

# AN IMMUNOELECTRON MICROSCOPIC STUDY : HELIX POMATIA LECTIN GOLD COMPLEX IN BREAST CANCER

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**SUMMARY :** *Abnormal cellular glycolysation demonstrated by the binding of Helix Pomatia Lectin (HPL) to paraffin-embedded sections has been shown in several studies to be associated with aggressive biological behavior and poor longterm prognosis in breast cancer. We investigated the relation between HPL staining of primary infiltrative ductal breast cancer and the presence of axillary lymph node (AX) metastases at the ultrastructural level by means of postembedding labeling. Breast cancer tissue fragments were fixed mild glutaraldehyde and embedded in Agar resin 100. Helix Pomatia Lectin Gold Complex (HPL-GC) was chosen as a marker. A satisfactory labeling and fine structure preservation were obtained. Dense HPL binding sites were observed over the plasma membrane and the cytoplasm of cancer cells. Absorbing lectin with appropriate sugar established the specificity of binding and allowed HPL-GC to be used as a sensitive and specific reagent. The results of this study suggest that expression of HPL binding sites in breast cancer cells is of prognostic significance and may reflect the ability of tumor to invade and metastasize.*

**Key Words :** *Helix Pomatia Lectin, Breast Cancer, Postembedding.*

## INTRODUCTION

Lectins are the sugar binding proteins and glycoproteins of non-immune origin (11). Their high affinity and specificity for certain carbohydrate residues have led to their use in histochemistry as discriminative sugar labels. Their role is analogous to the antigen recognizing role of monoclonal antibodies, and the technical details of histochemical methods with both types of reagent, are similar. Lectins are isolated from a wide range of plant and animal sources and are now readily available commercially in a highly purified form.

Currently, differences in cell surface carbohydrate expression are detected using Lectins and in a number of studies with human breast cancer tissues

such differences have been shown to be of prognostic significance (1, 2, 4, 5, 9, 10, 27, 29). Among these lectins, Helix Pomatia Lectin (HPL) isolated from edible snail shows binding specificity for the monosaccharide N-acetyl-galactosamine (GalNAc) and recognizes a wide range of glycoconjugate bearing GalNAc moieties in tissue sections (3, 13).

In breast cancer HPL appears to recognize abnormally glycosylated GalNAc bearing molecules. The prognostic significance of altered glycosylation, as detected by HPL binding, is unlikely to be through an association with proliferative rate, degree of anaplasia or cellular ploidy, but may rather be through a direct association with the

presence of nodal metastases (1, 4, 21).

Light microscopic studies concerning binding of lectins to human mammary carcinoma have been performed by several groups mentioned above. But there are very few ultrastructural data regarding the relationship between HPL binding and primary breast cancer (7).

Based on the knowledge of light microscopic studies we decided to perform an ultrastructural study in order to better localize HPL binding sites in human breast cancer cells, keeping in mind that this study has a different scope using post embedding labeling of HPL-GC. Light microscope study is more appropriate for routine screening; it is easy and less time consuming and overall evaluation of large areas of tissue can be made. The ultrastructural study does not have these advantages, but then lectin binding sites can be demonstrated precisely on different cell types and in interstitial tissue.

Since its introduction in immune electron microscopy by Faulk and Taylor in 1971 (8), colloidal gold has proven to be one of the best electron-dense markers in cytochemistry, displaying several major advantages when compared to the marker such as peroxidase. Because its particulate nature, very accurate identification and delineation of the labeled structure is possible without masking them. Roth and Wagner (26), were the first to demonstrate that lectin-gold complexes were valuable reagents not only for all surface labeling, but also for studies on internalization. Since then a whole variety of ligands and lectins have been successfully conjugated to colloidal gold and applied in pre-and post-embedding labeling of numerous animal and plant tissues (24, 25).

HPL-GC and postembedding electron microscope technique have not been examined in any previous study. By combining all these data we think that HPL-GC can be used as highly sensitive and specific reagent for infiltrating ductal breast carcinoma demonstrating HPL binding sites on cancer cells.

