® Postepy Hig Med Dosw (online), 2015; 69: 447-451
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 2013.11.25
Accepted: 2015.02.05
Published: 2015.04.08

Breast cancer metastasis – insight into selected molecular mechanisms of the phenomenon

Przerzutowanie w raku piersi – analiza wybranych mechanizmów molekularnych zjawiska

Jarosław Kozłowski[1,3], Aleksandra Kozłowska[1,2], Janusz Kocki[1]

[1] Department of Clinical Genetics, Medical University of Lublin,
[2] Department of Radiotherapy, St. John's Cancer Center, Lublin,
[3] Department of Oncological Surgery, St. John's Cancer Center, Lublin,

Summary

Metastasis is a complex, multistep biological process, involving a multitude of genes and biomolecules. Despite the successful therapeutic management of breast cancer, including surgery, chemotherapy and radiation therapy, that can control primary tumor growth, metastatic disease remains the greatest clinical challenge in oncology, as these methods are still not very effective in preventing relapses or in the management of breast cancer metastases. The knowledge of its mechanisms is still fragmentary and needs to be broadened in order to improve our therapeutic approach and influence on the long-term control of breast cancer progression. Despite the constant progress in understanding of breast cancer progression, it remains a major health problem around the world. Novel therapeutic modalities are being tested and developed, but the incidence and mortality rates are still frightening. In this paper, we review selected aspects of breast cancer metastasis, including the metastatic cascade and models of dissemination, tumor angiogenesis, disaggregation and migration of cells from the primary tumor, breaking the vascular wall, adaptation to a new environment, organotropism and dormant cells. The interactions between cancer cells and normal host cells contributing significantly to the metastatic cascade are highlighted, and a wide range of signaling and stimulating biomolecules and genes involved in the process are introduced.

Key words: **breast cancer • dormant cells • metastasis • molecular mechanisms • metastatic cascade • models of dissemination • organotropism • 'seed and soil' hypothesis • tumor angiogenesis.**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1148710

Word count: 2520
Tables: –
Figures: –
References: 38

Author's address: lek.med. Jarosław Kozłowski, Department of Clinical Genetics, Medical University of Lublin, 11 Radziwillowska St, 20-080 Lublin; e-mail: aj.kozlowscy@interia.pl

Introduction

Despite the constant progress in understanding of breast cancer progression, it remains a major health problem around the world. Novel therapeutic modalities are being tested and developed, but the incidence and mortality rates are still frightening. Tumor metastases are responsible for approximately 90% of cancer-related deaths [31]. It is established that between 25% and 50% of breast cancer patients will eventually develop deadly metastases, occurring even decades after the time of diagnosis and primary tumor removal. Some histological subtypes of breast cancer and molecular marker expression are known as strong prognostic and predictive factors. For instance, the ER, PGR and HER2 negative cancers, called 'triple negative', are associated with significantly increased risk of progression and dissemination [21]. Unfortunately, the prognosis for patients with metastatic disease is generally unfavorable, with an average 5-year survival rate of about 25% [27,36]. The therapeutic options used in clinical practice, including surgery, chemotherapy and radiation therapy, can control primary tumor growth. However, these methods are still not very effective in preventing relapses and in the management of breast cancer metastases. In order to improve long-term survival of cancer patients, it is essential to focus on prevention and treatment of metastatic disease by targeting specific steps governing the metastatic cascade, blocking the colonization of secondary organs and eradication of established metastases using novel modalities in addition to current treatment [8].

