Modified Citrus Pectin:

Shekhar, M. P., P. Nangia-Makker, et al. (2004). "Alterations in galectin-3 expression and distribution correlate with breast cancer progression: functional analysis of galectin-3 in breast epithelial-endothelial interactions." Am J Pathol **165**(6): 1931-41.

> To define the role of galectin-3 in breast cancer progression, we have used a novel three-dimensional co-culture system that recapitulates in vivo reciprocal functional breast epithelial-endothelial cell-cell and cell-matrix interactions, and examined the expression of galectin-3 mRNA and protein in human breast tumors and xenografts. **Galectin-3 is required for the stabilization of epithelial-endothelial interaction networks because immunoneutralization with galectin-3 antibodies abolishes the interactions in a dose-dependent manner.** Co-culture of epithelial cells with endothelial cells results in increase in levels of secreted galectin-3 and presence of proteolytically processed form of galectin-3 in the conditioned media. In contrast, intracellular galectin-3 predominantly exists in the intact form. This difference in sensitivity to proteolytic processing of secreted versus intracellular galectin-3 probably arises from differences in accessibility of protease-sensitive sites, levels, and/or type of activated protease(s), and may be indicative of different functional roles for intact and processed galectin-3. To determine whether the proteolytically cleaved galectin-3 retains its ability to bind to endothelial cells, binding assays were performed with the full-length and matrix metallopeoteinase-2-cleaved recombinant galectin-3. Although a dose-dependent increase in binding to human umbilical vein endothelial cells was observed with both full-length and cleaved galectin-3, proteolytically cleaved galectin-3 displayed approximately 20-fold higher affinity for human umbilical vein endothelial cells as compared to the full-length protein. Examination of galectin-3 expression in breast tumors and xenografts revealed elevated levels of galectin-3 mRNA and protein in the luminal epithelial cells of normal and benign ducts, down-regulation in early grades of ductal carcinoma in situ (DCIS), and re-expression in peripheral tumor cells as DCIS lesions progressed to comedo-DCIS and invasive carcinomas. These data suggest that galectin-3 expression is associated with specific morphological precursor subtypes of breast cancer and undergoes a transitional shift in expression from luminal to peripheral cells as tumors progressed to comedo-DCIS or invasive carcinomas. Such a localized expression of galectin-3 in cancer cells proximal to the stroma could lead to increased invasive potential by inducing novel or better interactions with the stromal counterparts.

Honjo, Y., P. Nangia-Makker, et al. (2001). "Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells." Clin Cancer Res

7(3): 661-8.

Galectin-3 is an endogenous beta-galactoside-binding protein with specificity for type I and II ABH blood group epitopes and poly-N-acetyllactosamine glycan-containing cell surface glycoproteins and is the major nonintegrin cellular laminin-binding protein. Galectin-3 is expressed at an elevated level in a wide range of neoplasms, and expression was shown to be associated in some tumor cell systems with metastases**. Here we determined the functional consequence of blocking galectin-3 expression in highly malignant human breast carcinoma MDA-MB-435 cells.** Inhibition of galectin-3 expression led to reversion of the transformed phenotype as determined by altered morphology, loss of serum-independent growth, acquisition of growth inhibition properties by cell contact, and abrogation of anchorage-independent growth. **The blockage of galectin-3 expression led to a significant suppression of tumor growth in nude mice.** These results provide direct evidence that galectin-3 expression is necessary for the maintenance of the transformed and tumorigenic phenotype of MDA-MB-435 breast carcinoma cells.

Nangia-Makker, P., Y. Honjo, et al. (2000). "Galectin-3 induces endothelial cell morphogenesis and angiogenesis." Am J Pathol **156**(3): 899-909.

