

## ***Parkia pendula* lectin as histochemistry marker for meningeothelial tumour**

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Lectins have been intensively used in histochemical techniques for cell surface characterization. These proteins are involved in several biological processes and their use as histochemical markers have been evaluated since they can indicate differences in cell surfaces. *Parkia pendula* lectin (PpeL) was evaluated as histochemical marker for meningeothelial meningioma biopsies. Tissue slices were incubated with PpeL conjugated to horseradish peroxidase (PpeL-HRP) and Concanavalin A-HRP (ConA-HRP) and the binding visualized with diaminobenzidine and hydrogen peroxide. The lectin-tissue binding was inhibited with D-glucose. PpeL showed to be a useful tool for the characterization of meningeothelial tumour and clinico-pathological diagnosis.

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**M**eningiomas belong to a group of predominantly benign tumours of adults. They develop from the meningeothelial cells of the arachnoid, although they are most intimately associated with the dura. This type of brain tumour accounts for the most frequent primary intracranial neoplasms in adults. In 1993, no less than 14 variants of this meningeothelial tumour were included in the revised edition of the World Health Organization (WHO) histological classification of central nervous system tumours and characterized meningiomas with an increased propensity to recur (WHO, 2000).

Plant lectins, especially those purified from seeds, have found a great number of applications in medicine and biological processes such as infection, cell differentiation and metastasis (Goldstein et al., 1980; Barondes, 1988; Sharon and Lis, 1990, 1993). As histochemical marker, lectins have been used to characterize mammary (Beltrão et al., 1998), uterine (Remani et al., 1994) and cerebral neoplastic tumours (Schumacher et al., 1995; Marafioti et al., 1994; Salmon et al., 1996, Wiley and Kline, 2000; Nishimura et al., 2000).

Lectin histochemistry has been used as an extra marker besides the traditional immunohistochemistry to characterize normal and transformed tissues. Human meningiomas were studied with lectins such as Peanut agglutinin (PNA), *Canavalia ensiformis* (Con A), *Ulex europaeus* 1 (UEA-1), *Dolichos biflorus* (DBA) and *Triticum vulgare* (WGA) (Marafioti et al., 1994). Histiocytic and xanthoma cells were detected differentially to vascular elements within meningiomas using lectin histochemistry (Kleinert and Radner, 1987; Herling et al., 2000).

The cytochemical binding intensity of some lectins is of value in distinguishing between different me-

ningioma subgroups. Fibroblastic meningioma cells are more strongly recognized by WGA and UEA-1 than the meningothelial subtype (Salmon et al., 1996).

*Parkia pendula* lectin (PpeL) is extracted from the seeds of a native tree from Atlantic Forest of the State of Pernambuco, Northeast Brazil. The tree belongs to the family Fabaceae and sub-family Mimosoideae. PpeL recognizes glucose and mannose and belongs to the same family of Concanavalin A (Con A), purified from *Canavalia ensiformis* seeds and the Isoform 1 of *Cratylia mollis* (Cra Iso 1) lectin, a seed lectin purified from a native forage legume from the Semiarid Region of the State of Pernambuco (Correia and Coelho, 1995).

The high incidence of meningothelial tumours diagnosed in the Neurology Sector of the Clinic Hospital at the Federal University of the State of Pernambuco (UFPE) raised the interest to investigate this brain pathology using lectin histochemistry.

In this work *Parkia pendula* lectin is evaluated as histochemical tool to help the clinico-pathological evaluation of meningiomas.

## Materials and Methods

### Lectins

PpeL was purified from seeds of *Parkia pendula* tree at the Laboratory of Glycoprotein at the Department of Biochemistry - UFPE. PpeL was labeled with horseradish peroxidase (HRP) as described by Avrameas et al. (1976). Concanavalin A-HRP (Con A-HRP) was purchased from Sigma Company. Lectin binding inhibition assays were accomplished using D-glucose from Sigma Company.

### Meningioma specimens

Five formalin-fixed and paraffin-embedded meningioma biopsies were obtained from the Neurology Sector of the Clinic Hospital at the Federal University of Pernambuco – UFPE. Patient ages varied between 40 and 50-year-old at the time of surgery.

