12 *ABSTRACT*

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Regulation of Cytokine Receptor Endocytosis and Signal Transduction by N-Acetylglucosaminyltransferase V (Mgat5)

profile

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Dr. Dennis is currently a Senior Investigator at the Samuel Lunenfeld Research Institute, and a Professor of Molecular Genetics and Microbiology at the University of Toronto. Dr. Dennis received his Ph.D. from the Department of Biochemistry, Queen's University, Kingston, and did postdoctoral work at the German Cancer Research Center in Heidelberg, and at the Hospital for Sick Children in Toronto with Dr. H. Schachter. Appointed Assistant Professor of Pathology at Queen's University in 1982, then moved to the Samuel Lunenfeld Research Institute, Mount Sinai Hospital in 1985. Dr. Dennis held a competitive salary award of Senior Research Scientist of the National Cancer Institute of Canada from 1991 to 1999, and currently hold the Chair in Glycobiology, sponsored by Ontario Challenge Fund.

Dr. Dennis was a founder of GlycoDesign Inc. in Toronto, serving as VP of Research between 1995-1998. GlycoDesign Inc is a drug discovery research that currently employs 60 people. Dr. Dennis was awarded the Friesen-Rygiel Prize for transfer of knowledge academic to commercial enterprise. Dr. Dennis holds grants from the National Cancer Institute of Canada, and National Science and Engineering council of Canada, the US Army Breast Cancer program. He is an Associate Editor of Glycobiology J. and Glycobiology.

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UDP-N-acetylglucosamine:α-6-D-mannoside β1,6 N-acetylglucosaminyltransferase V (Mgat5) is required for the biosynthesis of β1,6GlcNAc-branched N-glycans found on many cell-surface and secreted glycoproteins. Mgat5-modified N-glycans are preferentially elongated with poly N-acetyllactosamine, which serve as ligands for the galectin family of mammalian lectins. Mgat5-modified glycans in breast and colorectal carcinomas correlated with poor prognosis and reduced survival time[1,2]. Transformation of fibroblast and epithelial cells by oncogenes *v-src*, T24-*H-ras*, *v-fps*, or infection with polyomavirus or Rous sarcoma virus up-regulate Mgat5 activity[3-6]. Mgat5 gene transcription is stimulated by the Ras-Raf dependent activation of Ets transcription factors[7,8]. Furthermore, over-expression of Mgat5 in cultured epithelial cells results in loss of contact inhibition, increased cell motility, morphological transformation, and tumor formation upon inject of the cells into athymic nude mice[9], and enhanced metastasis[10].

We generated Mgat5-deficient mice by targeted gene mutation in ES cells to study the role of Mgat5 in development and cancer progression[11]. *Mgat5*$^{-/-}$ mice are viable and deficient in L-PHA reactive N-glycans, but display kidney autoimmune disease, enhanced delayed type hypersensitivity, and increased susceptibility to experimental autoimmune encephalomyelitis[12]. In addition, mutant mice display age-related osteoporosis, weight loss, and early mortality. Antigen recognition induces clustering of T cell receptors into cholesterol-rich rafts, a process that amplifies signal transduction and promotes receptor internalization. The Mgat5-modified glycans on naïve T cell receptor bind to galectin-3, which impeded receptor-clustering in response to agonists[12] . Following T cell activation, Mgat5 gene expression is up-regulation and may also regulate secondary response to antigen, and Th1/Th2 helper cells.

