1475 PROFILING CYTOKERATINS IN THE TUMOR MICROENVIRONMENT USING MULTIPLEX IMMUNOFLUORESCENCE

Danielle Fails*, Michael Spencer. *Fortis Life Sciences, Montgomery, TX, USA*

Background Cytokeratins have been regarded as a predictive disease marker and provide important clinical information such as tumor growth, potential metastases, and response to therapeutic agents.[1] For clinical purposes, it's important to capture the presence and/or absence of the correct cytokeratins in a tumor microenvironment, as each has distinct diagnostic purposes (i.e., cytokeratin 7 is useful in the distinguishment of ovarian and gastrointestinal carcinomas). This study profiled cytokeratins (types I and II) and the overall immune profile within the tumor microenvironments of numerous cancer tissues.

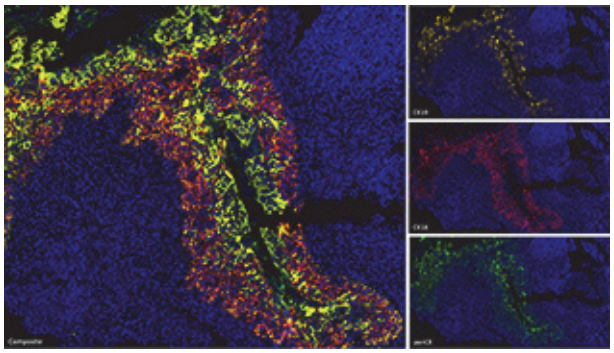
Methods Tissue microarrays containing multiple (80+) FFPE human tissue types (cancerous and normal) were stained with Bethyl Laboratories IHC-validated primary antibodies. A panel consisting of ten immune targets (CD3e, CD20, FoxP3, CD8a, PD-L1, panCK, PD-1, CD68, CD45, PCNA), three epithelial to mesenchymal transitionary (EMT) targets (Vimentin, E-cadherin, Zeb1), and five cytokeratins (CK7, CK14, CK18, CK19, CK20) was used to profile the tumor microenvironment. Optimization of antibody concentrations was performed for each target prior to multiplex staining. Antigen retrieval was done in the Epredia© PT Module using Tris-EDTA ph9 solution, at 100°C, for 1 hour incubation. Automated immunofluorescent staining and imaging of the samples was performed on the Lunaphore COMET™ system. Subsequent analysis of images for distinct phenotypes was done using the HORIZON™ software.

Results Cytokeratin profiles were examined in conjunction with immune phenotypes. Any samples that experienced a significant loss of tissue due to technical procedures were excluded from analyses. An increase in CK19 coupled with a decrease in CK14 points to enhanced potential for malignancies, whereas the reverse or relatively similar levels trend towards benign disease status (figure 1). In addition to this signature, the relationship between malignancy and/or metastasis and high expression of CK7 in was observed. Tissues that exhibited a PD-L1+/panCK+ phenotype, particularly lung and breast cancers, were linked to malignancies. Finally, high expression of Vimentin (EMT) was correlated with metastasis, and this has been shown to be a target indicative of poor prognosis.[2]

Conclusions Cytokeratins play many important roles in the body and are of significant clinical importance. Current, generalized diagnostic tests for these proteins are often inexpensive and yield fast presence/absence information to aid in the treatment of a variety of conditions. However, patients often have unique circumstances that require a more individualized approach. Examination of these structural markers in a patient's tumor microenvironment using multiplex immunofluorescence can provide additional information to further enhance clinical treatment plans.

REFERENCES

1. Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. *Clin Biochem*. 2004 Jul;**37**(7):529–40. doi: 10.1016/j.clinbiochem.2004.05.009. PMID: 15234234.
2. Katsarou SD, Messaritakis I, Voumvouraki A, Kakavogiannis S, Kotsakis A, Alkahtani S, Stournaras C, Martin SS, Georgoulias V, Kallergi G. Detyrosinated α-Tubulin, Vimentinand PD-L1 in Circulating Tumor Cells(CTCs) Isolated from Non-Small CellLung Cancer (NSCLC) Patients. *J.Pers. Med*. 2022;**12**:154. https://doi.org/10.3390/jpm12020154.



Abstract 1475 Figure 1 Tonsil tissue stained with panCK. CK14, and CK19 — indicators of benign/normal status and positive surface and crypt epithelium

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1475