The role of adhesive molecules in endometrial cancer: part II

Rola molekuł adhezyjnych w raku endometrium. Część II

Michał Wojciechowski[1,2], Tomasz Krawczyk[3], Janusz Śmigielski[4], Andrzej Malinowski[1,2]

[1]Department of Surgical and Endoscopic Gynaecology, Medical University of Łódź, Poland;
Head of Department: prof. dr hab. n. med. Andrzej Malinowski
[2]Department of Surgical and Endoscopic Gynaecology, Research Institute of the Polish Mother's Memorial Hospital in Łódź, Poland;
Head of Department: prof. dr hab. n. med. Andrzej Malinowski
[3]Department of Clinical Pathomorphology, Research Institute of the Polish Mother's Memorial Hospital in Łódź, Poland;
Head of Department: prof. dr hab. n. med. Andrzej Kulig
[4]Department of Informatics and Medical Statistics, Medical University of Łódź, Poland;
Head of Department: dr n. med. Radosław Zajdel

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Summary

The carcinogenesis is a result of both functional and structural disorders in the tissue. It initiates as a mutation in a gene encoding protein that is essential for cellular function. The subsequent cascade of events leads to accumulation of mutations and loss of cellular function. The cell loses its tissue-specific morphology, disconnects from other cells and extracellular matrix and migrates – the invasion begins. It is now clear that adhesive molecules are a key player in this cascade. These proteins of the cell membrane surface are responsible for attachment of the cells to each other and to the extracellular matrix. These interactions are crucial for both structural and functional tissue organization. Lack of this homeostasis destroys the tissue architecture and impairs its function and results in invasion. Abnormal expression of adhesive molecules was reported in all examined cancers, including endometrial cancer.

Endometrial cancer is the most common gynaecological cancer in developed countries. Although in many cases diagnosed and treated in early stages, and thus with good results, some patients cannot be cured. Complete knowledge of the pathogenesis of the disease will be helpful in identifying the patients with negative prognostic factors, increased risk of recurrence and, perhaps, to find other therapeutic options. In the paper we are trying to sum up the up-to-date knowledge of the role of adhesive molecules in pathogenesis of endometrial cancer.

Key words: adhesion, adhesive molecules, carcinogenesis, invasion, metastasis.

Streszczenie

Nowotworzenie to wynik zaburzenia struktury i funkcji tkanki na wielu poziomach. Proces rozpoczyna się od mutacji w kluczowym dla komórki genie kodującym istotne dla procesów komórkowych białka. Następuje kaskada wydarzeń prowadząca do kumulacji mutacji i zaburzająca funkcję komórki. Komórka traci charakterystyczną dla tkanki morfologię, ścisły kontakt z otoczeniem i zaczyna migrować – rozpoczyna się inwazja. Jak się okazuje, istotną rolę w tej kaskadzie odgrywają molekuły adhezyjne – powierzchniowe białka błon komórkowych, odpowiadające za połączenia komórek między sobą oraz komórek z przestrzenią międzykomórkową. Odgrywają one główną rolę zarówno w strukturalnej, jak i funkcjonalnej organizacji tkanek. Naruszenie wyznaczonego przez nie porządku prowadzi do zaburzenia struktury i funkcji tkanki i rozwoju fenotypu inwazyjnego. Wykazano nieprawidłową ekspresję wielu molekuł adhezyjnych we wszystkich badanych nowotworach, w tym w raku endometrium. Rak endometrium jest obecnie najczęstszym nowotworem złośliwym narządów płciowych kobiet w krajach rozwiniętych, w tym w Polsce. Choć w większości przypadków jest rozpoznawany we wczesnym stadium, kiedy rokowanie jest dobre, części pacjentek nie udaje się wyleczyć. Poznanie biologii tego nowotworu może ułatwić identyfikację pacjentek z rakiem endometrium o gorszym rokowaniu oraz, być może, znaleźć nowe opcje terapeutyczne. Poniższa praca jest próbą przedstawienia obecnego stanu wiedzy na temat roli adhezyn w patogenezie tej choroby.

Słowa kluczowe: adhezja, molekuły adhezyjne, karcynogeneza, inwazja, przerzut.

Address for correspondence:
Michał Wojciechowski, Department of Surgical and Endoscopic Gynaecology, Rzgowska 281/289, 93-338 Łódź, Poland

Immunoglobulin-like superfamily

Family of immunoglobulins is a group of adhesive molecules structurally similar to antibodies, with characteristic extracellular domain. Typical members of this group are: ICAM-1, ICAM-2, VCAM-1, PECAM-1, MAdCAM-1, NCAM or CEA.

