

## Commentary

# Metastases: the glycan connection

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Received: 2 May 2000  
Accepted: 31 May 2000  
Published: 7 July 2000

*Breast Cancer Res* 2000, **2**:321–323

Current Science Ltd (Print ISSN 1465-5411; Online ISSN 1465-542X)

### Abstract

An association between protein glycosylation and tumorigenesis has been recognized for over 10 years. Associations linking the importance of glycosylation events to tumor biology, especially the progression to metastatic disease, have been noted over many years. Recently, a mouse model in which  $\beta$ 1,6-*N*-acetylglucosaminyltransferase V (a rate-limiting enzyme in the N-glycan pathway) has been knocked out, was used to demonstrate the importance of glycosylation in tumor progression. By crossing mice lacking this enzyme with a transgenic mouse model of metastatic breast cancer, metastatic progression of the disease was dramatically reduced. These experiments provide *in vivo* evidence for the role of N-linked glycosylation in metastatic breast cancer and have significant implications for the development of new treatment strategies.

**Keywords:** breast cancer, glycosylation, metastases, MGAT5, transgenic mouse model

The progression of breast cancer to a metastatic, hormone-refractory stage heralds a poor prognosis, with less than 5% of patients having a long-term, complete response to treatment [1]. The cancer cell has to overcome many levels of regulation that maintain normal cellular growth for breast cancer to evolve to this advanced state. Multiple genetic alterations that enable the cancer cell to become self-sufficient (reviewed in [2]) in the production of growth signals have accumulated, such as the overexpression of *myc* (for a review, see [3]) and *her2/neu* [4], as well as losing the inhibitory effects of tumor suppressor genes such as p53 and Rb (reviewed in [5]). It is also clear that the normal protective apoptotic response to genomic damage is suppressed through the expression of survival factors, allowing such cells to accumulate and undergo further genetic aber-

ration. The reactivation of telomerase also ensures that tumor cells have limitless replicative potential [6]. However, it is the ability of cancer cells to spread to remote locations and grow without restraint that kills the patient. The metastatic spread of breast cancer involves the ability of the cancer cells to escape their normal tissue boundaries through invasion, engraftment and their ability to recruit a vascular supply (for a review, see [7]). Tissue invasion and metastasis are highly dependent on alterations in the extracellular matrix [8] and cell-cell interactions that, in part, involve structural changes in cell surface components, including glycosylation (for a review, see [9]). In the work of Granovsky *et al*, the importance of specific glycosylation events in mammary cancer metastases has been clearly demonstrated *in vivo* using genetically altered mice [10].

The N-linked glycoproteins have been shown to have important roles in cell–cell interactions including fertilization, inflammation, development and differentiation. These molecules are composed of a polypeptide backbone glycosylated in the endoplasmic reticulum with one or more carbohydrate chains through asparagine residues. The addition of the carbohydrate moiety occurs *en bloc* and, initially, the oligosaccharide chains of all glycoproteins are identical. Numerous Golgi enzymes (an estimated >200 glucosidases, mannosidases, *N*-acetylglucosamine [GlcNAc], and Gal transferases) are important in modifying glycoproteins to produce a diverse array of branching oligosaccharides. One subgroup composed of six Golgi enzymes is the *N*-acetylglucosaminyltransferases. The relation of Golgi  $\beta$ 1,6-*N*-acetylglucosaminyltransferase V (MGAT5; EC2.4.1.155) to tumorigenesis has been the focus of several recent studies (as reviewed in [9]). This enzyme catalyzes the addition of 1,6-GlcNAc to form tri- and tetra-antenna-like oligosaccharides [11]. Products of this reaction are often modified further to form polyacetosamines.

Malignant transformation has been associated with changes in the glycosylation of cell surface proteins. For example, the N-linked oligosaccharides containing the GlcNAc $\beta$ 1,6Man branch are increased after transformation in many cell types by a number of tumor viruses and oncogenes that induce the expression of MGAT5 [12]. Cells infected with MGAT5 express increased GlcNAc $\beta$ 1,6Man on integrins  $\alpha$ <sub>i</sub>,  $\alpha$ <sub>v</sub> and  $\beta$ <sub>1</sub> associated with increased cellular motility and decreased substratum adhesion [13]. Increased amounts of Mgat5 glycan products have also been reported in malignant tissue *in vivo*, and correlate with disease progression [12,14–20].

By generating mice with mutant alleles of Mgat5 by homologous recombination in embryonic stem cells, Granovsky *et al* were able to study the phenotypic effects produced by the absence of Mgat5 on both normal development and metastatic growth of mammary cancer. It has been demonstrated that while Mgat5 is not essential for development, it plays a critical role in metastatic progression of mammary tumors in the MMTV-polyoma middle-T (PyMT) transgenic mouse model [20].

