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Role of Galectins in Metastatic Breast Cancer

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Abstract: Galectins play a role in mammary morphogenesis and are expressed in all breast cancer subtypes. Galectins bind with glycoconjugates, as well as carbohydrate-independent targets in both the cytosolic and nuclear subcellular fractions. All tissues express galectins and galectins bind to numerous targets localized in different subcellular domains, including cell membrane, nucleus, mitochondria, and extracellular matrix. Galectin-3 and galectin-1 are associated with breast cancer progression and metastasis and will be the focus of this chapter. The chapter highlights mechanisms involved in galectins' role in modulating metastatic breast cancer phenotype. Immunocytochemistry analysis of galectin-3 in human breast cancer cells is used to illustrate galectin expression in the metastatic phenotype.

Keywords: adhesions; breast cancer; galectins in breast cancer; invasion; metastatic breast cancer

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INTRODUCTION

In 2020, an estimated 19.3 million new cancer cases developed worldwide with breast cancer accounting for 11.7% of all cancers (1). Metastatic cancer is the primary cause of cancer mortality. Therefore, metastatic breast cancer has attracted a significant amount of interest from the scientific and medical communities (2). Mammography is used for the early detection of breast cancer because it can visualize the greatest number and density of breast epithelial cells, fibroblasts and connective tissue (3). The progression of normal tissue to malignancy can be described in terms of initiation, promotion and progression. The Moolgavkar-Venzon-Knudson model describes two-stage clonal expansion of carcinogenesis that can be used to evaluate mammographs and quantify malignancy of breast epithelium (4). Briefly, the first stage consists of an epithelial cell obtaining an initial mutation to become an intermediate or precancerous cell that has the potential to gain further mutations to become a malignant cell (4). The second stage involves proliferation, clonal expansion and ultimately malignant transformation (4). This mathematical model considers changes in the microenvironment in the breast during menarche, parity, and menopause, which allows it to be predictive of the etiology of breast cancer. It is hypothesized that a combination of all the identified (and as yet unidentified) factors such as epithelial cell number, collagen density, growth factors associated with fibroblast, and extracellular matrix proteins play a role in cancer cell survival and progression (4). Various components may interact in mammographic dense areas of the breast to increase the number of fibroblasts that promote paracrine-dependent epithelial to mesenchymal transition (EMT) (5).

Although the exact mechanism mediating mammographic density promotion of EMT is not completely understood, the relationship between PTEN and Mgat5 in tumor cell survival is one possible explanation for breast cancer progression (6). PTEN is a lipid and protein phosphatase that deactivates PI3K/Akt signaling, while Mgat5 is a glycosyltransferase that catalyzes the addition of branched N-glycans on glycoproteins and increases cell adhesion-dependent PI3K/Akt signaling, cell spreading, and cell proliferation. Studies have shown that a single mutation in one of the alleles in the PTEN gene can increase Mgat5-dependent survival of mouse embryonic fibroblasts, which display the same morphology as migrating carcinoma cells (6). This mechanism could possibly facilitate the accumulation of mutations by activation of survival pathways and consequently increasing the risk of cancer cell progression (7). In one study, 43 patients with breast cancer were compared with 10 healthy individuals. Results showed that 44.2% of breast cancer patients had PTEN mutations associate with loss of function (8). Furthermore, these mutations encoded for a truncated form of PTEN that was associated with metastatic phenotypic behavior (8). Additional studies observed that patients with germline mutations in PTEN displayed an increased risk of breast cancer (9). PTEN promoter methylation has also been shown to influence the progression of breast cancer through the downregulation of PTEN and corresponding increase in Mgat5-mediated activation of PI3K/Akt signaling (9). Mgat5 expression in mammary epithelial tumor cells has been shown to be a rate limiting factor in EMT by increasing branched N-glycans on cytokine receptors, resulting in receptor binding to galectins and promoting galectin-dependent extracellular matrix remodeling (9).

