Indian Journal of Clinical Biochemistry, 2003, 18 (2) 169-180

CHARACTERIZATION OF LECTINS AND THEIR SPECIFICITY IN CARCINOMAS- AN APPRAISAL

Asma Farhat Sherwani, Sameena Mohmood, Fauzia Khan, Rizwan Hasan Khan* and Md. Asim Azfer

*Department of Zoology, AMU. Aligarh, 202 002 India, * Interdisciplinary Biotechnology Unit, AMU. Aligarh, 202 002 India*

ABSTRACT

Lectins, a group of specific glycoproteins present in animal as well as plant cells, are used as differentiating markers to study cancers and metastatic cell lines. This property of lectins depends on the process of cellular glycosylation. Glycosylation of some of the extracellular membrane proteins and lipids maintains the cell/cell and cell/matrix interactions. Chemical alterations in glycosylation play an important role in the metastatic behavior of tumor cells. Carbohydrate residues of the membrane glycoproteins can be detected using lectins due to their binding specificity to carbohydrates. Lectins, therefore have gained an importance in the field of cancer research. Galectins, a specialized group of lectin like proteins that are Ca+ independent and galactoside binding, are also considered as differentiation markers in some specific cancers like the carcinomas of thyroid.

Thus the use of lectins and galectins to identify specific carbohydrates present on cell surface help in invasion and metastasis processes.

KEY WORDS

lectins, metastasis, cellular glycosylation, prognostic markers, galectins.

INTRODUCTION

Cell division and differentiation of a single cell leads to the multicellularity and adulthood. Any change or aberration in this regulatory system of cell division and differentiation may lead to autonomous cell multiplicity giving rise to malignancy defining a cancerous/ neoplastic growth. The common neoplasms of breast cancer, lung cancer and colon cancer are a challenge in both the developed as well as developing countries of 20th century. In United States mortality associated with lung and bronchus cancer among women continues to increase and lung cancer is expected to account for 25% of all female cancer deaths in the year 2000 (1). Increased mortality rate due to cancer is also reported from various developing countries like Singapore (2), India (3), Thailand (4) etc.

Despite this degree of penetrance, cancer therapy is not successful mainly due to the lack of appropriate treatment once the cancer has spread. The formation of metastasis and it's proliferation, i.e., migration of neoplastic cells to different body parts via blood stream and lymph channels, is therefore most important in determining the fate of cancer patients. The process of metastasis formation is multistep (5) and each step involves cell to cell and cell to matrix interactions (6). Hence to understand and control this proliferative disease, new investigations have recently been shifted to these interactions (6). As both the outer surfaces of the cell membrane and the extra cellular matrix consist mainly of glycoconjugates, altered carbohydrate residues of these membrane glycoproteins due to neoplastic invasion can be detected using protein markers like lectins. This could create new insight into the process governing the metastatic cascade (6). In a study, inhibition of proliferation and induction of differentiation of glioma cells was found with a plant lectin, *Datura stramonium* agglutinin suggesting that this lectin may be useful as a new therapy for treating glioma without side effects (7). Modern research in this area showing some promising results is thus related to glycoproteins and lectins.

Address correspondence:

Dr. Md. Asim Azfer
Section of Genetics
Department of Zoology
AMU. Aligarh 202002, India
E-mail: azfer@mailcity.com

Lectins are indigenously found proteins or glycoproteins that are known for their carbohydrate binding specificities (8). This binding property makes them remarkable markers that are used in the studies involving histochemical, biochemical and functional techniques to characterize and differentiate cancer cells. In this review histochemically worked out lectin specificity to various carcinomas is taken into account using clinical material.

HISTOCHEMICAL IDENTIFICATION OF LECTINS:

Tumors derived from epithelial tissues showing glandular differentiation (tumor of colon, breast and female genetalia) are mostly used for the study of lectin histochemistry. Neoplasias of human breast and colon are mainly used to study the histochemical behavior of a plant lectin leukoagglutinin (L-PHA) and it was found that L-PHA-reactive beta 1-6 branched N-linked oligosaccharides are consistently increased in neoplasias of human breast and colon and that the level of L-PHA staining correlates with the pathological staging of the diseases (9).

Glands are differentiated as serous and mucous by analyzing their secretary product. Serous glands are negative for general carbohydrate stain and the Periodic Acid Schiff's reagent (PAS) reaction, while mucous glands are PAS positive. Their lectin binding patterns also reflect this property. Accordingly, lectins that recognize the N-linked sugars in the cell membrane glycocalyx bind with serous glandular cells. Such lectins include Concanavalin-A (Con A), Phytohaemagglutinin-L (PHA-L) etc. On the other hand lectins that identify O-linked glycoprotein bind to mucous cells. These lectins are either N-acetylgalactosamine specific (Soyabean agglutinin-SBA, *Dolichos biflorus* agglutinin-DBA and *Helix pomatia* agglutinin-HPA) or Fucose specific (*Ulex europaeus* agglutinin-UEA-I) (Table 1). Cell surface carbohydrate chains of human germ cell tumors were investigated histochemically using peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin-I (UEA-1), and anti- (Ma) antibody. Peanut agglutinin, a lectin specific for terminal beta-galactosyl residues, bound to the surface of tumor cells, *D. biflorus* agglutinin, was found to be specific for terminal alpha-N-acetyl galactosamine residues, and *U. europaeus* agglutinin, was tested to bound to terminal alpha-L-fucosyl residues (10). These lectins can thus be used as prescribed histochemical reagents for the study of mucin like carbohydrate residues in glandularly differentiated cancers.

