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PREDICTING THE SITE OF DISTANT METASTASES IN BREAST CANCER

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Abstract

Background. Distant organ tumor dissemination is a major cause of breast cancer-related deaths. Breast cancer can metastasize to several organs, and the most frequent metastatic sites include the bones, lungs and liver. There is a question what factors can influence the direction of spread of tumor cells to a particular organ. **Material and Methods.** We summarized the data available in the world literature on methods for prediction of the localization of distant metastases in breast cancer patients. **Results.** We divided the factors associated with the localization of distant metastases into two main groups: clinicopathological parameters of breast cancer patients and molecular features of tumor microenvironment and tumor cells (primary tumor and circulating tumor cells) or its derivates – exosomes. From our point of view, the most powerful clinicopathological factor predicting the distant metastasis site is a molecular subtype of primary tumor. We can conclude that luminal (HR+/HER2-) tumors are often characterized by single metastases and bones are the most common metastatic site, while TNBC and HER2-enriched tumors often metastasize to multiple sites, most commonly brain and liver. However, several authors did not reveal these associations in their studies. It likely indicates the existence of other factors that significantly affect the organotropism of metastasis. Numerous studies demonstrate the association of different molecules expressed on tumor cells with organotropic metastasis. However, these data are very fragmentary and rather contradictory. **Conclusion.** The found associations are common to all participants of metastatic cascade, but remains unclear which factors are essential and crucial in determining the direction of metastasis.

Key words: breast cancer, hematogenous metastasis, prognostic predictors.

ПРОГНОЗИРОВАНИЕ ЛОКАЛИЗАЦИИ ОТДАЛЕННЫХ МЕТАСТАЗОВ ПРИ РАКЕ МОЛОЧНОЙ ЖЕЛЕЗЫ

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Аннотация

Введение. Метастазирование опухоли в отдаленные органы является основной причиной смерти при раке молочной железы. Преимущественная локализация отдаленных метастазов при раке молочной железы – кости, легкие и печень. Возникает вопрос, какие факторы могут влиять на направление распространения опухолевых клеток в тот или иной орган. **Материал и методы.** Мы обобщили имеющиеся в мировой литературе данные о методах прогнозирования локализации отдаленных метастазов у больных раком молочной железы. **Результаты.** Факторы, связанные с локализацией отдаленных метастазов, мы разделили на две основные группы: клинико-патологические параметры больных раком молочной железы и молекулярные особенности микроокружения опухоли и опухолевых

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клеток (первичных опухолевых и циркулирующих опухолевых клеток) или их производных – экзосом. Представляется, что молекулярный подтип первичной опухоли является наиболее перспективным предиктором, предсказывающим место отдаленного метастазирования. Люминальные опухоли (HR+/HER2-) чаще характеризуются единичными метастазами, с преимущественной локализацией в костях, в то время как трижды негативные и HER2-обогащенные опухоли чаще образуют множественные метастазы, которые чаще локализуются в головном мозге и печени. Однако некоторые авторы не обнаруживают в своих исследованиях таких закономерностей, что, вероятно, свидетельствует о наличии других факторов, существенно влияющих на органотропность метастазирования. Многочисленные исследования демонстрируют ассоциацию различных молекул, экспрессируемых на опухолевых клетках, с органотропным метастазированием. Однако эти данные весьма фрагментарны и иногда весьма противоречивы. **Заключение.** Обнаруженные ассоциации относятся ко всем участникам метастатического каскада, но остается неясным, какие факторы являются существенными и решающими в определении направления метастазирования.

Ключевые слова: рак молочной железы, гематогенное метастазирование, предикторы прогноза.

Introduction

Cancer mortality is mainly caused by a metastatic relapse at distant sites. The time to development of hematogenous metastases depends on cancer type and could vary significantly. For instance, the metastatic recurrence in lung cancer can occur within a few weeks, while in colorectal cancer in a few years. The insidiousness of breast cancer is that the window of distant relapse in breast cancer can span from month to decades after surgery [1, 2]. Even though risk of distant metastasis in breast cancer patients is almost 10–20 % [3, 4], adjuvant systemic therapy is administered to all patients after surgery and radiation therapy to reduce the risk of distant metastases. Thus, most patients do not develop distant metastases and are likely to suffer needlessly from the systemic toxic side effects of chemotherapy.

For a long time, it was believed that the formation of metastases is a stochastic process, and the direction of metastasis is determined by the anatomical features of the blood and lymphatic outflow from the primary tumor. Indeed, anatomical features can fully explain, for example, the spread of tumor cells to regional lymph nodes or occurence of hematogenous metastases of intestinal cancer to the liver. However, the occurrence of metastases in the bone marrow in kidney cancer or in the lungs in breast cancer cannot be explained solely by the peculiarities of blood flow. The prediction of the localization of future metastasis will allow clinicians to focus attention on a specific organ for earlier detection of hematogenous metastasis. Therefore, the ability to assess the risk of occurrence and localization of future metastasis will provide a personalized approach to therapy of cancer patients.

In this review, we aim to summary the data available in the world literature on methods for prediction of the localization of distant metastases in breast cancer patients. We divided the factors associated with the localization of distant metastases into two main groups: clinicopathological parameters of breast cancer patients and molecular features of tumor cells (or its derivates – exosomes) and tumor microenvironment.

