Expression of CD24 in Human Tumors: New information From Histopathologic Studies

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BACKGROUND

•CD24 is a widespread cell surface marker with recently recognized importance in recognizing cell damage vs cell infection. For several years CD24 has also been recognized as a marker of malignancies, particularly in serious cases.

•CD24 has been shown to be a ligand for P-selectin on human tumor cells. The CD24:P-selectin binding is thought to be important to tumor spread and metastasis because "stabilized platelets-tumor thrombi can physically protect tumor cells from destruction as well as help and promote tumor extravasation and tissue penetration" (Aigner, Sthoeger et al. 3385-3395).

•Our objective for this Sarah Morrison funded study is to correlate CD24 presence in pulmonary adenocarcinoma vs squamous cell carcinoma of the lung and to relate the type and severity of the malignancy (as coded by pathologists during surgery) with such staining.

•This study selected two human lung tumor types: Adenocarcinoma and Squamous Cell Carcinoma

- Each tumor type has different localization in the lung
- Frequency of occurrence for both is approximately 30% of cases
- Recent work on the genetic background of these malignancies proposes that they both arise from a common stem cell progenitor

METHODS

- 16 causes of human lung adenocarcinoma and 16 cases of squamous cell (lung) carcinoma were chosen
- With appropriate clearances, surgical pathology reports on each tumor type were summarized
- Slides were prepared from the tumor paraffin blocks
- Staining was performed with **CD24 monoclonal SN3B***
- The stained areas were measured
  - For each slide, ten sequential low power fields were graded by three skilled observers on percent of CD24 stain in the field. Data were averaged and entered into STATISTICA

•CD24 staining and tumor size were compared by tumor type. Other grading parameters were incomplete and could not be statistically compared.

**SN3B monoclonal recognizes the sialic acid-O linked glycans on CD24 cells*

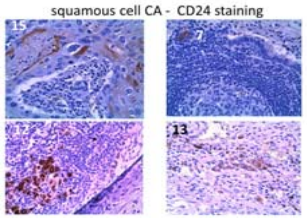
METHODS: Staining Procedure

•All cases were coded with a letter or number and the TMC patient number removed from working documents and slides.

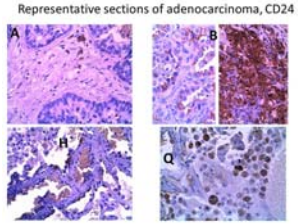
•Sections of the human tumors were made and antigen exposure was done (citrate buffer, 95^0C)

•The monoclonal (SN3B) was diluted to 1:50 with a mouse-directed ABC kit as a secondary antibody and counterstain

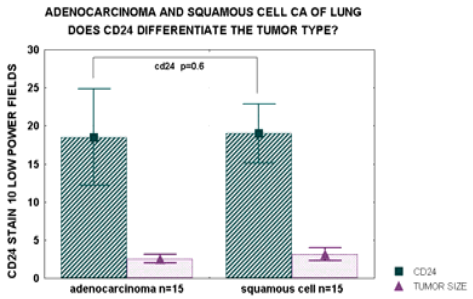
RESULTS



Representative photos of the 15 Squamous cell CA cases that were stained for CD24 using the glycan monoclonal SN3B. CD24 in these cases showed minimal staining (brown-tan) with blue counterstain. Case 7, for example, is largely inflammatory cells. 100x



Adenocarcinoma cases were coded with letters; case B shows two fields of the same tumor section illustrating the high variability from field to field 100x



CD24 staining was scored by three researchers (pathologist, student, researcher) giving an arbitrary score of percent coverage by CD24 stain in a single low power microscope field. Ten sequential fields were scored on each slide randomly selecting an area.
Hatched graph shows the means of all fields for all 15 tumor sections with each diagnosis (150 data points each). The "whisker" is the standard deviation of the data.
The short bar is the tumor size in cm from the surgical pathology/biopsy report.

CONCLUSIONS

- The future direction of this study includes: staining the samples with protein-directed antibodies and analyzing how the results relate to treatment and tumor prognosis.
- The CD24 cells on tumors may be detected by both protein-directed antibodies and glycan directed antibodies. Tumors may express one or the other CD24 antigens. The SN3B antibody used here stains glycans. These 30 tumor samples need to be stained with a CD24 protein directed antibody to clarify my original hypothesis.
- A weakness is the small sample size, and the absence of staging data on the surgical pathology reports which, if present, would allow more in-depth comparison of staining to tumor character

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