METASTATIC DISSEMINATION WITH SPECIAL EMPHASIS ON BREAST CANCER CUTANEOUS METASTASES

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ABSTRACT

Metastatic development is a complex, multistage process involving modulation of cell phenotype, cell migration, and dynamic homeotypic as well as heterotypic cell-cell interactions. In breast cancer, MUC1 mucin has been emerging as a key molecule involved in metastasis. MUC1 is a large transmembrane glycoprotein expressed by mammary normal epithelial cells but it is overexpressed and underglycosylated in cancer cells.

Cutaneous metastasis is a neoplastic lesion localized at the dermis or subcutaneous cellular tissue, which is not contiguous to the primary tumor, with an overall incidence of 5.3%. The most common tumor to metastasize to the skin is breast cancer with an incidence of 24%.

We present a minireview on metastatic dissemination with special emphasis on breast cancer cutaneous metastases and the role that MUC1 plays on it.

Keywords: metastases; cutaneous metastases; breast cancer; MUC1.

Glossary:

CCL21: cognate ligand of CCR7

CCR7: CC-chemokine receptor 7

CXCR4: CXC-chemokine receptor 4

MMPs: Matrix metalloproteinases

SDf-1/CXCL12: Stromal Derived Factor-1 (SDF-1, CXCL12), cognate ligand of CXCR4

VEGF: Vascular Endothelial Growth Factor

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Introduction

Breast cancer is the leading cause of death among solid tumors in women. It affects 12% of all women and about 40% of patients will die from metastatic disease [1].
Metastatic development is a complex, multistage process involving modulation of cell phenotype, cell migration, and dynamic homeotypic as well as heterotypic cell-cell interactions [2].
Cutaneous metastasis is a neoplastic lesion localized at the dermis or subcutaneous cellular tissue, which is not contiguous to the primary tumor [3, 4]. Cutaneous metastasis of internal malignancies is not a frequent event, with an overall incidence of 5.3%. The most common tumor to metastasize to the skin is breast cancer with an incidence of 24% [5].
In recent years, new concepts of how metastases form have begun to be developed. Special interest has been directed to the timing of dissemination, the complexity of routes of dissemination, and the interrelationship of the cellular and molecular mechanisms that regulate where disseminating tumor cells may lodge [6]. In breast cancer, MUC1 mucin has emerged as a key molecule involved in metastasis [7].

Routes of dissemination

A malignant tumor may disseminate directly or by metastatic spread. Metastatic dissemination involves hematogenous and lymphatic spreading; through lymphatics, malignant cells may gain the skin by the thoracic duct and the subclavian vein reaching the general blood circulation, or by emboli formation which would block lymphatic vessels and allow retrograde extravasation of malignant cells to the skin [4, 8]. Alternatively, tumor cells may access distant sites by their transportation into the blood stream and then exit by the venous system [9].
Nevertheless, it is difficult to differentiate between lymphatic and hematogenous routes because they are connected via anastomoses; in consequence, tumor cells may move from one to another system and produce synchronically lymphatic and hematogenous metastases [4].