Galectins are soluble lectins that bind to β-galactoside residues on glycoconjugates. TGF-β signaling regulates the expression of the TGF-β receptor and other cytokine receptors and promotes the addition of branched N-glycans on receptors. The downstream signaling of these cytokine receptors has been shown to regulate the transcription of Mgat5 to maintain a EMT phenotype. Regulation of receptor endocytosis is dependent on galectin-3 binding and oligomerizing with branched N-glycans on glycoproteins on the cell membrane and in the extracellular matrix (10). Branched N-glycans mediate cell migration and matrix remodeling (11). Taken together, these data suggest that the glycobiology of mammary tissue may facilitate mutated cell survival and further accumulation of mutations.

GLYCOBIOLOGY

Alterations in glycosylation of extracellular proteins have been characterized in metastatic breast cancer. These structures form the glycocalyx that is composed of membrane proteins, extracellular proteins, and lipids bound together by complex carbohydrates (12). Glycans consist of complex carbohydrates of varying lengths, composition, and branching. Glycans are covalently linked to glycoproteins, glycolipids or proteoglycans, and cover the surface of all mammalian cells and extracellular matrix components (12). N-glycans are attached to the amide nitrogen (N-linked glycosylation) on asparagine moieties in proteins that result in an increase in the branching, sialylation, and fucosylation during metastasis (13). Galectins bind the N-acetyllactosamine residues on branched N-glycans that are covalently attached to glycoproteins (integrins, growth factors, fibronectin, etc.). These multivalent glycoproteins vary in the number of N-glycans per protein and can induce the oligomerization of some galectins (14). This is of particular interest because galectin binding to Mgat5 promotes mammary tumor cell proliferation, migration, fibronectin matrix remodeling and metastasis (11, 15, 16). While Mgat5 is highly expressed in cancer, it is not the only glycosyltransferase that plays a role in malignancy. Glycosyltransferases, located in the Golgi, participate in post-translational modification of glycoproteins. The N-glycan profile on SKBR-3 breast cancer cells blocks the binding of the monoclonal antibody, trastuzumab, as well as displayed decreased sensitivity to doxorubicin (17). Following treatment with tunicamycin, an inhibitor of N-linked glycosylation, SKBR-3 cells displayed an increase sensitivity to doxorubicin and corresponding decreased sensitivity to mitogenic growth factors (17). Therefore, the glycocalyx appears to structurally inhibit the action of anticancer treatments. Upregulation of the glycocalyx enhances cancer cell adhesion, growth, and survival signaling (18). Specifically, chondroitinase ABC digestion of the glycocalyx chondroitin sulfate, leads to a decrease in MCF-7 breast cancer cellular adhesion, suggesting that glycocalyx regulates integrin ligation and activation by recruiting integrins to adhesion sites (18). This is possible because integrins contain an active binding site for chondroitin sulfate glycosaminoglycan (19). Galectins are localized in the glycocalyx where they interact with mucins to form a transcellular barrier for epithelial cells that is dependent on glycan binding and galectin oligomerization (20, 21). Galectins have been shown to interact with chondroitin sulfate (-A, -B, and -C), as well as other components of the glycocalyx to play a role in modulating mobility of tumor cells (22, 23).

