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Progress Report
From 8-15-2006 to 8-14-2007

DOD Concept Award BC046411 "Filling in the gap in galectin-1 export"

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Introduction: Many of the eukaryotic secreted proteins generally contain a leader- or export sequence, which directs their synthesis to the endoplasmic reticulum (ER). The secreted proteins are cotranslationally inserted into the ER lumen and are then trafficked to the Golgi and transport vesicles, which fuse with the plasma membrane to release their content to the extracellular space. Interestingly, galectins, fibroblast growth factor (FGF) 1 and 2 (1), interleukin (IL) 1b (2), the human immunodeficiency virus-1 (HIV-1) transactivating protein TAT (3) and the Herpes virus structural protein VP22 (4) are examples of proteins that do not contain leader sequence. While the mechanism of secretion of proteins lacking signal sequence is unknown, IL-1b, FGF-1 and FGF-2 appears to utilize shedding of membrane as vesicles as modules in their secretion (5). HIV-1 TAT, however, seems to be transported directly across the plasma membrane. However, the mechanism by which galectin-1 is secreted is unknown, which is described "non-conventional".

Galectins are a family of lectins that are widely expressed in the animal kingdom, ranging from *Caenorhabditis elegans* to human (6-8). As many as 15 mammalian galectins, termed galectin-1, -2, -3 and others have been identified and characterized in different cancers (8). A closer inspection of the NCBI database indicates that many of these galectins are transcribed as different isoforms, perhaps splice-variants, thus markedly increasing the number of galectins expressed in cells. Galectins are relatively small proteins with molecular masses ranging from 14.5 kDa to 38 kDa, and characteristically contain conserved ~130 amino acid stretch of carbohydrate recognition domain (CRD) (9, 10). Among all the known galectins, galectin-1 is well characterized, and is a prototype of the galectin family. In addition to the carbohydrate-binding ability, galectin-1 associates with proteins through protein-protein interactions and participates in a variety of oncogenic processes including transformation and proliferation, cell cycle regulation, cell adhesion, metastasis, inhibition and promotion of programmed cell death and also promotion of apoptosis in activated T cells (11, 12). Numerous ligands for galectin-1 have been described in different tissues and cells that include actin, laminin, fibronectin, vitronectin, integrins, CA-125, H-ras, CD45 and gemin-4 (13-17). Galectin-1 is expressed in a variety of cell types including breast epithelial cells, thymic epithelial cells, endothelial cells, dendritic cells, macrophages, fibroblasts and bone marrow cells (7, 18-22). There is an increased galectin-1 expression in many cancer types including colon, breast, ovary and prostate carcinomas and aggressive gliomas and increased accumulation of galectin-1 in stroma surrounding tumors in ovarian and prostate carcinoma (7, 23-27). Other galectins, specifically galectin-3 and galectin-8, are also involved in a variety of cellular and carcinogenic processes similarly as galectin-1.

Galectins are found intracellularly, extracellularly as well as in the nucleus.

Galectins have distinctly different and contradictory roles in cancers. Galectins bind to a wide array of glycoproteins and glycolipids both on the cell surface and in extracellular matrices. Thus the physiological roles of the extracellular galectins include cell-cell adhesion, cell-extracellular matrix adhesion, and metastasis. Studies indicate that galectins have important roles in cancer as these molecules contribute to neoplastic transformation, tumor cell survival, angiogenesis and tumor metastasis. Galectins are shown to modulate the immune and inflammatory responses and might have a key role helping tumors to escape immune surveillance by the cytotoxic tumor infiltrating T cells.

Since galectins are important molecules involved in the above-mentioned oncogenic processes, we proposed to identify the molecular mechanisms involved in the galectin-1 secretion.

BODY:

The specific aim of the proposed research was to identify traffic proteins that interact with galectin-1 protein by the yeast two-hybrid analysis. To accomplish this goal, the proposed experiments were carried out, which are described below.

