and expression of IL-8 genein endometrial cells of uterine stroma (33). Independently of this, activation of NF-kB plays a key role in TNF-α-induced expression of IL-6 in the cells (29, 33). It was also noted that due to the activity of macrophages and fibroblasts in blood vessel-surrounding ECM, and due to a disturbed equilibrium between pro- and anti-angiogenic factors, an excessive proliferation may develop polyclonal endothelial progenitor cells (haem Ecs or haemangioma endothelial cells) resulting in development of angioma (26).

Mutations of growth factor receptors result in activation of tyrosine kinase receptor or in mutations on RAS protein, which induce loss of its ATPase activity and its stable activity after binding to GTP (15). The transmembrane receptor kinase receives signals from PDGF, EGF, NGF, and other substances while cytoplasmic kinase receives them from receptors of the *src* (sarcoma) family and from *JAK* (Janus kinase) (22). Both kinase forms phosphorylate proteins, which subsequently bind to fragments of SH2 or SH3 (sarcoma homology, *src* represents the gene of Rous sarcoma virus, coding for tyrosine kinase), to the protein of phospholipase C and to the RAS signalling protein, with the resulting transmission of the signal (22). The proteins may activate MAPK/ERK kinases, which activate nuclear transcription factors, including transcription of protooncogenes, and stimulate cell proliferation. For example, the mutated RAS protein is stably activated and leads to persistent cell stimulation, as instanced by the mitogenic cascade of *RAF-MAP* kinase (15). The mutation of *RAS* gene involves the most frequent abnormality noted in oncogenes of human tumours. In addition, the *RAS* oncogene controls trans-cription of VEGF while activation of the gene increases production of VEGF (22).

In the process of phenotypic transformation cells of certain malignant tumours may change their shape, which frequently reflects a decreased expression of assembled cytoskeleton proteins. For example, cells of rat sarcoma carrying thin and short actin filaments prove to be more motile than the untransformed cells, which promote development of their metastases. Moreover, in such a tumour the level of thymosin β-4 is lower than in a tumour yielding no metastases (30). In a similar manner, fibroblasts transformed with SV-40 virus evidence a decreased level of α-actinin, while following transfection with cytoskeleton-coding cDNA their ability to form metastases decreases (30). Therefore, it can be concluded that expression of cytoskeleton-forming proteins provides significant information on tumour metastatic potential.

Neoplastic transformation of ECM cells

A neoplastic tumour mainly consists of neoplastic cells with a mutated genome, in line with Virchow's principle of "*omnis cellula a cellula eiusdem generis*" and, additionally, of normal cells forming vascular endothelium, of proper ECM cells (fibroblasts, fibrocytes, myofibroblasts, plasma cells, mast cells, and eosinophils) and occasionally of cells representing inflammatory infiltrate or immune response cells. Therefore, a developed tumour represents not a static but a dynamic structure and it may change during its development, also under the effect of the applied therapy. This pertains especially to cells of malignant tumours, which are particularly heterogenous. Their polymorphism makes ~~to~~ encountering two identical cells within such a structure impossible. Nevertheless, there is still a theory arising from the tumour histogenesis approach, which states that in tumours originating from a zygote 100% of totipo-tential cells can be categorised into only two types: *zygotoma benignum et zygotoma malignum*, *i.e.* benign and malignant tumours (26). A similar division is applied to tumours originating from mesenchymal tissue, *i.e.* those originating from mature mesenchymal tissue, which are benign (*mesenchymomata benigna*), and those originating from immature mesenchymal tissue, considered malignant (*mesenchymomata maligna*) (Fig. 2).

In turn, the development of teratomata involves a prototypic neoplastic cell, which exhibits all characteristics of the cells before their differentiation into three germinal layers (ectoderm, endoderm, and mesoderm) (26). The resulting creation is formed of tissue territorially foreign to a teratoma and composed of the aforementioned germinal layers. A teratoma

involves either ~~a~~ complete tissue chaos or, paradoxically, a complex of rudimentary organs, as happens in an adult teratoma (*teratoma adultum*). Additionally, the teratoma should not be confused with mixed tumours, *e.g*. tumours of salivary glands with a polymorphic appearance, or tumours containing more than one type of neoplastic cells and usually originating from a single germinal layer.