CELLULAR ADHESIONS

Adhesions form cell-cell and cell-extracellular matrix connections through a group of glycoproteins called cell adhesion molecules. Cell adhesion molecules are made up of two subgroups classified as calcium-independent and calcium-dependent adhesion molecules (24). Calcium-independent adhesion molecules include the immunoglobulin superfamily and the lymphocyte homing receptors, whereas calcium-dependent adhesion molecules include integrins, cadherins, and selectins (24). Integrins are transmembrane receptors that form a bridge between the actin cytoskeleton and the extracellular matrix. This bridge functions to relay mechanical and chemical signals from the outside to inside of the cell. Integrins are heterodimeric molecules composed of an α and β subunit that are noncovalently bonded. Presently, eighteen α subunits and eight β subunits have been found in humans that have the capacity to form twenty-four different integrins with different ligand binding specificities (25). The direct binding of the cytoplasmic domains of α and β subunits are responsible for sustained inactivation of integrins. Integrin receptor activation occurs through both inside-out and outside-in signaling (25). From inside the cell, the cytoplasmic domain of the β subunit can be phosphorylated by downstream signaling of G-protein coupled receptors to inhibit the binding of α and β subunits that causes the activation of the integrin. From outside the cell, extracellular ligand binding induces conformational changes that render the cytoplasmic domain of integrins available to interact with plaque proteins. Plaque proteins include focal adhesion kinase, talin, vinculin, tensin, paxillin, integrin linked kinase, α-actinin and others. Activated integrins and intracellular plaque proteins form a complex protein network of about 90 proteins on the plasma membrane known as focal adhesions (25). Focal adhesions are present in all adherent cells, but differ in size, morphology, and signaling. Focal adhesions are stimulated by RhoA signaling and are formed at the end of stress fibers. The most common integrins found in focal adhesions are the α5β1 fibronectin and αVβ3 vitronectin receptors. Both receptors play a vital role in matrix remodeling and cell migration (26). Although, integrin receptors lack enzymatic activity, many plaque proteins are kinases and phosphatases that play a role in signal transduction. This signaling complex activates survival, proliferation, and motility pathways that protect cells from death that may result from the loss of adhesion or anoikis (26). After integrin activation, the autophosphorylation of focal adhesion kinase creates a binding site for the SH2 domain of Src family tyrosine kinases. This leads to additional tyrosine phosphorylation of focal adhesion kinase (FAK) and recruitment of the adaptor protein GRB2/SOS that leads to the activation of Ras/Raf/MEK/MAPK and myosin light chain kinase (26). Activated FAK also leads to the activation of PI3K/Akt survival signaling and NF-κB-mediated increase in caspase inhibitors (27). Activation of the β1 integrin subunit can also increase the level of phosphorylated Akt by sequestering protein phosphatase 2A and preventing its ability to inactivate Akt (28).

Cell-extracellular matrix contacts are called fibrillar adhesions and display an elongated morphology compared to square focal adhesions. Prior to developing into mature adhesions, nascent adhesions display a dotlike morphology (29–31). Mature fibrillar adhesions are composed of α5β1 integrins ligated to fibronectin (Figure 1). The α5β1 integrins cluster in fibrillar adhesions after fibronectin

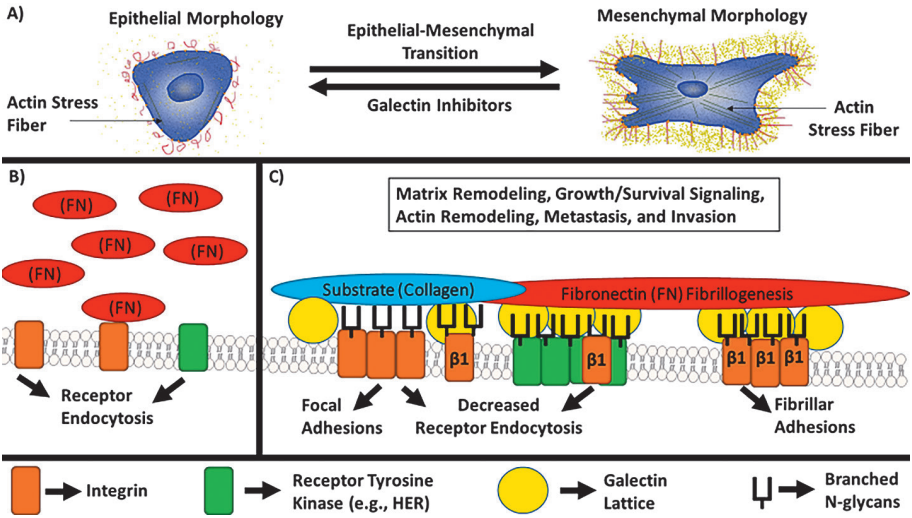

Figure 1. Morphology and molecular interactions of extracellular galectins. A) Morphological changes during epithelial-mesenchymal transition (EMT) and galectin inhibition (nucleus is dark blue, cell membrane is light blue, fibronectin is red, and galectin is yellow). In the absence of galectin inhibition, fibronectin fibrillogenesis and actin polymerization occur and promote EMT. In contrast, galectin inhibition result is the blockade of EMT. **B)** In the absence of branched N-glycans there is reduced integrin and receptor tyrosine kinase receptor binding to fibronectin and absence of galectin binding. **C)** In the presence of branched N-glycans, there is increased binding of galectins to receptor tyrosine kinases, integrins and fibronectin, and ultimately promotes fibrillogenesis, EMT and metastatic phenotypic behavior. When branched N-glycans are present, the galectin lattice clusters membrane receptors, preventing endocytosis, and stabilizes adhesions during matrix remodeling, actin remodeling, receptor signaling, invasion, and metastasis.