It has become apparent that lectins with similar carbohydrate specificities can show different patterns of reaction when applied to different tissue sections. This alteration may result due to slight differences in specificity and /or structural characteristics of the tissue glycocalyx or the lectin (11). In tumor cells alterations in cellular glycosylation may play a key role in metastatic behavior of tumor cells. The three studied metastatic cell lines, LOX (malignant melanoma), FEMX (malignant melanoma) and MA-11 (mammary carcinoma) have a very different metastatic behavior In vivo, and different glycans have been suggested to bring about these differences (12). Glycosylation, the most frequent biochemical alteration associated with tumorogenesis, modulates cell adhesion regulated by the integrins, the oncodevelopmental carbohydrate epitopes, which are altered in their expression as well as properties when transformation occurs. In the studied cell lines (T-24, Hu456, HCV29T-from human bladder carcinoma and HCV29, HCV29T- from human normal ureter and bladder epithelium) alpha3beta1 integrins were glycosylated, although in general each subunit is glycosylated differently (13). Positive reaction of alpha3beta1 integrin with *Phaseolus vulgaris* agglutinin and *Datura stramonium* agglutinin was found in cancerous cell lines (T-24, Hu456) as well as in normal bladder epithelial cells (Hu609). The changes in glycosylation profile attributed to invasive phenotype are associated with alpha3 not beta1 subunit (13).

MARKER LECTINS AND THEIR SPECIFICITIES TO GLANDULARLY DIFFERENTIATED CANCERS:

Various lectins have been studied for their identificational associations with different types of glandularly differentiated cancers like breast cancer, colorectal cancer, hepatopancreatic carcinomas, cancers of genitourinary tract, prostrate and lung cancers etc. (Table 2). This identification is proved to be very useful in detecting and distinguishing different types of adenomas and carcinomas thus to add a help in their treatment therapy.

1. Breast cancer specific lectins

Lectins are good markers and are well suited to the study of changes in carbohydrate expression in the

development of breast cancer. Incomplete glycosylation is considered to be the cause of carbohydrate alteration. The changed expression of a carcinoma-associated carbohydrate core epitope (like Tn) could be due to blockage at the earliest steps of oligosaccharide chain synthesis (14).

Klein et al. (1981, 1983) worked out the lectin histochemical association with breast cancer for the first time (15, 16). These studies indicate that the expression of binding sites for Peanut agglutinin (PNA) in breast cancer was correlated with the steroidal hormone receptor estimation (15). Some latter studies of breast cancer did reveal differences in lectin binding between normal and hyperplastic breast tissue and that in breast cancer (17), PNA – binding, as a prognostic indicator was not accepted widely.

Roman snail lectin, *Helix pomatia* agglutinin (HPA) when tested was proved to be quite successful histochemically (Table 2). Specific correlation between HPA-binding and axillary lymph node metastasis was reported (18, 19) and then clarified in two subsequent abstracts. Leathem's group published two more papers (20, 21) showing a correlation between HPA-binding to breast cancer cells and patient's prognosis. SCID (severe combined immunodeficient) mice were used as test systems to workout the correlation between metastatic potential and HPA-binding during the study (22). For the prognosis, the status of the lymph node is most important i.e., metastases are present in the local breast draining lymph nodes. The ability of the metastatic cells to bind HPA set a positive result as HPA-binding glycoconjugates are also found in most brain metastasis (23) and a similar correlation between the occurrence of metastatic tumor cells in local lymph nodes and HPA-binding of the primary tumor was found for colorectal cancer. This particular feature of PHA was not found with other lectins (24). HPA-binding shows similarities with the result of the Duke's classification that also uses involvement of lymph node status as a risk assessment factor (25).

Several groups propose HPA as prognostic lectin for human breast cancer (26 – 28) or at least of limited prognostic value (29, 30), but it was not accepted widely (31 – 33). Applying Kaplan Meir Analysis HPA-binding to tumor cells is correlated with survival time. These studies thus prove HPA-binding to be a non-suitable prognostic marker in tumor of breast (28, 34 – 36), colorectum (25, 37), stomach (38) and esophagus (39) due to this time factor.

From above studies the inference drawn is amazing that receptors for HPA are expressed in both the normal lactating breast and breast cancers that are metastasized to the local lymph node but not in those that do not metastasize. In the normal lactating breast HPA-binding was confined to the apical part tissue epithelium. SDS-PAGE and Westerning of human milk fat globule membrane protein revealed that only a limited number of glycoproteins react HPA (40). There is an inter-individual variation in the number of HPA-positive bands in SDS-PAGE, but recent data reveal maximum number of six bands (40). Later studies have shown that in human HPA-positive breast cancer cell lines (MCF7 and T47D) expressing metastatic abilities in immmunologically suppressed animals (SCID mice), almost all glycoproteins are HPA-positive. Thus, a simple overexpression of one or all of the HPA-positive glycoprotein of milk fat globule membrane is not an expression for the fact that HPA-positive breast cancer is metastatic (unpublished data, Schumacher U) and that the glycosylation of cell membrane proteins has changed. The particular HPA-binding glycoconjugates responsible for metastasis in breast cancer cells is not yet known (41).

The HPA-binding and breast cancer metastasis studies were based on long-term survival time. In short term survival studies, Wheat germ agglutinin (WGA), *Lotus tetragonolobus* agglutinin (LTA), Peanut agglutinin (PNA) and *Ulex europaeus*-I agglutinin (UAE-1) gave no further prognostic information than that already provided by histology (42).

Transformed mammary tissues were also studied for the specificity. Altered carbohydrate-binding patterns were investigated into transformed mammary tissues when treated with Isoform-1 from *Cratylia mollis* lectin (Cra Iso-1) utilizing histochemical techniques. This assay with Cra Iso-1 indicates a possible utilization of the lectin to characterize normal and transformed cells (43).