Association of clinicopathological parameters of breast cancer patients with sites of distant metastasis

Numerous studies demonstrate strong association of clinicopathological parameters with risk of occurrence and preferential localization of distant metastases. The main factors associated with hematogenous metastases are the molecular subtype, tumor size, nodal status and patient's age at diagnosis. It is noteworthy that data obtained in different studies are rather contradictory. The association of each of these factors with the specific localization of distant metastases will be discussed in detail below.

1.1 Molecular subtype of primary tumor

The molecular subtype of breast cancer largely determines the predominant spread of the tumor to the specific distant site. Some authors interpreted the data obtained according to the 50-gene expression classifier (PAM50) which allows the identification of luminal A, luminal B (HER2+/-), HER2-enriched, basal-like (triple-negative) subtypes, while others used classification based on three clinical subtypes: hormone-receptor (HR)-positive (HR+; ER+, PR+/- and HER2-), HER2-positive (HER2+) and triple-negative (TN; ER-, PR- and HER2-). Thus, Xiao et al. (2018) identified 295,213 patients with invasive breast cancer from 2010 to 2014 using the Surveillance, Epidemiology and End Results database. Multivariate analysis showed that HR+/HER2+ subtype significantly correlated with increased bone metastasis risk compared to HR+/HER2- subtype. Both HER2+ subtypes (HR+/HER2+ and HR-/HER2+) were significantly associated with higher rates of liver, brain, and lung metastases, and the risk of liver metastases was the highest. The TNBC subtype had a higher rate of brain (OR, 1.95 [95 % CI, 1.61–2.35]), liver (OR, 1.35 [95 % CI, 1.20–1.51]), and lung metastases (OR, 1.34 [95 % CI, 1.21–1.47]), but a significantly lower rate of bone metastases (OR, 0.64 [95 % CI, 0.59–0.69]) than HR+/HER2 subtypes [5]. So, the authors established the association of HR+/HER2+ subtype (which corresponds to luminal B (HER2+)) with bone metastasis, while

HER2+ subtype (which includes luminal B (HER2+) as well as HER2-enriched subtype tended to exhibit the highest incidence of liver metastases followed by brain and lung metastases.

In the study of I.A. Molnár et al. (2017), luminal A tumors also metastasized predominantly to bones in contrast to HER2+ and TNBC, which more often affected the brain. The second frequent metastatic sites of HR+ tumors were the lung and liver, whereas the brain was the most affected organ in HR- cases [6]. Moreover, single metastases were observed in 59 % of patients with luminal A tumors, while 79 % patients with HER2-enriched tumors had multiple metastases. Furthermore, in 59 % of patients with luminal A tumors, single metastases were observed, while 79 % patients with HER2-enriched tumors have multiple metastases.

In the study of S.U. Kunikullaya et al., in 143 patients with distant metastases, bone was the most common metastatic site in luminal A/B(HER2-) (53.3 %) and luminal B (HER2+) (57.1 %) tumors. Brain and liver were the most frequent sites of metastasis in HER2-enriched subtype (30.3 % and 45.5 %, respectively), while the incidence of brain metastasis was comparatively lower in luminal A/B subtype [7].

A. Soni et al. confirmed that luminal tumors were significantly associated with bone metastases, less frequently observed in lung, brain, and pleura, and less likely to be associated with multiorgan relapse. The HER2-enriched subtype demonstrated a significant liver-homing characteristic. Interestingly, there are probably some ethnic features of hematogenous metastasis in breast cancer patients. According to authors' observation, African Americans were significantly less likely to have CNS-only metastasis [8].

The complexity of investigating the association between the localization of metastases and the tumor characteristics lies in the fact that patients are often diagnosed with lesions in different organs simultaneously. In this regard, the study conducted by J. Diessner et al. (2016) is of great interest. The retrospective multicenter study including 9,625 patients with primary breast cancer demonstrated that breast cancer subtypes have the strongest influence on the development of bone-only metastases [9]. Authors established that one third of luminal A or luminal B patients had bone-only metastases, while in cases of triple negative BC (TNBC) or HER2-overexpressing tumors, only 10 % of patients demonstrated bone-only metastasis ($p<0.001$).

Despite numerous studies confirming the association of the molecular subtype with the risk and specific localization of distant metastases, there are some observations, which revealed no association of molecular subtype with distant metastasis. Thus, C. Boutros et al. conducted the study including 2,059 patients and developed a nomogram for prediction of distant metastasis in newly diagnosed patients with breast cancer [10]. The authors reported tumor size and nodal status as the only significant predictors of distant metastasis. These results are consistent with findings obtained in another study. B. Ali et al. showed that biologically aggressive variants of breast cancer, such as grade III, HER2-enriched and triple-negative tumors were not predictive of breast cancer metastasis [11].

In general, we can conclude that luminal (HR+/HER2-) tumors are characterized more often by isolated metastases. In case of multiple metastasis, the first site is more often localized in the bones. TNBC and HER2-enriched tumors often form multiple metastases and are more often localized in the brain and liver. However, several authors have not found this association in their studies. It likely indicates the existence of other factors that significantly affect the organotropism of metastasis.