ligation and the β1 cytoplasmic tail organizes tensin near actin filaments. This fibronectin-α5β1-tensin-actin complex functions to form fibronectin fibrils (fibrillogenesis) translocating towards the center of the cell, stretching fibronectin and to allow for polymerization. It is thought that tensile forces produced by the intracellular cytoskeleton provides the force needed for fibrillogenesis. Tensin inhibition has been shown to block α5β1 integrin translocation and fibronectin fibrillogenesis without affecting focal adhesions (32). Focal and fibrillar adhesions are required for tumor cell growth, migration, and matrix assembly. In the context of tissues, most adhesions resemble fibrillar adhesions are further elongated to form three-dimensional matrix adhesions that are composed of both focal and fibrillar adhesion components. These complex adhesion sites are associated with increased proliferation and faster migration compared to cells on polystyrene culture surfaces (33). Integrin-dependent migration works in a concerted manner with cadherin cell-cell contacts to coordinate collective cell migration in metastatic breast cancer (33). Cadherins are homotypic adhesion molecules that connect cells together in the presence of calcium to form adherens junctions. Intracellularly, cadherins have a catenin-binding domain that connects extracellular homotypic cadherin complexes with the actin cytoskeleton (33). Cadherins were initially named for the location they were found, such as E-cadherin were

found in epithelial tissue, whereas N-cadherin were found in neural tissue and not found in normal breast tissue. However, the transition from E-cadherin to N-cadherin represents an important cellular marker of EMT in metastatic breast cancer (34). Furthermore, N-cadherin has been shown to promote migration and invasion, regardless of the level of E-cadherin expression in various breast cancer cell lines (34).

Studies have also shown that extracellular galectin-3 binds Mgat5-modified N-glycans on N-cadherin and GM1 ganglioside, a glycolipid on mammary carcinoma cell-cell junctions, to increase the mobility of these glycoconjugates within the membrane and resulting in the destabilization of cell-cell junctions and the promotion of cancer cell migration (35). Other studies showed that combined treatment with swainsonine (Mgat5 inhibitor) and β-lactose (galectin competitive inhibitor) resulted in the significant inhibition of galectin-3 binding to branched N-glycans on integrins, focal adhesion kinase and PI3K activation, translocation of β1 integrins to fibrillar adhesion sites, cell migration on fibronectin substrates, fibronectin matrix remodeling, and f-actin turnover (35). In addition, treatment with monoclonal antibodies for the galectin-3 N-terminal domain, but not the carbohydrate recognition domain, had no effect (35). These data suggest that the oligomerization of extracellular galectins function as a transient framework that has the plasticity required to support the organization of protein complexes associated with matrix remodeling and cell migration.

Taken together, the data suggest that galectins may decrease the force of focal adhesions enough to allow β1 integrins to translocation to form fibrillar adhesions. This suggestion is further supported by the finding that galectin-3 decreases tumor cell adhesion to fibronectin, collagen, and laminin, but increases fibrillogenesis (11, 36). Therefore, extracellular galectin-3 may decrease the force needed for cells to migrate through microscopic structures (basement membrane) to subsequently promote invasion. Fibronectin matrix remodeling is thought to organize sites for collagens to initiate collagen fibrillogenesis, which is dependent on fibronectin and integrin activation (37). Collagen density plays an important role in triggering breast cancer initiation, progression, and invasion, as well as to inducing resistance to apoptosis caused by loss of cell anchorage (anoikis) in mammary tumor cells (38, 39). It is now firmly established that cellular adhesions play an essential role in survival, growth signaling, cell migration, invasion, angiogenesis, and EMT in metastatic breast cancer (40).