Metastatic cascade and models of dissemination

Our understanding of metastatic mechanisms is still fragmentary. To date, the hypothesis of 'seed and soil', coined by the English surgeon Stephen Paget in 1889, provides possibly the most appropriate definition of the process. The author made an observation that 'organ-specific metastases are due to the dependence of the seed (the cancer cell) on the soil (the secondary organ) [25]. Thus, the subsequent growth of cells 'seeded' to an organ depends on the compatibility of the 'seed' and the 'soil' encountered in the target organ. Currently, it is determined that the formation of a metastasis is a complex, multistep and multifunctional biological event. This process results from a sequential molecular cascade through which cancer cells spread from the primary tumor to distant anatomical sites, where they can proliferate and create secondary neoplastic foci [32]. This cascade of events includes the following steps: 1. angiogenesis, that is the development of new blood supply to the growing tumor, 2. the escape of tumor cells from the primary tumor mass, 3. the invasion of and migration through the basement membrane and extracellular matrix surrounding the tumor epithelium, 4. subsequent invasion of the basement membrane supporting the endothelium of local blood vessels or lymphatics, 5. the intravasation of the tumor cells into the blood or lymphatic vessels, 6. the adhesion of the circulating tumor cells to the endothelium of capillaries of the target organ site, 7. the extravasation and invasion of tumor cells through the endothelial cell layer, surrounding basement membrane and target organ tissue, 8. the growth of secondary neoplastic foci at the target organ site [5,10]. It is believed that the metastatic phenomenon is attributable to low efficiency. Only a small fraction of cancer cells entering the circulation will successfully generate secondary tumors. That results from tumor cell death in the blood circulation, caused by exposure to stress, hemodynamic forces, induction of anoikis due to lack of adhesion and immune attack by cytotoxic effector cells [22,23].

There are two major spatial-temporal models of tumor cell dissemination described [19,26]. Traditionally, it is stated that formation of metastases occurs late, at a time when the primary lesion has already reached a considerable volume. This model, called the linear model of metastasis, is generally acceptable for most cancers, as the primary tumor volume represents the risk factor for dissemination. It is assumed that genetic modifications accumulate progressively in cancer cells. Consequently, cells with advantageous mutations will survive and expend through clonal evolution. According to this model, the process leads to separation of a small cell subpopulation with acquired metastatic capacities within the primary tumor which, when disseminated, are more effective in colonizing distant organs. Based on this model, metastasis-driving mutations should occur rarely in the primary tumor, but frequently in the distant metastases [6]. Numerous studies have confirmed a similar molecular signature of the metastases and their corresponding primary tumors, which was interpreted as evidence for the linear model [2,28]. However, this model has recently been challenged by the parallel model of metastasis, proposed as an answer to observations suggesting that cancer cells, particularly in breast cancer, might disseminate early during tumor progression, even at pre-malignant stages of disease. This model implies independent evolution of disseminated cells. Some pieces of research demonstrate that genetic alterations found in breast cancer cells disseminated into the bone marrow often differ significantly from those present in the primary tumor [4]. Accordingly, the genetic profile of disseminated tumor cells should be more relevant in predicting response to therapy in metastatic disease.

Tumor angiogenesis

The first step in the metastatic cascade is formation of new blood vessels from pre-existing vasculature. When the process of angiogenesis occurs physiologically, for example during wound healing or pregnancy, there is a controlled balance between angio-inhibitory and pro-angiogenic factors [29]. The impairment of these delicate controls favoring inappropriate angiogenesis is observed in cancer, as well as in many other disorders, including diabetic retinopathy, rheumatoid arthritis and psoriasis [5,11]. The currently well-accepted theory that the growth and survival of malignant tumors and the

