Utilizing Multiplex Immunofluorescence to Explore the Epithelial-Mesenchymal Transition in Breast, Ovarian Cancers

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Background Epithelial-mesenchymal transition (EMT) is instrumental during embryonic development—assisting in extensive movement and differentiation of cells. However, during metastasis and tumorigenesis, this process is hijacked. The disruption of this developmental process, and subsequent acquisition of a mesenchymal phenotype, has been shown to increase therapeutic resistance and often leads to poor prognosis in breast cancer. Using bioinformatic resources and current clinical data, we designed a panel of biomarkers of value to specifically observe this epithelial/mesenchymal transition.

Methods Human breast cancer FFPE tissue samples were stained with Bethyl Laboratories IHC-validated primary antibodies, followed by Bethyl HRP-conjugated secondary antibodies, and detected using Akoya Opal™ Polaris 7-color IHC kit fluorophores (Akoya Biosciences [NEL861001KT]). The panel consisted of beta-Catenin, E-Cadherin, Ki67, CD3e, PD-L1, and FOXP3. Antibody staining order was optimized using tissue microarray serial sections, three slides per target, and stained in either the first, third, or sixth position via heat-induced epitope retrieval (HIER) methods. Exposure time was maintained for all three slides/target and cell counts, signal intensity, background, and autofluorescence were analyzed. The final optimized order was then tested on the breast cancer microarray in seven-color mIF. Whole slide scans were generated using the Vectra Polaris® and analyses performed using InForm® and R® Studio.

Results Two integral EMT targets, E-Cadherin and beta-Catenin, were used to observe a key occurrence in this transition. Under tumorigenic circumstances, when released from the complex they form together (E-cadherin-B-catenin complex), Beta-catenin can induce EMT. This disjunction/activation of EMT can be seen in the invasive ductal carcinoma below (figure 1). The disorganized E-cadherin cells are in direct contrast to normal, non-cancerous cells in similar tissue. Total CD3e cell counts were down (2%), with 35% cells restricted to the stroma vs. the 1% seen intra-tumorally. Coupled with the elevated presence of Ki67 (10%), a level of rapid cancer growth and potential metastasis (Invasive Ductal Carcinoma Grade II) can be observed.

Conclusions The presence of EMT in breast cancers is often indicative of a poor prognosis, so the need for reliable markers is imperative. E-Cadherin and beta-Catenin are both up-and-coming clinical targets that can serve to outline this transition within the tumor microenvironment. By utilizing these markers in mIF, closer spatial examination of proteins of interest can be achieved. The application of this mIF panel has the potential to provide invaluable insights into how tumor infiltrating lymphocytes behave in cancers exhibiting the hallmarks of EMT.

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REFERENCES

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