Experiments:

Bait. The human Galectin-1 cDNA was kindly given by Dr. Linda Baum (UCSF). Since the N-terminus of secreted and membrane proteins that are being synthesized interacts with the signal recognition particle, translocon and other proteins in the traffic machinery in the ER, we predicted that the N-terminal region of galectin-1 will be recognized by these proteins. Thus, we chose the first 40 amino acid stretch of galectin-1 as bait. The cDNA encoding the 1-40 amino acid stretch of galectin-1 was amplified using forward primer, 5'-GACCTGCATATGGCTTGTGGTCTGGT-3' and reverse primer, 5'-GGCCGCTGCAGGGCTGTCTTTGCCAGGT-3' and the human Galectin-1 cDNA as template. The amplified 120 bp fragment codes for the 1-40 amino acid stretch of galectin-1 (Gal1-40) was then subcloned into pGBKT7 vector that contained the Gal4 DNA binding domain (Gal4 DB). This plasmid DNA was transformed into yeast strain, AH109, and plated on SD without tryptophan (*trp*⁻) plates and isolated the bait strain. Expression of Gal4-BD-Gal1-40 protein was confirmed by western blotting. Self-activation was not found with the Gal1-40 in the yeast, which suggested that the bait is suitable for the yeast hybrid analysis.

Prey library and preparation. The human mammary gland cDNA library subcloned into pGADT7 vector containing Gal4 activating domain and transformed into E.coli was purchased from Clontech. Large scale DNA was prepared from this E.coli using Qiagen plasmid DNA preparation kit (Qiagen, CA).

Yeast 2-Hybrid analysis. To screen for the possible interactive proteins, we used the MatchMaker 2-Hybrid system 3 kit from Clontech, by following the protocol supplied by the manufacturer. Briefly, the above mentioned bait strain was retransformed with human mammary gland cDNA plasmid library, prepared as above. The transformants were plated on a quadruple (*Leu*⁻, *Trp*⁻, *Ade*⁻ and *His*⁻) dropout SD plates (a total of 50) and

grown at 30° C for 3-6 days. About 59 positive interactive clones with transcriptional activation were isolated from the plates. All of these positive clones were re-plated on SD-quadruple dropout agar plates. All the clones were able to grow again on these plates. The library plasmid DNAs from these clones were prepared using Yeast plasmid Isolation kit (YEASTMAKER from Clontech), followed by amplification in E.coli. Although we proposed slightly different methodology for identifying proteins involved in the protein trafficking, we adapted the conventional method of sequencing every plasmid obtained in this study. The main reason for this change in the methodology is that we did not obtain large number of interactors. Since 59 plasmids can be easily sequenced, we sent out all of the library plasmid DNAs for sequencing to the DNA sequencing Facility at the University of Nebraska, Lincoln, NE, on pay per service basis. A homology search in the NCBI database was performed using the DNA-DNA BLAST service to identify the full length interactive proteins and predict their biological roles.

Results.

Sequencing the library plasmids and homology search with NCBI database revealed few interesting proteins that shed new light on the biology of galectin-1, which are described below.

Identifier (accession number)	Protein	Known function	Possible role in galectin- biology
BC006206	NIK and IKK(b) binding protein (NIKb)	NF-kB activation	Provides clues on the role of galectin- in apoptosis
AK223038	Cathepsin C isoform	unknown	unknown
BC003672	Fatty acid binding protein 4 (FABP4)		unknown
NM_001863	Cytochrome c oxidase subunit VI polypeptide 1		unknown
BC001693	Galectin-1	Cell-cell and –matrix interactions, immunosuppression and other cellular functions	Suggests that galectin-1 is a dimmer
AB064059	IgK light chain		unknown
BC0667111	Filamen A alfa (actin binding protein)		unknown
NM_001457	Filamen B beta (actin binding protein 278)		unknown
NM_001039919	Zinc finger protein 384		Unknown
XP_511331	Epsin 2	Protein trafficking	Unknown

NM_080545.1	Adaptor-related protein complex 1, gamma 2 subunit (AP1G2), variant 2	components of clathrin-coated vesicles transporting ligand-receptor complexes from the plasma membrane or from the trans-Golgi network to lysosomes.	Unknown
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NIK and IKK β binding protein (NIBP). The NF-kB is a transcription factor, and plays an important role in the expression of numerous genes involved in biological responses such as inflammation, immunity and apoptosis. Recently, it was shown that a protein termed NIBP, interacts with NIK and IKK β and potentiates NF-kB activation. The observation that galectin-1 interacts with NIBP raised a distinct possibility that galectin-1 is an upstream molecule, regulating the NF-kB pathway. To determine the specificity of galectin-1-NIBP interactions, we carried out the yeast 2-hybrid analysis using different bait vectors. Fig.1 shows that the galectin-1 and NIBP interactions are specific. Further studies on characterizing these interactions and the role of galectin-1 in NF-kB pathway will be addressed in a grant application.