progress of metastatic disease are dependent on oxygen and nutrients supplied by newly created vessels was based on pioneering research of Folkman in 1971. Nowadays, microvessel density, a measure of angiogenesis, is known as a prognostic factor predicting the survival of cancer patients [37]. It is determined that hypoxia is a trigger factor for the molecular cascades underlying angiogenesis [9]. Under hypoxic conditions, the hypoxia inducible factor 1 (HIF-1) transcriptional complex is stabilized and consequently genes coding for angiogenesis- inducing factors, anaerobic metabolism, cell motility and resistance to apoptosis are activated [15]. HIF-1 induces expression of the chemokine receptor CXCR4, which may mediate organ-specific dissemination, and it also interacts with lysyl oxidase (LOX), which is involved in collagen maturation [9]. Tumor cells and surrounding stromal cells stimulate angiogenesis by production of soluble proinflammatory and pro-angiogenic cytokines, such as tumor necrosis factor alpha (TNFα), vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). They diffuse into nearby tissues and bind to receptors on the endothelial cells of pre-existing blood vessels in the vicinity of the tumor. As a result, adhesion molecules, including integrins, proteases and plasminogen activators, are secreted, and inhibitors, such as thrombospondin, are down-regulated [3]. Therefore, the stimulated endothelial cells begin their proliferation and degrade the surrounding stroma with the aid of proteases. The adhesive and de-adhesive interactions with stromal components, mediated predominantly by integrins, are responsible for their migration through the area of degraded stroma. These migrating endothelial cells form new, immature vessels, which undergo canalization and create a complete vascular network supplying blood to tumor cells. The intratumoral vessels are characterized by increased permeability, enabling the cancer cells to enter the systemic circulation. The blockage of tumor angiogenesis by means of inhibitors targeting the tumor-associated endothelium is already used in clinical practice (endostatin, bevacizumab, etc.) [12].

Disaggregation and migration of cells from the primary tumor

The migratory and invasive abilities are the crucial parameters of cancer cells. Two fundamentally different patterns of cancer cell invasion are defined: single cell invasion and collective cell invasion [31]. The majority of cancers originate from epithelial tissues. In order to leave the primary tumor and invade the surrounding tissue, these tumor cells need to diminish their tight cell-cell adhesion. This process permits disaggregation of tumor cells from the primary site and aids initial dissemination. It is suggested that in most cases, single cells leaving the primary tumor undergo epithelial to mesenchymal transition (EMT), mediated by such molecules as TGFβ, MAP kinases, transcription regulators Twist and Snail (SNA, SNAI1), Wnt, Notch and Hedgehog [35]. The opposite process, MET-mesenchymal to epithelial transition, takes place when the metastasis is being formed in a specific organ. The mechanism of EMT, involving the loss of epithelial polarity and the achievement of a mesenchymal morphology, remains currently a major focus in metastasis research [33]. The disruption of cell-cell contacts and the acquisition of a fibroblastoid spindle-shape morphology connected with increased invasiveness and slower rates of cell division result in the release of single cells from a solid epithelial tumor. Despite the fact that the actual presence of EMT in patients is still debated, there is increasing evidence for this process in various human cancers, including breast cancer [30]. It is demonstrated that the expression of vimentin, a mesenchymal marker, in epithelial cells of breast cancer is a factor indicating shorter postoperative survival of patients [34]. Moreover, the highly invasive metaplastic and claudin-low subtypes of breast cancer have been characterized by attenuated E-cadherin expression and elevated levels of the EMT-inducing transcription factors Twist and Slug [16]. The data mentioned above suggest that epithelial to mesenchymal transition plays an important role in the process of metastasis and thus needs to be dissected apart. The significance of cadherins and catenins in the process of disaggregation is highlighted [5]. Cadherins are a large group of adhesion molecules that interact with the actin component of the cytoskeleton via the cytosolic proteins catenins. In this way, they are involved in maintaining tight intercellular adhesion interactions. The alteration in cadherin/catenin expression results in the loss of cell-cell adhesion, which is demonstrated to be associated with metastatic phenotype [7].

Breaking the vascular wall

After escaping from the primary tumor site and entering the circulation, the breast cancer cells begin their migration towards distant organs. The first obstacle encountered by circulating tumor cells is the endothelial lining of the vascular wall. In some organs, such as bone marrow or the liver, the microvessels are comprised of sinusoids, which are highly permeable and represent weak barriers for cancer cells [21]. In contrast, in most organs, including the brain, the endothelial lining is tight and prevents easy penetration by the cells. It is established that leucocytes and blood platelets facilitate metastasis, forming complexes with tumor cells by L- or P-selectins. Increased expression of selectin ligands is attributable to metastatic progression and poor prognosis [20]. The extravasation is encouraged by the expression of genes responsible for remodeling the vasculature to increase permeability (e.g. the *ANGPTL4* gene). Furthermore, also the chemokines expressed in a target tissue account for the migration of cancer cells, by interacting with their receptors. Chemokines are small cytokine-like proteins that elicit directional cell migration and activate signaling pathways regulating cytoskeletal rearrangement and adhesion. It is determined, for example, that the CXCR4 and CCR7 receptors and their ligands CXCL12 and CCL21, respectively, are used by breast cancer cells to arrest and migrate to secondary organs [24].

