RESOLVING HISTOLOGICAL HETEROGENITY BY MORPHOLOGY GUIDED DIGITAL SPATIAL PROFILING PROVIDES NEW CLUES TO RATIONALIZE IMMUNOTHERAPY APPLICATION IN MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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Malignant Pleural Mesothelioma (MPM) is a deadly and heterogeneous disease. Morphological heterogeneity is evidenced by the existence of three distinct phenotypes: epithelioid (E-MPM), sarcomatoid(S-MPM) and biphasic MPM (biph-MPM), the latter consisting of a mix of both phenotypes. Degree of differentiation reflects clinical aggressiveness with S lesions being the most lethal, making histology the most credited criteria for treatment choice. However, due to their mixed phenotype, biph-MPMs remain difficult to diagnose in pre-surgical biopsies as well as to manage, due to the lack of definitive guidelines, making urgent the development of biomarkers to overcome these limitations. Resolving tumor heterogeneity at the molecular level is essential to improve clinical management of these lesions.To this end, we employed a morphology-guided gene expression profiling approach using the GeoMx™Digital Spatial Profiler (DSP), to map intra-patient evolution of biph-MPM. A total of 188 Area of Interest (AOIs) in the E (n=89) and S (99) components from a retrospective cohort of 8 surgically resected biph-MPMs were selected and profiled for the expression of 1800 genes of the Cancer Transcriptome Atlas. Differential analysis revealed that transition from E to S phenotype is marked by expression loss of many epithelial distinctive elements including Cadherins, Keratins and Desmosomes components and by gain of processes like angiogenesis and matrix remodeling that usually underline cancer aggressiveness.Also, transcriptional mediators of EMT including ZEB1, ZEB2, SNAI2 and TWIST1 were increased in S-components in support of a driving role of this process in biph-MPM. Noticeably,S-component profiles showed signs of inflammation and immune activation, evocative of a potential difference in the immune-microenvironment between E and S phenotype. Analysis on a panel of selected genes by Nanostring nCounter in a validation cohort of 84 MPMs confirmed these differences.

Overall, these data demonstrated that morphology guided transcriptional profiling offers a high-fidelity picture of the tumor spatial heterogeneity providing new insights into the pathobiology of biph-MPM. Our results point out for the first time to a histotype-specific structural difference in the immune comportment of MPM. In light of the fact that a different response was observed in patients with different histotype, this observation holds important implication to improve the use of immunotherapy in MPM.