## MATERIALS AND METHODS

A 68 year old woman with infiltrating ductal breast carcinoma having axillary lymph node metastases was studied. She underwent total mastectomy with complete axillary lymph node clearance.

Tumor tissue fragments were fixed immediately in 1 % glutaraldehyde in PBS for 2 hours and then washed in PBS. Free aldehyde groups were blocked by 0.5M NH<sub>4</sub>Cl in PBS for 1 hour at room temperature. After washing PBS, tissue samples were dehydrated in ethanol series and embedded in Agar Resin 100 (Agar Scientific Ltd. UK). Ultrathin sections were picked up on 200 mesh-uncoated nickel grids.

HPL-GC (Sigma UK) having 14 nm gold particle diameter was the marker selected for the ultrastructural study, using one-step lectin gold labeling method. Specific soluble sugar was obtained from Sigma and stored desiccated at -20°C until used. HPL was diluted in 0.1M sugar solution in PBS as control to compete for binding sites.

### Control for lectin

#### Negative control

As negative controls the lectin was incubated with the section in the presence of 0.1 M N-acetyl galactosamine, the simple sugar which the lectin is reported to bind with greatest affinity (3, 13).

### Staining protocol

Incubations were always performed at room temperature. Initially all the grids with attached ultrathin section were placed on PBS for 5 minutes.

### One step labeling method

Thin sections were incubated for 60 minutes with HPL-GC diluted with 0.02 % polyethylene glycol (PEG) in PBS (1 : 2). Afterwards sections were washed with PBS and bidistilled water and counterstained with 3 % aqueous uranyl acetate and lead citrate. All sections were examined under Carl Zeiss 95-2 transmission electron microscope.

## RESULTS

### Light microscopic observations

A tumoral tissue, displaying duct-like structures, composed of distinct pleomorphic atypical cells with large oval or rounded vesicular nuclei having prominent nucleoli surrounded by a thin rim of cytoplasm was observed in semithin sections stained with alcian blue-pyronin-borax (Fig. 1). The nuclei were isolated within fibrous tissue or formed groups.

### Electron microscopic observations

Use of specific sugar to block lectin binding

provided satisfactory negative controls. High HPL binding sites appeared on the carcinoma cells and labeling was restricted to the cytoplasm and membrane surfaces of cells (Fig. 2).

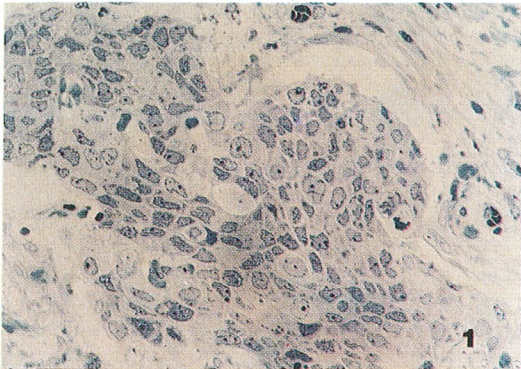


Fig - 1 : Tumoral tissue is observed, composed of distinct pleomorphic typical epithelial cells with large oval or rounded vesicular nuclei having high mitotic activity. The nuclei form groups or seen isolated within fibrous tissue in semithin section. Toluidin blue-borax-pyronin. X 440.

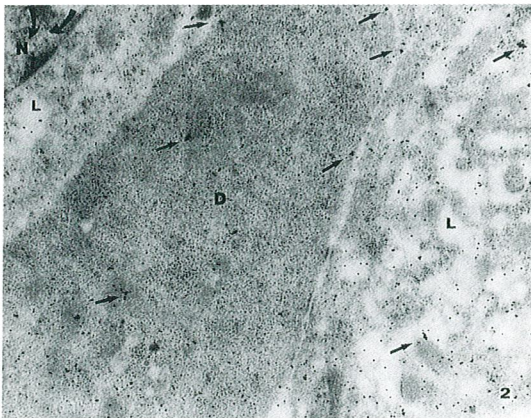


Fig - 2 : High Helix Pomatia Lectin (HPL) binding sites represented by black gold particles are observed on the Dark (D) and Light (L) infiltrative ductal carcinoma cells. Labeling is restricted to the cytoplasm and membrane surfaces of cells (thin arrows). Nucleus (N, thick arrows). X 33,250.