1.2 Tumor stage and nodal status

Numerous studies point to the association of larger tumor size and involvement of lymph nodes with a high risk of distant metastases in breast cancer patients [12–14]. However, some studies do not confirm such association. J. Diessner et al. (2016) showed that the established breast cancer prognostic markers such as the presence of lymph node metastases, large size of primary tumor and loss of histopathological differentiation (grade) have no influence on the development of bone-only metastases [9]. Probably, tumor stage and lymph node status are non-specific factors that reflect tumor aggressiveness and are associated with the risk of metastasis but can hardly provide information about the association with the possible site of future metastasis. We found only one study that revealed association of T and N stage with metastasis to specific organ. Y. Yao et al. (2019) examined 26,863 patients with primary triple-negative breast cancer, and 1,330 (5.0 %) of them presented with distant metastasis and declared that higher clinical stage T and lymph node involvement were independent risk factors for bone metastasis in primary triple-negative breast cancer [15].

1.3 Patient's age at diagnosis of breast cancer

There is a lot of evidence that age at diagnosis is associated with risk of hematogenous metastases. It was found that women younger than 50 years at diagnosis had the highest risk of distant metastasis [15–17]. It is likely that patients of this age group had significantly more aggressive disease than patients of other age groups: more frequent HER2+ (25.7 *vs* 15.3 % in group >60 years old) and triple negative subtypes (27.4 *vs* 14.6 % in group >60 years old) [18]. The study of A. Purushotham et al. confirmed that patients above 40 years old at diagnosis had a significant decrease in the risk of developing distant metastases [19].

According to J. Diessner at al., molecular subtypes and age at primary diagnosis were the most important parameters influencing the development of bone metastases [9]. The greatest influence of age was shown in the case of bone-only metastases between women

younger than 65 years and women older than 65 years ($p<0.001$). Only one of five women under the age of 65 developed bone metastases, while every third woman over 65 suffered from bone-only metastases. These findings are consistent with those of a study of Y. Yao et al., who revealed that age over 50 years was an independent risk factor for distant metastasis of primary TNBC [15].

Molecular markers of tumor cells predicting the site of potential metastasis

The most inspiring results were demonstrated in a study conducted by A. Hoshino et al. (2015) [20]. The authors showed that metastasis could be targeted by integrins – adhesion molecules expressed on tumor-derived exosomes. During the experiment, the authors obtained subpopulations of breast cancer cell line preferentially metastasizing to the lung, liver and brain. At the same time, tumor-derived exosomes demonstrated the same tropism to the target organs as each subpopulation of cancer cells. Moreover, "education" of premetastatic niche by lung-tropic exosomes redirected the spread of bone-tropic tumor cells to the lung. Data obtained by A. Hoshino et al. indicate the possibility of the existence of a mechanism for targeting metastases to the specific organ. Thus, search for molecules associating with organ-specific metastasis appears to be a promising area of research. Data on the association of molecular markers of tumor cells, tumor-derived exosomes, and circulating tumor cells (CTCs) with the possible localization of distant metastases are presented below.

2.1 Molecular markers of tumor cells in primary site

H. Shito et al. analyzed 234 primary tumors of metastatic breast cancer patients for 17 proteins using immunohistochemistry. It was found that primary tumors that gave first rise to bone metastases expressed frequently ER and SNAI1 and rarely COX2 and HER2. Tumors with first metastases in the liver, vice versa expressed rarely SNAI1. Tumors with lung metastases expressed frequently the EGFR, cytokeratin-5 and HER2, and infrequently PR. In the few samples with early skin metastases authors detected E-cadherin expression rarely. Breast tumors with first metastases in the brain expressed nestin, prominin-1 and cytokeratin 5 and infrequently ER and PR [21].

S. McFarlany et al. established that hyaluronan receptor CD44 correlated with increased tumor spread to the skeleton in patients with lymph-node positive status or large tumors [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4484469/]. CD44 knock-down increased survival and decreased overall tumor burden in the bones *in vivo* [22].

A. Leontovitch et al. identified an association between Notch3 expression and the development of metastases in luminal and triple-negative breast cancer (TNBC) models [23]. Knockout of Notch3 expression significantly reduced the self-renewal and invasive capacity of both breast cancer lines. Furthermore, forced expression of the mitotic Aurora kinase A (AURKA), which promotes breast cancer metastases, failed to restore the invasive capacity of Notch3-null cells, demonstrating that Notch3 expression is required for an invasive phenotype. However, the authors failed to identify relationship between the expression of markers and metastasis to certain organs.

M. Oshi et al established 4-genes score (DOK 4, HCCS, PGF, and SHCBP1) with genes upregulated in LM2-4 cell line, a metastatic variant of MDA-MB-231. Authors found that the 4-gene scores were significantly elevated in tumors that subsequently developed metastasis to the brain or lung, but not to bone alone [24].

2.2 Tumor-derived exosomes in predicting distant metastatic sites

Tumor-derived exosomes (TDEs) are actively produced and released by tumor cells, allowing them to communicate with distantly located cells. TDEs transfer wide spectrum of bioactive molecules from host cells, namely, RNAs, DNAs, proteins, lipids and metabolites. TDEs can recruit bone marrow-derived cells (BMCs) to distant organs, form the premetastatic niche, and provide metastatic tumor growth in the secondary site. In addition, it turned out that TDEs can bind various resident cells in the distant organs, thereby directing metastasis to a specific site. This phenomenon was most fully and comprehensively described in the study conducted by A. Hoshino et al. using animal models [20]. The exosome-derived integrins α6β4 and α6β1 were associated with lung metastasis *in vivo*, while integrin αvβ5 was responsible for the occurrence of metastases in the liver. The authors also demonstrated the role of exosomal integrin β3 in brain metastasis. G.Y. Chen carried out a study on 75 lung cancer patients with brain metastasis and found that after whole-brain radiotherapy patients who had higher levels of circulating exosomal integrin β3 had worse overall survival [25]. One can suppose that the organotropism of integrin-mediated metastasis can be the universal mechanism for different carcinomas. T. Luo et al. in the study published as a preprint found that integrin α6 and β4 were overexpressed in highly tumorigenic and metastatic colorectal carcinoma (CRC) cell lines HCT116 and SW620, as well as in their exosomes compared to the low tumorigenic and non-metastatic CRC cell lines [26]. The authors used the same approach as A. Hoshino et al., with the only difference that the CRC cell line was used instead of breast cancer cell line. As a result, the exosomes derived from highly metastatic counterpart SW620 could significantly increase the lung metastasis of SW480 cells, validating a role of exosomal integrin α6 and β4 in the lung metastasis of CRC.