2. Colorectal cancer specific lectins

PNA is the most widely used lectin in colon cancer studies, but the detection depends largely on the sensitivity of the method used. A sequential progression in case of metastatic adenoma follows the following path:

NORMAL COLON CELLS → INCREASED CELL GROWTH →
EARLY STAGE ADENOMA → INTERMEDIATE STAGE ADENOMA →
LATE STAGE ADENOMA → CARCINOMA → METASTASIS

With a sensitive biochemical assay a definite, though slight, reaction of PNA with normal colonic mucins could be detected which would have been difficult to show by fluorescence microscopy (44). SDS-PAGE of normal and tumor samples revealed four distinct carcinoma associated glycoproteins giving positive reactions with PNA (26,32,35 and 50kD MW) in addition to the four glycoproteins also positive with PNA which were common to both normal and neoplastic tissues (29,30,33 and 36kD MW).

Dietary lectins can also alter the proliferation of colonic carcinoma. One of the dietary lectin *Vicia faba* agglutinin (VFA), the lectin present in broad beans, stimulates differentiation of undifferentiated colon cancer cells. The colon cancer cell line (LS174T) is differentiated into gland like structure in the presence of VFA. The epithelial adhesion molecule epCAM is found to be involved in this. Studies show that dietary or therapeutic VFA may slow progression of colon cancer (45).

Human mannose-binding lectin (MBL) preferentially binds to some human colon adenocarcinoma cell lines (Colo205, Colo201, and DLD-1) expressing high amount of Lewis A and Lewis B antigens. This binding was analyzed by flow cytometry using specific antibodies against MBL and was found to be sugar specific and calcium dependent (46).

In normal mucosa and adenocarcinoma samples from 43 colorectal cancer patients, the expression of different sialoglycoconjugates and fucoglycoconjugates was investigated by using specific lectins and applying semiquantitative analysis. A significant increase in the staining with *Sambucus nigra* lectin (SNA-1), which binds to alpha (2,6)- linked sialic acid residues, was detected in the epithelial cells as well as in the mucins from tumors. The intensity of staining with SNA-1 could be a valid parameter for predicting recurrence in colorectal cancer (47). There are almost always identical lectin binding patterns between the primary tumors and local recurrences indicating that these may develop from remnant cells of the primary tumor left after surgery. The frequency of high-grade dysplasia was significantly greater in older patients and in samples with UEA-I positivity was worked out without neuraminidase pretreatment. UEA-I-reactive adenomas were generally characterized by high cell proliferation rates. A statistical model based on patient's age and UEA-I binding without neuraminidase treatment can generally predict grade of dysplasia in 83% of adenomas and particularly high-grade dysplasia in up to 93% of adenomas. So, such a model may be potentially useful for the early detection of neoplasia, for instance in exfoliative cells from the large intestine (48).

3. Hepatocellular (hepatopancreatic) carcinoma specific lectins

For the screening and prognosis of pancreatic carcinoma several investigators proposed the histochemical use of the PNA lectin. The parameter for the selection of PNA was due to an increased intensity in PNA-binding of secreted mucins in the pancreatic carcinoma.

The analysis of PNA-positive mucins was later refined by carbohydrate analysis of the PNA-positive glycoproteins detected in the sera of cancer patients (49). Furthermore, despite the non-binding of SBA by normal ductal epithelium, cancer cell lines, derived from pancreatic cancer (49), showed a positive reaction with this lectin.

Detection of lectin- binding with *Bauhinia purpurea* agglutinin (BPA) or *Vicia villosa* agglutinin (VVA) in addition to PNA on cancer- associated mucins from patients with pancreatic and gastric cancer has been considered a useful approach in the diagnosis of pancreatic cancer (50, 51). Changes in glycosylation of normal pancreas during malignant transformation are not limited to mucin alone but also found in the glycan side chain of gammaglutamyltranspeptidase (52).

Aleuria aurantia lectin (AAL) was invesgated in concern with liver cirrhosis (LC) and hepatocellular carcinoma. In liver cirrhosis (LC) and hepatocellular carcinoma *Aleuria aurantia* lectin (AAL)- reactive serum cholinesterase (chE) activity increases. This reactivity is used to discriminate liver cirrhosis from chronic hepatitis (53).

The long-term prognosis of hepatocellular carcinoma is predicted utilizing blood concentration levels of alpha-fetoprotein like the *Lens culinaris* agglutinin-reactive fraction (AFP-L3) as the useful markers. AFP-L3 is a positive indicator for small advanced hepatocellular carcinoma (54).

Elevated serum levels of *Tricosanthes japonica* agglutinin-1 (TJA-1)- binding alkaline phosphatase (ALP) in serum were higher in cases of liver cirrhosis and hepatocellular carcinoma compared with chronic heptitis. This analysis is clinically useful for differentiating liver cirrhosis from chronic hepatitis and that altered sugar chain expression in ALP (55).

4. Genitourinary tract cancer specific lectins

Some lectins can be used to investigate the carbohydrate composition of transitional cell carcinoma and thus can be considered as markers of differentiation. Differentiation of tumors of urothelium from the normal tissues can thus be investigated using lectins. Lectins like *Ricinus communis* agglutinin (RCA-120), Wheat germ agglutinin (WGA), *Griffonia simplicifolia* agglutinin (GSA-II) can be bound by all urothelial cell in the normal tissue in large amounts, while some lectins like PNA, Con A, DBA and SBA show only slight binding. Other lectins, such as *Maclura pomifera* agglutinin (MPA), UEA-I, GSA-I and DBA show an increased binding from basal to superficial cell layers (56).