GROWTH FACTORS

Growth factor activation and signaling of receptor tyrosine-kinases are initiated following ligand binding, receptor dimerization and autophosphorylation in various pathways including JAK/STAT, PI3K/Akt, and Ras/Raf/MAPK. Normally, these receptor tyrosine kinases play an important role in cell proliferation and differentiation, whereas excessive activation or dysregulation of these receptors is associated with the promotion and progression of a variety of cancers (41). Therefore, targeting growth factor receptors is of great interest in the treatment of cancer. Various approaches have been developed to either block ligand binding and dimerization or inhibit the tyrosine kinase activity in these receptors.

The first treatment to be approved was the humanized monoclonal antibody called trastuzumab (Herceptin), which binds to the extracellular domain of human epidermal growth factor receptor 2 (HER2) and blocks ligand binding and activation (41). Specifically, trastuzumab blocks HER2 homo- and hetero-dimerization with other EGFR/HER1, HER2, HER3, and HER4 receptors (41). While initially these treatments appear somewhat promising, various forms of cancer have been found to circumvent these therapies. However, recent advancements in glycomics have led to a greater understanding of the function of glycoconjugates and their role in promoting cancer cell survival and progression. For example, the glycans on HER2 receptors and mucins (mucins bind all four human epidermal growth factor receptors) are thought to block the binding of trastuzumab, and removal of these glycans significantly increases trastuzumab binding (41). Since glycan removal also results in an increased in sensitivity to doxorubicin in several human breast cancer cell lines (17), it is possible that galectin-bound glycoconjugates (growth receptors and mucins) form a barrier that blocks anticancer treatments in the same way that galectins form a barrier on the apical membrane of epithelial cells to block the activity of microorganisms, drugs, and vaccines (21). HER2 has been shown to induce breast cancer cell migration, matrix remodeling, and actin reorganization by stimulating galectin-3/phospho-caveolin-1-dependent activation of β1 integrins (21). These processes are known to be dependent on Src and ROCK/RhoA intracellular signaling and illustrates the crosstalk that occurs between growth factor and integrin receptors (42).

GALECTINS

During mammary morphogenesis, galectin-1 plays a role in epithelial migration and branching structure formation, whereas galectin-3 is upregulated in epithelial cells during involution and is associated with anti-apoptotic properties of these cells (43, 44). Lectin binding to complex carbohydrates in the glycocalyx and extracellular matrix has been characterized as a topological cellular storage and transfer network with bites of information expressed in the complexity of the carbohydrate (e.g., branched N-glycans) (45). Galectins are the most expressed lectin in the human body and have been implicated in almost all forms of cancer (46). Galectins were first identified as soluble S-type lectins. Three types of galectins have been found in humans and are classified as chimera-type, proto-type, and tandem repeat-type galectins. Chimera-type galectin-3 is the only galectin of this type to be identified and contains a C-terminal carbohydrate recognition domain and an intrinsically disordered N-terminal domain responsible for the oligomerization of galectin-3 (47). Proto-type galectins have one carbohydrate recognition domain and can dimerize. Proto-type galectins consists of galectin-1, galectin-2, galectin-5, galectin-7, galectin-10, galectin-11, galectin-13, galectin-14, and galectin-15 (47). Tandem repeat-type galectins have two carbohydrate recognition domains connected by a linker peptide and consists of galectin-4, galectin-6, galectin-8, galectin-9, and galectin-12. Galectins differ from each other in how they recognize carbohydrate residues. It is thought that some tandem repeat-type galectins may recognize different carbohydrates in both carbohydrate recognition

domains. Galectins bind the N-acetyllactosamine residues on carbohydrates. Therefore, galectin binding and oligomerization is dependent on the number of N-acetyllactosamine residues on glycans and the extent of glycosylation on glycoproteins (15). Galectins bind with relatively weak affinities when binding to a single saccharide. However, the oligomerization of galectins and the multivalency of glycoproteins interact to form a lattice structure with high avidity (14, 46). Galectins have been shown to form lattice structures as determined by liquid-liquid phase separation. Under these conditions, soluble galectins are in a state of equilibrium between monomeric form and oligomer form that can self-associate depending on the carbohydrates present (48–50). The lattice structure of galectins helps to organize protein complexes and alters the function of glycoconjugates that the galectin lattice is a part of. The galectin lattice can prolong the activation of cytokine receptors in breast cancer cells by decreasing constitutive endocytosis of these receptors that acts to promote tumor cell motility, EMT and metastasis (10).