Adaptation to new environment

Physiologically, cells are able to survive and proliferate only within their specific original tissue. However, the disseminated cancer cells can form growing metastases in tissues differing significantly from their origin. That is why the cancer cells must adapt to their new environment. At the time of initial invasion the tumor cells develop a bidirectional relationship with the surrounding host stroma. Consequently, these interactions lead to progression. Tumor cells (the 'seeds') exert an impact on many physiological events of colonized tissue stroma (the 'soil'), resulting in promotion of tumor growth. They twist and polarize inflammation, suppress the immune response, favor angiogenesis, and influence growth, survival and release of motility factors [14]. As a result, the stromal cells can alter their transcriptome in order to express genes creating an adequate environment for the disseminated cancer cells. Interestingly, scientific observations showed that tissue modifications occur prior to tumor cell seeding. These findings led to the introduction of the pre-metastatic niche concept. According to this model, bone marrow derived cells (BMDCs), mobilized by factors released by the primary tumor, create a suitable microenvironment for metastatic colonization before the arrival of disseminated tumor cells. There are numerous molecules promoting the pre-metastatic niche, including VEGFR1, LOX, periostin, tenascin-C, chemokines such as CXCL12, and many others [17].

Organotropism

Cancers arising at different primary sites show distinct and typical patterns of metastatic spread. This striking phenomenon has fascinated researchers since Paget's 'seed and soil hypothesis' [25]. In 1928 Ewing proposed an alternative rule of mechanical entrapment, suggesting that circulating tumor cell aggregates become trapped in the first vascular bed they encounter. Some metastatic lesions do support this idea, but many types of cancer metastasize to sites that cannot be explained by simple blood pattern, for instance the propensity of breast cancer to metastasize to the long bones, brain and adrenal glands [5]. There are many examples of organ-selective seeding. It is noted that breast cancers expressing the *ERB-B* oncogenes preferentially form metastases in the central nervous system, where heregulins and neuregulins, their binding ligands, are expressed, while breast cancer cells expressing CXCR2 tend to metastasize to tissues such as the lungs, which are rich in CXCL12 [38]. To date, several genes and molecules mediating metastatic colonization of breast cancer to bone, lung and brain have been identified. The expression of these genes in the primary tumor seems to predict organ-specific progression in patients [28]. However, the mechanism of re-seeding recently discovered in experimental tumors, suggesting that the metastatic cells can recirculate from the metastatic sites back to the primary tumor, alters the interpretation of previous observations [18]. If proven in human cancers, this mechanism could explain the origin of 'metastatic signatures' detected in primary tumors and weaken the predictive value of such signatures.

Dormant cells

The clinical observation of breast cancer patients reveals many cases of late relapses, appearing years or decades after surgical resection of primary lesions. The facts indicate the occurrence of the 'metastatic dormancy' phenomenon, associated with the presence of disseminated tumor cells of low proliferative and metabolism activities [13]. These cells are not capable of giving rise to a secondary tumor at the moment of their seeding, but remain viable and acquire invasive abilities through genetic modifications and interactions with the microenvironment. This state of dormancy could represent a major problem limiting the efficiency of adjuvant chemotherapy, aiming to target active and proliferative cells, but failing to eradicate dormant metastases [1]. Understanding metastatic dormancy is a crucial goal for science, as it could introduce novel therapies improving long-term control of cancer progression.