Blood group related carbohydrate antigen expression, which is detected by several specific lectins, has been used as a predictive parameter in these tumors. Indeed using cell suspensions from transitional cell carcinoma, aneuploid cells bound PNA more extensively and were less reactive with WGA than diploid cell population (57). Using tissue sections and comparing the classical markers of prognosis (aneuploidy and invasion) these changes correlated better with the loss of WGA-binding than the reduction in the PNA-binding (58), the latter have been characterized and found to be different to that of the blood group T-antigen (59).

Later, considering decrease in lectin binding during malignant progression as a parameter, several other lectins such as *Vicia faba* agglutinin (VFA), *Lens culinaris* agglutinin (LCA), PNA, WGA, *Griffonia simplicifolia* agglutinin (GSA-II), *Solanum tuberosum* agglutinin (STA), DBA and HPA were studied (60). But, with HPA as an exception, no binding sites were detected for other lectins (61).

Purified galactoside-specific lectin *Viscum album L.* (VAA) was investigated as a major biological response modifier in the low dose range. Its effect on N-butyl-N- (4-hydroxybutyl)-nitrosamine(BBN)-induced carcinogenesis in the urinary bladder of rats. But it seems highly unlikely that adjuvant treatment with VAA might favorably influence bladder cancer in patients by immunological effector mechanisms (62).

5. Prostate cancer specific lectin

In case of prostate carcinoma (PCA), Soya bean agglutinin (SBA) was found to distinguish between benign and malignant cells by binding only to the latter (63). Interestingly, later studies show a negative result for this binding (64, 65).

In addition to the primary tumors, bone metastases derived from prostate adenocarcinoma have been studied and the remarkable result of the investigation was that HPA expression was more common in cases with metastases in the bone or other organs (66). Studies on endometrial carcinoma show UEA-I and PNA to be useful indicators (67).

The asparagines (N)-linked sugar-chain structures of prostate-specific antigen (PSA) are altered in PCA and this may serve as diagnostic tool. This altered state can be investigated by passing the amounts of PSA through columns of Con A, PHA-E4 and PHA-L4. These amounts of PSA were significantly increased for PCA-derived PSA. It was proposed that the sugar moiety structure of PSA, which is increased in PCA, is a multiantennary complex type with branched N-acetylglucosamine beta (1->4) mannose (68).

6. Lung cancer specific lectins

Lung cancer cases with reference to any specific lectin are not yet worked out widely. Recent studies show 159 cases of lung cancer that were investigated for their capacity to bind *Viscum album* L. (VAA) and galectin-1 and for Lewis antigen reactivity (69). Approximately 70% of all tumors exhibited moderate to strong binding capacity for VAA. Adenocarcinomas and branchioalveolar carcinomas were more frequently labeled than squamous carcinomas. No correlation between VAA-binding capacity and survival was observed, whereas expression of galectin-1 binding sites revealed a

better prognosis than those lacking binding sites or showing a weak reactivity (p=0.0257 log rank test or Kaplan-Meier statistics) (69).

OTHER DISEASED TISSUES AND PROGNOSTIC LECTINS:

Varieties of other malignant tissues were also studied taking lectins into consideration to characterize the glycoconjugate content. *Cepaea hortensis* agglutinin (CHA-I) binds to O-glycosidically linked sialic acids with a characterized specificity. Thus CHA-I acts as a useful tool for detecting O-glycosidically linked sialic acid in formalin fixed tissues, and is a potentially powerful tool for the isolation and characterization of unknown sialomucin in normal and eventually in diseased tissues (70).

THE GALECTIN FAMILY OF PROTEINS AND METAGENESIS:

The galectins are a family of proteins that includes calcium independent galactoside binding lectins with the beta-strand topology of the jelly- roll and are distributed widely in all living organisms (71). This family has been implicated in many essential functions including development, differentiation, cell-cell adhesion, cell-matrix interaction, growth regulation, apoptosis, RNA splicing, and tumor metastasis (72). The functional versatility of galectin family explains the current interest in monitoring their expression in cancer research (71). Their contribution to human carcinogenesis has been suggested from experimental studies. In clinical research, they could be used as a differentiation markers, particularly in thyroid carcinomas and in certain lymphomas (73). Overexpression of one of the galectin, Galectin-7, is found to be associated with the apoptotic process in UVB- induced sunburn keratinocytes (74). This galectin is a beta-galactoside binding protein specifically expressed in stratified epithelia and notably in epidermis, but barely detectable in epidermal tumors and absent from squamous carcinoma cell lines. Further studies over this lectin of 14 kD molecular weight, show that it is a marker of all types of stratified epithelia (75). Another galectin, Galectin-1 an endogenous lectin, possesses galactose specific surface binding sites (69).

The isolation of Prostate Carcinoma Tumor Antigen-I (PCTA-I), a cDNA closely related to rat and human Galectin-8, as a surface marker associated with prostate cancer was achieved. It appears that PCTA-I expression is almost ubiquitous in normal human tissues and could alter in specific context such as transformation and metastasis (76). Galectin-8 is also reported to be a physiological modulator of cell adhesion. When immobilized, it functions as a matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering of cell surface integrin receptors (77).

Tandem-repeat-type Galectin-9 and its (probable) allelic variant, ecalectin (a potent eosinophil chemoattractant) are human leukocyte product. Their mRNA is expressed in 17 of 21 human colorectal cancer cell lines (71). This analysis identified the insertion of a single nucleotide into the coding sequence generating a frame-shift mutation, an event not so far reported for any other galectin. Thus mRNA for galectin-9-isoform or ecalectin is present in established human colon cancer cell lines (71).