To study the role of Mgat5 in cancer progression, transgenic mice expressing polyoma virus middle T (PyMT) oncoprotein were crossed with Mgat5-deficient mice[11]. PyMT is a substrate for c-Src and activates P85/PI3 kinase and Shc/Ras pathways causing multifocal mammary tumors *in situ.* Tumors in PyMT *Mgat5*$^{-/-}$ transgenic mice grew slower and metastasized less frequently compared to PyMT litermates expressing Mgat5. The intrinsic defect in *Mgat5*$^{-/-}$ cells appears to be a failure to fully activate PI3 kinase/Akt signaling as required for optimal PyMT-induced tumor growth. Furthermore, additional genetic event(s) in some PyMT *Mgat5*$^{-/-}$ tumors enabled a minority of these tumors to escape growth suppression, and this was accompanied by increased Akt activation. Motility and spreading of *Mgat5*$^{-/-}$ PyMT tumor cells on fibronectin was severely impaired, and could be rescued by infection with a retroviral vector for Mgat5 expression. A vector with the Lec 4a Mgat5 mutation (L188R) which fails to localize in the Golgi did not rescue cell motility.

Mgat5-modified glycans impede TCR interalization and may have similar effects on other receptors. Recent work in my laboratory by Emily Partride, a graduate student has examined cell surface retention and signaling by cytokine receptor in the PyMT tumor cells. *Mgat5*$^{-/-}$ cells were deficient in acute response to epithelial growth factor (EGF) and transformating growth factor - β (TGF-β1) cytokines. The levels of EGF and TGF-β receptors at the cell surface are dependent on Mgat5 glycosylation, which appears to slowed receptor internalized in tumor cells. Cell cycle progression, cell volume, and sensitivity to drugs were also altered in *Mgat5*$^{-/-}$ PyMT tumor cells consistent with the loss of responsiveness to extracellular signaling. Our results suggest Mgat5 N-glycans modify receptor interactions with galectins, and thereby regulates cell motility and responsivness to extracellular stimuli.

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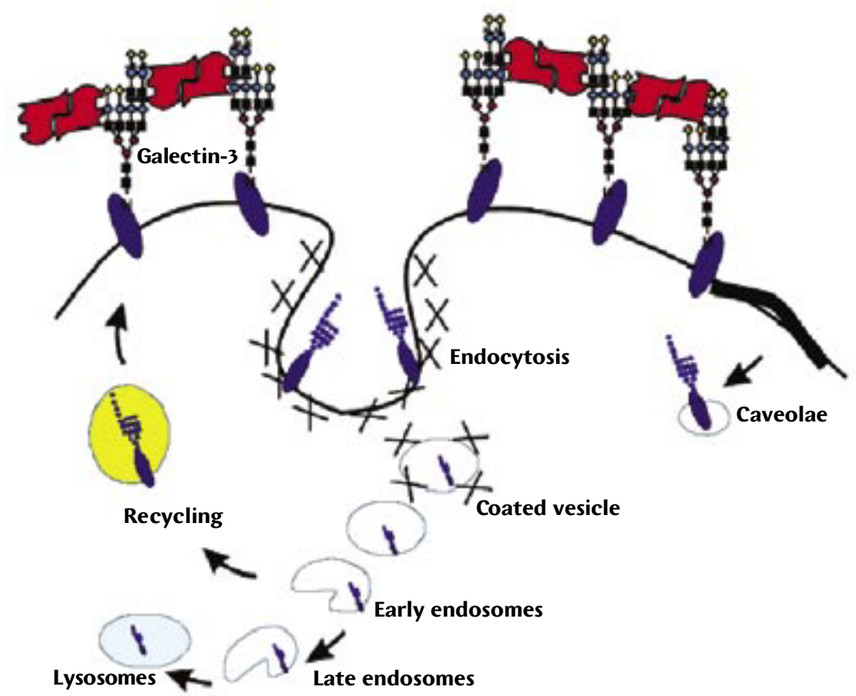

Figure 1. A simple scheme of receptor movement from cell surface into endosomes either through clathrin-coated pits or caveolae lipid rafts, followed by receptor destruction or re-cycling. Galectins (red) binds with greater affinity N-glycans modified by Mgat5, forming a glycoprotein-lectin multivalent lattice that retains receptors at the cell surface. Greater retention of receptors enhances responsiveness to cytokine ligands.

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