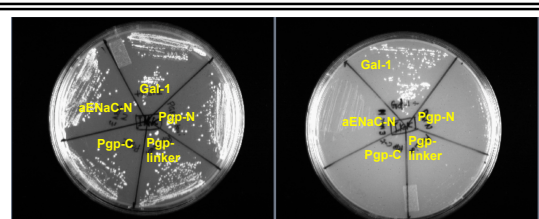


Fig.1. NIBP interactions with galectin-1. Various baits including galectin-1, N-terminus of Pgp (Pgp-N), linker region of Pgp (Pgp-linker), C-terminus of Pgp (Pgp-C), N-terminus of α -ENaC subunit (α -ENaC-N) were mated with NIBP in yeast. Growth in quadruple dropout plate (right) indicates interactions.

Adaptor-related protein complex 1, gamma 2 subunit (AP1G2), variant 2. Adaptor protein are important components of clathrin-coated vesicles transporting ligand-receptor complexes from the plasma membrane or from the trans-Golgi network to lysosomes/plasma membrane. To further confirm this interaction, we tested the specificity of galectin-1-AP1G2 interactions in yeast. Results presented in Fig.2 suggest that galectin-1 interacts with AP1G2. This observation suggests that galectin-1 is likely a secreted protein, which utilizes the secretory pathway. Further analysis will be carried out in the No Cost Extension period.

Epsin 2. Epsins are implicated in clathrin-mediated endocytosis. Epsin 1 and 2 are most similar in their NH₂-terminal region, which represents a module (epsin NH₂ terminal homology domain, ENTH domain) found in a variety of other proteins of the data base. The multiple DPW motifs, typical of the central region of epsin 1, are only partially conserved in epsin 2. Both proteins, however, interact through this central region with the clathrin adaptor AP-2. In addition, it has been shown

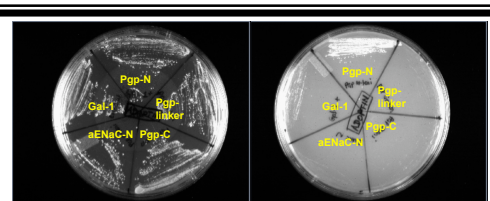


Fig.2. AP1G2 interactions with galectin-1. Various baits including galectin-1, N-terminus of Pgp (Pgp-N), linker region of Pgp (Pgp-linker), C-terminus of Pgp (Pgp-C), N-terminus of α -ENaC subunit (α -ENaC-N) were mated with AP1G2 in yeast. Growth in quadruple dropout plate (right) indicates interactions.

that both epsin 1 and 2 interact with clathrin. The three NPF motifs of the COOH-terminal region of epsin 1 are conserved in the corresponding region of epsin 2, consistent with the binding of both proteins to Eps15. Epsin 2, like epsin 1 is present in a brain-derived clathrin-coated vesicle fraction, is concentrated in the peri-Golgi region and at the cell periphery of transfected cells, and partially colocalizes with clathrin. Further analysis of galectin-1-epsin 2 interactions will be addressed.

Cathepsin C. cathepsin C, also known as dipeptidyl peptidase I (DPPI), is a lysosomal cysteine hydrolase expressed in most mammalian tissues. Studies have indicated a significant role for DPPI in thrombin regulation, fibronectin turnover, angiogenesis, acute experimental arthritis, cytotoxic lymphocyte-mediated apoptosis, and host immune defense. However, the role of galectin-1 in the function of DPPI is unclear at present.

Galectin-1 interacts with P-glycoprotein (Pgp).

Our laboratory has been investigating the mechanism of P-glycoprotein-mediated drug transport. To identify proteins that interact with Pgp, we carried out the yeast 2-hybrid analysis using prostate adenocarcinoma as a prey library. This analysis identified galectin-1 as an interactor of Pgp, specifically its N- and C-termini. Fig.3 shows the characterization of galectin-1 interactions with different regions of Pgp. The results show that galectin-1 interacts with N- and C-termini but not with the linker region. Since N- and C-termini are important for the function of Pgp, we hypothesize that these regions of Pgp form a part of the transport pore. If this is the case, then these results would suggest that galectin-1 is likely a transport substrate of Pgp. We will investigate this in the near future.