One of the most important and best known functions of molecules from this family is participation in inflammatory reaction – especially in activation and migration of leucocytes at the site of inflammatory reaction, along with integrins and selectins. For instance, ICAM-1 is present on leucocytes, monocytes, eosinophiles, hematopoietic cells, fibroblasts or vessel endothelium. Inflammation is the strongest inductor of ICAM-1 expression and leads to its upregulation on endothelium. Next, ICAM-1 binds with activated leucocyte surface integrins LFA-1 and Mac-1, leading to their attachment to endothelium. It is the first and crucial stage of leucocyte arrest and extravasation at sites of inflammation, analogous to the process of metastasis formation [4]. Similarly, MadCAM-1 is responsible for arrest and extravasation of leucocytes mainly in lymphatic tissue and mucosa. The most probable function of the immunoglobulin family in cancer invasion is regulation of interactions between leucocytes and neoplastic cells circulating in the bloodstream. It is also possible that immunoglobulin-like adhesive molecules (mainly ICAM and VCAM), together with selectins and integrins, are responsible – just like in the case of inflammatory reaction – for adherence of circulating cancer cells to endothelium (activated and presenting integrins VLA-4, LFA-1, Mac-1). Such an interaction would allow for attachment of cancer cells to the vessel wall and their extravasation. It was shown that an increased expression of ICAM-1 correlates with high metastatic potential in some neoplasms [4]. NCAM, present on cells of the nervous system, was originally suspected of participation in progression of some tumours, which has not been proven though yet. However, initially in colon cancer, and then also in breast, prostate, bladder and endometrial cancers, inactivation of a gene, subsequently called DCC (deleted in colorectal cancer) was reported. Its sequence is very similar to the sequence of a NCAM gene. It is believed that a product of the DCC gene could function as adhesive molecule forming homophilic junctions between cells, like E-cadherin. Loss of this adhesion would lead to dissemination of cancer [4].

There are not many reports on a potential role of molecules from immunoglobulin-like family in pathogenesis of endometrial cancer. It has been shown that, like in breast or colon cancer, neoplastic transformation in endometrium is accompanied by abnormal expression of C-CAM (CD66a), molecule from the CEA family. Loss of expression of this molecule as well as change of its cell membrane expression pattern, from polarized to dispersed, is observed along with tumour grade increase [26]. L1CAM, relatively recently known as a unfavourable prognostic marker in endometrial cancer, is not present on cells of healthy endometrium, whereas it is intensively expressed in type-2 (unendometrioid) endometrial cancer and in low-differentiated endometrioid cancers. Increased expression of L1CAM correlates with loss of E-cadherin and EandP receptors [27].

Selectins

Selectins (CD62) are a group of adhesive molecules, dependent on Ca^{2+}, that are mediators in a specific interaction between cells of endothelium, leucocytes and thrombocytes. They include L-selectin (CD62L), constantly present on most leucocytes, which binds leucocytes with lymphatic vessel endothelium; E-selectin (CD62E), expressed on the endothelium surface after activation by proinflammatory cytokines (interleukin 1 – Il-1, tumour necrosis factor – TNF), which binds neutrophiles, monocytes, memory T lymphocytes, eosinophiles and basophiles, and P-selectin (CD62P), present on endothelium and thrombocytes after their activation and degranulation, which binds neutrophiles, monocytes, memory T lymphocytes, eosinophiles and basophiles [5, 28, 29]. The role of endothelial P- and E-selectins is to arrest circulating leucocytes by the surface of endothelium in capillaries, at the site of inflammation. Their ligands, present on leucocytes and also – as it turns out – on neoplastic cells, are glycolipids and glycoproteins containing, crucial for binding, tetrasaccharide residues– sialyl LewisX (sLeX) [4, 5, 28, 29]. This binding is weak and unstable. But due to it, circulating leucocytes slow down (so called "rolling" phenomenon) and get in the vicinity of proinflammatory cytokines and undergo activation. It results in expression of β2-integrins (LFA-1), which bind with the family of endothelial immunoglobulins (ICAM-1) or β1-integrins (VLA-4) binding with VCAM-1. This binding is strong and permanently immobilizes leucocytes. A coordinated interplay of adhesive molecules and chemotactic factors makes leucocyte hit exactly the place of inflammation and, after attachment to the endothelium, it receives a signal to start diapedesis through endothelium monolayer. L-selectin, present on leucocytes, is responsible for their arrest by the wall of lymphatic vessels in lymphatic organs (lymph nodes, spleen) [29].

It is believed that selectins may play a crucial role in the metastatic cascade at the stage of attachment and extravasation of cancer cells in target tissues for metastatic deposits, exactly imitating adhesive sequence in inflammatory reaction. Cells of many human cancers, including cancers of colon, lung, breast, stomach, melanoma, neuroblastoma and others, present ligands for P- and E-selectin on their surface.