Tumours and carcinomas in particular are frequently accompanied by an inflammatory infiltrate in ECM, which may behave in two alternative ways: it may locally restrict or even block proliferation of the neoplastic cells, as happens in cases of urinary bladder carcinoma in humans treated with attenuated BCG bacilli or, inversely, it may be the source of proliferative signals for its own cells and induce formation of another tumour type, this time of a mesenchymatic character (13). An example of the latter type is histo-logically differentiated stromal tumours, originating from interstitial cells of Cajal in the gastrointestinal system (GIST or gastrointestinal stromal tumour) (10). The tumours are covered by an intact mucosa, which proves that they are located inside the wall of the alimentary tract. GIST-oma type tumours can be either benign or malignant. They express tyrosine kinase receptors membraneously as a product of *c-kit* proto-oncogene and, therefore, they are treated using an inhibitor of the enzyme, STI 571 (Gleevac). In turn, lymphoma of MALT-oma (neoplastically transformed mucosa-associated lymphoid tissue) type originates from lymphocytes of intestinal Peyer's patches. It should also be mentioned that fibroblasts present in intestinal lamina propria produce a cytokine, bone morphogenic protein, BMP-4. This protein inhibits expression of WNT protein, the product of *wnt* gene (22).

Other examples of an excessive proliferation involving cells of inflammatory response include the appearance of eosinophilic, mast cell, lymphatic, or plasma cell leukaemia. In such cases, the protective cells of the body become invasive to the host (19). Occasionally, as happens in gastric carcinoma ensuing from a chronic inflammation background condition (*e.g.*, induced by *Helicobacter pylori)*, this process is preceded by the earlier stage of intestinal metaplasia and dysplasia (at present substituted by the term intraepithelial neoplasia). In the development of some types of lymphoma, proliferation involves lymphoid follicles and dispersed lymphocytes of GALT due to gastritis itself, which is an example of primary involvement of inflammatory infiltrate cells in neoplastic progression (26). Eradication of *Helicobacter pylori* infection may result in regression of the tumour since its growth is induced by cytokines of T lymphocytes, produced in response to infection with the bacteria. It should be mentioned that *Helicobacter pylori* stimulates production of cytokines of IL- 1, IL- 6, IL – 8, and TNF not only with cells of gastric epithelium, but also with inflammatory cells. In exceptional cases, a secondary lymphoma may develop, which consists of B lymphocytes ($CD5^-$, $CD10^-$, $CD23^-$), *MALT-oma,* or MALT cells), which frequently accompany stomach carcinoma. Such reactive lymphocytes frequently demonstrate genetic instability