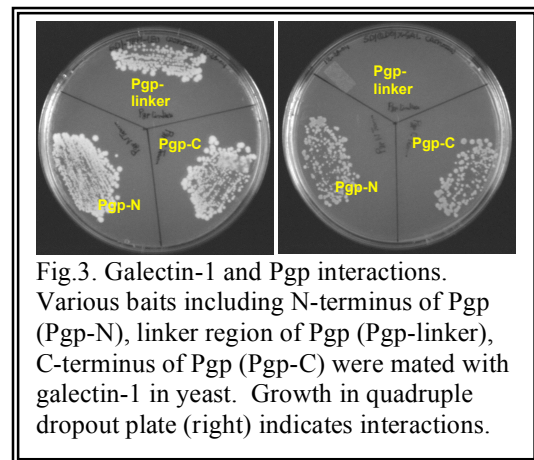


Fig.3. Galectin-1 and Pgp interactions. Various baits including N-terminus of Pgp (Pgp-N), linker region of Pgp (Pgp-linker), C-terminus of Pgp (Pgp-C) were mated with galectin-1 in yeast. Growth in quadruple dropout plate (right) indicates interactions.

Interaction between translocon-delta subunit and galectin-1. In the yeast 2-hybrid analysis, we isolated translocon subunit delta, as an interactor of Pgp. This interaction with translocon is not unexpected, as Pgp is a membrane protein and interacts with protein trafficking machinery during its biosynthesis. To test whether galectin-1 interacts with translocon, we carried the interactions between galectin-1 and translocon. Fig.4 shows that galectin-1 in fact interacts with translocon, as Pgp does, suggesting galectin-1 may be a secreted protein. Further characterization of galectin-1 interactions with trafficking machinery will

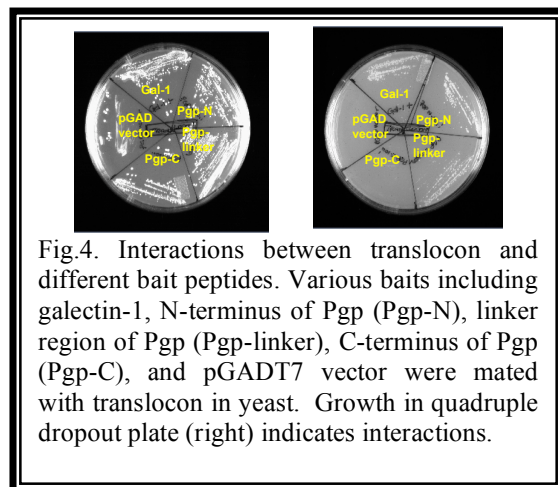


Fig.4. Interactions between translocon and different bait peptides. Various baits including galectin-1, N-terminus of Pgp (Pgp-N), linker region of Pgp (Pgp-linker), C-terminus of Pgp (Pgp-C), and pGADT7 vector were mated with translocon in yeast. Growth in quadruple dropout plate (right) indicates interactions.

be proposed in a new grant application.

Work carried out from August 15 2006 to August 14, 2007.

Following the above study, we focused on identifying a suitable cancer cell model system for studying galectin-1 secretion. Toward this end, we screened several cancer cell lines for the expression of galectin-1 as well as other galectin forms, and the results are summarized below.

Analysis of galectin expression in different cancers. We examined the expression of different galectins in several established cell lines using the RT-PCR. Representative amplified DNA fragments were subcloned into pGEMTeasy cloning vector and sequenced to confirm their identity. Fig.5 shows the amplification of ~0.4 kb galectin-1 cDNA and 0.3 kb galectin-3 cDNA, which were present in nearly similar amounts in all of the cancer cell lines. Galectin-4 cDNA was readily amplified from HT-29 cells, which was not detectable in other cell lines, perhaps due to low abundance. All of these cancer cell lines although contained galectin-7 transcript, the expression levels varied among the cell lines. Finally, the galectin-8-specific primers used in the PCR amplified two transcripts of 1077 and 951 bp in all of the cancer cell lines, and their amounts were nearly equal. DNA sequencing and alignment with NCBI database using BLAST server identified these two DNA fragments as galectin-8 isoform 1 (NM_201543) and isoform 2 (NM_201544), respectively.

To evaluate the expression levels of different galectins in each cell line, the RT-PCR mixtures of different galectins prepared as above were separated by agarose gel electrophoresis. Fig.6 shows the relative levels of different galectins in the cancer cell lines. The drug-sensitive MCF-7 cells and the drug-resistant MCF-7/Adr^R cells contained galectin-1, -3 and -8 significantly in higher amounts when compared to the levels of galectin-4 and -7. HT-29 cell line uniquely contained high levels of galectins-4 and -7 when compared to other cell lines. Galectins-4 and -7 were less abundant in HCT-116 and T98G cells. As controls, we analyzed the expression of these galectins in human embryonic kidney HEK-293 and fibroblast HFF-2 cells using the identical RT-PCR condition and the results show that these non-cancer cells transcribe galectins-1 and -3 but not other forms. Together, these results suggested that galectins are differentially transcribed in cancer cells and galectins-4, -7 and -8 are cancer-specific, which are absent in the non-cancer cell lines.