Galectin-1 and galectin-3 are the most studied and understood galectins and both have been implicated in the modulation of the molecular mechanisms mediating metastatic breast cancer (10). Galectin-1 and galectin-3 have been shown to bind glycans on GM1 gangliosides that organize raft domains on the plasma membrane (35, 51). Galectin-1 and galectin-3 have also been shown to bind glycans on β1 integrin and regulates fibronectin remodeling, cell migration, and drug resistance in MDA-MB-231 breast cancer cells (42, 52). In addition, galectin-1 and galectin-3 have been associated with the clustering and signaling of H-Ras and K-Ras in mammary tumor cells, respectively (53, 54). These intracellular and extracellular functions of galectins support the migration and survival of metastatic breast cancer cells. Galectin-3 serum levels have been shown to be significantly elevated in patients with breast cancer as compared to noncancerous individuals, suggesting a role for galectin-3 in intravasation and extravasation of cancer cells (55). Studies have shown that treatment with recombinant galectin-3 bind to the Thomsen-Friedenreich disaccharide on MUC1, which subsequently exposes adhesion receptors that can then interact with the endothelium (55). It appears that MUC1 can have both adhesive and anti-adhesive properties depending on the sialylation profile of MUC1. Galectins only bind to unsialylated glycans (56). Therefore, sialylated MUC1 will not be organized by the galectin-3 lattice and ultimately, cell adhesion sites will be blocked (56).

Along with cell-substrate and cell-extracellular matrix interactions, galectins play a role in cell-cell junctions during collective migration by regulating N-cadherin (35). Collective cell migration has been characterized in breast cancer as a multiple cell migration led by fibroblasts or macrophages in the tumor microenvironment along paths of extracellular matrix proteins (57). Fibroblasts and macrophages both express galectins, which function to mediate cellular activation and motility in hypoxic areas of inflammation (58–60). Galectin-3 and galectin-1 are up regulated during hypoxic conditions and act to promote EMT, collective migration, and ultimately breast cancer metastasis (61–63). The tumor environment that facilitates the activation of metastasis has been described as “a wound that never heals” and is consistent with the promotional role of galectins in wound healing and associated collagen organization (64–66). Current literature has shown that breast polyploid giant cancer cells or syncytial (multinucleated) cells,

are associated with increases in EMT, N-cadherin, invasion, and metastasis in patient tumor biopsies, as well as MCF-7 cells, and MDA-MB-231 breast cancer cells (67). Additional studies showed that hypoxia increased the fusion of non-metastatic breast cancer cells (MCF-7 and T47D) and multipotent stem/stromal cells, but this was not found to occur in highly metastatic MDA-MB-231 breast cancer cells (67). These finding suggest that hypoxia induces fusion of non-metastatic cells, but not necessarily in MDA-MB-231 cells because they already display cell fusion and metastatic phenotype (68). Breast polyploid giant cancer cells have also been shown to have higher mitochondrial content and increased production of reactive oxygen species that can be selectively targeted with antioxidants (69). Galectins are also upregulated during hypoxia and elevated galectin levels are a characteristic of metastatic breast cancer. In addition, galectin-1 has been implicated in cell fusion and syncytium formation in trophoblast tumor cells, whereas galectin-3 has been shown to mediate human trophoblast migration and invasion (70, 71). In summary, these findings suggest a direct role for the galectin lattice in the promotion of the dense microenvironment, receptor expression and activation, matrix remodeling, and actin cytoskeleton organization that supports migration and is associated with fusion of human breast cancer cells during progression and metastasis.