The PyMT mouse model consistently develops mammary tumors with a high incidence of associated lung metastases. No differences in the occurrence of very early mammary lesions were observed when PyMT transgenic mice carrying either the Mgat5<sup>+/+</sup>, Mgat5<sup>+/-</sup>, or Mgat5<sup>-/-</sup> genotype were analyzed; thus, the absence of Mgat5 did not appear to affect the initiation of early events involved in the PyMT-induced oncogenesis. However, once initiated, the rates of mammary tumor growth and metastases observed in PyMT-Mgat5<sup>-/-</sup> mice were considerably less than the rates in PyMT transgenic littermates expressing Mgat5. Northern analysis demonstrated that the decrease

in tumor growth was not a secondary effect due to reduced transgene expression since steady state levels of the oncogene were not changed in the absence of one or both MGAT5 alleles. Significantly, the incidence of lung metastases in PyMT-Mgat5<sup>-/-</sup> mice was approximately 5% of that seen in PyMT-Mgat5<sup>+/-</sup> and PyMT-Mgat5<sup>+/+</sup> mice. Since the design of the mutant Mgat5 construct leads to the expression of *lacZ* under the control of the endogenous Mgat5 regulatory sequences, the results also demonstrate that PyMT is a positive regulator of Mgat5 expression.

The authors explore two potentially related mechanisms of Mgat5 action by which 1,6-GlcNAc glycosylation may be involved in the metastatic phenotype. First, how this specific glycosylation may lead to alterations in interactions with adhesion proteins, cellular structure and cell–cell interactions. The second mechanism is the role by which Mgat5 may affect intracellular signaling activated by PyMT, and thus accelerate cell growth. PyMT-Mgat5<sup>+/-</sup> tumor cells exhibited impaired membrane ruffling compared with that of PyMT-Mgat5<sup>+/+</sup> cells. This appears to be due to an inhibition of PI3 kinase activation through the PyMT-c-Src pathway, leading to reduced phosphorylation of paxillin and its recruitment into focal adhesions. The authors conclude that the intrinsic defect in cells lacking Mgat5 is the inability to accelerate focal adhesion turnover and signaling through PI3 kinase/PKB by PyMT.

The precise location of the MGAT5 products on specific glycoprotein(s) and their role in regulating tumor growth and metastasis currently remains to be determined. Further work localizing MGAT5 products may lead to the identification of specific cell surface molecules that may be targets for inhibiting tumor development and metastases. Given the universal nature of glycosylation, determining specificity is important so as not to disrupt other, potentially important cellular functions. The abnormal phenotypes seen in adult Mgat5<sup>-/-</sup> animals, including abnormal inflammatory responses, glomeruli and nurturing behavior, illustrate this point. Experiments in which another glycosylation enzyme, Mgat1, was knocked out in mice led to embryonic lethality [21]. However, Mgat1 regulates early glycosylation events and presumably affects a larger array of glycosylated glycoproteins, which may account for why this enzyme is essential for embryonic development.

The article by Granovsky *et al* clearly illustrates the role of MGAT5 in tumor progression. However, the signal-transduction pathway through which PyMT induces tumor formation is different from other oncogene pathways that lead to cellular transformation. For instance, breast cancer is associated with the overexpression of several oncogenes including *myc* [3] and *her2/neu* [4], and with the loss of function of tumor suppressor genes such as p53, Rb [5], BRCA1 [22] and BRCA2 [23]. Since Mgat5

affects a particular part of the PyMT signaling pathway, a number of interesting questions remain in relation to how the absence of Mgat5 may influence the metastatic potential of tumors induced through other pathways. It will be interesting to determine whether crosses between Mgat5<sup>-/-</sup> and other transgenic mammary cancer models will similarly alter the metastatic phenotype. There are, unfortunately, currently few useful mammary cancer models in genetically engineered mice that develop a high rate of metastases. There are presently also no good transgenic models in which to study mammary metastases to the bone, liver or to the brain, organs that are frequently the sites of metastases in human breast cancer.

Interestingly, a small percentage of PyMT Mgat5<sup>-/-</sup> tumors acquired a fast-growth phenotype similar to that of PyMT Mgat5<sup>+/+</sup> tumors that was not the result of 1,6-GlcNAc glycosylation. This indicates that while Mgat5 plays an important role in metastatic growth, tumor cells may activate other mechanisms that lead to aggressive, metastatic growth even in the absence of Mgat5 activity. Since glycosylation events occur within the Golgi in the cell, it remains unclear whether therapeutic targets to inhibit Mgat5 glycosylation will be efficacious if delivered to the extracellular environment or will require transport or expression within the cell.

