

## **DIFFERENTIAL PROTEIN EXPRESSION PROFILING BY ITRAQ-2DLC-MS/MS OF HUMAN BLADDER CANCER EJ138 CELLS TRANSFECTED WITH THE METASTASIS SUPPRESSOR KISS-1 GENE**

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The use of isobaric tags for relative and absolute quantization (iTRAQ) followed by multidimensional liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis is emerging as a powerful methodology for biomarker and drug target discovery. KiSS-1 is a metastasis suppressor gene that has been reported to be involved in the progression of several solid neoplasias. The loss of KiSS-1 gene expression has been shown to be inversely correlated with increasing tumour stage and poor overall survival in bladder tumors. Moreover, cases developing distant metastases displayed complete loss of KiSS-1 expression. In order to identify the molecular pathways associated with the metastasis suppressor role of KiSS-1 in bladder cancer, we carried out a proteome discovery analysis of bladder cancer cells (EJ138) transiently transfected with a vector encompassing the full length KiSS-1 gene using an iTRAQ approach. Protein extracts collected after 24h and 48h transfection were fractionated, digested with trypsin and treated with iTRAQ reagents. The labelled peptides were separated through Strong Cation Exchange (SCX) and Reversed Phase LC and analysed by MALDI TOF/TOF MS. Three software packages were utilized for data analysis: ProteinPilot for identification and quantification of differentially expressed proteins, Protein Center for gene ontology (GO) analysis and Ingenuity Pathway to provide insight into biological networks. Comparative analysis among transfected, mock and empty vector exposed cells have identified more than 800 proteins with high confidence (>99%), showing high correlation rates among replicates (>70%). The involvement of the identified proteins in biological networks has served to characterize molecular pathways associated with KiSS-1 expression and to select critical candidates for validation analyses by Western Blot using independent transfected replicates. As part of complementary clinical validation strategies, immunohistochemical analyses of potential metastasis-related biomarkers in bladder cancer progression have been performed in metastatic bladder tumours spotted onto tissue microarrays (n=78). In summary, our study not only has served to reveal molecular mechanisms associated with the metastasis suppressor role of KiSS-1 in bladder cancer, but also to identify novel potential metastatic biomarkers for patients affected with bladder tumors.