Increasing evidence suggests that carbohydrate-binding proteins play an essential role in tumor growth and metastasis. However, conflicting results on their function in the regulation of cell proliferation and differentiation during angiogenesis have been reported. We have examined the role of galectin-3 in the regulation of human umbilical vein endothelial cell proliferation, differentiation, migration, and neovascularization. Galectin-3, a carbohydrate-binding protein, with specificity for type 1 and 11 ABH blood group epitopes and polylactosamine glycan containing cell surface glycoproteins, is the major nonintegrin cellular laminin-binding protein. Because galectin-3 expression was shown to be associated in some tumor systems with metastasis, we questioned whether it induces endothelial cell morphogenesis. Here we show that galectin-3 affects chemotaxis and morphology and stimulates capillary tube formation of HUV-EC-C in vitro and angiogenesis in vivo. **Endothelial cell morphogenesis is a carbohydrate-dependent process, as it is neutralized by specific sugars and antibodies. These findings demonstrate that endothelial cell surface carbohydrate recognition event(s) can induce a signaling cascade leading to the differentiation and angiogenesis of endothelial cells.**

Bresalier, R. S., N. Mazurek, et al. (1998). "Metastasis of human colon cancer is altered by modifying expression of the beta-galactoside-binding protein galectin 3." Gastroenterology **115**(2): 287-96.

BACKGROUND & AIMS: Galectin 3 is a beta-galactoside-binding protein

whose expression has been correlated with advanced tumor stage in the colon, but direct evidence for a role in metastasis is lacking. The current study was designed to more directly establish the role of galectin 3 in colon cancer metastasis. METHODS: Galectin 3 levels were manipulated in human colon cancer cells using eukaryotic expression constructs designed to express the complete galectin 3 complementary DNA in either the sense or antisense orientation. Liver colonization was assessed in athymic mice after splenic-portal inoculation or after spontaneous metastasis during cecal growth. RESULTS: Introduction of galectin 3 antisense into metastatic colon cancer cells (LSLiM6, HM7) resulted in a significant reduction in galectin 3-specific messenger RNA and total and cell surface galectin 3 protein. Conversely, stable integration of galectin 3 in the sense orientation resulted in an increase in cellular and cell surface galectin 3 in cells of low metastatic potential (LS174T). Reduction in galectin 3 levels was associated with a marked decrease in liver colonization and spontaneous metastasis by LSLiM6 and HM7 cells, whereas up-regulation of galectin 3 resulted in increased metastasis by LS174T cells. CONCLUSIONS: **This study provides direct evidence that galectin 3 plays an important role in colon cancer metastasis.**

Warfield, P. R., P. N. Makker, et al. (1997). "Adhesion of human breast carcinoma to extracellular matrix proteins is modulated by galectin-3." Invasion Metastasis **17**(2): 101-12.

In this report, we have analyzed the adhesive interactions of a breast carcinoma cell line, BT-549, and its galectin-3-transfected subclone 11-9-1-4 with laminin, collagen IV and fibronectin. We determined that 11-9-1-4 cells adhered much more rapidly (within 1 h of plating) to laminin- and collagen IV-coated wells than the galectin-3 null expressing BT-549 cells. However, after 24 h, both cell lines fully adhered to laminin and collagen IV. Both cell lines also achieved maximum adhesion to fibronectin within 30 min. Not only did 11-9-1-4 express galectin-3 in the usual punctate pattern on its cell surface, it demonstrated a higher surface expression of alpha 6 beta 1 integrin compared to BT-549. The 11-9-1-4 cells were able to invade through matrigel-coated polycarbonate filters at approximately 3 times the rate of BT-549 parental cells. **Our data suggest that galectin-3 is essential for adhesion to laminin and collagen IV but not fibronectin by breast carcinoma cells. In addition, galectin-3 expression may modulate the surface expression of some of the integrins specific for laminin and collagen IV adhesion and invasion of basement membrane by breast carcinoma cells.**

Inohara, H. and A. Raz (1994). "Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions." Glycoconj J **11**(6): 527-32.