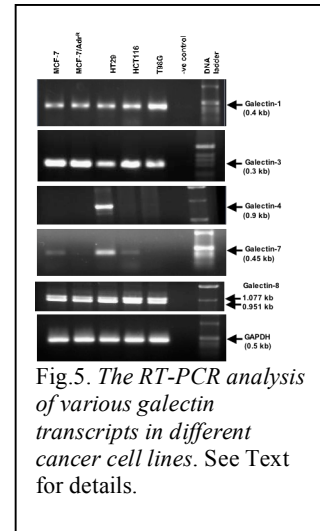


Fig.5. The RT-PCR analysis of various galectin transcripts in different cancer cell lines. See Text for details.

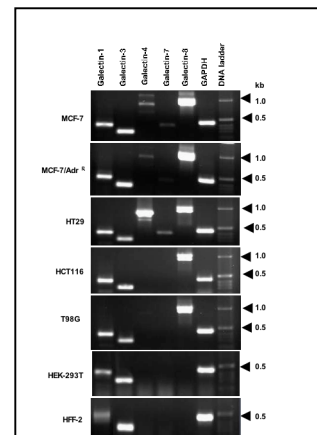
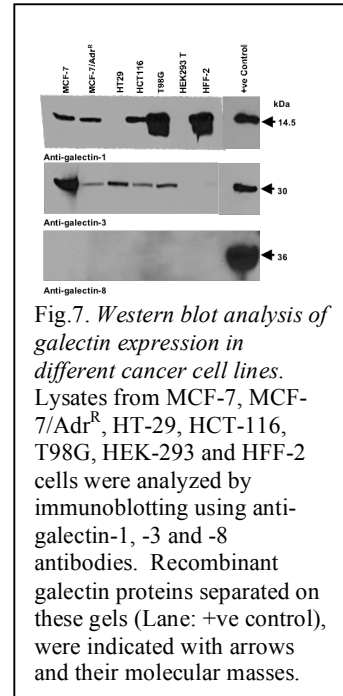


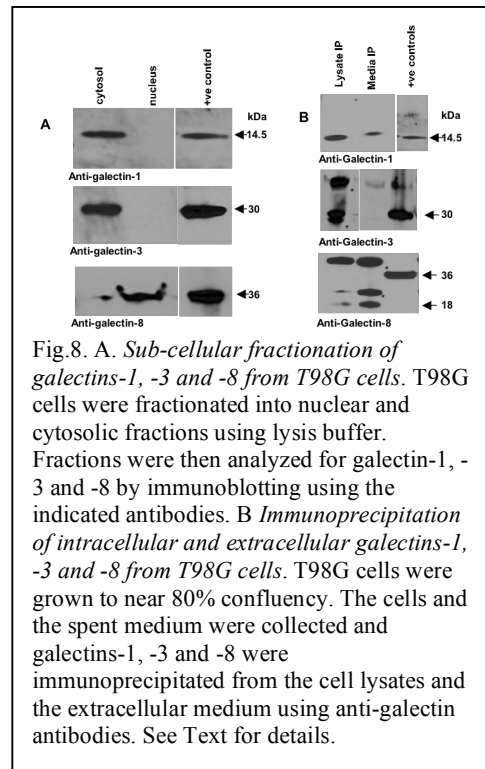
Fig.6. Comparative analysis of various galectins showing their relative amounts in different cancer cell lines. The RT-PCR mixtures of different galectin obtained from each cancer cell line shown in Fig.1 were separated on 1% agarose gel electrophoresis and the images were captured.

Immunoblotting analysis of galectins: To further determine the expression of galectins in the above cells, we analyzed the expression of galectins-1, -3 and -8, because recent studies indicate that these lectins play key roles in the oncogenic processes (28-30). Fig.7 shows that MCF-7 and MCF-7/Adr^R cells and HCT-116 cells contained the expected 14.5 kDa galectin-1 in nearly equal amounts, corroborating the above RT-PCR studies. Notice the significantly high levels of galectin-1 expression and its resolution into two species with molecular masses of ~14.5 and 13.5 kDa in T98G and HFF-2 cells, despite the similar levels of steady state gene transcripts in these cells, compared to that of other cell lines. This suggests that either the half-life or efficiency of translation of galectin-1 in T98G and HFF-2 cells might be higher. On the other hand, galectin-1 could not be detected in HEK-293 cells, although these cells contained significant levels of transcript, perhaps indicating the existence of translational repression mechanisms in these cells.



