Spring 5-20-2022

Antigen staining for detection of MUC13 and MUC16 expression in carcinoma tissue

Jose A. Benitez
The University of Texas Rio Grande Valley, jose.benitez02@utrgv.edu

Antigen staining for detection of MUC13 and MUC16 expression in carcinoma tissue

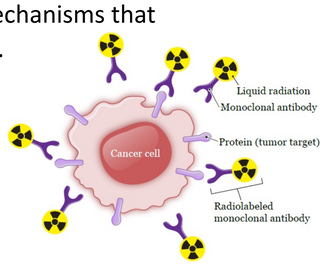
Jose Benitez
MEDI-9331
UTRGV School of Medicine

Abstract

- MUC13 and MUC16 are epithelial expressed proteins implicated in various carcinomas. Overexpression of these biomarkers appear to play a role in tumor growth; this discovery has paved a road for multiple studies discussing the potential of targeting mucin proteins and optimize immunotherapy approaches against carcinomas.
- Our study serves to investigate the level of expression of MUC13 and MUC16 in cancerous and normal tissue and to discuss the implications our findings may have for the utilization of these biomarkers for cancer therapy.

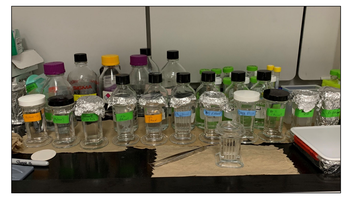
Introduction and background

- Radioimmunotherapy (RIT) is a relatively new anti-neoplastic therapeutic approach that combines high dose radiation to cancer cells with antigen specific targeted therapy with monoclonal antibodies.
- Over the past few decades, radiosensitivity for solid tumors has become more known and subject of recent immunotherapy research.
- RIT allows high-dose radiation to be more effectively targeted against tumor specific cells, therefore optimizing deposition of radiation in tumors and allowing a safer method of immunotherapy approach that spares innocent cells.
- Multi-antigen immunotherapy has been shown to increase effectiveness against tumors vs. a single antigen approach, indicating the significance of further detection and research of neoplastic associated antigens.
- Mucin 13 (MUC13) and Mucin 16 (MUC16) are proteins expressed in epithelial mucosal surfaces whose overexpression has been linked to certain epithelial carcinomas.
- Epidermal growth factor receptor (EGFR) protein is heavily involved in cell signaling that regulates cell mitosis, growth, and lifespan. EGFR has been extensively studied and identified as another prevalent tumor biomarker.
- In previous studies, overexpression of these biomarkers has been heavily linked to increased cancerous cell proliferation and inhibiting immune mechanisms that protect cells from becoming cancerous.