POSSIBLE TARGETS IN THE TREATMENT OF METASTATIC BREAST CANCER

There are currently no clinically approved galectin inhibitors for the treatment of breast cancer. However, there are several galectin inhibitors that have produced promising preclinical results. One promising compound appears to be a peptide with galectin-3 specificity called G3-C12. This compound has been shown to decrease lung metastasis of MDA-MB-231 human breast cancer cells after injection into athymic nude mice (72). Another promising study utilized a high-affinity antibody that disrupts mucin-galectin interactions, resulting in the suppression of metastasis in mucin expressing breast cancer cells (73). There are many other potential galectin inhibitors being developed, but most are peptide and/or sugar moieties that are designed to disrupt the carbohydrate recognition domain of galectins. An exception to this approach is a salicylic acid derivative called salirasib. Salirasib inhibits the activation of farnesylated Ras by binding to the hydrophobic (farnesyl) binding pocket of galectin-1 and galectin-3, thereby disrupting galectin-dependent clustering of various isoforms of Ras on the inner leaflet of the plasma membrane of cancer cells, suggesting that galectin inhibition in metastatic breast cancer can be achieved through both hydrophobic binding and hydrophilic binding (74–76).

Given that salirasib and γ-tocotrienol have similar chemical structure and lipophilicity, studies were conducted to determine the role of galectin-3 in mediating the anticancer effects of γ-tocotrienol in metastatic breast cancer cells. Previous studies have demonstrated the anticancer effects of γ-tocotrienol (77). γ-tocotrienol is a rare natural isoform of vitamin E that displays potent apoptotic and anti-metastatic activity against a wide range of breast

cancer cells. Specifically, γ-tocotrienol inhibits breast cancer cell EMT, lipid raft integrity, migration, invasion, and Hedgehog/Wnt/RTK signaling at treatment doses that have little or no effect on normal mammary epithelial cell growth or viability (77). MDA-MB-231 human breast cancer cells have been shown to undergo cell fusion to produce polyploid giant cancer cells that display metastatic properties (67, 68). Polyploid giant cancer cells display increased mitochondrial content and elevations in reactive oxygen species, and these characteristics are blocked by treatment with antioxidants (69). Results in Figures 2 and 3 show that elevations in galectin-3 is associated with the formation of multinucleated MDA-MB-231 cells and this effect is blocked following treatment with γ-tocotrienol. It appears that γ-tocotrienol treatment induced a decrease in f-actin stress fibers and focal adhesions, as identified by vinculin and f-actin positive staining (Figures 2 and 3). Since galectins levels show a positive correlation with metastatic behavior and γ-tocotrienol significantly reduces galectin-3 levels, these finding provide strong evidence that γ-tocotrienol may provide significant benefit in the prevention and treatment of metastatic breast cancer.