There are studies which revealed other exosomal molecules that contributed to organ-specific metas-

tasis. Liver is the most common metastatic sites for gastrointestinal tumors. So, liver stromal cells act as recipient for gastric cancer-derived exosomal EGFR which leads to effective activation of hepatocyte growth factor (HGF) by suppressing miR-26a/b expression [27]. After that upregulated paracrine HGF binds the c-MET receptor on the migrated cancer cells, facilitating the landing and proliferation of metastatic cancer cells in liver.

B. Costa-Silva et al. have found that Kupffer cells uptake pancreatic ductal adenocarcinoma-derived exosomal macrophage migration inhibitory factor (MIF) which supports PMN formation and promotes liver metastasis [28]. J. Sun et al. demonstrated that metalloproteinase 17 (ADAM17) transported via exosomes increased the ability of colorectal cancer cells to metastasize to liver *in vivo* [29].

A study conducted by C. Zhang et al. demonstrated that cancer-derived exosomal HSPC111 promoted colorectal cancer liver metastasis by reprogramming lipid metabolism in cancer-associated fibroblasts *in vivo* [30]. In vivo experimental data was confirmed on clinical samples. Colorectal cancer patients with liver metastasis had higher level of HSPC111 in serum exosomes, primary tumors, and cancer-associated fibroblasts (CAFs) in liver metastasis than those without.

2.3 CTCs molecular markers predicting future metastatic site

Circulating tumor cells (CTCs) are essential and informative objects for understanding the mechanisms of carcinoma metastasis [31]. CTCs are cells that have been able to detach from the primary tumor and enter the bloodstream. Previously considered that CTCs are stopped at branch points in vessels due to low shear stress and size limitations, as the diameter of CTCs is up to 20 μm compared to ~3–7 μm capillaries [32]. Indeed, such explanation is competent, for spread of tumor cells to the liver in intestinal cancer. But how one can explain the appearance of brain metastases, suggesting the migration of tumor cells through the blood-brain barrier? It is likely that spread of tumor cells is not a stochastic but a directional process requiring a molecular apparatus to target specific organs. Thus, Q. Chen et al. (2011) report that vascular cell adhesion molecule-1 (VCAM-1) expressed by disseminating breast cancer cells binds to integrin α4 expressed by leukocytes, which promotes lung metastasis *in vivo*. This binding activates Akt signaling in lung-metastasizing cancer cells, thereby protecting them from apoptosis [33].

P.M. McGowan et al. had shown the role of Notch1 signaling in formation of brain metastasis *in vivo* [34]. Originally it was found that Notch1 involved in differentiation of astrocytes, but now more and more evidence that dysregulated Notch1 signaling has been observed in many human cancers, including endometrial cancer (19), colon cancer (20), lung cancer (21) and breast cancer [35, 36]. Notch1 knockdown reduced the expression of $CD44^{hi}/CD24^{lo}$ phenotype by ~20 %. Authors found that 231-BR tumor cells (brain metastatic variant of MDA-MB-231 cells) with the $CD44^{hi}/CD24^{lo}$ stem phenotype form 2 times more macrometastases compared to non-stem $CD44^{lo}/CD24^{hi}$ cells *in vivo*. Furthermore, inhibition of Notch1 signaling pathway in two different ways, namely, administration of DAPT (γ-secretase inhibitor; MK-0752) and using of shRNA resulted in inhibition of 74–79 % of brain metastasis. Results of phase 4 clinical trial of MK-0752 combined with either tamoxifen or letrozole to treat early-stage breast cancer were published in 2019 [37].

L. Zhang et al (2013) confirmed the role of Notch1 expression in brain metastasis of breast cancer [38]. Group of authors identified in EpCam-negative CTCs a potential signature of brain metastasis comprising "brain metastasis selected markers (BMSMs)" HER2+/EGFR+/HPSE+/Notch1+. Three CTC lines (CTC-1, CTC-2, CTC-3) were established, and their metastatic potential was evaluated *in vivo*. Although all CTC lines (CTC-1, CTC-2, CTC-3) could form lung metastasis, only CTC-1 promoted relevant brain colonization. Furthermore, CTC-1 line was the most invasive, with approximately 25 % more cells penetrating the Matrigel barrier than the highly invasive 231-BR cells. The authors suggest that the reason for this is the fact that the CTC-1 were obtained from a TNBC patient. The authors also compared the expression of BMSMs in tumors induced by CTC in the brain and found an increased expression of proteins HER2, EGFR, HPSE and Notch1 versus lung metastasis which suggests the relevance of the BMSM CTC signature to brain metastasis.