These findings underscore the importance of glycosylation events in tumor biology, and have significant implications for understanding and treating malignant disease. While additional mechanisms involved in metastatic progression will undoubtedly be identified in the future, perhaps inhibitors of specific glycosylation enzymes will prove to be useful in halting tumor progression and metastasis. Additional studies on the relationship between glycosylation and metastases should provide important insights into mechanisms of cell-cell interactions and tumor progression to the metastatic stage.

## References

- Greenberg PA, Hortobagyi GN, Smith TL, Ziegler LD, Frye DK, Buzdar AU: **Long-term follow up of patients with complete remission following combination chemotherapy for metastatic breast cancer.** *J Clin Oncol* 1996, **14**:2197-2205.
- Hanahan D, Weinberg RA: **The hallmarks of cancer.** *Cell* 1999, **100**:57-70.
- Callahan R, Campbell G: **Mutation in human breast cancer: an overview.** *J Natl Cancer Inst* 1989, **81**:1780-1786.
- Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al: **Studies of the Her-2/NEU proto-oncogene in human breast and ovarian cancer.** *Science* 1989, **244**:707-712.
- Band V: **The role of retinoblastoma and p53 tumor suppressor pathways in human mammary epithelial cell immortalization.** *Int J Oncol* 1998, **12**:499-507.
- Harley CB, Kim NW: **Telomerase and cancer.** *Important Adv Oncol* 1996, **12**:57-67.
- Kerbel RS: **Tumor angiogenesis: past, present and the near future.** *Carcinogenesis* 2000, **21**:505-515.
- Curran S, Murray GI: **Matrix metalloproteinases in tumor invasion and metastasis.** *J Pathol* 1999, **189**:300-308.
- Dennis JW, Granovsky M, Warren CE: **Glycoprotein glycosylation and cancer progression.** *Biochem Biophys Acta* 1999, **1473**:21-34.
- Granovsky M, Fata J, Pawling J, Muller WJ, Khokha R, Dennis JW: **Suppression of tumor growth and metastasis in Mgat5-deficient mice.** *Nat Med* 2000, **6**:306-312.
- Cummings RD, Trowbridge IS, Kornfeld S: **A mouse lymphoma cell line resistant to the leukoagglutinating lectin from *Phaseolus vulgaris* is deficient in UDP-GlcNAc: alpha-D-mannoside beta-1,6-N-acetylglucosaminyltransferase.** *J Biol Chem* 1982, **257**:13421-13427.
- Buck CA, Glick MC, Warren L: **Effect of growth on the glycoproteins from the surface of control and Rous sarcoma virus transformed hamster cells.** *Science* 1971, **172**:169-174.
- Dennis JW, Kosh K, Bryce DM, Breitman ML: **Oncogenes conferring metastatic potential induce increased branching of Asn-linked oligosaccharides in rat2 fibroblasts.** *Oncogene* 1989, **4**:853-860.
- Le Marer N, Laudet V, Svensson EC, Cazlaris H, Van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P: **The c-Ha-ras oncogene induces increased expression of beta-galactoside alpha-2,6-sialyltransferase in rat fibroblast (FR3T3) cells.** *Glycobiology* 1992, **2**:49-56.
- Demetriou M, Nabi IR, Coppolino M, Dedhar S, Dennis JW: **Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase V.** *J Cell Biol* 1995, **130**:383-392.
- Seelentag WK, Li WP, Schmitz SF, Metzger U, Aeberhard P, Heitz PU, Roth J: **Prognostic value of beta1,6-branched oligosaccharides in human colorectal cancer.** *Cancer Res* 1998, **58**:5559-5564.
- Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS: **Beta-1,6 branching of Apn-linked oligosaccharides is directly associated with metastasis.** *Science* 1987, **236**:582-585.
- Fernandes B, Sagman U, Auger M, Demetrio M, Dennis JW: **Beta-1,6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia.** *Cancer Res* 1991, **51**:718-723.
- Chen L, Zhang W, Fregien N, Pierce M: **The her-2/neu oncogene stimulates the transcription of N-acetylglucosaminyl transferase V and expression of its cell surface oligosaccharide products.** *Oncogene* 1998, **17**:2087-2093.
- Guy CT: **Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease.** *Mol Cell Biol* 1992, **12**:954-961.
- Metzler M, Gertz A, Sarkar M, Schachter H, Schrader JW, Marth JD: **Complex asparagine-linked oligosaccharides are required for morphogenic events during post implantation development.** *EMBO J* 1994, **13**:2056-2065.
- Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE: **Risks of cancer in BRCA1 mutation carriers.** *Lancet* 1994, **343**:692-695.
- Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Manko FH, Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, Cannon-Albright LA, Peto J, Ponder BAJ, Skolnick MH, Easton DF, Goldgar DE, Stratton MR: **Localization of a breast cancer susceptibility gene BRCA-2 to chromosome 13q12-13.** *Science* 1994, **2655**:2088-2090.

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