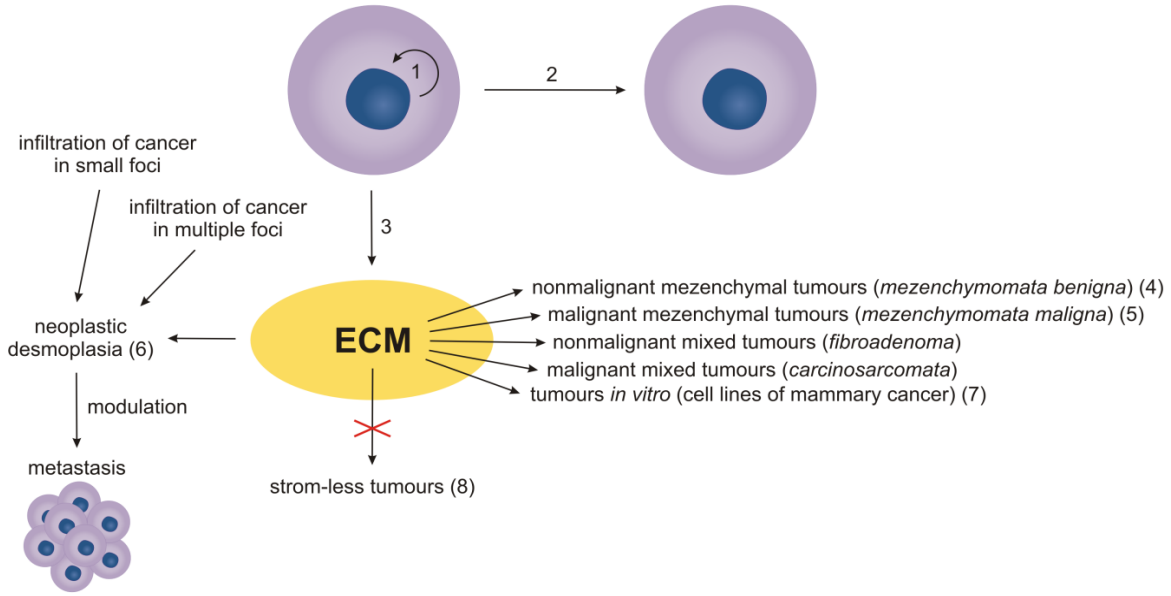

Fig. 2. Extracellular matrix (ECM) as an additional source of neoplastic lesions. 1 –disturbed intracellular contacts, 2 – disturbances in intercellular communication, 3 – disturbances in extracellular communication, 4 – *fibroma, histiocytoma, xanthoma, and angioma*, 5 – *fibrosarcoma*, *angiosarcoma etc*., 6 – fibres produced by fibroblasts, GAG (glycosaminoglycans), stromelysines (MMT – metalloproteinase), 7 – "luminal epithelial-like", "weakly luminal epithelial-like", "mesenchymal-like", 8 – *chordoma, chorioepithelioma*, "body cavity-based lymphoma" (detailed description in the text)

and cytogenetic aberrations, *i.e.* translocations, trisomias, mutations of *TP53,* and *c- myc* genes. The process begins as an inflammatory polyclonal lesion (*pseudolymphoma*), from which over time a monoclonal tumour develops showing a variable grade of malignancy (26). This is the essence of the difference between monoclonal lymphoma and the polyclonal inflammatory infiltrate of lymphoid cells. A similar phenomenon, *i.e.* neoplastic transformation of atypically behaving cells of inflammatory infiltrate, and transformation of B lymphocytes into lymphoma cells, takes place in Hashimoto-type thyreoditis (*thyreoiditis lymphocytaria*) (26). The syndrome of gluten-sensitive enteroopathy also increases the risk of development of T cell lymphoma in the intestines (EATL or enteropathy-associated T-cell lymphoma), consisting of atypical cells with expression of CD3 and CD7 antigens, with genome amplification in the region of 9q33 – 34, or del 16q 12.1, and also with additional 1q and 5q chromosomal fragments in humans (9). Similarly in salivary glands, *i.e.* in the parotid and submandibular salivary glands, the developmental abnormality can be encountered in the form of small accumulations of lymphoid tissue, which frequently provide the source for lymphoma development (22). Finally, it should be noted that according to Hanahan and Weiberg (13) tumour ability to induce inflammation promotes tumour growth.

Cell infiltrates more frequently accompany fibroplasia and consist of lymphocytes and plasma cells or only of plasma cells, particularly in carcinomas of low differentiation. Cell infiltrate plays a significant role in damaging neoplastic cells, with particular activity manifested by T cytotoxic lymphocytes and NKs (natural killers). These produce granzymes (esterases) and perforins, incorporate them into the wall of the target cells, and perforate them. In parallel, they produce protectin, which protects lymphocytes from the action of perforins. Fibrosis-activating factors produced by macrophages (TNF, PDGF, IGF1 – insulin–like growth factor, fibronectin), recruit fibroblasts (29). Macrophages also synthesise pro-inflammatory factors (LTB4 – leukotriene, IL - 8, IL - 6, TNF, MIP-1 α, and macrophage inflammatary protein 1 α), which additionally recruit and activate inflammatory cells (24). Thus, the cellular infiltrate proves that the tumour is recognised by host's immune system. In cases of necrotic foci in the tumour, neutrophilic granulocytes may also appear, or they reflect infection of the tumour with bacteria. The inflammatory process may exert a favourable role, leading to at least partial destruction of neoplastic cells, but it may also facilitate migration of neoplastic cells causing metastases. This reflects the fact that tumours carry specific antigens, capable of inducing immune reactions of both cell-mediated and humoral types (14). The inflammatory reaction evoked by such antigens may lead to oedema and destruction of tissues, in this manner paving the way for neoplastic cells in ECM. This is favoured by the slow flow of lymph and presence of small lymphatic vessels in ECM, on the valves of which tumour emboli form. In such situations, lactic acid, fatty acids, and amino acids accumulate in ECM, and its pH decreases to around 6, which leads to an increase in osmotic pressure, while the lost integrity of cell membranes results in the release to ECM of lyzosomal enzymes, histamines and prostaglandins (23).