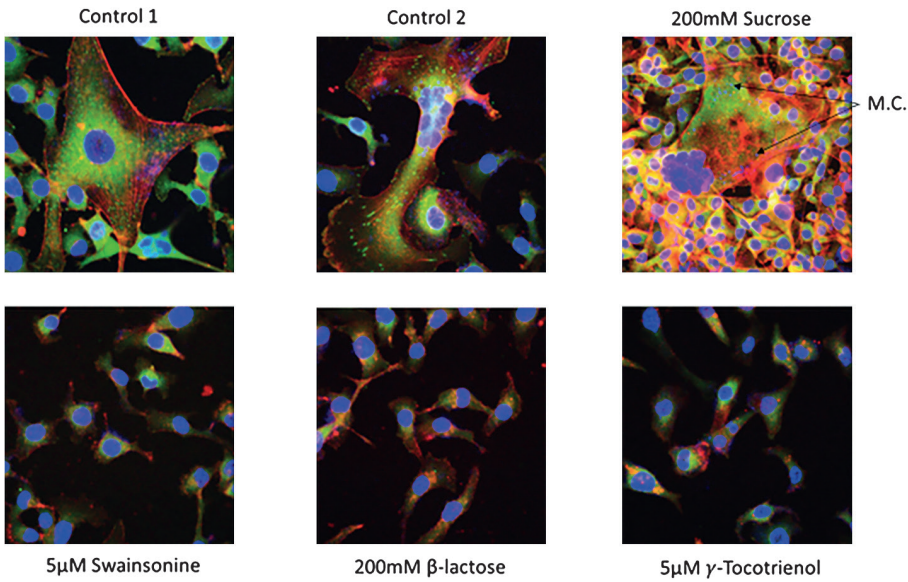

Figure 2. Changes in MDA-MB-231 morphology following a four-day treatment with various anti-metastatic agents. Vinculin (green), f-Actin (red), DAPI (blue). Control 1 and Control 2 show MDA-MB-231 cells can produce giant cells with f-actin stress fibers attached to focal adhesions and appears to be polypoidal, whereas treatment with swainsonine (golgi inhibitor) or β-lactose (a competitive inhibitor for galectin-carbohydrate binding) eliminated the appearance of giant cells. Cells in the 200mM Control Sucrose group (a treatment that stimulates giant and polypoidal cell expression) also shows a polyploid giant cancer cell migrating above the other MDA-MB-231 cells. This giant cell also contains f-actin stress fibers attached to focal adhesions and an increase in mitochondrial content (MC) that is believed to be due to increased oxidative metabolism, and treatment with 5μM γ-tocotrienol completely blocked these characteristics in MDA-MB-231 cells.

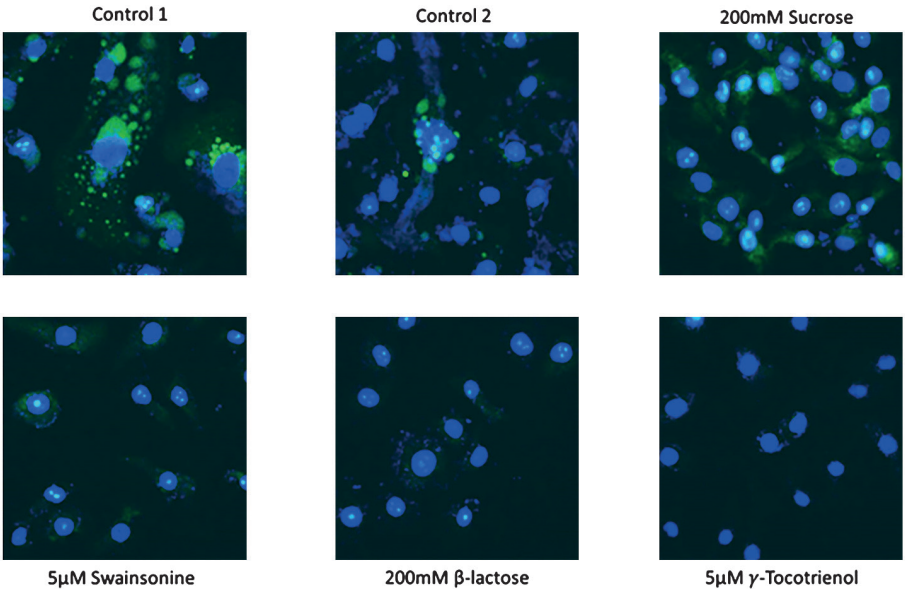

Figure 3. Changes in MDA-MB-231 galectin-3 expression following a four-day treatment with anti-galectin agents. Galectin-3 (green), DAPI (blue). Control 1, Control 2 and 200mM Control Sucrose groups all contain galectin-3 expressing cells with different nuclear sizes (DAPI staining) and a polyploid giant cancer cell with increased galectin-3 expression compared to other cells in the image. Treatment with 5μM Swainsonine, 200mM β-lactose, or 5μM γ-tocotrienol induced a large decrease in galectin-3 expression.

CONCLUSION

Extracellular galectins form a framework for protein networks to localize in specific cellular domains. Galectins have been shown to be associated with almost all aspects of breast cancer metastasis such as migration, matrix remodeling, action turnover, drug resistance, angiogenesis, invasion, intravasation, and extravasation. Cancer cells can fuse with other cancer cells and/or other cells, such as macrophages to produce polyploid giant cancer cells, which can result in these polyploidal cells obtaining properties from each cell and is coordinated in one giant cell. These cells are associated with breast cancer metastasis. Galectin expression is increased as polyploid giant cancer cells are generated. Results also showed that metastatic breast cancer cells exposed to γ-tocotrienol were found to display a large decrease in galectin-3 expression and a large inhibition in the development of giant polyploid breast cancer cells. The exact molecular mechanism by which γ-tocotrienol inhibits galectin-3 expression needs future investigation. However, these findings strongly suggest that targeting galectins may provide significant benefit in the treatment of metastatic breast cancer.

Conflict of Interest: the authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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