Other study carried out by R. Klotz et al. (2020) also provide the experimental evidence of the promising role of CTCs as a prognostic factor for site-specific metastasis [39]. The authors established four CTCs lines derived from luminal-type breast cancer patient, with differential metastatic tropism. The authors enriched subpopulation of tumor cells tropic to brain by additional rounds of *in vivo* selection, resulted in significant increase in brain metastatic activity (50 % of mice in generation 3 compared to 5 % of mice in parental CTC line). Copy-number variation analysis revealed an amplification of chromosome 9q (chr9q13–34) in brain-tropic subpopulation of tumor cells. Among identified genes residing on chromosome 9q, for which the expression is altered in tumor cells with a preferential tropism for the brain, *SEMA4D* (gene encoding Semaphorin 4D) was associated with a significant decrease of metastasis-free survival in the brain, but not in lung and bones. Protein overexpression of Semaphorin 4D was observed in 7 out 12 samples obtained after surgical resection from breast cancer patient with brain metastasis. An important note is the fact that intracardiac inoculation of CTCs in mice did not show an exact one-to-one match of the patient's metastases, which is probably due to significant differences in microenvironment.

M. Clements et al. (2020) postulated PREX1, Rac-pathway activator, as key driver of spontaneous dissemination of tumor cells from the primary site to the bone marrow *in vivo* [40]. The authors established novel model of spontaneous bone metastasis derived from human ER+ MCF7 cells, which obtained increased migratory, invasive, and adhesive behavior *in vitro* compared to parental MCF7 cells. Using shRNA blocking PREX1 expression, the authors confirmed that more aggressive phenotype was mediated by PREX1.

One can concludes that characterization of CTCs is a rather difficult task due to their small number and the absence of clear phenotypic characteristics. Often, the investigation of metastasis mechanisms is carried out using model systems, which hardly provide a complete understanding of the molecular mechanisms of the spread of carcinoma tumor cells at the level of the whole organism.

Tumor microinvironment in predicting organ-specific metastasis

The tumor microenvironment is a set of cellular and molecular components in which primary tumor originates, evolves, and intravasate into vessels. Cellular components represented by stromal and immune cells make a significant contribution at all stages of metastasis. Initially, microenvironment and structure of specific organ influence metastatic niche formation and further interactions between cancer cells and local resident cells, regulating the survival of cancer cells and formation of metastatic lesions [41]. For example, A.M. Gil-Bernabé et al. demonstrated the essential role of monocytes/macrophages in premetastatic niche establishment in mice [42]. Tissue factors (TF) expressed by tumor cells induced clot formation which enhanced tumor cell survival after arrest in the lung *in vivo*. It turned out that such phenomena were realized by recruiting macrophages characterized by CD11b, CD68, F4/80, and CX3CR1 expression. The use of any inhibition variant (anticoagulant treatment or induction of TF pathway inhibitor expression) abrogated macrophage recruitment and tumor cell survival. Neutrophils are also involved in tumor progression as they can form projections called "neutrophil extracellular traps" (NETs) in response to pro-inflammatory signals generated by the primary tumor [43]. NETs are net-like structures composed of DNA-histone complexes and proteins released by activated neutrophils and involved in wide spectrum of pathophysiological processes, as well as autoimmune diseases, coagulation disorders, thrombosis, diabetes, atherosclerosis, vasculitis. NETs can catch CTCs thereby providing secondary adhesion to distant organs [44]. Furthermore, NETs are required for dormant cancer cells to awake in the lung [45]. J. Albrengues et al. revealed two NET-associated proteases, neutrophil elastase and matrix metalloproteinase 9, sequentially cleaved laminin. As a result, the proteolytically remodeled laminin induced proliferation of dormant cancer cells by activating integrin 31 signaling. It was shown that blocking antibodies against NET-remodeled laminin prevented awakening of dormant cells in lungs.

Conclusion

The totality of above facts indicates the existence of certain molecular patterns responsible for the direction of metastasis to certain organs. However, these data are very fragmentary and the found associations are common to all participants of metastatic process. In this regard, the question remains to be answered: which of the factors are more determining, the phenotypic characteristics of tumor cells or the parameters of the microenvironment? Which of the factors are limiting in the spread of tumor cells to a certain distant organ? Understanding of the role of each of the participants in metastasis, as well as their cumulative effect on the organotropic metastasis of breast cancer, has a great potential for new biological discoveries with genuine clinical applications.

ЛИТЕРАТУРА/REFERENCES

1. *Riggio A.I., Varley K.E., Welm A.L.* The lingering mysteries of metastatic recurrence in breast cancer. Br J Cancer. 2021; 124(1): 13–26. doi: 10.1038/s41416-020-01161-4.

2. *Gomis R.R., Gawrzak S.* Tumor cell dormancy. Mol Oncol. 2017; 11(1): 62–78. doi: 10.1016/j.molonc.2016.09.009.

3. *Malmgren J., Hurlbert M., Atwood M., Kaplan H.G.* Examination of a paradox: recurrent metastatic breast cancer incidence decline without improved distant disease survival: 1990-2011. Breast Cancer Res Treat. 2019; 174(2): 505–14. doi: 10.1007/s10549-018-05090-y.

4. *Holleczek B., Stegmaier C., Radosa J.C., Solomayer E.F., Brenner H.* Risk of loco-regional recurrence and distant metastases of patients with invasive breast cancer up to ten years after diagnosis – results from a registry-based study from Germany. BMC Cancer. 2019; 19(1): 520. doi: 10.1186/s12885-019-5710-5.