Table 1
Marker lectins and their histochemical specifity

LECTINS	SOURCE	SEROUS/MUCOUS SPECIFICITY	CARBOHYDRATE SPECIFICITY
Con-A Concanavalin-A	Plant	Serous	N-linked sugar
PHA Phytohaemagglutinin	Plant	Serous	N-linked sugar
SBA Soya bean agglutinin	Plant	Mucous	O-linked sugar mucin
DBA *Dolichos biflorus* agglutinin	Plant	Serous	N-linked sugar Nacetylgalactosamine
HPA *Helix pomatia* agglutinin	Animal	Serous	N-linked sugar Nacetylgalactosamine
UEA-I *Ulex europaeus* agglutinin	Plant	Serous	N-linked sugar (Fucose)
PVA *Phaseolus vulgaris* agglutinin	Plant	Serous	N-linked sugar α-3 beta I integrin
DSA *Datura stramonium* agglutinin	Plant	Serous	N-linked sugar α-3 beta I integrin
MBL Mannose binding lectin	-	-	Mannose specific
SNA-I *Sambucus nigra* lectin-I	Plant	Mucous	α (2,6)-linkedsialic acid residues

Table 2
Marker lectins and their sources

S.NO.	TYPE OF CANCER	BINDING LECTIN	SOURCE OF LECTIN	SITE OF ACTION	REFERENCES
1.	Breast cancer	HPA	Roman snail (*Helix pomatia)*	Membrane glycoprotien	18-41
2.	Transformed mammary cells	Cra Iso-I	*Cratylia mollis*	Carbohydrate binding patterns	43
3.	Colon cancer	PNA, VFA	Peanut, *Vicia faba*	Clonic mucin	44 45
4.	Colorectal cancer	SNA-I	*Sambucus nigra*	Epithelial cells, mucin	47
5.	Pancreatic& Gastric cancer	PNA, BPA VVA	Peanut,*Bauhinia purpurea, Vicia villosa*	Sera glycoprotien, Secreted mucin	49 50,51
6.	Hepatocellular carcinoma	AAL, AFP-L3, TJA-I	*Aleuria aurantia, Lens culinaris, Trichosanthes japonica*	Blood Serum	53-55
7.	Genitourinary tract tumors	UEA-I, PNA, HPA etc.	*Ulex europaeus,* Peanut, *Helix pomatia*		56 60, 61
8.	Bladder carcinoma	VAA	*Viscum album L.*		62
9.	Prostate cancer	SBA, HPA	Soya bean *Helix pomatia*	Tissues Bones	63 66
10.	Endometrial carcinoma	UEA-I, PNA	*Ulex europaeus,* Peanut		67
11.	Lung cancer	VAA HPA UEA-I, PVL	*Viscum album L., Helix pomatia, Ulex europaeus Phasoeolus vulgaris*		69, 71

REFERENCES

1. Greenlee, R. T., Murray, T., Bolden, S. and Wingo, P. A. (2000) Cancer statistics. Cancer J. Clin. 50(1), 7-33.
2. Du, W. B., Chia, K. S., Sankaranarayanan, R., Sankila, R., Seow, A. and Lee, H. P. (2002) Population-based survival analysis of colorectal cancer patients in Singapore, 1968-1992. Int. J. Cancer 99, 460-5.
3. Sen, U., Sankaranarayanan, R., Mandal, S., Ramanakumar, A. V., Parkin, D. M. and Siddiqi, M. (2002) Cancer patterns in eastern India: the first report of the Kolkata cancer registry. Int. J. Cancer 100, 86-91.
4. Thongsuksai, P., Sriplung, H., Phungrassami, T. and Prechavittayakul, P. (1997) Cancer incidence in Songkhla, southern Thailand, 1990-1994. Southeast Asian J. Trop. Med. Public Health 28 Suppl 3, 1-10.
5. Hart, I. R., Goode, N. R. and Wilson, R. E. (1989) Molecular aspects of the metastatic cascade. Biochim. Biophys. Acta 989, 65-84.
6. Hart, I. R. and Saini, A. (1992) Biology of tumour metastasis. Biol. Tum. Metab. 339, 1453-61.
7. Sasaki, T., Yamazaki, K., Yamori, T. and Endo, T. (2002) Inhibition of proliferation and induction of differentiation of glioma cells with *Datura stramonium* agglutinin. Br. J. Cancer 87, 918-23.
8. Goldstein, I. J. and Hayes, C. E. (1978) The lectins: Carbohydrate – binding proteins of plants and animals, Adv. Carbohydr. Chem. Biochem. 35, 127 – 340.
9. Fernandes, B., Sagman, U., Auger, M., Demetrio, M. and Dennis, J. W. (1991) Beta 1-6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. Cancer Res. 51, 718-23. Comment in: Cancer Res. (1994). 54, 306-8.
10. Teshima, S., Hirohashi, S., Shimosato, Y., Kishi, K., Ino, Y., Matsumoto, K. and Yamada, T. (1984) Histochemically demonstrable changes in cell surface carbohydrates of human germ cell tumors, Lab. Invest. 50, 271-7.
11. Vijayan, K. K., Remani, P., Beevi, H., Ankathil, R., Vijaykumar, T., Radendran, R., Augustine, J. and Vasudevan, D. M. (1987) Tissue binding patterns of lectins in premalignant and malignant lesions of the oral cavity. J. Exp. Pathol. 3, 295-304.
12. Bironaite, D., Nesland, J. M., Dalen, H., Risberg, B. and Bryne, M. (2000) N-Glycans influence the in vitro adhesive and invasive behaviour of three metastatic cell lines. Tumour Biol. 21, 165-75.
13. Litynska, A., Przybylo, M., Ksiazek, D. and Laidler, P. (2000) Differences of alpha3 beta1 integrin glycans from different human bladder cell lines. Acta Biochim. Pol. 47, 427-34.
14. Hull, H., Sugarman, E., Spielman, J. and Carraway, K. (1991) Biosynthetic maturation of an ascites tumor cell surface sialomucin. Evidence for O – glycosylation of cell surface glycoprotein by the addition of new oligosaccharides during recycling. J. Biol. Chem. 266 (21), 13580-13586.
15. Klein, P. J., Vierbuchen, M., Wurz, H., Schulz, K. D. and Newman, R. A. (1981) Secretion associated lectin – binding sites as a parameter of hormone dependence in mammary carcinoma. Brit. Jr. Can. 44,476-78.
16. Klein, P. J., Vierbuchen, M., Fischer, J., Schulz, K. D., Farrar, G. and Uhlenbruck, G. (1983) The significance of lectin receptors for the evaluatin of hormone dependence in breast cancer. Jr. Str. Biochem. 19, 839-44.
17. Luis, C. L., Sztynda, T., Cheng, Z. H. and Wyllie, R. G. (1983) Lectin – binding affinities of human breast tumors. Cancer. 52, 1244-50.
18. Leathem, A., Dokal, I. and Atkins, N. (1984) Carbohydrate expression in breast cancer as an early indicator of metastatic potential. Jr. Path. 142, A32.
19. Leathem, A. J., Atkins, N. and Eisen, T. (1985) Breast cancer metastsis, survival and carbohydrate expression associated with lectin binding. J. Path. 145,73A.
20. Brooks, S. and Leathem, A. J. C. (1991) Prediction of lymph node invovement in breast cancer by detection of altered glycosylation in the primary tumour. Lancet. 338,71-74.
21. Leathem, A. J. and Brooks, S. A. (1987) Predictive value of lectin binding on breast cancer recurrence and survival. Lancet. 1054-1056.