Two different cell types dark and light, were shown in the infiltrative ductal carcinoma tissue sections. Dark cells, or cells with higher electron density, contained irregular, pleomorphic, a indented nuclei with prominent perinuclear ring of dense

chromatin and one or two nucleoli. The light cells, with lower electron density, exhibited round or occasionally slightly indented nuclei containing homogeneously dispersed smaller granules of chromatin and clearly defined nucleoli. The cytoplasm was relatively less dense. Dark cell types were predominately seen in the sections. Both cell types showed all ultrastructural details characteristics of epithelial mammary tumor cells (18).

## DISCUSSION

Intense binding is seen predominantly on the carcinoma cells, and labelling is restricted to the cytoplasm and membrane surfaces of the cells. Vascular endothelium also show lectin staining pattern (4, 14, 27).

Calafat and Jansen (7) found lectin receptors on the cytoplasm and cell membrane of cancer cells as well as on the T-lymphocytes, plasma cells and vascular endothelium in their ultrastructural study.

In our study, we observed well-preserved tissue structure in both semithin and ultrathin sections, using mild glutaraldehyde and Agar Resin 100 embedding material. Ultrathin sections incubated with direct HPL-GC displayed high HPL binding sites on the cytoplasm and cell membrane of the infiltrating ductal breast cancer cells.

Brooks and Leatham (6) reported that staining of primary breast cancers cells for HPL was an "excellent predictor of long-term patient prognosis" and that was a "possible staging adjunct in patients apparently lymph-node negative".

HPL staining has been found to be significantly associated with tumor size, axillary lymph node (AX) and internal mammary lymph node (IM) metastases, but not significantly related to age, menopausal status and histologic type (21).

Alterations in cell surface carbohydrates have been found to be related with metastatic potential of experimental tumors and correlated with high and low metastatic sublines (2, 29). Brooks and Leatham (5) suggested that HPL might provide valuable information in breast cancer patients in whom AX dissection had not been performed, because there was a significant relationship between HPL staining and AX metastases (1, 9, 10, 15, 16).

HPL binding specificity for terminal N-acetylgalactosamine (GalNAc) residues and elevated

level of Gal Nac expression in breast cancer cells suggests that abnormality probably lies in the glycosylation pathways. Alteration in protein glycosylation has previously been related with malignancy (20, 28, 30).

In malignant cells, the content of sialic acid-rich carbohydrates is increased (27). HPL<sub>+</sub>-positive cells tend to metastasize readily in part due to an incomplete synthesis of cancer cell sugar chains (10).

Mehta et al (18) have established two different malignant breast carcinoma cell lines, designated as UIISO-BCA-1 and UIISO-BCA-2, from metastatic pleural effusions of a 71-years old woman with a previously confirmed history of infiltrative ductal carcinoma. Both cell lines developed uniform monolayers in tissue culture flask shortly after seeding. Even though these cell lines actively grouped in culture, they were not extensively subcloned and retained their original morphological and biochemical characteristics. At the ultrastructural level these cell lines showed (two different cell types, dark and light. Similar cell types have been reported in malignant and non-malignant tissues (12), although their functional significance in malignant tumors is still speculative. The dark and light cells are thought to represent tissue components of different histogenetic and functional states of cells showing different degrees of differentiation (12, 17).

The dark cells are often thought to be dying degenerating cells (12). Recently, detailed electronmicroscopic investigation of a tumor-derived cell line (8701-BC) (19) and of breast tumor biopsies (22, 23) suggested that these two distinct cytotypes were transformed counterparts of luminal cells, usually present in the normal ductal epithelium of mammary.

We also found two types of cells, dark and light at the ultrastructural level in the infiltrating ductal breast cancer tissue sections. Their ultrastructural features were similar to cells established by Mehta et al (18).

We conclude that expression of HPL binding sites on the breast cancer cells having abnormal glycosylation is of prognostic significance and may reflect the ability of a tumor to invade and metastasize.

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