5. *Xiao W., Zheng S., Yang A., Zhang X., Zou Y., Tang H., Xie X.* Breast cancer subtypes and the risk of distant metastasis at initial diagnosis: a population-based study. Cancer Manag Res. 2018; 10: 5329–38. doi: 10.2147/CMAR.S176763.

6. *Molnár I.A., Molnár B.Á., Vízkeleti L., Fekete K., Tamás J., Deák P., Szundi C., Székely B., Moldvay J., Vári-Kakas S., Szász M.A., Ács B., Kulka J., Tőkés A.M.* Breast carcinoma subtypes show different patterns of metastatic behavior. Virchows Arch. 2017; 470(3): 275–83. doi: 10.1007/s00428-017-2065-7.

7. *Kunikullaya S.U., Poddar J., Sharma A.D., Patel S.* Pattern of distant metastasis in molecular subtypes of carcinoma breast: An institutional study. Indian J Cancer. 2017; 54(1): 327–32. doi: 10.4103/ijc.IJC_177_17.

8. *Soni A., Ren Z., Hameed O., Chanda D., Morgan C.J., Siegal G.P., Wei S.* Breast cancer subtypes predispose the site of distant metastases. Am J Clin Pathol. 2015; 143(4): 471–8. doi: 10.1309/AJCPYO5FSV3UPEXS.

9. *Diessner J., Wischnewsky M., Stüber T., Stein R., Krockenberger M., Häusler S., Janni W., Kreienberg R., Blettner M., Schwentner L., Wöckel A., Bartmann C.* Evaluation of clinical parameters influencing the development of bone metastasis in breast cancer. BMC Cancer. 2016; 16: 307. doi: 10.1186/s12885-016-2345-7.

10. *Boutros C., Mazouni C., Lerebours F., Stevens D., Lei X., Gonzalez-Angulo A.M., Delaloge S.* A preoperative nomogram to predict the risk of synchronous distant metastases at diagnosis of primary breast cancer. Br J Cancer. 2015; 112: 992–7. doi: 10.1038/bjc.2015.34.

11. *Ali B., Mubarik F., Zahid N., Sattar A.K.* Clinicopathologic Features Predictive of Distant Metastasis in Patients Diagnosed With Invasive Breast Cancer. JCO Glob Oncol. 2020; 6: 1346–51. doi: 10.1200/GO.20.00257.

12. *Sopik V., Narod S.A.* The relationship between tumour size, nodal status and distant metastases: on the origins of breast cancer. Breast Cancer Res Treat. 2018; 170(3): 647–56. doi: 10.1007/s10549-018-4796-9.

13. *Park H., Chang S.K., Kim J.Y., Lee B.M., Shin H.S.* Risk factors for distant metastasis as a primary site of treatment failure in early-stage

breast cancer. Chonnam Med J. 2014; 50(3): 96–101. doi: 10.4068/cmj.2014.50.3.96.

14. *Min Y., Liu X., Hu D., Chen H., Chen J., Xiang K., Yin G., Han Y., Feng Y., Luo H.* Risk Factors, Prognostic Factors, and Nomogram for Distant Metastasis in Breast Cancer Patients Without Lymph Node Metastasis. Front Endocrinol (Lausanne). 2021; 12. doi: 10.3389/fendo.2021.771226.

15. *Yao Y., Chu Y., Xu B., Hu Q., Song Q.* Risk factors for distant metastasis of patients with primary triple-negative breast cancer. Biosci Rep. 2019; 39(6). doi: 10.1042/BSR20190288.

16. *Chen M.T., Sun H.F., Zhao Y., Fu W.Y., Yang L.P., Gao S.P., Li L.D., Jiang H.L., Jin W.* Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. Sci Rep. 2017; 7(1): 9254. doi: 10.1038/s41598-017-10166-8.

17. *Colzani E., Johansson A.L., Liljegren A., Foukakis T., Clements M., Adolfsson J., Hall P., Czene K.* Time-dependent risk of developing distant metastasis in breast cancer patients according to treatment, age and tumour characteristics. Br J Cancer. 2014; 110(5): 1378–84. doi: 10.1038/bjc.2014.5.

18. *Frank S., Carton M., Dubot C., Campone M., Pistilli B., Dalenc F., Mailliez A., Levy C., D'Hondt V., Debled M., Vermeulin T., Coudert B., Perrin C., Gonçalves A., Uwer L., Ferrero J.M., Eymard J.C., Petit T., Mouret-Reynier M.A., Patsouris A., Guesmia T., Bachelot T., Robain M., Cottu P.* Impact of age at diagnosis of metastatic breast cancer on overall survival in the real-life ESME metastatic breast cancer cohort. Breast. 2020; 52: 50–7. doi: 10.1016/j.breast.2020.04.009.

19. *Purushotham A., Shamil E., Cariati M., Agbaje O., Muhidin A., Gillett C., Mera A., Sivanadiyan K., Harries M., Sullivan R., Pinder S.E., Garmo H., Holmberg L.* Age at diagnosis and distant metastasis in breast cancer--a surprising inverse relationship. Eur J Cancer. 2014; 50(10): 1697–705. doi: 10.1016/j.ejca.2014.04.002.

