Lectin Histochemistry as a Predictor of Dysplasia Grade in Colorectal Adenomas

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Lectins are sugar-binding proteins that bind to specific cellular carbohydrates, commonly affecting cellular physiology. Phaseolus vulgaris leucoagglutinin (PHA), ulex europaeus isoagglutinin-I (UEA-I), wheat germ agglutinin (WGA) and peanut agglutinin (PNA) are among the most well studied lectins in various tissues. The purpose of this study was to detect the above lectins' binding sites and so examine alterations in glycoconjugate expression in neoplastic cells of 52 colorectal adenomas with various clinicopathologic characteristics and proliferation rates. Lectin histochemistry was performed in paraffin sections with and without neuraminidase treatment. Proliferative fraction was determined by immunolabelling for Proliferating Cell Nuclear Antigen. PHA was the more frequently positive lectin in the examined specimens; however, it was simultaneously detected in normal colonic mucosa and so was WGA. The frequency of high grade dysplasia was significantly greater in older patients and in samples with UEA-I positivity without neuraminidase pretreatment. UEA-I-reactive adenomas were generally characterized by high cell proliferation rates. A statistical model based on patients' 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. (Pathology Oncology Research Vol 6, No 4, 265–271, 2000)

Keywords: colorectal adenomas, lectin binding, phaseolus vulgaris leucoagglutinin, ulex europaeus, wheat germ agglutinin, peanut agglutinin

Introduction

Colorectal carcinogenesis is known to involve multiple steps from hyperproliferative epithelium through adenoma formation to cancerous stages. As concerns sporadic carcinogenesis of the large intestine, no consistent genetic abnormalities have so far been defined. Most cancers of the colon are preceded by an adenomatous polyp (adenoma). The well established risk factors for an adenoma's malignant transformation (i.e. max. diameter >2cm, vil-

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Abbreviations: PHA: Phaseolus vulgaris leucoagglutinin, UEA-1; Ulex europaeus isoagglutinin-I, WGA: Wheat germ agglutinin, PNA: Peanut agglutinin, PBS: Phosphate buffer saline

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acting as lectin receptors. As such, lectins are useful tools to study the glycoprotein and glycolipid cellular structure. Altered glycosylation detected by lectin binding has been found after mitogenic stimulation of quiescent cells, induction of cell differentiation, malignant transformation, tumor progression and acquisition of the metastatic phenotype. Some lectins are mitogenic themselves and have, therefore, been used as growth stimulators in cell culture systems.

The lectin staining properties within individual tissues, may have a relevance to the neoplastic process or may reflect the biological nature of the tumor. Phaseolus vulgaris agglutinin (PHA) is a common dietary lectin, isolated from red kidney beans, which binds to β1,6 branched oligosaccharides and has been associated with poor prognosis in human breast and colon cancers. The lectin of Ulex europaeus isoagglutinin I (UEA-I) combines with the H antigen that corresponds to blood group O and binds mainly to endothelial cells. Wheat germ agglutinin (WGA) is a dietary lectin and binds to colonic receptors. Peanut agglutinin (PNA) increases proliferation of the colonic mucosa and has been shown on tissue sections to bind selectively to a mucin glycoprotein, the T antigen, produced by colon cancers. It is theorized that increasing expression of the T antigen and other abnormal surface antigens on increasingly dysplastic tissues reprises the adenoma to carcinoma sequence. The sugar moieties recognized by the above mentioned lectins are shown in Table 1.

Materials and Methods

Fifty-two patients with sporadic adenomas were enrolled in our study [mean age (range): 73 (48,88), male/female ratio: 34/18]. As regards the location of the adenomas, 9 were located at the right colon, 29 at the left colon and 14 at the rectum. Thirty-two samples measured less or equal to 10 mm and the rest had a maximum diameter of more than 10 mm. All tissues were originally fixed in buffered formalin and embedded in paraffin in the routine fashion. Histological diagnosis was reached by evaluation after conventional haematoxylin and eosin staining. Apart from the adenomatous tissue, the majority of sections contained contiguous, histologically normal mucosa. Histologically, the breakdown of the examined adenomatous polyps was as follows: four villous adenomas, thirteen tubulovillous adenomas and thirty-five tubular adenomas. Tumors and moderate cytologic atypias and architectural abnormalities (n = 29) were categorized in the group of low grade (G1) dysplasia while those samples with severe epithelial atypias including those with focal areas of "intraepithelial" and "intramucosal" carcinoma were grouped as high grade (GII) dysplastic adenomas.

![Figure 1. UEA-I binding at the surface of columnar neoplastic cells of a villous adenoma with low grade dysplasia (UEA-I histochemistry, original magnification x200).](image)

Table 1. Major sugar binding specificities of lectins used in this study

<table>
<thead>
<tr>
<th>Lectin from</th>
<th>Abbreviation</th>
<th>Carbohydrate specificity</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaseolus vulgaris</td>
<td>PHA</td>
<td>N-Acetyl D-Galactosamine</td>
<td>N-Acetyl D-Galactosamine</td>
</tr>
<tr>
<td>Ulex europaeus I</td>
<td>UEA-I</td>
<td>α-L-Fucose</td>
<td>L-Fucose</td>
</tr>
<tr>
<td>Triticum vulgaris</td>
<td>WGA</td>
<td>N-Acetyl D-Glucosamine, N-Acetyl neuraminic acid</td>
<td>N-Acetyl D-Glucosamine</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>PNA</td>
<td>Galactosyl β (1,3)-N-Acetyl D-galactosamine</td>
<td>D-Galactose</td>
</tr>
</tbody>
</table>