Conclusions and future directions

It is now recognized that metastasis is an extremely complex and multistep biological event. There is evidence that interactions between cancer cells and normal host cells contribute significantly to the metastatic cascade. A wide range of signaling and stimulating biomolecules and genes involved in the process are identified. However, there are still many questions awaiting answers. When, where and how do metastatic cells appear within the primary tumor? Can we identify circulating tumor cells with metastatic capabilities among the whole population of these cells? The solution of these and other problems could help us to understand the limitations of current therapies and to implement more effective drugs and therapeutic approaches. Thus, despite great advances in exploring the mechanisms of metastasis, this phenomenon remains the greatest clinical challenge in oncology.

References

[1] Aguirre-Ghiso J.A.: Models, mechanisms and clinical evidence for cancer dormancy. Nat. Rev. Cancer, 2007; 7: 834-846

[2] Albini A., Mirisola V., Pfeffer U.: Metastasis signatures: genes regulating tumor-microenvironment interactions predict metastatic behavior. Cancer Metastasis Rev., 2008; 27: 75-83

[3] Avraamides C.J., Garmy-Susini B., Varner J.A.: Integrins in angiogenesis and lymphangiogenesis. Nat. Rev. Cancer, 2008; 8: 604-617

[4] Braun S., Vogl F.D., Naume B., Janni W., Osborne M.P., Coombes R.C., Schlimok G., Diel I.J., Gerber B., Gebauer G., Pierga J.Y., Marth C., Oruzio D., Wiedswang G., Solomayer E.F. et al.: A pooled analysis

of bone marrow micrometastasis in breast cancer. N. Engl. J. Med., 2005; 353: 793-802

[5] Brooks S.A., Lomax-Browne H.J., Carter T.M., Kinch C.E., Hall D.M.: Molecular interactions in cancer cell metastasis. Acta Histochem., 2010; 112: 3-25

[6] Chaffer C.L., Weinberg R.A.: A perspective on cancer cell metastasis. Science, 2011; 331: 1559-1564

[7] Christofori G., Semb H.: The role of the cell-adhesion molecule E-cadherin as a tumor-suppressor gene. Trends Biochem. Sci., 1999; 24: 73-76

[8] Ding L., Ellis M.J., Li S., Larson D.E., Chen K., Wallis J.W., Harris C.C., McLellan M.D., Fulton R..S, Fulton L.L., Abbott R.M., Hoog J., Dooling D.J., Koboldt D.C., Schmidt H. et al.: Genome remodeling in a basal-like breast cancer metastasis and xenograft. Nature, 2010; 464: 999-1005

[9] Erler J.T., Bennewith K.L., Nicolau M., Dornhöfer N., Kong C., Le Q.T., Chi J.T., Jeffrey S.S., Giaccia A.J.: Lysyl oxidase is essential for hypoxia--induced metastasis. Nature, 2006; 440: 1222-1226

[10] Fidler I.J.: The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat. Rev. Cancer, 2003; 3: 453-458

[11] Folkman J.: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med., 1995; 1: 27-31

[12] Folkman J.: Antiangiogenesis in cancer therapy - endostatin and its mechanisms of action. Exp. Cell. Res., 2006; 312: 594-607

[13] Goss P.E., Chambers A.F.: Does tumor dormancy offer a therapeutic target? Nat. Rev. Cancer, 2010; 10: 871-877

[14] Hanahan D., Weinberg R.A.: Hallmarks of cancer: the next generation. Cell, 2011; 144: 646-674

[15] Harris A.L.: Hypoxia - a key regulatory factor in tumor growth. Nat. Rev. Cancer, 2002; 2: 38-47

[16] Hennessy B.T., Gonzalez-Angulo A.M., Stemke-Hale K., Gilcrease M.Z., Krishnamurthy S., Lee J.S., Fridlyand J., Sahin A., Agarwal R., Joy C., Liu W., Stivers D., Baggerly K., Carey M., Lluch A. et al.: Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res., 2009; 69: 4116- 4124

[17] Kaplan R.N., Psaila B., Lyden D.: Bone marrow cells in the 'pre--metastatic niche': within bone and beyond. Cancer Metastasis Rev., 2006; 25: 521-529