20. *Hoshino A., Costa-Silva B., Shen T.L., Rodrigues G., Hashimoto A., Tesic Mark M., Molina H., Kohsaka S., Di Giannatale A., Ceder S., Singh S., Williams C., Soplop N., Uryu K., Pharmer L., King T., Bojmar L., Davies A.E., Ararso Y., Zhang T., Zhang H., Hernandez J., Weiss J.M., Dumont-Cole V.D., Kramer K., Wexler L.H., Narendran A., Schwartz G.K., Healey J.H., Sandstrom P., Labori K.J., Kure E.H., Grandgenett P.M., Hollingsworth M.A., de Sousa M., Kaur S., Jain M., Mallya K., Batra S.K., Jarnagin W.R., Brady M.S., Fodstad O., Muller V., Pantel K., Minn A.J., Bissell M.J., Garcia B.A., Kang Y., Rajasekhar V.K., Ghajar C.M., Matei I., Peinado H., Bromberg J., Lyden D.* Tumour exosome integrins determine organotropic metastasis. Nature. 2015; 527(7578): 329–35. doi: 10.1038/nature15756.

21. *Sihto H., Lundin J., Lundin M., Lehtimäki T., Ristimäki A., Holli K., Sailas L., Kataja V., Turpeenniemi-Hujanen T., Isola J., Heikkilä P., Joensuu H.* Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. Breast Cancer Res. 2011; 13(5): R87. doi: 10.1186/bcr2944.

22. *McFarlane S., Coulter J.A., Tibbits P., O'Grady A., McFarlane C., Montgomery N., Hill A., McCarthy H.O., Young L.S., Kay E.W., Isacke C.M., Waugh D.J.* CD44 increases the efficiency of distant metastasis of breast cancer. Oncotarget. 2015; 6(13): 11465–76. doi: 10.18632/oncotarget.3410.

23. *Leontovich A.A., Jalalirad M., Salisbury J.L., Mills L., Haddox C., Schroeder M., Tuma A., Guicciardi M.E., Zammataro L., Gambino M.W., Amato A., Di Leonardo A., McCubrey J., Lange C.A., Liu M., Haddad T., Goetz M., Boughey J., Sarkaria J., Wang L., Ingle J.N., Galanis E., D'Assoro A.B.* NOTCH3 expression is linked to breast cancer seeding and distant metastasis. Breast Cancer Res. 2018; 20(1): 105. doi: 10.1186/s13058-018-1020-0.

24. *Oshi M., Katsuta E., Yan L., Ebos J.M.L., Rashid O.M., Matsuyama R., Endo I., Takabe K.* A Novel 4-Gene Score to Predict Survival, Distant Metastasis and Response to Neoadjuvant Therapy in Breast Cancer. Cancers (Basel). 2020; 12(5): 1148. doi: 10.3390/cancers12051148.

25. *Chen G.Y., Cheng J.C., Chen Y.F., Yang J.C., Hsu F.M.* Circulating Exosomal Integrin β3 Is Associated with Intracranial Failure and Survival in Lung Cancer Patients Receiving Cranial Irradiation for Brain Metastases: A Prospective Observational Study. Cancers (Basel). 2021; 13(3): 380. doi: 10.3390/cancers13030380.

26. *Luo T., Huang J., Wu C., Huang Q., Zhong H., Qiu G., Liu Y., Liu J., Tang W.* Cancer Exosome-derived Integrin α6 and Integrin β4 Promote Lung Metastasis of Colorectal Cancer. Research Square. 2020. https://doi.org/10.21203/rs.3.rs-91600/v1.

27. *Zhang H., Deng T., Liu R., Bai M., Zhou L., Wang X., Li S., Wang X., Yang H., Li J., Ning T., Huang D., Li H., Zhang L., Ying G., Ba Y.* Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. Nat Commun. 2017; 8. doi: 10.1038/ncomms15016.

28. *Costa-Silva B., Aiello N.M., Ocean A.J., Singh S., Zhang H., Thakur B.K., Becker A., Hoshino A., Mark M.T., Molina H., Xiang J., Zhang T., Theilen T.M., García-Santos G., Williams C., Ararso Y., Huang Y., Rodrigues G., Shen T.L., Labori K.J., Lothe I.M., Kure E.H., Hernandez J., Doussot A., Ebbesen S.H., Grandgenett P.M., Hollingsworth M.A., Jain M., Mallya K., Batra S.K., Jarnagin W.R., Schwartz R.E., Matei I., Peinado H., Stanger B.Z., Bromberg J., Lyden D.* Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015; 17(6): 816–26. doi: 10.1038/ncb3169.

29. *Sun J., Lu Z., Fu W., Lu K., Gu X., Xu F., Dai J., Yang Y., Jiang J.* Exosome-Derived ADAM17 Promotes Liver Metastasis in Colorectal Cancer. Front Pharmacol. 2021; 12: 734351. doi: 10.3389/fphar.2021.734351.

30. *Zhang C., Wang X.Y., Zhang P., He T.C., Han J.H., Zhang R., Lin J., Fan J., Lu L., Zhu W.W., Jia H.L., Zhang J.B., Chen J.H.* Cancer-derived exosomal HSPC111 promotes colorectal cancer liver metastasis by reprogramming lipid metabolism in cancer-associated fibroblasts. Cell Death Dis. 2022; 13(1): 57. doi: 10.1038/s41419-022-04506-4. PM.

31. *Tayoun T., Faugeroux V., Oulhen M., Aberlenc A., Pawlikowska P., Farace F.* CTC-Derived Models: A Window into the Seeding Capacity of Circulating Tumor Cells (CTCs). Cells. 2019; 8(10): 1145. doi: 10.3390/cells8101145.

