

iTRAQ-Facilitated Proteomic Analysis of Human Prostate Cancer Cells Identifies Proteins Associated with Progression

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The unpredictable behavior of prostate cancer presents a major clinical challenge during patient management. In order to gain an insight into the molecular mechanisms associated with prostate cancer progression, we employed the shot-gun proteomic approach of isobaric tags for relative and absolute quantitation (iTRAQ), followed by 2D-LC-MS/MS, using the poorly metastatic LNCaP cell line and its highly metastatic variant LNCaP-LN3 cell line as a model. A total number of 280 unique proteins were identified ($\geq 95\%$ confidence), and relative expression data was obtained for 176 of these. Ten proteins were found to be significantly up-regulated (≥ 1.50 fold), while 4 proteins were significantly down-regulated (≥ -1.50 fold), in LNCaP-LN3 cells. Differential expression of brain creatine kinase (CKBB), soluble catechol-*O*-methyltransferase (S-COMT), tumor rejection antigen (gp96), and glucose regulated protein, 78 kDa (grp78), was confirmed by Western blotting or independent 2D-PAGE analysis. Additionally, iTRAQ analysis identified absence of the lactate dehydrogenase-B (LDH-B) subunit in LNCaP-LN3 cells, confirming our published data. The clinical relevance of gp96 was assessed by immunohistochemistry using prostate tissues from benign ($n = 95$), malignant ($n = 66$), and metastatic cases ($n = 3$). Benign epithelium showed absent/weak gp96 expression in the basal cells, in contrast to the moderate/strong expression seen in malignant epithelium. Furthermore, there was a statistically significant difference in the intensity of gp96 expression between benign and malignant cases ($p < 0.0005$, Mann-Whitney U). Our study is the first to report the application of iTRAQ technology and its potential for the global proteomic profiling of prostate cancer cells, including the identification of absent protein expression.

Keywords: benign hyperplasia • shot-gun proteomics • 2D-PAGE • immunochemistry • tumor rejection antigen (gp96)