Application of Targeted Proteomics to Track Epithelial to Mesenchymal Transition in Adenocarcinomas

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Epithelial to mesenchymal transition (EMT) naturally occurs during embryogenesis and tissue repair but is also involved in cancer progression and metastasis. EMT induces complex cellular and microenviromental changes resulting in loss of epithelial phenotype and acquisition of mesenchymal properties, which promotes migratory and invasive capabilities to cells. EMT can be triggered by several factors, including TGF-β, HGF and PDGF or by overexpression of some transcription factors such as ZEB1, TWIST1, SNAIL and SLUG. We studied EMT induction using a targeted proteomics approach in different cancer tissues, represented by breast, ovarian, pancreatic and prostate cell lines. Proteotypic peptides representing 103 relevant proteins, selected from high throughput proteomic study of EMT, were selected and synthesized in light and heavy forms to compose our molecular signature of EMT. Using a 30 minutes MRM method developed in a UPLC-XEVO TQs platform (Waters), we quantified this multiplex EMT molecular signature using a in adenocarcinoma cell lines that were induced to EMT by treatment with growth factors or overexpression of the transcription factor SNAIL. Fifty micrograms of total extracts and conditioned media of cell lines induced (or not) to EMT were processed to obtain tryptic peptide digest for subsequent MRM analysis. The method reached a sensitivity of around 50fmol injected, allowing quantification of proteins present at nanograms/ml in samples. Our data not only validates previous experiments where some EMT markers were regulated in one ore more cancer cell lines, but also demonstrates important protein alterations that can be considered tissue specific. For instance, HE4, a promising biomarker for ovarian cancer was extensively modulated in several cell lines during EMT. With this MRM method we expect to monitor large patient sample sets in order to validate EMT-related proteins as potential targets for cancer diagnosis.
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