



Commentary

Downregulation of PDZ Domain Containing 1 (PDZK1) is a Poor Prognostic Marker for Clear Cell Renal Cell Carcinoma



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In this issue of *EBioMedicine*, Zheng et al., (2016), used a proteomic profiling approach to report the identification and validation of PDZ domain containing 1 (PDZK1) as a poor prognostic marker for clear cell renal cell carcinoma (ccRCC). This work elegantly highlights the use of quantitative proteomics for cancer biomarker discovery and further validation using multiple patient cohorts.

The demand for the identification of novel prognostic markers for ccRCC is urgent. Currently, there are no molecular markers that can help predict if a tumor will remain indolent or become aggressive. Presently, stage and grade are the best prognostic indicators, however these broad groups do not account for tumor heterogeneity, which can significantly alter the course of disease and subsequently, patient prognosis, even among patients within the same classification. Molecular markers that can help predict which tumors will become aggressive will help shape precision medicine for kidney cancer patients.

Zheng et al. (2016), aimed to identify prognostic molecular biomarkers to identify patients in need of early aggressive ccRCC management. Using a combined approach of quantitative proteomics analysis using isobaric tags for relative and absolute quantitation (iTRAQ) with LC-MS/MS, they compared ccRCC tumor tissue to adjacent normal tissue from the same patient in Stages I, II, III and IV. After statistical analyses, they found 38 significantly upregulated (>1.5 fold over normal, $p < 0.05$) and 174 significantly downregulated (<0.67 fold over normal, $p < 0.05$) proteins. GO annotation in addition to KEGG pathway analysis and GSEA analyses showed downregulated proteins were mainly related to lipid metabolism. Furthermore, two of these downregulated proteins, fatty acid binding protein 1 (FABP1) and PDZK1, were also found related to cell proliferation and consequently selected for further investigation.

mRNA expression of *FABP1* showed no correlation to T stage, however, *PDZK1* was significantly downregulated in ccRCC compared to normal adjacent tissue from the same patient. Furthermore, immunohistochemical analyses showed low PDZK1 protein expression was correlated with a shorter overall survival time ($p < 0.001$) and could discriminate between good and poor prognosis with AUC 0.877. PDZK1 is a 70-kDa adapter protein with four PDZ-interacting domains and is believed to regulate levels of the scavenger receptor class B, type 1 (SR-BI) in a post-transcriptional manner (Kocher et al., 2003), and thus its relation to lipid metabolism. PDZK1 is also a member of the Na^+/H^+ exchange regulatory factor (NHERF) family. NHERFs have been shown to be associated with malignant cell transformation (Yao et al., 2012). PDZK1 was reported by Masui et al. (Masui et al., 2013), to be downregulated in two of six primary ccRCC tumors and one of six metastatic tumors, however it did not pass the criteria to be considered significantly differentially expressed.

On the other hand, Kocher et al. (1999), reported PDZK1 was upregulated in human carcinomas. More specifically, PDZK1 had increased expression in breast cancer tissues, and ectopic expression of PDZK1 stimulated cell growth and enhanced E2-promoted growth of the breast cancer cell line MCF-7 (Kim et al., 2013). Moreover, PDZK1 knockdown in MCF-7 cells blocked estrogen receptor-dependent growth and reduced c-Myc expression (Kim et al., 2013). PDZK1 was also shown to regulate the breast cancer resistance protein in the small intestine (Shimizu et al., 2011).

PDZK1 is highly expressed in the apical membrane of the renal proximal tubular cells. Downregulation of PDZK1 in ccRCC, while upregulation in other cancers, suggests PDZK1 has an alternative mechanism of regulation in ccRCC and may play different biological roles in different cancers. Interestingly, normal breast tissue stains negative for PDZK1 and hence a role for PDZK1 in normal breast tissue has not been identified.

Proteins differentially expressed in tumors compared to normal kidney tissues or different ccRCC stages, were identified using iTRAQ labeling and tandem LC-MS/MS. This method offers the ability to multiplex and quantify several samples simultaneously. In this case, eight samples, including pooled samples from ccRCC patients Stage I to Stage IV, and their adjacent normal tissues, were pooled and subsequently quantified. One of the drawbacks of this method is due to the requirement for enzymatic digestion of proteins prior to labeling which increases sample complexity and therefore needs a powerful multidimensional fractionation method of peptides before MS identification (Chandramouli and Qian, 2009).

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One of the major strengths of this paper was the selection and use of three independent patient cohorts; 112 primary ccRCCs and adjacent normal tissue (Beijing Friendship Hospital 2013–2014), 90 primary ccRCCs and adjacent normal tissue (Beijing Friendship Hospital 2006–2008); and 532 ccRCCs with 72 adjacent normal tissues (TCGA database). The discovery set consisted of 18 pairs of ccRCC and normal adjacent tissue and were used for iTRAQ analysis (selected from the 112 ccRCC tumor set). Validation of the discovery set initial findings was carried out by analyzing the publically available TCGA data set for mRNA expression levels and outcome data including, disease free survival and overall survival. Use of this data is very valuable as publically available databases provide a plethora of molecular information at the mRNA, protein and chromosome levels. Fully characterized data sets including clinicopathological and outcome data allow online data cohorts to be used as validation sets for biomarker discovery. In this case, the authors analyzed mRNA levels for many genes in hundreds of samples and were able to perform survival analysis with the included complete clinicopathological information. They also validated 112 ccRCC tumors and 19 adjacent normal tissues by immunohistochemical analyses and chose 38 pairs of ccRCC and normal adjacent tissue from that cohort for western blot analysis. Finally, 90 pairs of ccRCC tumors and normal adjacent tissues were used for tissue microarray construction and overall survival analysis. In total, the authors used 734 ccRCC samples to support their findings.

There are still many challenges to overcome using quantitative proteomics for biomarker discovery including the reproducibility of labeling-based quantification, patient intra- and inter-variability, tumor heterogeneity, and the determination of cutoff levels that will be able to discriminate between two groups. Also, due to the presence of biases that may exist in a single institution, external validation on at least one independent set of tumors, preferably more, is warranted.

Disclosure

The author declared no conflicts of interest.

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