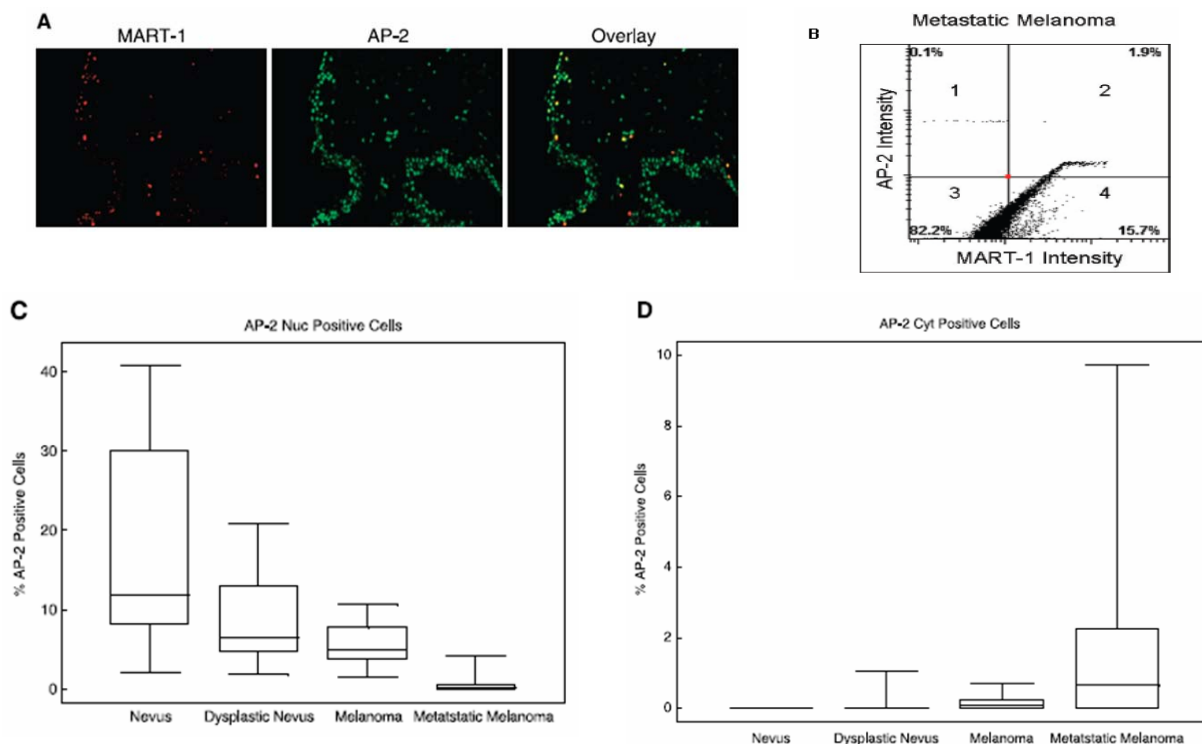


Case Study #10 - High Complexity Cellular Localization Study Identifies Melanoma Survival Protein

Tissue microarrays (TMAs) provide a high-throughput approach to simultaneously screen hundreds of patient samples on a single slide in a uniform fashion, thereby reducing the technical variability between specimens. Automated quantitative analysis systems can be used to generate accurate, reproducible measurement of protein, DNA or RNA. In addition, quantitative analysis provides continuous scoring as opposed to pathology-based categorical scoring. This capability is crucial for identifying subtle differences in expression, particularly for validating biomarkers with high variation in expression in a large number of samples. Scientist's at ApoCell designed and developed a high complexity analytical method for quantifying nuclear and cytoplasmic protein levels in a melanoma TMA. The results showed that a high level of AP-2 expression in the cytoplasm relative to the nucleus correlates with poor prognosis and the loss of nuclear AP-2 expression is associated with malignant transformation and progression of melanoma.



A, Laser-generated image of a melanoma TMA immunofluorescently stained for MART-1 and AP-2. Optimization of staining is crucial for accurate flow analysis of each biomarker. **B**, During laser scanning, quantitative information is systematically acquired for nuclear or cytoplasmic compartments based on the fluorescent intensity of each probe. Scattergram shows the intensity of AP-2 expression in MART-1 positive cells. **C**, Nuclear expression of AP-2 from each case on the TMA was analyzed. A box plot displays the median (middle line), and the minimum and maximum range (outside lines) of MART-1/AP-2-positive cells from each diagnosis group. **D**, Cytoplasmic expression of AP-2. Levels of AP-2 expression were analyzed using a Kruskal-Wallis test.

Prior to this study, the literature indicated that loss of AP-2 expression was associated with tumorigenicity and metastatic potential in melanoma cells. Scientist's at ApoCell astutely recognized the peculiar staining pattern while analyzing the melanoma TMA. Subsequently, a systematic multiplex assay was developed to accurately quantify nuclear and cytoplasmic AP-2 expression in MART-1-positive cells. This assay was crucial for demonstrating that the distribution of the AP-2 protein, and not the loss, correlates with progression of melanoma and poor prognosis.

Reference

1. Cancer Res. 2005 Dec 1;65(23):11185-92