Occasionally, the infiltrate of neoplastic cells induces atrophy by compression of healthy cells, which stimulates connective tissue to form a capsule around the tumour together with connective tissue of its stroma, which also participates in formation of the capsule. In cases of malignant tumours, the compressed tissue gives the impression of a connective tissue pseudocapsule.

An excessive proliferation of stromal connective tissue (*desmoplasia*) is noted in certain carcinomas, termed desmoplastic carcinomas (*carcinoma fibrosum s. durum s. scirrhosum s. desmopalasticum*). Fibroplasia or rejuvenation of connective tissue accompanies anaplastic, or poorly differentiated tumours, while in highly differentiated tumours their stroma resembles much more the mature connective tissue. An example may be provided by nephroblastoma or Wilms' tumour of the kidney, in which the stroma is formed of immature mesenchymal tissue with the appearance of a foetal mesenchyme and cells differentiating to fibroblasts or, seldom, to transversely striated myocytes. On the other hand, cells of tumour parenchyma differentiate in the epithelial direction, forming immature glomeruli and renal tubules (26). Such behaviour of the stroma affects the manner in which cancer cells penetrate ECM: the more compact the stroma is, the higher tendency is observed for a dispersed infiltration or infiltration, with formation of small foci, while loosening of the ECM structure leads to infiltration in the form of multiple foci. In cases when a malignant tumour destroys ECM, blood vessels loose their continuity, haemorrhages develop, and essential input to the tumour becomes impoverished, which is manifested by its degeneration and necrosis (26).

Hyperplasia of cells in endometrial stroma, *i.e.* hyperplasia of uterine multipotential mesenchymal cells without traits of atypia, is called *stromatosis s. hyperplasia stromalis endometrii* (26). In the case of insignificant atypia and individual mitoses, tumourous lesions may develop, but they always have a benign character. An increase in the number of mitoses in cells points to the development of stromal sarcoma (*sarcoma stromale*). In turn, the mixed mesodermal tumour (*tumor mixtus mesodermalis malignus*) contains both epithelial and connective tissue component. It may contain squamocellular carcinoma, adenocarcinoma, anaplastic carcinoma, as well as myo-, chondro-, or osteosarcoma, tissues foreign to a normal uterus (26). In the uterus adenosarcoma at a developed stage may

consist of a malignant stroma and benign hyperplasia of epithelium. Around uterine carcinoma cells, investigators detected existence of a dissipative (dispersing) space, characterised by a gradually increasing expression of metallothionein (MT) over decreasing distance from the tumour, not only in cells of a healthy endometrium, but also in its stromal cells (16). Although the aforementioned cells had not reached the neoplastic phenotype yet, they already showed alterations, which might have lead toward such a phenotype. Thus, the observation seems to confirm the thesis that a tumour involves a progeny of mutated cells, which regulate the general development of new, better-organised dissipative structures (16). Stromal tumours, formed by fibroblasts and smooth myocytes, can also be encountered in the prostate gland.