[18] Kim M.Y., Oskarsson T., Acharyya S., Nguyen D.X., Zhang X.H., Norton L., Massagué J.: Tumor self-seeding by circulating cancer cells. Cell, 2009; 139: 1315-1326

[19] Klein C.A.: Parallel progression of primary tumors and metastases. Nat. Rev. Cancer, 2009; 9: 302-312

[20] Laubli H., Borsig L.: Selectins promote tumor metastasis. Semin. Cancer Biol., 2010; 20: 169-177

[21] Lorusso G., Ruegg C.: New insights into the mechanisms of organ--specific breast cancer metastasis. Semin. Cancer Biol., 2012; 22: 226-233

[22] Lorusso G., Ruegg C.: The tumor microenvironment and its contribution to tumor evolution toward metastasis. Histochem. Cell Biol., 2008; 130: 1091-1103

[23] Luzzi K.J., MacDonald I.C., Schmidt EE, Kerkvliet N., Morris V.L., Chambers A.F., Groom A.C.: Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am. J. Pathol., 1998; 153: 865-873

[24] Muller A., Homey B., Soto H., Ge N., Catron D., Buchanan M.E., McClanahan T., Murphy E., Yuan W., Wagner S.N., Barrera J.L., Mohar A., Verastegui E., Zlotnik A.: Involvement of chemokine receptors in breast cancer metastasis. Nature, 2001; 410: 50-56

[25] Paget S.: The distribution of secondary growths in cancer of the breast. Cancer Metastasis Rev., 1989; 8: 98-101

[26] Pantel K., Brakenhoff R.H.: Dissecting the metastatic cascade. Nat. Rev. Cancer, 2004; 4: 448-456

[27] Rabbani S.A., Mazar A.P.: Evaluating distant metastases in breast cancer: from biology to outcomes. Cancer Metastasis Rev., 2007; 26: 663-674

[28] Ramaswamy S., Ross K.N., Lander E.S., Golub T.R.: A molecular signature of metastasis in primary solid tumors. Nat. Genet., 2003; 33: 49-54

[29] Reynolds L.P., Redmer D.A.: Angiogenesis in the placenta. Biol. Reprod., 2001; 64: 1033-1040

[30] Sarrio D., Rodriguez-Pinilla S.M., Hardisson D., Cano A., Moreno--Bueno G., Palacios J.: Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. Cancer Res., 2008; 68: 989-997

[31] Spano D., Heck C., De Antonellis P., Christofori G., Zollo M.: Molecular networks that regulate cancer metastasis. Semin. Cancer Biol., 2012; 22: 234-249

[32] Steeg P.S.: Tumor metastasis: mechanistic insights and clinical challenges. Nat. Med., 2006; 12: 895-904

[33] Thiery J.P., Acloque H., Huang R.Y., Nieto M.A.: Epithelial-mesenchymal transitions in development and disease. Cell, 2009; 139: 871-890

[34] Thomas P.A., Kirschmann D.A., Cerhan J.R., Folberg R., Seftor E.A., Sellers T.A., Hendrix M.J.: Association between keratin and vimentin expression, malignant phenotype, and survival in postmenopausal breast cancer patients. Clin. Cancer Res., 1999; 5: 2698-2703

[35] Tse J.C., Kalluri R.: Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment. J. Cell Biochem., 2007; 101: 816-829

[36] Valastyan S., Weinberg R.A.: Tumor metastasis: molecular insights and evolving paradigms. Cell, 2011; 147: 275-292

[37] Weidner N., Folkman J., Pozza F., Bevilacqua P., Allred E.N., Moore D.H., Meli S., Gasparini G.: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J. Natl. Cancer Inst., 1992; 84: 1875-1887

[38] Weil R.J., Palmieri D.C., Bronder J.L., Stark A.M., Steeg P.S.: Breast cancer metastasis to the central nervous system. Am. J. Pathol., 2005; 167: 913-920

The authors have no potential conflicts of interest to declare.