As expected from the transcription levels seen in Figs.5 and 6, the ~30 kDa galectin-3 was detectable in all the cancer cell lines at relatively similar amounts except for MCF-7 cells. However, galectin-3 was not detectable either in HEK-293 or HFF-2 cells, despite the presence of corresponding mRNA. Finally, the immunoblotting using monoclonal antibody to galectin-8 or the polyclonal antibodies did not indicate the presence of ~36 kDa galectin-8 in any of the cell lines, even after extended chemiluminescence. Since the recombinant galectin-8 was easily immunoreactive in these blots, these results suggest the absence of galectin-8 protein in any of the cell lines. From this analysis it is apparent that cancer cells exhibit fine control of lectin translation, and it is therefore essential to combine the RT-PCR analysis with immunoblotting profiles for assessing the function of the individual galectins.

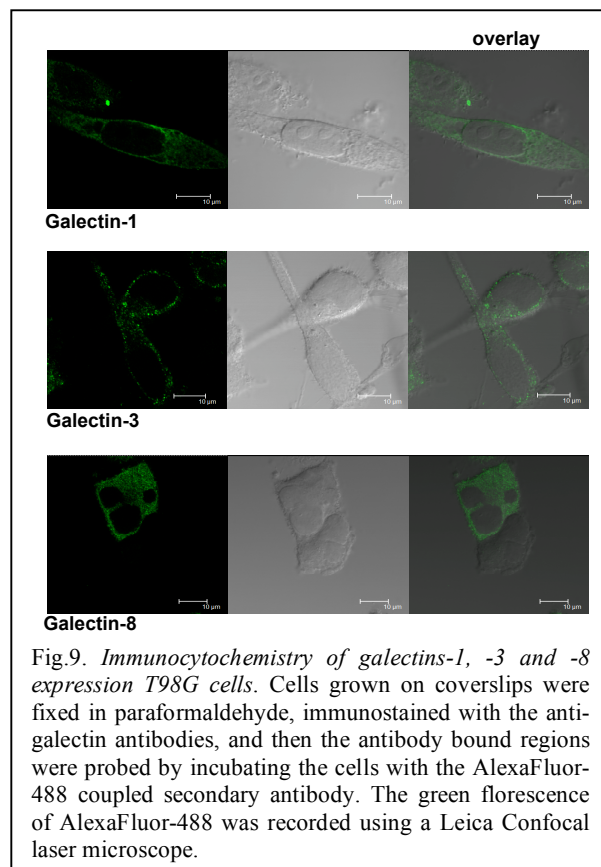
Cellular localization of galectins. Galectins are known to be localized in the cytosol, nucleus and in the extracellular space. Therefore, we characterized the subcellular location of galectins in T98G cells, which was used as a model for this analysis. T98G cells grown to ~80% confluency were fractionated into cytosol and nuclear fractions, and then analyzed



for the presence of different galectins by immunoblotting. Fig.8. shows that galectin-1 and -3 were present in the cytosolic fraction and absent in the nuclear fraction. Interestingly, when these subcellular fractions were analyzed for the presence of galectin-8, it was surprising to detect the ~36 kDa protein predominantly in the nuclear fraction, and to some extent in the cytosolic. The reasons for our failure to detect galectin-8 in the whole cell lysates by direct SDS-PAGE/immunoblotting analysis are unclear at present. Identification of galectin-8 in the cytosolic and nuclear fractions indicates that a cell fractionation procedure is a prerequisite for the detection of galectin-8.