22. Schumacher, U., Adam, E., Brooks, S. A. and Leathem, A. J. (1995) Lectin binding properties of human breast cancer cell lines and human milk with particular reference to *Helix pomatia* agglutinin. J. Histochem. Cytochem. 43, 275-281.

23. Schumacher, U., Kretzschmar, H., Brooks, S. and Leathem, A. (1992) Helix pomatia lectin binding pattern of brain metastasis originating from breast cancers. Path. Res. Prac. 188, 284-86.

24. Krogerus, L. and Andersson, L. C. (1990) Different lectin – binding patterns in primary breast cancers and their metastasis. Cancer 66, 1802-9.

25. Schumacher, U., Higgs, D., Loizidou, M., Pickering, R., Leathem, A. and Taylor, I. (1994) The lectin Helix pomatia agglutinin is a good prognostic marker in colon cancer. Cancer 74, 3104 -3107.

26. Alam, S. M., Whitford, P., Cushley, W., George, W. D. and Campbell A. M. (1990) Flow cytometric analysis of cell surface carbohydrates in metasattic human breast cancer. Brit. Jr. Can. 62, 238-42.

27. Fenlon, S., Ellis, I. O., Bell, J., Todd, J.H., Elston, C. W. and Blamey, R. W. (1987) Helix pomatia and Ulex europeus lectin binding in humanbreast cancer. Jr. Path. 152, 169-76.

28. Fukutomi, T., Itabashi, M., Tsugane, S., Yamamoto, H., Nanasawa, T. and Hirota, T. (1989) Prognostic contributions of Helix pomatia and carcinoembryonic antigen staining using histochemical techniques in breast carcinomas. Jpn. Jr. Clin. Onco. 19, 127-134.

29. Noguchi, M., Thomas, M., Kitagawa, H., Kinoshita, K., Ohta, N., Nagamori, M. and Miyazaki, I. (1993) Further analysis of predictive value of Helix pomatia lectin binding to primary breast cancer of axillary and internal mammary lymph node metastasis. Br. Jr. Can. 67, 1368- 71.

30. Thomas, M., Noguchi, M., Fonseca, L., Kitagawa, H., Kinoshita, K. and Miyazaki, I. (1993) Prognostic significance of Helix pomatia lectin and c – erbB – 2 oncoprotein in human breast cancer. Br. Jr. Can. 68, 621-26.

31. Galea, M. H., Ellis, I. O., Bell, J., Elston, C. W. and Blamey, R. W. (1991) prediction of lymph node involvement in breast cancer. Lancet. 338, 392-93.

32. Taylor, C. W., Anbazhagan, R., Jayatilake, H., Adams, A., Gusterson, B. A., Price, K., Gelber, R. D. and Goldhirsch, A. (1991) Helix pomatia in breast cancer. Lancet. 338, 580.

33. Gusterson, B. A. and International (Ludwig) Breast Cancer Study Group. (1993) Prognostic value of Helix pomatia in breast cancer. Br. Jr. Can. 68, 146-50.

34. Leathem, A.J. and Brooks, S.A. (1987) Predictive value of lectin binding on breast cancer recurrence and survival. Lancet. 1054 -1056.

35. Brooks, S. A. and Leathem, A. J. C. (1991) Prediction of lymph node invovement in breast cancer by detection of altered glycosylation in the primary tumour. Lancet. 338,71-74.

36. Alam, S. M., Whitford, P., Cushley, W., George, W.D. and Campbell, A. M. (1990) Flow cytometric analysis of cell surface carbohydrates in metasattic human breast cancer. Brit. Jr. Can. 62, 238-42.