Models of metastatic dissemination

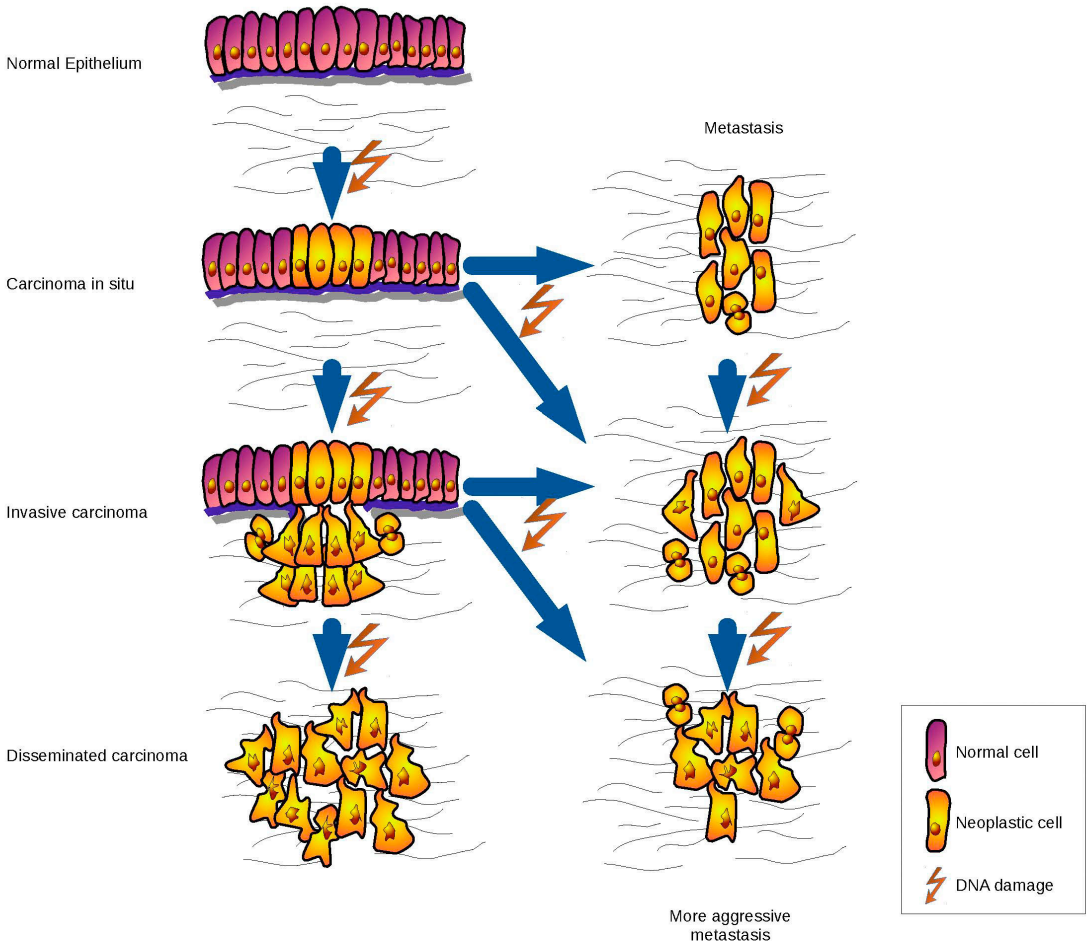

Figure 1. Two Models of Cancer Development and Progression. On the left, the classic model of human cancer development, where metastasis corresponds to the final step of the tumorigenic cascade. On the right, the new, alternative model, which predicts that the dissemination of primary tumor cells may occur at any time during cancer development.

The classic, linear model of cancer development proposes that metastasis constitute the last of a series of sequential steps [1, 6]. During the process of tumor development, the appearance of a metastatic clone would depend on the activation of oncogenes and loss of tumor suppressor genes, which can occur in any cell of the primary tumor.
A novel model has been proposed on the basis of tumor growth rate measurements and single cell genomics on dormant tumor cells that seed metastases [6]. In this context, metastatic phenomenon is an early event, and can even occur at premalignant stages of tumor development [1, 6]. Following this hypothesis, the ability of cutaneous dissemination would be early present in the primary tumor.

Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is a biologic process that allows an epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes migratory capacity, invasiveness, elevated resistance to

apoptosis, and extracellular matrix (ECM) components highly increased production. Several distinct molecular processes are engaged in order to initiate an EMT and enable it to reach completion. These include activation of transcription factors, expression of specific cell-surface proteins, reorganization and expression of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs [10].

A spectrum of signaling agents that contribute to EMT of cancer cells are released from the tumor-associated stroma. They appear to be responsible for the induction or functional activation in cancer cells of a series of EMT-inducing transcription factors which are related to decrease E-cadherin expression, which favors tumor invasiveness and migration. Growing evidence has demonstrated that EMT can be induced in human mammary epithelial cells leading to the acquisition of stem cell properties [11].

One of the essential factors that induce EMT is MUC1 mucin. MUC1 is a large transmembrane glycoprotein expressed by mammary normal epithelial cells but it is overexpressed and underglycosylated in cancer cells. MUC1 is translated as a single polypeptide that undergoes autocleavage into two subunits (MUC1-N and MUC1-C) in the endoplasmic reticulum, and forms a stable heterodimer at the apical membrane of normal epithelial cells [12] (Figure 2). With transformation and loss of polarity, MUC1 is found at the cytosol and over the entire plasmatic membrane of carcinoma cells; it is overexpressed up to at least 10-fold in tumor cells, mainly breast cancer cells [13, 14]. MUC1 cytoplasmic tail translocates to the nucleus in association with β-catenin, represses E-cadherin expression, and upregulates expression of EMT inducers [15]. As a consequence, the adherent junctions are destabilized and profound cytoskeleton rearrangement occurs, reducing contacts between cancer cells, and facilitating basement membrane invasion [16]. MUC1 also induces EMT at post-transcriptional level by modulating the expression of miRNAs that control EMT-related gene expression [11].

