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Extracellular Matrix in muscle-invasive Bladder Cancer Identification and validation of ECM related mechanisms in MIBC: A multi-omics analysis of a novel interpreted 3D *in vitro* model

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Bladder cancer (BC) is the second leading cause of cancer death in the world. Treatment options for muscle-invasive bladder cancer (MIBC) are limited, with poor prognosis and incomplete molecular tumor classification posing a significant problem. BC onset and progression is highly dependent on extracellular matrix (ECM) remodeling. Moreover, the use or development of translational 3D *in vitro* models is rare in BC research, thus the mechanistically background underlying the interaction between MIBC tumor cells and ECM, as well as the identification of novel therapeutic targets and biomarker needs to be further investigated.
In this study, the BC cell lines (UROtsa, RT4, T24/83, SCaBER) were applied to the 3D *in vitro* ultra-low-attachment (ULA) method under Matrigel-free conditions. Intact spheroids were analyzed by a transcriptomic microarray and proteomic approach, and were compared to the regular used 2D model. Based on the protein level, gene set enrichment analysis was performed and pathways related to ECM proteins were identified. Resulting targets were further validated by public available mRNA sequencing data from MIBC patients. The targets gene expression was related to clinicopathological parameters, overall survival (OS), and disease-free survival (DFS).
Intact spheroid formation and growth was realized for T24/83 and RT4, while T24/83 needed the addition of 50% unconditioned fibroblast basal medium (UCM) or fibroblast conditioned medium (CM). A combination of ECM-based and -interacting targets (n=10) were significantly increased on protein level and mRNA level for the invasive T24/83 spheroids, compared to 2D culture. 6(10) targets were elevated under CM (CALU, CD109, FBN1, HTRA1, LAMC1, LRP1), 2(10) in both (P4HA2 and PLOD1), and 2(10) under UCM condition (DPYSL3 and SUMF2). Furthermore, increased levels of these targets correlated with worse OS, DFS and advanced MIBC features. Mechanistically, these targets were found to act in epithelial to mesenchymal transition (EMT), embryonic stem cell signature (ESC), neurological activity, cancer pathways, and posttranslational modification (PTM). For RT4, no ECM-related target was valid.
Spheroids of the invasive T24/83 BC cell line expressed malignant features more abundant compared to the 2D standard condition. Under CM, more targets were validated compared to UCM conditions, indicating more distinct MIBC properties under CM condition. Thus, T24/83 spheroids cultured under CM conditions can serve as a valid *in vitro* 3D model to study invasive BC mechanisms (EMT, ESC, RAF1/MAP2K1/ERK pathway, ERBB2) and treatment options for future projects. Furthermore, follow up the 10 identified ECM-related targets could lead to promising molecular biomarker or future therapeutic targets, which is urgent to improve personalized stratification, and therapy for MIBC patients.