37. Ikeda, Y., Mori, M., Adachi, Y., Matsushima, T. and Sugimachi, K. (1994) Prognostic value of the histochemical expression of helix pomatia agglutinin in advanced colorectal cancer. A univariate and multivariate analysis. Dis. Colon Rectum. 37, 181-184.

38. Kakeji, Y., Tsujitami, S., Mori, M., Maehara, Y. and Sugimachi K. (1991) Helix pomatia agglutinin binding activity is a predictorof survival time for patients with gastric carcinoma. Cancer 68, 2438 - 2442.

39. Yoshida, Y., Okamura, T. and Shirakusa, T. (1993) An immunohistochemical study of *Helix pomatia* agglutinin binding on carcinomas of the esophagus. Surg. Gynecol. Obstet. 177, 632.

40. Schumacher, U. (1990) Vergleichende histologische, histochemische und ultrastrukturelle untersuchungen zum Nachweis und zur Bedeutung von kohlenhydrathaltigen Verbindungen in der Mamma: Drusenepithel, Milchfettkugelmembran, Bindegewebe. Habilitapionschrift. Thesis, University of Munchen.

41. Walker, R. A. (1993) Helix pomatia and prognosis of breast cancer. Br. J. Can. 68, 453 - 54.

42. Walker, R. A. (1990) Assesment of milk fat

gobule membrane antibodies and lectins as markers of short-term prognosis in breast cancer. Br. J. Can. 62, 462 - 66.

43. Beltrao, E. I., Correia, M. T., Figueredo-Silva, J. and Coelho, L. C. (1998) Binding evaluation of Isoform 1 from Cratylia mollis lectin to human mammary tissues. Appl. Biochem. Biotechnol. 74(3): 125-34.

44. Boland, C. R. and Roberts, J. A. (1998) Quantitation of lectins. Jr. Histochem. Cytochem. 36, 1305 - 07.

45. Jordinson, M., El-Hariry, I., Calnan, D., Calam, J. and Pignatelli, M. (1999) *Vicia faba* agglutinin, the lectin present in broad beans, stimulates differentiation of undifferentiated colon cancer cells. Gut. 44(5): 709-14.

46. Muto, S., Sakuma, K., Taniguchi, A. and Matsumoto, K. (1999) Human mannose – binding lectin preferentially binds to human colon adenocarcinoma cell lines expressing high amount of Lewis A and Lewis B antigens. Biol. Pharm. Bull. 22(4): 347-52.

47. Fernandez-Rodriguez, J., Feijoo-Camero, C., Merino-Trigo, A., Paez de la Cadena, M., Rodriguez-Berrocal, F. J., de Carlos, A., Butron, M. and Martinez-Zorzano, V.S. (2000) Immunohistochemical analysis of sialic acid and fucose composition in human colorectal adenocarcinoma. Tumor Biol. 21(3): 153-64.

48. Ching, C. K. and Rhodes, J. M. (1990) Purification and characterization of a peanut agglutinin binding pancreatic - cancer - related serum mucous glycoprotein. Intr. Jr. Can. 45,1022-27.

49. Nishimura, K., Watnabe, A. and Sasaki, H. (1993) Newly established human pancreatic carcinoma cell lines and their lectin binding properties. Intr. Jr. Panreat. 13, 31-41.

50. Kawa, S., Kato, M., Oguchi, H., Kobayashi, T., Furuta, S. and Kanai, M. (1991) Preparation of pancreatic cancer – associated mucin expressing CA19 – 9, CA50, Span- 1, sialyl SSEA- 1, and Dupan- 2. Scand. Jr. Gastroentero. 26, 981-92.

51. Kawa, S., Kato, M., Oguchi, H., Hsue, G. L., Kobayashi, T., Koiwai, T., Tokoo, M., Furuta, S., Ichikawa, T. and Kanai, M. (1992) Clinical evaluation of pancreatic cancer – associated mucin expressing CA19 – 9, CA50, Span- 1, sialyl SSEA- 1, and Dupan- 2. Scand. Jr. Gastroentero. 27, 635-43.

52. Ohta, H., Sawabu, N., Odani, H., Kawakami, H., Watanabe, H., Toya, D., Ozaki, K. and Hattori, N. (1990) Characterization of gamma – glutamyltranspeptidase from human pancreatic cancer. Pancreas. 5, 82-90.

53. Hada, T., Kondo, M., Yasukawa, K., Amuro, Y. and Higashino, K. (1999) Discrimination of liver cirrohosis from chronic hepatitis by measuring the ratio of *Aleuria aurantia* lectin – reactive serum cholinesterase to immunoreactive protein. Clin. Chim. Acted. 281(1-2): 37-46.

54. Kumada, T., Nakano, S., Takeda, I., Kiriyama, S., Sone, Y., Hayashi, K., Katoh, H., Endoh, T., Sassa, T. and Satomura, S. (1999) Clinical utility of *Lens culinaris* agglutinin – reactive alpha – fetoprotein in small hepatocellular carcinoma: special refrence to imaging diagnosis. J Hepatol. 30(1): 125-30.

55. Fukushima, K., Hada, T., Higashino, K. and Yamashita, K. (1998) Elevated serum levels of *Tricosanthus japonica* agglutinin-I binding alkaline phosphatase in relation to high – risk groups for hepatocellular carcinomas. Clin. Cancer. Res. 4(11): 2771-7.

56. Ward, G. K., Steward, S. S., Price, G. B. and Mackillop, J. W. (1987) Cellular heterogeneity in normal human urothelium: quatitative studies of lectin binding. Histochem. Jr. 19, 337-44.