Metastatic foci encompass development of both parenchyma and stroma, but parenchyma consists of neoplastic cells originating from its primary focus, while the stroma originates from the stroma of an organ in which metastasis develops. The conclusion follows that in a new metastatic focus its stroma "provides consent to proliferation of neoplastic cells". Normal human fibroblasts were shown to divide *in vitro* around 50 times (in accord with Hayflick's phenomenon) and to die subsequently by apoptosis; on the other hand, neoplastic fibroblasts continue to divide and become immortal cells. The phenomenon is linked to the abnormal TP53 protein, which does not stop the cell cycle, nor exerts any control over the system of DNA damage repair (15). The ECM fibroblasts may develop to fibromata, their malignant forms of *fibrosarcomata* and fibroblastic outgrowths of fibromatosis type, while vascular endothelial cells may yield angiomata and angiosarcomata, respectively. ECM histiocytes may give rise to *histiocytoma*. A lipid accumulation rendering the cells foamy in appearance indicates xanthoma (*xanthoma s. xanthofibroma*). In tumours of *fibroma* or *histiocytoma* type, their structure resembles the pattern of fibrous connective tissue, which means that at their beginning their stroma is affected, but not their parenchyma, as in the majority of tumours. Parallel proliferation of stroma and epithelium, *e.g.* in adenoma, results in development of fibroadenoma. Occasionally, in this tumour a thick histological section imitates in appearance a high number of stromal cells, erroneously indicating that this is sarcoma and, inversely, sarcoma on such occasions may be mistaken with fibroma (26).

Certain tumours contain no stroma at all and, therefore, they contain no blood vessels, *e.g.* chordoma and chorionepithelioma. The primary effusion lymphoma or body cavity-based lymphoma in humans, sometimes accompanying AIDS, particularly upon parallel infections with HIV and Kaposi sarcoma virus (KSHV), also represent stroma-less tumours. In the latter case, no tumour can be detected nor involvement of serous membranes while the tumour cells freely flow in body cavity, which occasionally is termed "the natural culture of neoplastic cells" (26).

In the thymus, in turn, the stroma is formed by epithelial tissue, which represents a unique situation, since other lymphoid organs manifest ECM consisting of the proper connective tissue. Cells of thymic stroma may provide a site for development of thymomata, *i.e.* lymphoepithelial thymoma, epithelial thymoma, fusocellular thymoma, and carcinoma of the thymus (26).

A separate problem results from the presence of neoplastic cells in blood, in which ECM is liquid (plasma) and not, as it is in other tissues, in the form of gel. The cells mature inhibitedly at various stages of haemocytopoiesis (leukaemias), or they actively penetrate the vascular bed as neoplastic metastases. In leukaemias precursors of leukocytes or erythrocytes appear in the blood, while the numbers of mature cells and thrombocytes become reduced. The term "neoplasia without a tumour" also exists: *e.g.* the intravascular lymphoma, consisting of large B cells, is formed by atypical cells filling vein lumen in the brain and skin of elderly individuals (26).

A neoplastic process, due to impoverished blood supply, is frequently accompanied by hypoxia, acidaemia, or hypoglycaemia, which is followed by alterations in gene expression and metabolism of neoplastic cells (30). This may also lead to mechanical stress, which is given sign of by an increased mechanical pressure within the tumour, causing cell stretching and compression, as well as shear pressures, acting on cell surface (32). The changes in pressures also affect expression of cytoskeleton proteins and ECM-modifying proteins. It is even assumed that the latter processes facilitate penetration of tumour cells to vascular lumen, and increase their metastasing potential, as an alternative to their destruction by free radicals formed *in situ* in the tumour (31).

Carcinosarcoma is a specific form of a tumour, which may be of a collisional type, with the development of two tumours independent from each other, *i.e.* a complex one, formed of both epithelial cells and stromal cells, and a combination type, in which a single stem cell gives both carcinoma and sarcoma, *e.g.* clear cell carcinoma of the kidney transforming toward renal sarcoma (26). In immunohistochemical tests the carcinosarcoma yields a positive reaction for cytokeratin in the epithelial (carcinoma) component and a positive reaction for vimentin in the connective tissue (sarcoma) component, which was demonstrated in humans (1, 5, 6, 11, 25), and animals (2, 12). At the same time, it should be underlined that cancerous cells in the tumour much more frequently yield metastases to lymph nodes than sarcomatous cells do. The recent studies complicated the problem even more. According to Lacroix and Leclercq (17), cell lines of mouse mammary carcinoma are divided into: a – luminal epithelial-like with typical markers of epithelial cells (E-cadherin, desmoplaquin I/I), b – weakly luminal

epithelial-like cells with markedly weakened expression of epithelial antigens, and c – mesenchymal-like cell lines with expression of proteins typical for mesenchymal cells (vimentin, N-cadherin) and manifesting no expression of antigens typical for epithelium. In a similar manner, Vineis *et al.* (31) are of the opinion that spontaneous carcinomas may originate exclusively from epithelial tissue or from epithelium and tumour stroma.