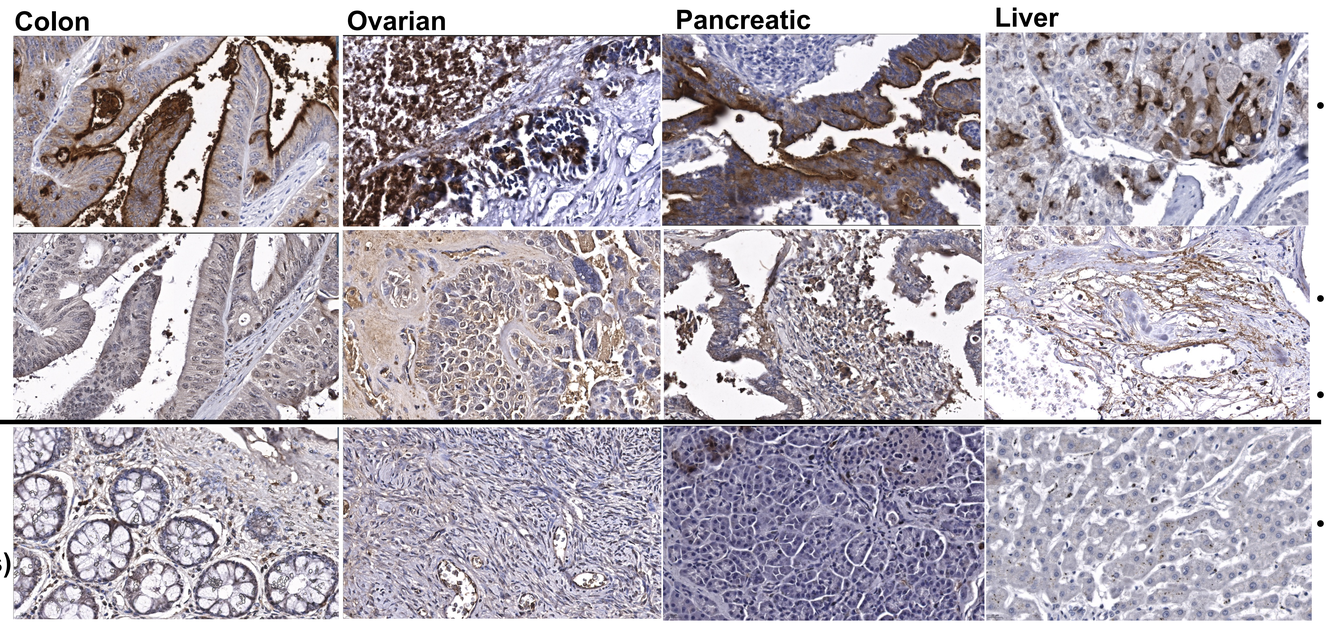


Materials and Methods

- Immunohistochemistry is a laboratory method that allows the visualization of specific antigens in tissue samples through antibody-antigen enzymatic interaction.
- We used IHC to investigate the level of protein expression of MUC13 and MUC16 in tissue microarray with different types and stages of cancers (TMA samples of patients with confirmed cancer diagnosis were used).
- Cancerous tissues were of pancreatic, ovarian, colon, and liver origin; samples underwent either single or double IHC staining to detect antigen (MUC13 & MUC16) expression.
  - ❑ After proper processing, samples were incubated with primary antibody (i.e., MUC13, MUC16) overnight.
  - ❑ TMAs were incubated with secondary antibody and stained using 3, 3'-diaminobenzidine (DAB) and hematoxylin.
- Slides were analyzed using a 3D-Histech Slide Scanner; samples were visualized at 40X magnification.



Results



- Immunostaining with DAB (brown highlights) demonstrating MUC13 and MUC16 expression in multiple types of carcinomas.
  - ❑ Bottom row shows non-cancerous tissues
- DAB staining appeared prominent in cytoplasmic and apical cellular regions.
- Cancerous tissue samples that underwent double staining showed more expression of both MUC proteins (not pictured here).
- Multi-antigen labeling clearly demonstrated higher proportion of stained cancer cells per tissue samples.

Discussion

- MUC16 and MUC13 expression was more prevalent in cancerous tissue. The level of expression also varied among different types and stages of cancer (comparisons not pictured above).
- Expression of MUC proteins was evident in normal tissue indicating heterogeneity of protein expression. This indicates the potential need to target multiple tumor antigens to optimize exclusivity of immunotherapy against tumor cells.
- Further research is needed to elucidate on other relevant protein biomarkers that can be synergistically targeted along with MUC proteins for effective immunotherapy approach against carcinomas.

References

Chauhan, S., Vinayek, N., Maher, D., Bell, M., & Dunham, K. (2007). Combined Staining of TAG-72, MUC1, and CA125 Improves Labeling Sensitivity in Ovarian Cancer: Antigens for Multi-targeted Antibody-guided Therapy. *Journal of Histochemistry & Cytochemistry*, 867-875.

Felder, M., Kapur, A., Gonzalez-Bosquet, Jesus, Horibata, S., Heinz, J., . . . Fass, L. (2014). MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. *Molecular Cancer*, Article number: 129.

Sheng, Y. H., S, T., R, W., Das, I., Gerloff, K., Florin, T. H., . . . McGuckin, M. A. (2012). MUC1 and MUC13 differentially regulate epithelial inflammation in response to inflammatory and infectious stimuli. *Mucosal Immunology*, 557-568.