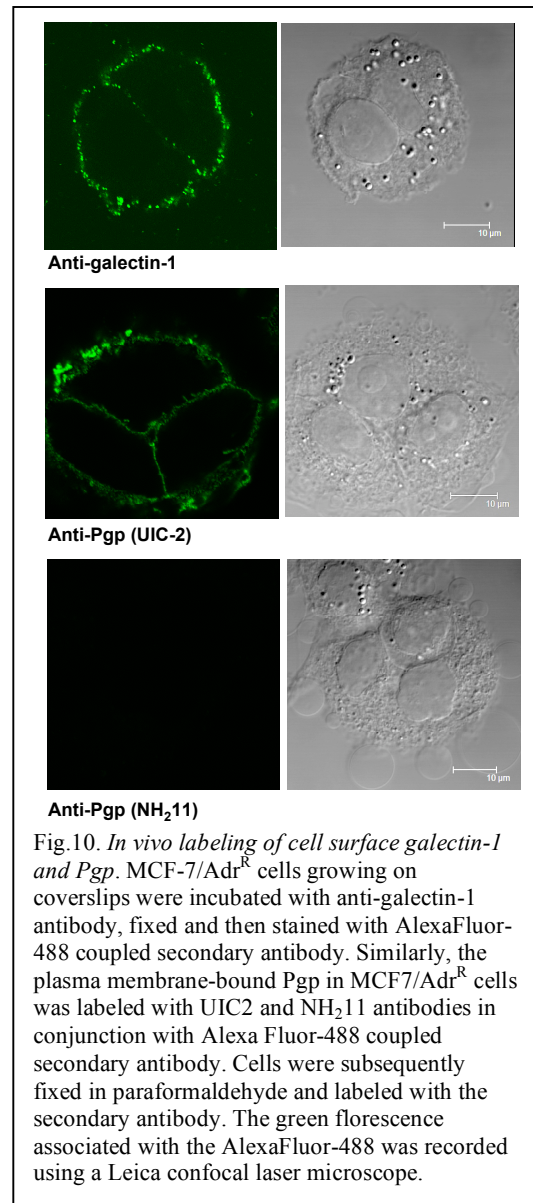
To determine if galectins are secreted into the extracellular space, T98G cells were grown to near 80% confluency and the spent medium was collected. Galectins in the spent medium were immunoprecipitated using anti-galectin antibodies. Fig.8 also shows that a significant amount of ~15 kDa protein immunoreactive with anti-galectin-1 was detectable in the spent medium, which is slightly higher in molecular mass, compared to the size of the galectin-1 immunoprecipitated from the total cell lysates. Since no protein was immunoprecipitated by anti-galectin-1 from the fresh growth medium (not shown), the ~15 kDa protein is either a different form or post-translationally modified form of galectin-1. On the other hand, the 30 kDa galectin-3 was not detectable in the immunoprecipitates obtained from the spent medium, which was however immunoprecipitated from the cell lysates. Although the ~36 kDa galectin-8 was not detected in the immunoprecipitates prepared either from the cell lysates or the spent growth medium, an ~18 kDa anti-galectin-8 immunoreactive protein was consistently detectable in both the cell lysates and extracellular medium. The identity of this ~18 kDa protein is unclear at present. These results together suggest that the intracellular galectins-1, -3 and -8 have the expected molecular masses. Because galectins detected in the extracellular medium are different from that of the expected, it is likely that the secreted galectins undergo further modification before or after secretion.

Immunocytochemistry of galectins. To further characterize the cellular localization of galectins-1, -3 and -8, T98G cells were grown on coverslips and fixed using paraformaldehyde. Cells were immunostained with anti-galectin antibodies in conjunction with AlexaFluor-488-coupled secondary antibody and then analyzed by



confocal laser microscopy. Fig. 9 shows that the AlexaFluor-488 fluorescence associated with galectins-1 and -3 was predominantly cytosolic and absent in the nucleus, supporting the results shown in Fig.4. Staining of cells with anti-galectin-8 indicated the presence of galectin-8 in different locations of different cells. Some cells contained galectin-8 exclusively in the cytosol, whereas, some cells contained immunofluorescence both in the nuclei and cytosol. Interestingly, some cells did not immunostain at all suggesting the absence of galectin-8 expression. Although the reasons for the complete absence of galectin-8 in some cells and, its differential subcellular distribution in different cells of the same cell line are unclear, it is likely that the expression of galectin-8 is finely regulated and appears cell cycle stage-dependent.