Summary

It can be assumed that the source of additional neoplastic outgrowths, in parallel to the already existing tumour, may involve the cells present in its stroma, transforming spontaneously or undergoing stimulation by the tumour due to intercellular signals, *e.g.*, through Shh (sonic hedgehog) protein. Glycoproteins present in ECM, *e.g.* fibronectin or laminin, bind to cell surface integrins. Furthermore, the cells of inflammatory infiltrate, frequently accompanying cancers, may behave differently depending on the context: they may restrict tumour proliferation or, inversely, they may provide a source for induction and proliferation of another tumour type, the tumour of a mesenchymal type.

Despite the numerous histogenetic classifications of tumours (*zygotoma benignum et zygotoma malignum,* or *mesenchymomata maligna et mesenchomomata benigna*), currently in oncological diagnosis the view prevails that the direction of tumour differentiation, and its degree of histologic malignancy (grading) are more important than the histogenesis of the tumour. The evaluation should also include the stage of clinical advancement reached by the tumour (staging), according to the TNM system (T - tumour, N –lymph nodes, M – metastases).

Until now, the mechanisms controlling communication between tumour cells and between other cell types and ECM have not been entirely clarified. Such a limited knowledge impedes therapy, particularly targeted therapy, *e.g.* using antibodies specific for proteins encoded by oncogenes, such as Her-2 neu, which allows physicians to spare patients toxic complications. This also hampers detection of the "so called" residual disease, or detection of infrequent neoplastic cells remaining after the treatment of neoplastic disease (lymphomas, leukaemias), which might prevent relapse and neoplastic metastases (32).