### Staining procedure

Four-micrometer paraffin sections were cut and microscope slides were prepared. Sections were deparaffinized in xylene and hydrated through graded alcohol (100-70%). Tissue slices were

treated with a 0.1% (w/v) trypsin solution at 35°C, for 2 min, followed by 0.3% (v/v) methanol-hydrogen peroxide for 30 min at room temperature and then incubated with PpeL-HRP (75 µg/mL) and Con A-HRP (60 µg/mL) overnight. All solutions were prepared in 10 mM phosphate buffer, containing 0.15M NaCl (PBS), pH 7.2. After each step, the slices were immersed in PBS for two 5-min washes. Peroxidase was visualized using diaminobenzidine (DAB) solution containing hydrogen peroxide for 5-8 min. Sections were counterstained with haematoxylin. Lectin binding was evaluated on an Olympus BH-2 light microscope. Assays for lectin binding inhibition were accomplished incubating each lectin solution with D-glucose (0.02 to 0.5 M) for 15 min prior to its incubation with the tissue. Following steps were as described previously for the binding protocol.

## Results and Discussion

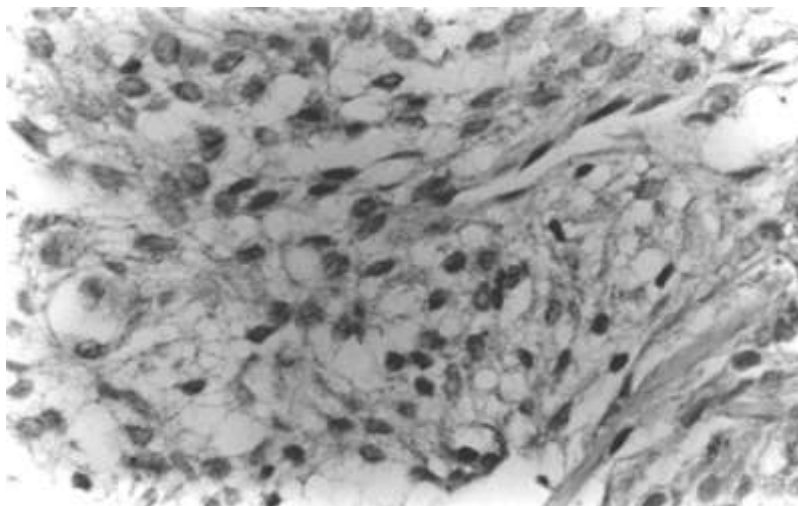
Lectins bind to carbohydrates of cell surfaces in oligosaccharides or glycoconjugates (Goldstein et al., 1980; Barondes, 1988; Sharon and Lis, 1990, 1993; Kennedy et al., 1995).

Qualitative and quantitative changes in the glycosylated components of cell surface could be significant in the development and progression of pathologic processes, including neoplastic ones (Karuna et al., 1992; Brooks, 1995; Astoul et al., 2000; Nishimura et al., 2000). Beltrão et al. (1998) evaluated normal and transformed mammary tissues with Cra Iso 1 and Con A. Their results support the use of lectins as histochemical tool for tissue differentiation/characterization since the more malignant the more intense lectin staining was observed.