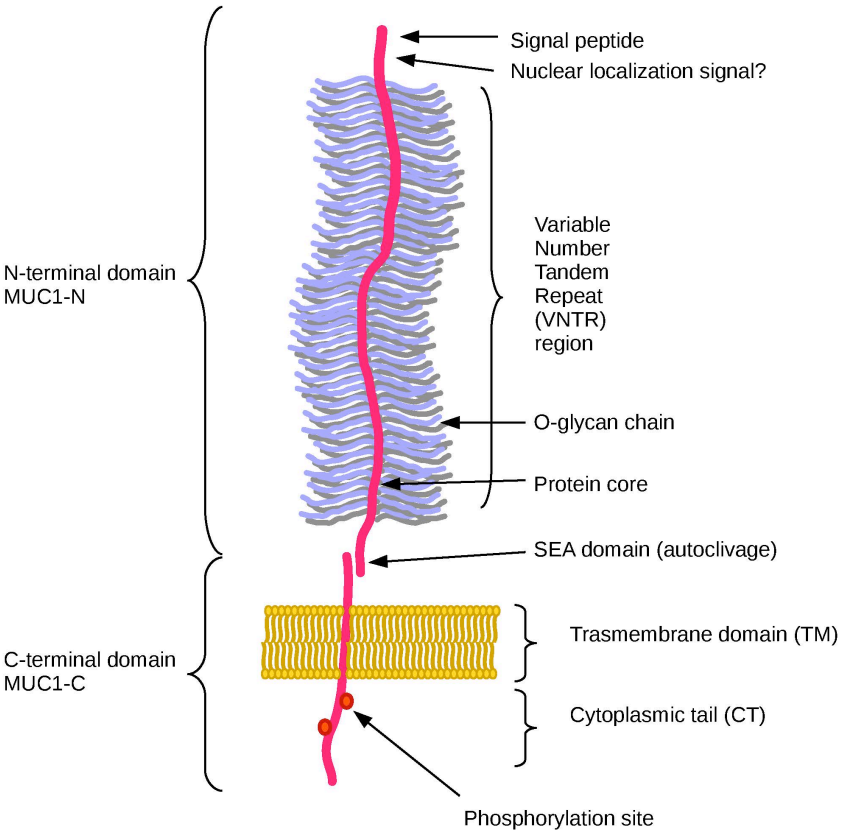

Figure 2. Schematic representation of the structure of MUC1; the N-terminal subunit (MUC1-N) and C-terminal subunit (MUC1-C) of MUC1 associate around the SEA domain, forming a stable heterodimeric complex. MUC1-N contains the signal peptide, the variable number tandem repeat (VNTR) region, which is highly glycosylated, and the SEA domain. The C-terminal domain (MUC1-C) consists of the extracellular domain (ECD), transmembrane domain (TMD), and the Cytoplasmic Tail (CT).

Tumor microenvironment

The main focus of cancer research for the past four decades has been on the malignant cancer cell, seeking to understand the dominant oncogenes and tumor suppressor genes related to carcinogenesis. However, the importance of the tumor microenvironment (TME) is now increasingly accepted, based on the concept that cancer cells do not manifest the disease alone, but rather resident and recruited normal cell types contribute to form the tumor stroma. Collaborative interactions between neoplastic cancer cells and their supporting stroma create the adequate conditions to cell proliferation, invasion and metastatic development [17]. Three types of cells mainly constitute the tumor microenvironment: angiogenic vascular cells, infiltrating immune cells, and cancer associated fibroblasts [17, 18, 19].

Cutaneous metastasis development

Distant metastasis development requires the initial invasion of primary tumor microenvironment, crossing the basal membrane and neighboring stroma to gain the lymphatic system and blood vessels.

