Rev Esp Patol 1999; Vol. 32, Nº3: 261-262

Keynote Lecture 2

Tumor-associated glycoconjugates and cell adhesion molecules: *In vivo* veritas?

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Introduction

In vitro studies on various rodent and human tumor cell lines and in transgenic mouse models have demonstrated an association between altered carbohydrate structures of glycoproteins and aberrant cell adhesion molecule expression and invasive growth behavior, as well as acquisition of metastatic potential. A prototype example of structural and compositional changes of N-glucosidically linked oligosaccharide side chains of glycoproteins consists in the increased synthesis of N-acetylglucosamine ß1 ,6 mannose structures, the so-called 1,6 branches (1). Functionally, an increased number of ß1,6 branches was found to be associated with reduced contact inhibition of growth, reduced cell-substratum adhesion, enhanced cellular motility and metastatic potential (2, 3).

Another type of tumor-associated aberrant glycosylation is due to simplified synthesis of O-glucosidically linked oligosaccharides resulting, for instance, in the presence of the Thomsen-Friedenreich glycotope (4, 5). Although its functional implications tor the social behavior of cells are not known, the Thomsen-Friedenreich glycotope has been proposed to represent a general carcinoma autoantigen (6, 7) and therefore to be of diagnostic and prognostic value. Furthermore, active specific immunotherapy using synthetic Thomsen-Friedenreich glycoconjugates for the development of tumor vaccines has been proposed (8-10). Among the cell adhesion molecules, CD44 and the E-cadherin/catenin complex have attracted special interest since their involvement in tumor progression and metastasis was demonstrated (11-15). This lecture will focus on the above mentioned selected tumor-associated glycoconjugates and cell adhesion molecules, providing a comparative analysis of *in vitro* data and results from transgenic animals with human carcinomas, with regard to their prognostic value and their use as general tumor markers.

Increased ß1 .6 branching of oligosaccharides

To date the most direct *in vitro* evidence for the association between increased number of ß1,6 branches and altered cellular growth properties was obtained by transfection ot the immortalized lung epithelial cell line Mvl Lu with the glycosyltransferase synthesizing this structure (16). A correlation with tumor stage and progression in colon and breast carcinoma has been reported (17,18). Furthermore, quantitative differences in human colon carcinoma cell lines differing in metastatic potential have been reported (19). A recent study has also demonstrated the prognostic value of ß1,6 branches in human colorectal cancer (20). The carcinoma staining index for ß1 ,6 branches was highly associated with the disease-free survival and overall survival and with the presence of lymph node metastases.

Can the results obtained for colorectal carcinoma be extended to other carcinomas

In this context the presence of ß1,6 branches in normal tissues ot humans has to be considered (21). For instance, pancreatic ß cells showed only taint staining. Notwithstanding, evaluation of well-documented human insulinomas showed no relation to malignancy and metastasis formation (22). Experimental evidence indicated an association with metastasis formation of melanoma cells (3). However, in clinically well-documented superficial spreading and nodular melanomas, no association between the ß1,6 staining index and melanoma type, as well as presence of metastases, was found (22).

Thomsen-Friedenreich glycotype

The Thomsen-Friedenreich glycotope has been proposed to represent a general carcinoma-associated antigen and a candidate for development of a tumor vaccine. A recent study in human lung, however, demonstrated that it represents a differentiation antigen, rather than a carcinoma-associated antigen (23). It was restricted to undifferentiated elements of developing lung and to pneumocytes in adult lung, whereas its sialylated form was found in all epithelial structures of developing and adult lung and in well-differentiated lung carcinomas. Similar results were obtained for human kidney. Thus, the Thomsen-Friedenreich glycotope is of limited value in the diagnosis of lung and renal carcinomas and there is no rationale for a Thomsen-Friedenreich glycotope based immunotherapy of lung renal carcinoma.

CD44

Theme is a whole body of evidence that both CD44s and CD44v isoforms may play a role in tumor progression and metastasis of various human tumors (12). However, an immunohistochemical study on the expression of CD44 standard and variant exons (CD44v3, CD44v4, CD44v5, CD44v6, CD44v9) in benign, semimalignant and malignant human epithelial skin tumors demonstrated that qualitative and quantitative changes in CD44 splice-variant expression did not correlate with invasive and metastatic potential nor with cellular proliferation (24). They were rather related to the degree of tumor differentiation. A similar study in human malignant melanoma compared superficial spreading melanoma and nodular melanoma (25). Although the former showed a significant stronger staining for CD44 standard than the nodular melanomas, metastasizing melanomas could not be distinguished from nonmetastasizing

ones based on CD44 immunostaining. In another study from our laboratory, the potential value of CD44 immunohistochemistry in identifying patient-groups with pTa and pTI transitional cell carcinoma of the bladder at risk for tumor recurrence could be demonstrated. Noninvasive pTa carcinomas displaying areas with loss of staining for both CD44 v3 and v6 had a shorter recurrence-free survival. This relation was not observed for minimally invasive pT1 carcinomas suggesting a different behavior of pTa and pT1 urothelial carcinomas.

E-cadherin/catenin complex

Various experimental and clinical studies have revealed that the adhesive function of E-cadherin is frequently lost in many human carcinomas (13-15). In a transgenic mouse model of insulinoma, it could be shown that loss of E-cadherin expression was a cause for progression from benign to malignant insulinoma (26). In this tumorigenesis model, the rat insulin promoter was utilized to direct expression of SV4O large T antigen to the pancreatic beta cells. Our studies in a series of benign and malignant human insulinomas failed to establish such a correlation, By immunohistochemistry and Western blotting, both benign and malignant human insulinomas expressed E-cadherin. Furthermore, no correlation existed with regard to the expression of alpha and ß catenin.

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