Since galectins are secreted into the extracellular space, it is likely that these lectins are bound to the carbohydrate moieties of the cell surface proteins and lipids. Thus, we tested the possibility that galectin-1 is bound to the extracellular surface by *in vivo* labeling of MCF-7/Adr^R cells. Pgp-expressing MCF-7/Adr^R cells growing on coverslips were incubated with anti-galectin-1 at 37° C for 45 min. Cells were fixed in buffer containing paraformaldehyde but lacking detergents and then immunostained with anti-galectin-1 in conjunction with AlexaFluor-488 coupled secondary antibody. Fig.10 shows that AlexaFluor-488 immunofluorescence was predominantly localized at the cell surface of MCF-7/Adr^R cells, suggesting that these cells secrete galectin-1, which was bound to the cell surface. To determine if the *in vivo* labeling methodology used here detects only the extracellular galectin-1, we immunostained these cells for Pgp, a plasma membrane protein using two well characterized antibodies: UIC2 and NH₂11. UIC2 antibody is known as a conformation-specific antibody that binds to the extracellular epitope (31), whereas, NH₂11 antibody recognizes an epitope in Pgp that is intracellularly located (32, 33). Thus, MCF-7/Adr^R cells growing on coverslips were immunostained with UIC2 and NH₂11 antibodies for 45 min as described above, and the location of these antibodies was determined by confocal laser microscopy analysis. Fig. 6 shows that UIC2-bound immunofluorescence was present exclusively at the cell surface. On the other hand, no



immunofluorescence was detectable when NH₂11 was used under identical experimental conditions. We have previously shown that immunostaining of Pgp with NH₂11 requires a prior permeabilization of cells with detergents (32), and failure to immunostain Pgp with NH₂11 in the absence of detergents, together suggested that immunostaining procedures used in this study selectively immunostains protein epitopes present at the exposed cell surface. These data also indicate that the antibodies are not internalized. Taken together, these results suggested that these cancer cells secrete galectin-1, which is bound to the extracellular surface.

Key Research Accomplishments:

- Adaptor-related protein complex-1, gamma 2 subunit (AP1G2); epsin-2 and translocon subunit delta were isolated as interacting proteins of galectin-1.
- The N- and C-termini of P-glycoprotein interact with galectin-1.
- NIBP, an intermediate of NF- κ B pathway interacts with galectin-1.
- Several cancer cell lines secrete galectin-1.
- Several cancer cell lines are identified as potential model systems for studying galectin-1 secretion.

Reportable outcomes:

Poster and oral presentations:

“Differential expression of galectins and confocal analysis of galectin-1 in the human cancer cell lines” Arun Satelli and U.S. Rao (2007) Phage Therapy week – Research Days Texas Tech University Health Sciences Center, Lubbock, TX. (poster presentation)

“Differential expression of galectins in cancer” Arun Satelli and U.S. Rao (2007) Cancer Symposium, Texas Tech University Health Sciences Center, Lubbock, TX. (poster presentation)– Lubbock.

“Varied expression and localization of multiple galectins in different cancer cell lines” Arun Satelli and U.S. Rao (2007) Research Days, Texas Tech University Health Sciences Center, Amarillo, TX. (poster presentation).

Toward elucidating the role of galectin-1 in cancer. Arun Satelli and U.S. Rao (2007) oral presentation. Texas Tech University Health Sciences Center, Amarillo, TX.

Manuscripts:

Varied expression and localization of multiple galectins in different cancer cell lines
Arun Satelli, P.S. Rao, P.K. Gupta, P.R. Lockman, K.S. Srivenugopal, and U.S. Rao.
Submitted.

Conclusions:

As mentioned in the Introduction, the mechanism of galectin-1 secretion is unknown. Our observations that galectin-1 interacts with several proteins that include (i) translocon subunit delta, (ii) adaptor-related protein complex 1, gamma 2 subunit (AP1G2), and (iii) epsin-2 clearly suggest that we have identified a potential pathway of galectin-1 secretion. This pathway is likely the conventional ER-Golgi secretory pathway. Interestingly, this ER-Golgi secretory pathway was not found to be the pathway in the galectin-1 secretion, in the earlier studies. While we are cognizant of limitations associated with yeast two hybrid analysis, the identification of secretory pathway intermediate proteins such as translocon, epsin and adaptor proteins, raises a distinct possibility that galectin-1 may utilize the conventional secretory pathway. As a future study, we will determine if galectin-1 secretion employs the conventional secretory pathway.

Importantly, we also observed that galectin-1 interacts with P-glycoprotein, a drug pump that is known to extrude small peptides. This is important observation, which suggests a mechanism by which galectin-1 is secreted directly from the plasma membrane.

Taken together the above observations suggest that galectin-1 may adapt different mechanisms of secretion, depending on the presence and absence of active transporter in the cancers.

The other observations that galectin-1 interacts with NIBP provides insights into the mechanisms by which galectin-1 regulates NF-kB pathway. It is therefore likely that galectin-1 regulates the apoptotic and proliferative pathways by regulating the function of NIBP intermediate in this signaling pathway.

We now identified several cancer cell lines expressing different galectins, which will serve as model systems for studying galectin secretion.

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Appendices:

None.