P-selectin, and also E-selectin, would be responsible for initial arrest of cancer cells by endothelium and thus starting the process of their extravasation [4, 28, 29].

An increased expression of ligands for L-selectin (present on the surface of blastocyst cells) was revealed in endometrium in peri-implantation phase, which can beneficially affect the course of the process of implantation. However there are no explicit reports on a potential role of selectins in oncogenesis of endometrial cancer.

CD44

CD44 is an adhesive molecule, which does not match any primary family of adhesive molecules. Actually it is a group of molecules that are the most important receptors for hyaluronate, consisting of a standard form (CD44s) and so-called variants (CD44v). Initially it was described as a surface particle of lymphocytes, participating in their activation and recirculation. Variant forms of CD44 (CD44v) are products of alternative splicing of the transcript of CD44 gene. Cytoplasmic domain of CD44 binds with cytoskeleton proteins: ankyrin, esrin, moesin and radixin, which determines their participation in outside-inside cell signalling pathways and cell migration [4].

Particle CD44s is present on the surface of majority of healthy cells. Expression of variants is very limited. They are present on hematopoietic cells, mononuclear blood cells – activated lymphocytes and lymph node cells and proliferating epithelial cells.

As it was mentioned, the CD44 molecule is the main membrane receptor for hyaluronate – a very widespread polysaccharide of extracellular matrix. Other CD44 ligands are osteopontin, chondroityn, collagen, laminin, fibronectin, fibrinogen, vascular adresins, MHC II, L- and E-selectin. A large amount of ligands and variant forms of CD44 is the reason why the particle participates in numerous cellular processes. The basic role of the CD44 adhesive molecule is interaction in keeping a three-dimensional tissue structure. As a result of binding with hyaluronate and other ligands this molecule is responsible for attaching the cell to the extracellular matrix and forming a scaffolding for the tissue. Through binding with the hyaluronic acid CD44 also participates in adherence of neighbouring cells. Homophilic junctions CD44-CD44 are also possible. Interaction of CD44 with its ligands, especially HA, is also crucial for other functions of this adhesive molecule. The expression of both CD44 and HA is increased during cell proliferation while regeneration of epithelia, wound healing, myelopoiesis and lymphopoiesis, proliferation and migration of cells during angiogenesis, embryogenesis and cancer invasion as well. Hyaluronic acid strongly binds water, forming a semi-liquid environment in which cell migration is easier. Simultaneously, a strong interaction between CD44 and HA stimulates proteins of cell cytoskeleton, their rearrangement and cell movement. Thus, the molecule CD44 is responsible for cell migration in HA-rich environment [7].

As it was already mentioned, an important function of the molecule CD44 (also called lymphocyte homing receptor) is its participation in lymphocyte circulation and function. Activation of T lymphocytes through a specific antigen leads to intensification of CD44 expression, which then allows for reaction of lymphocyte with endothelium presenting HA. This interaction is responsible for arrest and diapedesis of lymphocytes through a vessel wall into the place of inflammatory reaction or to the lymphatic tissue. This interaction is stimulated by pro-inflammatory cytokines TNF-α, IL-1β. In organisms devoid of CD44, lymphocyte migration and delayed type immune hypersensitivity response are impaired. The CD44-HA interaction, which occurs in inflammation, is analogous to the previously described interaction of 'naive' lymphocytes with endothelium through L-selectin. Just like in the case of selectins, the binding between CD44-HA is weak and, after activation, a stronger interaction between integrins and adhesive molecules from the family of immunoglobulins is created.

The CD44 molecule, especially its variants, have been shown to be able to play a significant role in cancer progression and metastatic cascade [4]. In experimental research on a model of nonmetastatic pancreatic cancer in rats, transfection of cDNA encoding CD44 (CD44v6) led to formation of metastases, which in turn was inhibited by administration of antibodies against CD44v6. It was also shown that lymphoma cells presenting CD44v molecules disseminated to the lymph nodes faster than with the standard CD44 form. Administration of specific antibodies against CD44v or hyaluronidase inhibits occurrence of new, metastatic tumours and lymph node metastases. An increased expression of CD44, mainly variant forms, is observed on cancer cells, particularly in neoplasms with high invasive potential: non-Hodgkin lymphomas, malignant melanoma, cancers of colon, stomach, thyroid, breast, pancreas, uterine cervix, ovary, lung and endometrium. Over-expression of CD44, particularly untypical variants, specific of each cancer, is usually associated with worse prognosis and more invasive phenotype. Most often an increased expression of CD44v6 variant form is observed. This phenomenon can have two reasons. Either variant forms of the CD44 particle take part in carcinogenesis or neoplastic transformation mainly concerns the cells of quickly proliferating epithelial layers, which strongly express CD44v.