57. Orntift, T. F., Petersen, S. E. and Wolf, H. (1988) Dual – parameter flow cytometry of transitional cell carcinomas. Quantitation of DNA content and binding of carbohydrate ligands in cellular subpopulations. Cancer. 61, 963-70.

58. Langkilde, N. C., Wolf, H. and Orntoft, T. F. (1989 a) Binding of wheat and peanut lectins to human transitional cell carcinomas. Cancer. 64, 849-53.

59. Langkilde, N. C., Wolf, H., Clausen, H. and Orntoft, T. F. (1992) Human urinary bladder carcinoma glycoconjugates expressing T – (Gal beta (1-3) GalNac alpha 1 – O – R) and T – like antigens: a comparitive study using peanut agglutinin and poly – and monoclonal antibodies. Cancer Research. 52, 5030-36.

60. Langkilde, N. C., Wolf, H. and Orntoft, T. F. (1989 b) Lectinohistochemistry of human bladder cancer: loss of lectin binding structures in invasive carcinomas. APIMS.

97, 367-73.

61. Ward, G. K., Steward, S. S., Dotsikas, G., Price, G. B. and Mackillop, W. J. (1992) Cellular heterogeneity in human transitional cell carcinoma: an analysis of optical properties and lectin binding. Histochem. J. 24, 685-94.

62. Kunze, E., Schulz, H., Adamek, M. and Gabius, H.J. (2000) Long term administration of galactoside – specific mistletoe lectin in an animal model: no protection against N-butyl- N- (4 – hydroxybutyl)- nitrosamine – induced urinary bladder carcinogenesis in rats and n induction of a relevant local cellular immune response. J Cancer Res. Clin. Oncol. 126, 125- 38.

63. Soderstrom, K. O. (1987) Lectin binding to prostatic adenocarcinoma. Cancer 60, 1823-31.

64. Loy, T.S., Kyle, J. and Bickel, J. T. (1989) Binding of soyabean agglutinin lectin to prostatic hyperplasia and adenocarcinoma. Cancer 63, 1583-86.

65. McNeal, J. E., Alroy, J., Villers, A., Rewine, E. A., Friha, F. S. and Stamey, T. A. (1991) Mucinous differentiation in prostatic adenocarcinoma. Human Pathology. 22, 979-88.

66. Shiraishi, T., Atsumi, S. and Yatani, R. (1992) Comparitive study of prostatic carcinoma bone metastasis among Japanese in Japan and Japanese Americans and whites in Hawaii. Advan. Exp. Medi. Bio. 324, 7-16.

67. Aoki, D., Nozawa, S., Iizuka, R., Kawakami, H. and Hirano, H. (1990) Diffences in lectin binding patterns of normal endometrium and endometrial adenocarcinoma, with special reference to staining with Ulex europeus agglutinin – 1 and peanut agglutinin. Gynae. Onco. 37, 338-45.

68. Sumi, S., Arai, K., Kitahara, S. and Yoshida, K. (1999) Serial lectin affinity chromatography demonstrates altered asparagines - linked sugar- chain structures of prostate specific antigen in human prostate carcinomas. J. Chromatogr. B. Biomed. Sci. Appl. 727(1-2): 9-14.

69. Fritz, P., Seizer-Schmidt, R., Murdter, T. E., Kroemer, H. K., Aulitzky, W., Andre, S., Gabius, H. J., Friedel, G., Toomes, H. and Siegle, I. (1999) Ligands for Viscum album agglutinin and Galectin – I in human lung cancer. Is there any prognostic relevance? Acta Histochem. 101, 239-53.

70. Fischer, E., Wagner, M. and Bertsch, T. (2000) *Cepaea hortensis* agglutinin-I, specific for O-glycosidically linked sialic acids, selectively labels endothelial cells of distinct vascular beds. Histochem J. 32, 105-9.

71. Lahm, H., Hoeflich, A., Andre, S., Sordat, B., Kaltner, H., Wolf, E. and Gabius H (2000) Gene expression of galectin – 9/ ecalectin, a potent eosinophil chemoattractant, and/ or the insertional isoform in human colorectal carcinoma cell lines and detection of frame-shift mutations for protein sequence truncations in the second functional lectin domain. Int. J. Oncol. 17, 519-24.

72. Danguy, A., Camby, I. and Kiss, R. (2002) Galectins and cancer. Biochim Biophys Acted. 1572(2-3): 285-93.

73. Bassen, R., Brichory, F., Caulet-Maugendre, S., Delaval, P. and Dazord, L. (2000) Vertebrate galectins: structure and function, role in tumoral process. Bull. Cancer 87, 703-7.

74. Bernerd, F., Sarasin, A. and Magnaldo, T. (1999) Galectin – 7, overexpression is associated with the apoptotic process in UVB – induced sunburn keratinocytes. Proc. Natl. Acad. Sci. USA. 96, 11329-34.

75. Magnaldo, T., Fowlis, D. and Darmon, M. (1998) Galectin-7, a marker of all types of stratified epithelia. Differentiation. 63, 159-68.

76. Gopalkrishnan, R. V., Roberts, T., Tuli, S., Kang, D., Christiansen, K. A. and Fisher, P.B. (2000) Molecular characterization of prostate carcinoma tumor antigen – I, a human galectin – 8 related gene. Oncogene 19, 4405-16.

77. Levy, Y., Arbel-Goren, R., Hadari, Y. R., Eshhar, S., Ronen, D., Elhanany, E., Geiger, B. and Zick, Y. (2001) Galectin-8 functions as a matricellular modulator of cell adhesion. J Biol *Chem.* 276,31285-95.