Citrus pectin (CP) and pH-modified citrus pectin (MCP) are highly branched and non-branched complex polysaccharides, respectively, rich in galactoside residues, capable of combining with the carbohydrate-binding domain of galectin-3. We reported previously that intravenous injection of B16-F1 murine melanoma cells with CP or MCP into syngeneic mice resulted in a significant increase or decrease of lung colonization, respectively (Platt D, Raz A (1992) J Natl Cancer Inst 84:438-42). Here we studied the effects of these polysaccharides on cell-cell and cell-matrix interactions mediated by carbohydrate-recognition. MCP, but not CP, inhibited B16-F1 melanoma cells adhesion to laminin and asialofetuin-induced homotypic aggregation. Both polysaccharides inhibited anchorage-independent growth of B16-F1 cells in semisolid medium, i.e. agarose. These results indicate that carbohydrate-recognition by cell surface galectin-3 may be involved in cell-extracellular matrix interaction and play a role in anchorage-independent growth as well as the in vivo embolization of tumour cells.

Pienta, K. J., H. Naik, et al. (1995). "Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin." J Natl Cancer Inst **87**(5): 348-53.

BACKGROUND: Prostate cancer is the most common cancer diagnosed in U.S. men and remains incurable once it has metastasized. **Many stages of the metastatic cascade involve cellular interactions mediated by cell surface components, such as carbohydrate-binding proteins, including galactoside-binding lectins (galectins). Modified citrus pectin (pH-modified), a soluble component of plant fiber derived from citrus fruit, has been shown to interfere with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules.** PURPOSE: The aim of this study was to determine whether modified citrus pectin, a complex polysaccharide rich in galactosyl residues, could inhibit spontaneous metastasis of prostate adenocarcinoma cells in the rat. METHODS: The ability of modified citrus pectin to inhibit the adhesion of Dunning rat prostate cancer MAT-LyLu cells to rat endothelial cells was measured by 51Cr-labeling. Modified citrus pectin inhibition of MAT-LyLu cell anchorage-independent growth was measured by colony formation in agarose. The presence of galectin-3 in rat MAT-LyLu cells and human prostate carcinoma was demonstrated by immunoblotting and immunohistochemistry. One million MAT-LyLu cells were injected subcutaneously into the hind limb of male Copenhagen rats on day 0. Rats were given 0.0%, 0.01%, 0.1%, or 1.0% (wt/vol) modified citrus pectin continuously in their drinking water (from day 4 until necropsy on day 30). The number of MAT-LyLu tumor colonies in the lungs were counted. RESULTS: Compared with 15 or 16 control rats that had lung metastases on day 30, seven of 14 rats in the 0.1% and nine of 16 rats in

the 1.0% **modified citrus-pectin group had statistically significant (two-sided; P < .03 and P < .001, respectively) reductions in lung metastases.** The lungs of the 1.0% modified citrus pectin-treated rats had significantly (two-sided; P < .05) fewer metastatic colonies than control groups (9 colonies +/- 4 [mean +/- SE] in the control group compared with 1 colony +/- 1 in the treated group). **Modified citrus pectin had no effect on the growth of the primary tumors.** In vitro, modified citrus pectin inhibited MAT-LyLu cell adhesion to rat endothelial cells in a time- and dose-dependent manner as well as their colony formation in semisolid medium. CONCLUSIONS: **We present a novel therapy in which oral intake of modified citrus pectin acts as a potent inhibitor of spontaneous prostate carcinoma metastasis in the Copenhagen rat.** IMPLICATIONS: Further investigations are warranted to determine the following: 1) the role of galectin-3 in normal and cancerous prostate tissues and 2) the ability of modified citrus pectin to inhibit human prostate metastasis in nude mice.

Guess, B. W., M. C. Scholz, et al. (2003). "Modified citrus pectin (MCP) increases the prostate-specific antigen doubling time in men with prostate cancer: a phase II pilot study." Prostate Cancer Prostatic Dis **6**(4): 301-4.

This trial investigated the tolerability and effect of modified citrus pectin (Pecta-Sol) in 13 men with prostate cancer and biochemical prostate-specific antigen (PSA) failure after localized treatment, that is, radical prostatectomy, radiation, or cryosurgery. A total of 13 men were evaluated for tolerability and 10 for efficacy. Changes in the prostate-specific antigen doubling time (PSADT) of the 10 men were the primary end point in the study. **We found that the PSADT increased (P-value<0.05) in seven (70%) of 10 men after taking MCP for 12 months compared to before taking MCP. This study suggests that MCP may lengthen the PSADT in men with recurrent prostate cancer.**