
Altered glycosylation of cell surface glycoproteins commonly accompanies malignant transformation. In rodent tumors, presence of larger N-linked oligosaccharides branched at the trimannosyl core is associated with the metastatic phenotype. In turn, drugs which inhibit branching of these complex-type N-linked oligosaccharides, e.g., swainsonine, block metastasis (1). Particular aberrant patterns of glycosylation can be used as markers for tumor progression, and lectins with known specificity are useful tools for probing such patterns.

The lectin L-PHA1 requires both galactose and the β1–6-linked antennae for high affinity binding. Synthesis of the latter is dependent upon the activity of N-acetylgalactosamine transferase V and favors the formation of poly-N-acetyllactosamine extensions, ultimately associated to the malignant phenotype. Following this rationale, Fernandes et al. (2) analyzed L-PHA reactivity in colon and breast cancer specimens at different pathological stagings. Malignant lesions were consistently more L-PHA reactive than their benign counterparts. Their findings prompted us to evaluate the prognostic value of L-PHA reactivity in breast cancer specimens.

We analyzed 69 cases randomly taken from a series of 235 consecutive female patients with primary, nonmetastatic breast cancer admitted to the Mastology Department of the A. C. Camargo Hospital, São Paulo, Brazil, from January 1983 to December 1984, as described in a previous report from our group (3). L-PHA epithelial reactivity was evaluated by means of lectin histochemistry, as described by Fernandes et al. (2). Staining patterns were essentially the same as discussed in their paper. Tumors were either negative or positive for staining. The staining was diffuse cytoplasmic, sometimes concentrated in the Golgi area, or at the plasma membrane. Besides epithelial staining, stromal areas were also weakly positive, and this was more intense around epithelial cells. Vascular endothelial cells were consistently positive.

L-PHA reactivity alone on epithelial cells was not significantly predictive of either disease-free or overall survival of these patients (Fig. 1). The most striking finding was the negative association between tumor L-PHA reactivity and the presence of axillary lymph node metastases at the time of diagnosis. Lymph node status is the most accurate predictor of long-term prognosis in breast cancer. Of the 50 patients with a L-PHA-positive tumor only 15 (30%) had lymph node involvement, whereas 11 of 19 (58%) L-PHA-negative tumors presented axillary metastasis (P = 0.0327, χ² test). A similar finding was already suggested by Fernandes et al. as a trend in 12 cases. Despite that, the authors suggested a correlation with breast cancer progression, which was not observed on an epidemiological basis. Recently, an independent group had showed that L-PHA-binding oligosaccharides were unrelated to progression of mammary neoplasia in spontaneous and experimental metastasis models (4).

Characterization of the glycoproteins which bear L-PHA-reactive oligosaccharides and an assessment of their functions might help explain the failure to ascribe a prognostic value for L-PHA reactivity in breast cancer. Dennis and Laferte (5) have shown previously that among the glycoproteins from human breast cancer tissue there are two major polypeptides which carry L-PHA-reactive oligosaccharides. These polypeptides have an apparent molecular weight of 75,000 and 130,000. The same polypeptides appear to be present in different cultured mammary epithelial cells, as analyzed in a lectin blot (Fig. 2e). MCF-10 is a “normal” human mammary cell line. T47-D, MDA MB231, MDA MB453, MDA MB361, and SKBr3 are human breast tumor-derived cell lines. HC11 is a normal murine mammary epithelial cell line.

The HC11 cell line constitutes an interesting model, since the cells can be differentiated in vitro. In the presence of lactogenic hormones the cells produce milk proteins protein including β-casein (6). β-Ca-sein synthesis is dependent upon the presence of a specific transcription factor named mammary gland factor (7). HC11 transformation with ras or raf oncogenes renders the cells unresponsive to lactogenic hormones, due to lack of synthesis or activation of mammary gland factor (8). We have analyzed the presence of L-PHA reactive glycoproteins (gp130 and gp75) in different transfectants of HC11 cells stimulated to differentiate (Fig. 2b). Cells which underwent differentiation, therefore producing β-casein as analyzed by Western blots, expressed high amounts of glycosylated gp130, whereas neither gp130 synthesis nor its glycosylation were observed in lactogenic hormone induced ras- and raf-transfected cells.

Coordinated expression of β-casein and L-PHA reactive gp130 suggests that the latter is a differentiation marker and is apparently unrelated to tumor progression. Although L-PHA reactivity is often increased in breast carcinomas when compared to normal breast, as shown by Fernandes et al. (2), this does not correlate with other

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1 The abbreviation used is: L-PHA, leukoagglutinin from Phaseolus vulgaris.

Fig. 1. Overall (a) and disease-free survival (b) curves according to L-PHA reactivity in primary breast carcinoma.
Fig. 2. L-PHA lectin blot of glycoproteins from (A) different human breast-derived cell lines and HC11, a normal mammary epithelial cell line from BALB/c mice; (B) different HC11-transfected cells stimulated to differentiate with lactogenic hormones; genes transfected are listed at the bottom of the figure. In B, expression of β-casein and mammary gland factor (MGF) is indicated with − or + signs; 100 µg of protein extracts were loaded in each lane.

markers of breast carcinoma progression. Given the importance of properly identifying prognostic factors in breast cancer we feel that this issue deserves further examination.

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References


Reply

We have read with interest the findings of Chammas et al. relating to the expression of branched oligosaccharides in human breast cancer. They have addressed an important issue regarding the relationship between L-PHA1 binding by mammary carcinoma and survival of these patients. However, we have serious reservations regarding the methodology that they have utilized. In evaluating the L-PHA staining by tumor cells, they have characterized tumors as either “negative” or “positive.” We have previously shown that weak staining for L-PHA may be seen in some normal breast epithelial cells while in neoplastic breast epithelial cells the staining intensity was variably increased. In addition within tumors there is heterogeneity of staining with some

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