Analysis of Protein Expression in Human Renal Cell Carcinoma

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ABSTRACT

Protein expressions in eleven pairs of renal cell carcinoma (RCC) primary cell cultures and patient-matched normal kidney primary cell cultures were profiled by two-dimensional polyacrylamide gel electrophoresis. The protein expression profiles were analyzed and twenty-three proteins differentially expressed in some of the RCC cell cultures were identified using mass spectrometry. Among the 23 proteins, manganese superoxide dismutase, αβ-crystallin and annexin IV are over-expressed in more than 50% of the primary RCC cell cultures. Their over-expressions in RCC tissues were identified using immunoblot analysis. Interestingly, none of the 23 proteins was universally differentially expressed in all RCC cell cultures, indicating the heterogeneity of the protein expressions in RCC.

CATEGORY

- Proteomic Data Analysis

Keywords: Protein alterations, Renal Cell Carcinoma, 2-D PAGE.

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1. INTRODUCTION

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) is the major technique used to separate hundreds to thousands of polypeptides from cultured cells and tissues [1, 2] and mass spectrometry (MS) is a powerful technique to identify proteins. Together, these techniques can be used to characterize proteomic alterations and modifications associated with disease processes. Characterizing the alterations of protein expression profiles in cancer cells could provide insights into the understanding of the changes in the functional pathways and thus the fundamental mechanisms of cancer development at the molecular level. In this study, we profiled protein expressions in eleven pairs of primary cell cultures derived from RCC tissues and patient-matched normal kidney tissues utilizing 2-D PAGE. We analyzed the expression profiles and identified twenty-three differentially expressed proteins in RCC by MS.

2. MATERIALS AND METHODS

RCC tissues along with their patient-matched normal kidney tissues were obtained from patients at the Cleveland Clinic Foundation after nephrectomy. Each tissue was cut into small pieces, digested with collagenase in tissue culture media RPMI 1640 to release cells and plated out to obtain primary cell cultures. Proteins from primary cell cultures and tissues were extracted by homogenizing samples in the lysis buffer (0.5% SDS, 25mM Tris, 2.5mM $MgCl_2$) with protease inhibitor, phenymethyl sulfonyl fluoride (PMSF). Each protein sample dissolved in rehydration buffer (7M urea, 2M thiourea, 1% DTT, 1% Chaps, 1% ampholytes, 1% Triton) was actively rehydrated into an immobilized pH-gradient strip (11cm, pH 5-8) and isoelectric focusing was performed. The second dimension separations of proteins were performed in 12.5% SDS/polyacrylamide gel. Coomassie Brilliant Blue stained gels were scanned using a Epson perfection 1650 scanner (Epson) and the protein spots on the gels were analyzed visually and by PDQuest software (Bio-Rad). Gel slices of selected protein spots were analyzed and identified using MS. Immunoblot analysis was performed following standard methods.

3. RESULTS AND DISCUSSION

The overall patterns of protein expressions in every paired normal and cancer cell cultures were highly similar to each other on 2-D PAGE gels (Fig. 1), which indicates cancer cells and normal cells are of the same type. About 500 Coomassie blue stained protein spots were detected on each 2-D PAGE gel. Twenty-three differentially expressed proteins were identified using MS. Among

them, sixteen of the proteins were over-expressed in some of the RCC primary cell cultures and seven were under-expressed. These proteins cover many functional groups including metabolism, cellular morphology, heat shock response, cell growth and cell motility and adhesion.