Migration is a process in which cells are guided both by internal and external signals, mechanical and chemical. Mechanical signals depend on stiffness and density of the extracellular matrix, and they are sensed by cell adhesion molecules, mainly integrins [2]. Chemical signals are sensed by chemokine and growth factor receptors present on tumor cell surface [2]. The epithelial-mesenchymal transition favors tumor cell disruption due to loss of cell-cell connections and confers migratory capacity and invasiveness. Once the tumor cell loses its connections with other cells and the basement membrane, it adheres to the matrix diverse hydrolytic enzymes. MMPs are key regulators on infiltration process and high levels of MMPs correlate positively with invasive capacity [11, 17]. Then, malignant cells gain entry into blood or lymphatic vessels, crossing the vessel walls with proteolytic enzymes. An important factor on intravasation constitutes neovascularization, which is stimulated by angiogenic factors like VEGF, mainly VEGF-A, which is the most potent breast cancer angiogenic factor [20]. MUC1 overexpression in breast cancer was found to upregulate VEGF, thereby promoting endothelial migration and tube formation [7].

Once in the circulation, malignant cells must survive to mechanical attack by homotypic aggregation (formation of multicellular emboli) or heterotypic aggregation (tumor emboli covered with fibrin) which protect tumor cells from mechanical damage as well as from immune injury [6]. Recently, it has been shown that interaction of TF antigen on cancer-associated MUC1 with the circulating galectin-3 promotes metastasis by enhancing tumor cell heterotypic adhesion to the vascular endothelium and also by increasing tumor cell homotypic aggregation [21].

The following phenomenon is extravasation of circulating tumor cells; mechanical entrapment of the tumor cells as they enter capillaries with a lumen smaller than their diameter accounts for initial arrest; also, an active attachment to endothelial cells is reported as the primary mode of arrest [22]. At the molecular level, selectins have emerged as key adhesion molecules in the extravasation process. Selectin ligands are expressed on the surface of many types of tumor cells and correlate with poor prognosis [6].

Although multiple E-selectin ligands have been described, MUC1 plays an active role on rolling and adhesion of breast cancer cells to activated endothelium since it has been demonstrated that MUC1 may be a carrier of sialyl Lewis x, which has been frequently

detected at metastatic sites [23]. We have reported that 27% of breast cancer primary tumors express sialyl Lewis x [14].
An important step on metastatic development is gaining entrance into organ parenchyma, which means the formation of tumor cell colonies at the metastatic site. Most cutaneous metastases are localized at the dermis, which determines that tumor cell interaction with dermic factors plays a crucial role [8]. Metastatic cells require an environment similar to the primary tumor where they have high survival possibilities. Galactoforous ducts derive from the embryonic ectoderm and they are considered as modified sweat glands; in consequence, the dermis constitutes a favorable environment to colonization and survival of malignant breast cancer cells [8].
Chemokines and their receptors have been implicated in organ-specific dissemination. Breast cancer cells express CXCR4 and CCR7; their cognate ligands SDF-1/CXCL12 and CCL21 are expressed in sites to which breast cancer cells metastasize [24]; extracellular matrix changes at the metastatic site are also crucial [6].
Once tumor cells successfully extravasate, the available evidence suggests that if they do not grow out as metastases, they either die or remain dormant. Dormant tumor cells are thought to be in a quiescent state typified by G1 arrest; they may remain inactive during long periods but they may be responsible of late relapse even many years after the first diagnosis. Breast cancer is especially known for long asymptomatic periods up to 25 years with no evidence of disease, followed by relapse [25].
Immune surveillance has also recently been implicated in regulating dormancy, as depletion of cytostatic CD8+T-cells promoted outgrowth of visceral metastases [26].

Location

Frequently, cutaneous metastases are located near the primary tumor but without contiguity [27]. The anterior chest wall and the abdomen are the most commonly affected sites in breast cancer cutaneous metastases [28]. This location is probably determined by tumor cell lymphatic dissemination, since lymphatic vessels present anastomosis with areola, axilla, upper and lower anterior chest walls, and contralateral breast [29]