References

1. Akoluk A.A., Barazani Y., Slova D., Shah S., Tarren B.: Carcinosarcoma of the bladder: case report and reviev of the literature. Canad Urol Assoc J 2011, 5, 69–73.
2. Almes K.M., Heaney A.M., Anderews G.A.: Intracardiac ectopic thyroid carcinoma in dog. Vet Pathol 2008, 45, 500–504.
3. Baines C.P.: Role of the mitochondrium in programmed necrosis. Front Physiol 2011, 1, 156–164.
4. Brocker C., Vasiliou V., Nebert D.W.: Evolutionary divergence and functions of the ADAM and ADAMS gene families. Human Genomics 2009, 4, 43–55.
5. Del Carmen M.G., Birrer M., Schorge J.O.: Carcinosarcoma of thy ovary: a review of the literature. Gynec Oncol 2012, 125, 271–277.
6. Cirocci R., Trastulli S., Desiderio J., Grassi V., Barillaro I., Santoro A., Boselli C., Avena N., Noya G., De Sol A.: Gastric carcinosarcoma. A case report and review of the literature. Oncol Lett 2012, 4, 53–58.
7. Cirri P., Chiarugi P.: Cancer–associated–fibroblasts and tumour cells: a diabolic liaison driving cancer progression. Cancer Metastasis Rev 2012, 31, 195–208.
8. Edwards D.R., Handsley M.M., Pennington C.J.: The ADAM metalloproteinases. Mol Aspects Med 2008, 29, 258–289.
9. Frazier K., Williams S., Kothapalli D., Klapper H., Grotendorst G.R.: Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 1996, 107, 404–411.
10. Farell R.J., Kelley C.P.: Celiae sprue. N Engl J Med 2002, 346, 180–188.
11. Gelos M., Behringer D., Philippou S., Mann B.: Pancreatic carcinosarcoma. Case report of multimodal therapy and review of the literature. JOP J Pancreas 2008, 9, 50–55.
12. Grubor B., Haynes J.S.: Thyroid carcinosarcoma in a dog. Vet Pathol 2005, 42, 84–87.
13. Hanahan D., Weiberg R.A.: Hallmarks of cancer: the next generation. Cell 2011, 144, 646–674.
14. Kaczanowski A., Kaczanowska J.: Mechanisms of embryonic spatial differentiation. Post Biol Kom 2001, 28, 69–98.
15. Kawiak J., Zabel M.: Seminars cytophysiology. Urban & Partner, Wrocław, 2002.
16. Klimek R., Madej J.A., Sieroń A.: Cancer – tumors and neoplastic disease. Editor R. Klimek, Kraków 2006.
17. Lacroix M., Leclecq G.: Relevance of breast cancer cell lines as models for breast tumours: an update. Brest Cancer Res Treat 2004, 83, 249–280.
18. Madej J.A.: Pathomorphology of cumulative and proliferative non-neoplastic lesions in extracellular matrix. Med Weter 2008, 64, 977–983.
19. Madej J.A.: Outline of the natural history of tumours. Med Weter 2012, 68, 259–264.
20. Nyberg P., Salo T., Kalluri R.: Tumor microenvironment and angiogenesis. Front Biosci 2008, 13, 6537–6553.
21. Rumi E., Passamonit F., Della Porta M.G., Elena C., Arcaini L., Vanelli L., Del Curto C., Pietra D., Pascutto C., Cazzola M., Lazzarino M.: Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. J Clin Oncol 2007, 25, 5630–5635.
22. Sawicki W., Malejczyk J.: Histology. PZWL, Warszawa, 2012.
23. Schwartz E.P., Kelly M.G.: Malignant transformation of myomas: myth or reality? Obstet Gynecol Clin N Am 2006, 33, 183–198.
24. Sica A., Allavena P., Mantovani A.: Cancer related inflammation: the macrophage connection. Cancer Lett 2008, 267, 204–215.
25. Sokucu S.N., Kocaturk C., Urer N., Sonmezoglu Y., Dalar L., Karasulu L., Altin S., Bedirhan M.A.: Evaluation of six patients with pulmonary carcinosarcoma with a literature review. Sci World J 2012, 10, 1–5.
26. Stachura J., Domagała W.: Pathology – word about the disease. Vol II. General Pathology. PAU, Kraków, 2003.
27. St Croix B., Rago C., Velcukscu V., Traverso G., Romans K.E., Montgomery E., Lal A., Riggins G.J., Lengauer C., Vogelstein B., Kinzler K.W.: Gene expressed in human tumor endothelium. Science 2000, 289, 1197–1202.
28. Szala S., Radzikowski C.: Molecular basis of cancer angiogenesis. Nowotwory 1997, 41, 1–20.
29. Szlosarek P.W., Balkwill F.R.: Tumour necrosis factor alpha: a potential target for the therapy of solid tumors. Lancet Oncol 2003, 4, 565–573.

30. Vallee R.B., Sheetz M.P.: Targeting of motor proteins. Science 1996, 271, 1539–1545.
31. Vineis P., Schatzkin A., Potter J.D.: Models of cancerogenesis: an overview. Carcinogenesis 2011, 31, 1703–1709.
32. Xie K., Huang S.: Regulation of cancer metastasis by stress pathways. Clin Exp Metastasis 2003, 20, 31–43.
33. Yamaguchi Y., Yashikawa K.: Cutaneous wound healing: an update. J Dermatol 2001, 28, 521–531.
34. Yamanchi N., Harada T., Tanigucki F., Yoshida S., Iwabe T., Terakawa N.: Tumor necrosis factor-α induced the relase o interleukin – 6 from endometriotic stromall cells by the nuclear factor – kB and mitogen activated protein kinase pathways. Fert Steril 2004, 82 (suppl), 1023–1028.
35. Zarębski A.: Some inhibitors of intracellular signal transduction in the inhibition of cytokines synthesis. Post Biol Kom 2001, 28 (suppl), 15–22.
36. Zhou J., Mochizuki T., Smeets H., Antignac C., Laurila P., de Paepe A., Triggvason K., Reeders S.T.: Deletion of the paired alfa 5 (IV) and alfa 6 (IV) collagen genes in inherited smooth muscle tumors. Science 1993, 261, 1167–1169.