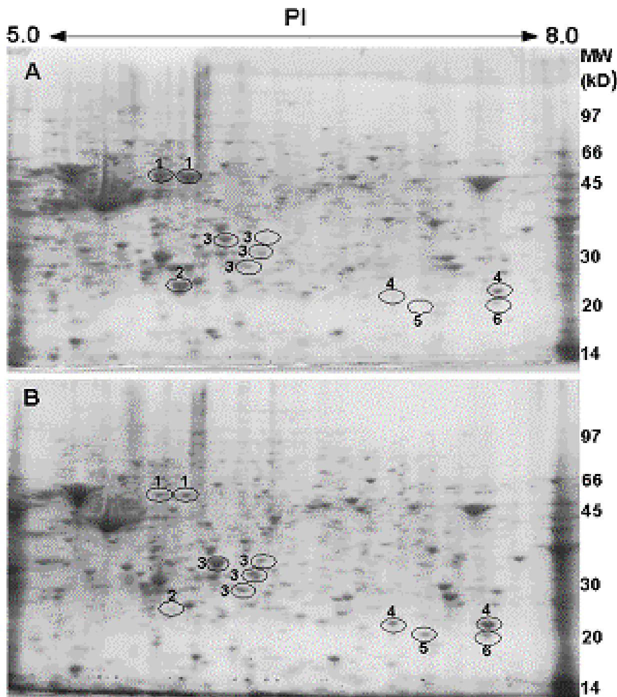

Figure 1AB. A pair of coomassie blue stained 2-D PAGE gels showing the protein expression profiles of primary cell cultures from a normal kidney tissue (A) and a RCC tissue (B). Differentially expressed protein isoforms are circled and labeled with numbers: 1. sarcolectin; 2. glutathione transferse; 3. annexin IV; 4. MnSOD; 5. transgelin; 6. αβ-crystallin.

Table 1. Differentially expressed proteins in RCC

Protein	Chg.	Freq.
Manganese Superoxide Dismutase	↑	7/11
Alpha-β Crystalline	↑	7/11
Annexin IV	↑	6/11
N-myc downstream regulated gene	↑	2/11
Aldehyde Dehydrogenase 1A1	↑	2/11
Phosphoglycerate kinase I	↑	2/11
Heat shock 27kDa protein	↑	2/11
Sarcolectin	↓	2/11

Table 1 shows the proteins that are differentially expressed in at least two RCC samples. The over-expression of manganese superoxide dismutase (MnSOD) and αβ-crystallin were observed in 7 out of 11 RCC cell cultures comparing with their patient matched normal samples. The expression levels have no obvious changes in the other 4 RCC samples. The expression level of annexin IV was found higher in 6 out of 11 RCC samples and no obvious changes in the other RCC samples. But none of the 23 identified proteins were differentially expressed in all the RCC tissue samples. This result is different from the results of some other studies that reported a clear-cut expression alteration of some proteins such as MnSOD [3].

To validate the 2-D protein expression results, we extracted proteins from seven pairs of original RCC tissues and patient-matched normal kidney tissues that were still available and performed immunoblot analysis to determine the protein expression levels of αβ-crystallin, MnSOD and annexin IV. The results of immunoblot analysis (data not shown) were consistent with those obtained from RCC primary cell cultures in the 2-D PAGE analysis.

2-D PAGE has been successfully used in profiling protein expressions in different types of cancers [4,5]. It has been widely used to analyze tumor proteomes and identify potential tumor markers. However, we found many of the tumor-associated protein alterations were not common and some of the protein alterations were detected in only one or two different individual RCC samples. There are a few possible reasons for not being able to detect universal protein alterations among all of the RCC samples examined in this study. First, universal protein alterations in RCC may exist, but they may be contained in certain compartments or membrane proteins, which makes them difficult to be extracted without using some special methods. Also possibly, these proteins are of very low abundance, and the scope and sensitivity of the 2-D methods used in this study cannot detect the possibly subtle, yet important differences in protein expression levels between cancer and normal tissues. Second, the biology of RCC may be very complex at the molecular level and there is no or a very few common changes at the protein level among very heterogeneous RCC patients. Similar results were reported in recent protein profiling study of prostate cancers [6].

We conclude that at the protein level, it may be very difficult to find a single protein whose expression level is significantly altered in all RCC patients. Therefore, a search for a panel of protein tumor markers instead of one universal protein tumor marker that can be used for diagnosis, prognosis and treatment of RCC in clinical setting may be a more feasible goal.

4. ACKNOWLEDGMENTS

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