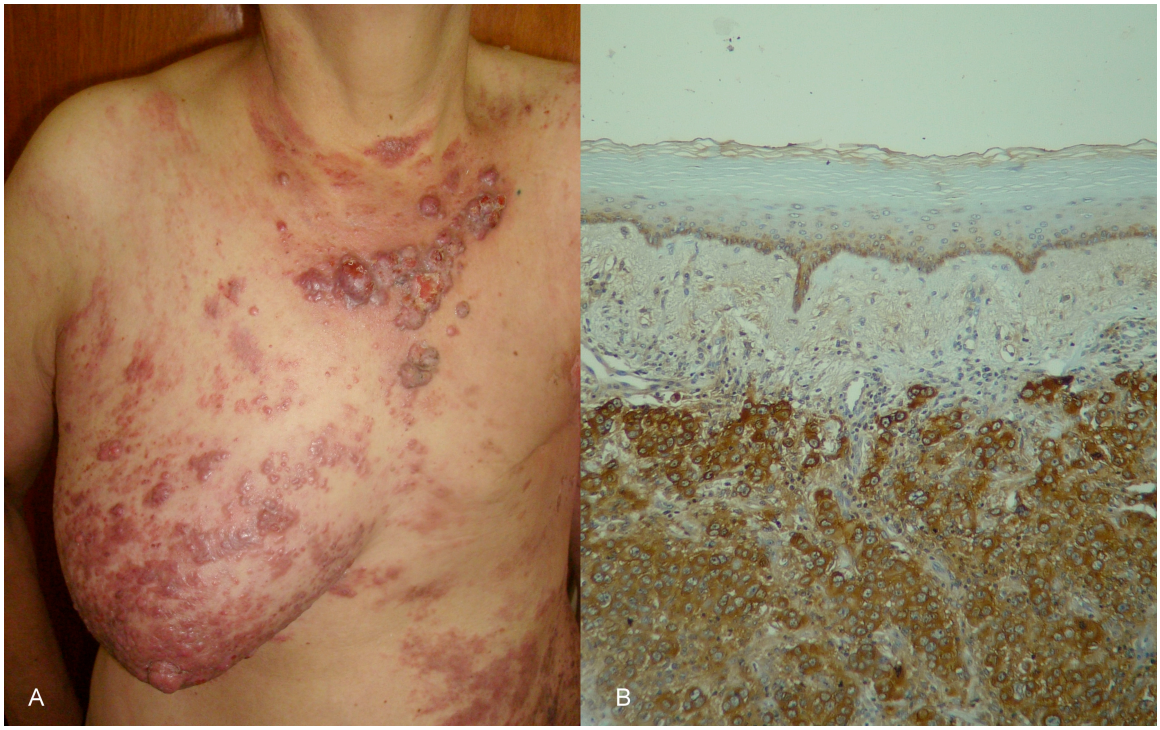

Figure 3. Breast cancer cutaneous metastasis. On the left, a patient with multiple metastatic nodules on the chest region, contralateral breast, and abdomen. On the right, microphotograph of an immunoperoxidase staining of a metastatic cutaneous lesion of the patient; neoplastic cells in the dermis show a strong reaction with an anti-MUC1-N monoclonal antibody.

In a study published by Lookingbill et al comprising 212 breast cancer patients with skin metastases [27], 192 (90,5%) had lesions at the thorax while Azcune et al [9] reported 94 patients with cutaneous metastases, 65 patients (69%) had breast cancer skin metastases that involved chest and mammary region in 56%, followed by back and scalp locations. It has been reported that in women, breast cancer is the most frequent tumor that presents skin metastases to scalp [30].

Conclusions

A confluence of factors influences whether primary tumors metastasize to a determined site. Differentiation programs innate to the primary tumor may predetermine the metastatic phenotype, though additional genetic or epigenetic changes may also affect a cell's ability to disseminate. Similar embryonic origins between breast and cutaneous tissues would probably affect active mechanisms for attracting breast tumor cells to the skin.
MUC1 overexpression in breast cancer is involved in multiple steps of tumor dissemination; study of MUC1 and associated carbohydrate antigens expressed at primary tumor, lymph nodes and cutaneous metastases would bring information about the dissemination process and may be useful to identify the primary tumor which would develop cutaneous metastasis, offering valuable clinical implications for prognosis and treatment.

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Amada Segal-Eiras developed her scientific career in immunological research at the Faculty of Medical Sciences, where she became Professor and scientific researcher at the CONICET. She was the recipient of numerous awards, author of many scientific contributions; organizer and participant of national and international scientific meetings; member and president of scientific societies. In 1996, she founded the CINIBA at the Faculty of Medical Sciences where she was the director of many pre and post-doc students and scientific researchers. After retirement age is keen to be involved and contribute to research; actually, she is the Director of CINIBA.