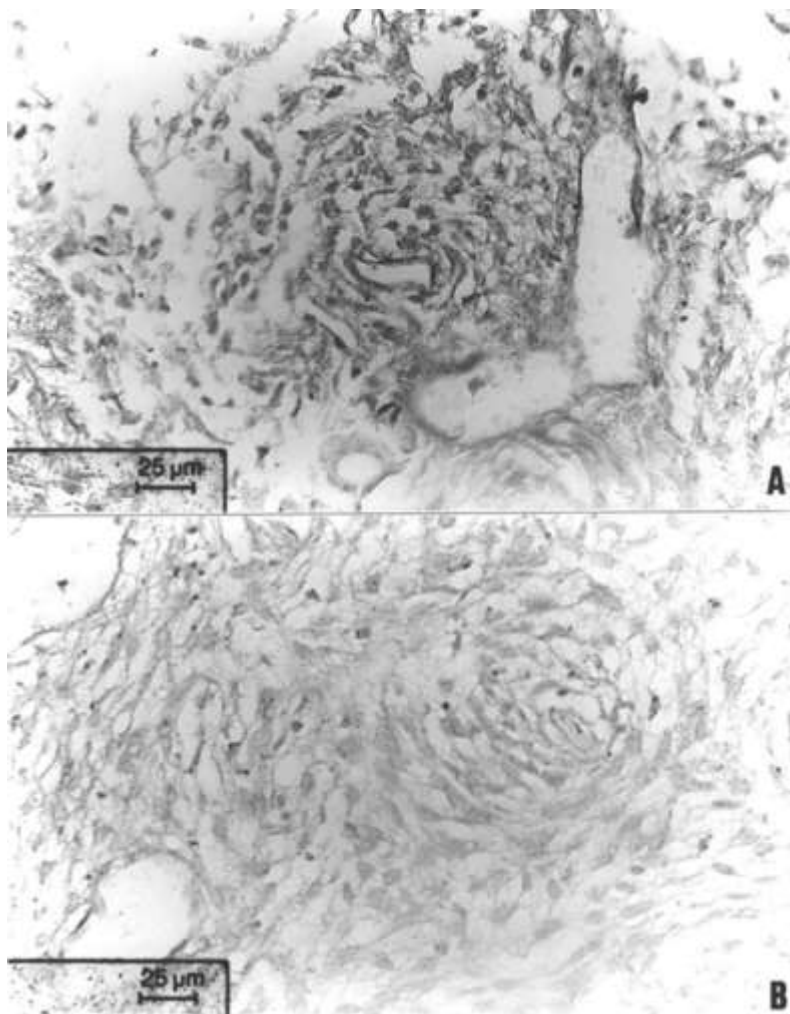
In the five specimens studied the staining of meningioma cells with PpeL (75 µg/mL) characterized the syncytial cell aspect in the tissue (Figure 1) and the outline endothelial blood vessels. Con A, used as a pattern of glucose/mannose binding lectin presented a similar staining. The PpeL and Con A binding were completely abolished with D-glucose at the concentration of 300 mM (Figure 2).

PpeL showed similar results to those observed using lectin histochemistry for human meningiomas (Marafioti et al., 1994; Salmon et al., 1996; Wiley and Kline, 2000; Nishimura et al., 2000).

Lectin binding pattern diversity allows different histological sub-types of meningioma to be distinguished although the biological significance of



**Figure 1.** Meningothelial tumour stained with PpeL-HRP. Syncytial aspect of meningeal cells is characterized by the lectin staining.



**Figure 2.** PpeL-HRP binding (A) and D-glucose inhibition (B) to meningothelial tumour.

binding patterns is unclear (Kleiner and Radner, 1987). Our findings show that PpeL presented reactivity patterns and expressed strong cytoplasmatic staining in a number of cells, which allowed

the characterization of meningothelial sub-type.

The bulk of evidence obtained from previous researches indicates that monosaccharide composition and biochemical metabolism in brain cells

change with age and dedifferentiation thus lectins may be also useful as histochemical markers for investigating senile changes in the brain (Kiati-pattanasakul et al., 1998; Yoshihara et al, 1999) and moreover, morphologic aspects of tumours (Jeong et al., 1996, Probst-Cousin et al., 1997).

In the current study, the benign cases of meningiomas used were followed for less than one year. Reasons for a relative lack of longer follow up periods in this population are unknown. However, this may be related to a better post-treatment prognosis than for the fact that malignant meningiomas are so hard to be diagnosed. The survival rate in patients with either condition is increased in those who have undergone surgical treatment.

Our results demonstrate that PpeL is a promising histochemistry marker to distinguish differentiation in meningioma tumours the meningothelial variant and to show details of cytoplasm, morphologic cell features and endothelial arrangement of blood vessels in the tumour studied. It also allowed characterization of meningothelial form indicating a relevant clinico-pathological differentiation.

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