There are many hypotheses which try to explain the role of the CD44 molecule in oncogenesis. An increased expression of CD44 in tumour, around which the HA is simultaneously over-expressed, leads to a strong cell

adhesion to HA-rich extracellular matrix, tearing off the neighbouring cells and the ECM that is poor in HA and migration. On the other hand, the CD44 molecule cooperates with hyaluronidase in internalization and degradation of hyaluronate by a cell, which allows it to release from the hyaluronate-rich milieu and cross cell membranes (basement membranes, vessel walls). Moreover, the expression of CD44 variants, absent in healthy tissues, of weaker affinity to ligands, would affect the adhesive cell profile, especially towards HA and disturb a proper cell-ECM interaction and thus promote invasion. As mentioned before, it was revealed that cancer cells with increased expression of the CD44 molecule, variants in particular, have a larger predisposition to metastases. Due to the CD44 molecule, they imitate migration of lymphocytes to lymph nodes and a multistep model of leucocyte extravasation: binding with HA on endothelium initiate a transient, weak interaction ("rolling" adhesion), followed by secondary (firm) adhesion, mediated by other adhesive molecules. This is the first step of the following extravasation and invasion. It is possible that variant forms of CD44 also function as ligands for E-selectin (endothelial selectin), which could also explain the role of CD44v in formation of metastasis at the stage of interaction between cancer and endothelial cells. In the target tissue, in HA-rich environment, the CD44 binds with extracellular matrix immobilizing the metastatic cells, which will form a secondary tumour. Once more the CD44-HA interaction is here crucial for the process of neoangiogenesis.

CD44 is present in endometrial epithelium, but the expression is subject to strong fluctuations depending on the cycle phase. In the proliferative phase neither standard (CD44s) nor variant forms (CD44v) were detected. However, in the secretory phase, even 2/3 of examined specimens were positive for CD44s, CD44v3 and v6, with inverse correlation with estrogen and progesterone receptors [7, 14]. It was suggested that the expression of CD44 can be inhibited by estrogens secreted in proliferative phase, whereas their presence only in secretory phase can be significant for implantation [7].

Expression of the CD44 adhesive molecule in endometrium with simple and complex hyperplasia is stronger than in healthy tissue but weaker than in atypical hyperplasia. In endometrial cancer, the expression of CD44s and especially isoforms v3 or v6 (although the data concerning the latter are contradictory) is the strongest [7, 14, our own research, not published yet]. Only one research revealed a decreased expression of CD44 in endometrial hyperplasia and cancer. However the literature lacks explicit data on the role of the CD44 molecule and its variants in endometrial cancer and their relation with clinical and pathological parameters and prognosis. They are often even contradictory. Increased expression of CD44, especially v3, turned out to be related to the vascular space involvement and deep myometrial invasion [14]. According to some reports, the CD44v6 form was expressed stronger [7], whereas in other researches – the expression was weaker [14], with various, often completely opposite effect on prognosis. In our unpublished research, CD44 was more intensively expressed in patients with endometrial cancer. The expression was significantly increased in papillary-serous type of cancer.

Conclusions

Adhesive molecules play an important role in maintaining the structural homeostasis in the tissue. They participate in numerous, significant for cell functioning, signalling pathways which control crucial cellular processes: proliferation, growth, tissue differentiation, migration or apoptosis. Disruption of the adhesion forces results in tissue structural disorganization, relaxation of intercellular junctions, detachment from the surrounding extracellular matrix and, as a consequence, impairment of its function along with loss of control over crucial cellular processes. These are phenomena typical for carcinogenesis. Complex interactions between adhesive molecules on cell surfaces and their ligands in the surrounding extracellular matrix determine migration of cancer cells, their penetration via biological membranes or vessel endothelium – events that are characteristic for invasion – a hallmark of malignancy. This diverse group of proteins, taking part in every stage of carcinogenesis, must have become a subject of intense research. There are many data about a crucial role of adhesive molecules in oncogenesis of all examined cancers. There is however a relatively small number of reports on endometrial cancer, although available results seem to be in accordance with observations concerning other tumours. Explanation of the role of particular adhesive molecules in pathogenesis of endometrial cancer could give us new, interesting diagnostic, prognostic and, perhaps, also therapeutic options. For a long time there have been attempts to use the cell adhesion phenomenon in therapy. Some of them, particularly based on monoclonal antibodies against adhesive molecules, proved efficient and became a standard therapeutic option in many diseases.

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