고포도당생물학의 프로테오믹분석 개선기술

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Improving Technology of Proteomic Analysis in High Glucose Biology

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Introduction : HG induced renal cell injury, profoundly investigated by mass profiling mRNA analysis, gives numerous informative knowledge in the field of oxidative stress and actin cytoskeleton. This analysis needs more integrated understanding in target molecules for the protein-protein reaction. Recently developed two dimensional (2D) protein analyses with computerized technology made easier visual comparison between two groups. Problems, which exist in 2D analysis because of over-saturation of silver staining or low detection by Coomassie blue staining, are now solved by the DIGE analysis. We investigated to improve a better methodology in HG-induced renal cell injury models, which may need a meticulous detection technology since the difference of two groups, normal glucose (NG) vs HG, is too small.

Methods : Mouse mesangial cells (MMCs), mouse tubular cells in normal glucose (NG; 5.6 mM) vs HG (30 mM) and db/m and db/db mouse renal tissues were analyzed. After 2D analysis, silver stained gels were compared by the ImageMaster. For DIGE, samples were labeled with Cy2, Cy3, and Cy5 minimal dyes (GE health), respectively. Labeled samples were combined and processed by 2D analysis same as the silver ones. The Cy2, Cy3, and Cy5 signals were individually imaged with excitation/emission wavelengths of 488/520, 532/580, and 633/670 nm, respectively, using Typhoon 9410 (GE health). DeCyder software (GE health) was used for pairwise comparisons of each dyes.

Results : After DIGE, we detected consistent results and found 9 decreased and 6 increased protein expressions in MMCs cultured in HG for 72h when compared with NG. Up-regulated proteins were ras-GTPase-activating protein SH2-domain binding protein (35%), tropomodulin (TPM) 3 (21%). And down-regulated proteins were gelsolin (23%), glutathion S tranferase (GST) π (31%). For the comparison, silver stained spots corresponding to the significant expressed molecules of DIGE were reanalyzed. Each molecule did not show the significance and revealed variable expressions in silver stained 2D gels, TPM 3 (-9-34%) and GST π (-22-37%).

Conclusion : We found various results of each protein expressions, impossible to decide the difference of changes, in silver stained 2D analysis in cell cultured samples in NG and HG and normal and diabetic mouse samples. DIGE gives better technology to detect significance in high glucose biology.