32. *Dianat-Moghadam H., Azizi M., Eslami-S Z., Cortés-Hernández L.E., Heidarifard M., Nouri M., Alix-Panabières C.* The Role of Circulating Tumor Cells in the Metastatic Cascade: Biology, Technical Challenges, and Clinical Relevance. Cancers (Basel). 2020; 12(4): 867. doi: 10.3390/cancers12040867.

33. *Chen Q., Zhang X.H., Massagué J.* Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. Cancer Cell. 2011; 20(4): 538–49. doi: 10.1016/j.ccr.2011.08.025.

34. *McGowan P.M., Simedrea C., Ribot E.J., Foster P.J., Palmieri D., Steeg P.S., Allan A.L., Chambers A.F.* Notch1 inhibition alters the CD44hi/CD24lo population and reduces the formation of brain metastases from breast cancer. Mol Cancer Res. 2011; 9(7): 834–44. doi: 10.1158/1541-7786.MCR-10-0457.

35. *Zhong Y., Shen S., Zhou Y., Mao F., Lin Y., Guan J., Xu Y., Zhang S., Liu X., Sun Q.* NOTCH1 is a poor prognostic factor for breast cancer and is associated with breast cancer stem cells. Onco Targets Ther. 2016; 9: 6865–71. doi: 10.2147/OTT.S109606.

36. *Miao K., Lei J.H., Valecha M.V., Zhang A., Xu J., Wang L., Lyu X., Chen S., Miao Z., Zhang X., Su S.M., Shao F., Rajendran B.K., Bao J., Zeng J., Sun H., Chen P., Tan K., Chen Q., Wong K.H., Xu X., Deng C.X.* NOTCH1 activation compensates BRCA1 deficiency and promotes triple-negative breast cancer formation. Nat Commun. 2020; 11(1): 3256. doi: 10.1038/s41467-020-16936-9.

37. *Study Of MK-0752* In Combination With Tamoxifen Or Letrozole to Treat Early Stage Breast Cancer (MK-0752) [Internet]. ClinicalTrials.gov Identifier: NCT00756717. URL: https://www.clinicaltrials.gov [cited 2021].

38. *Zhang L., Ridgway L.D., Wetzel M.D., Ngo J., Yin W., Kumar D., Goodman J.C., Groves M.D., Marchetti D.* The identification and characterization of breast cancer CTCs competent for brain metastasis. Sci Transl Med. 2013; 5(180): 180ra48. doi: 10.1126/scitranslmed.3005109. Erratum in: Sci Transl Med. 2013; 5(189): 189er5.

39. *Klotz R., Thomas A., Teng T., Han S.M., Iriondo O., Li L., Restrepo-Vassalli S., Wang A., Izadian N., MacKay M., Moon B.S., Liu K.J., Ganesan S.K., Lee G., Kang D.S., Walmsley C.S., Pinto C., Press M.F., Lu W., Lu J., Juric D., Bardia A., Hicks J., Salhia B., Attenello F., Smith A.D., Yu M.* Circulating Tumor Cells Exhibit Metastatic Tropism and Reveal Brain Metastasis Drivers. Cancer Discov. 2020; 10(1): 86–103. doi: 10.1158/2159-8290.CD-19-0384.

40. *Clements M.E., Johnson R.W.* PREX1 drives spontaneous bone dissemination of ER+ breast cancer cells. Oncogene. 2020; 39(6): 1318–34. doi: 10.1038/s41388-019-1064-3.

41. *Chen W., Hoffmann A.D., Liu H., Liu X.* Organotropism: new insights into molecular mechanisms of breast cancer metastasis. NPJ Precis Oncol. 2018; 2(1): 4. doi: 10.1038/s41698-018-0047-0.

42. *Gil-Bernabé A.M., Ferjancic S., Tlalka M., Zhao L., Allen P.D., Im J.H., Watson K., Hill S.A., Amirkhosravi A., Francis J.L., Pollard J.W., Ruf W., Muschel R.J.* Recruitment of monocytes/macrophages by tissue factor-mediated coagulation is essential for metastatic cell survival and premetastatic niche establishment in mice. Blood. 2012; 119(13): 3164–75. doi: 10.1182/blood-2011-08-376426.

43. *Najmeh S., Cools-Lartigue J., Rayes R.F., Gowing S., Vourtzoumis P., Bourdeau F., Giannias B., Berube J., Rousseau S., Ferri L.E., Spicer J.D.* Neutrophil extracellular traps sequester circulating tumor cells via β1-integrin mediated interactions. Int J Cancer. 2017; 140(10): 2321–30. doi: 10.1002/ijc.30635.

44. *Cools-Lartigue J., Spicer J., McDonald B., Gowing S., Chow S., Giannias B., Bourdeau F., Kubes P., Ferri L.* Neutrophil extracellular traps

sequester circulating tumor cells and promote metastasis. J Clin Invest. 2013; 123(8): 3446–58. doi: 10.1172/JCI67484.

45. *Albrengues J., Shields M.A., Ng D., Park C.G., Ambrico A., Poindexter M.E., Upadhyay P., Uyeminami D.L., Pommier A., Küttner V., Bružas E., Maiorino L., Bautista C., Carmona E.M., Gimotty P.A., Fearon D.T., Chang K., Lyons S.K., Pinkerton K.E., Trotman L.C., Goldberg M.S., Yeh J.T., Egeblad M.* Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science. 2018; 361(6409). doi: 10.1126/science.aao4227.

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