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Secretome proteomic analysis of renal cells treated with the profibrotic agents ANG II, TGFß1 and PDGF: A comparative study

Marwa Eltoweissy, Gerhard A Müller, Abdul-Rahman Asif and **Hassan Dihazi**
University Medicine of Göttingen, Germany

Secreted proteins are major factors in cell invasion, migration, motility, growth control, matrix degradation and adhesion. Inflammatory agents (cytokines and growth factors) activation results in impairment of secretome and can result in cell transformation. These aspects play an important role in renal fibrosis onset and progression. In the present study, we targeted to investigate the alteration of renal cell secretome upon treatment with pro-fibrotic inflammatory factors and to identify potential key proteins in renal fibrosis progression. For this purpose, renal fibroblasts (TK173) and renal epithelial cells (HK2) were cultured in FCS free medium for 24 h and then treated with TGFß-1, PDGF or ANG II for 72 h. Subsequently, the secretomes were collected and proteins were enriched. Two-dimensional secretome protein maps were generated using 2D-gel electrophoresis and the proteins were processed and identified using mass spectrometry analysis combined with data base search. Differential secreted proteins were annotated and bioinformatics functional analyses were carried out to investigate the potential role of secreted proteins in renal fibrosis progression. We optimized a protocol, which allowed us to achieve high purity in secretome protein for further analysis. 2-DE combined with peptide sequence analysis and database searches identified sets of 61, 41 and 66 non-redundant protein, which were differently secreted in TK173 cells under TGFß-1, PDGF and ANG II treatment respectively. Among the identified proteins, 15 were shared in all treatments. Treatment of renal epithelial cells HK2 with the same agents resulted in differential secretion of 48, 57 and 49 proteins respectively. In case of HK2 only 8 proteins were shared by all three treatments. Functional analysis of the identified proteins by DAVID Bioinformatics software packages identified 41.8% for TK173 and 38.6% for HK2 as secretory proteins, which could be attributed to classical and non-classical secretory pathways. Further functional classification of proteins ascribed to classical pathway verified the presence of proteins belonging to cytokines, receptors, membrane proteins, lysosomal proteins and proteins associated with specific sub-cellular localizations such as endoplasmic reticulum, mitochondria, nucleus, cytoplasm and ribosome. Our results shed light on a very interesting aspect in renal fibrosis progression. The investigation of secretome could help to understand the mechanism of acceleration of fibrosis progression and offer new insights in the pathogenesis of this disease.

Biography

Marwa Eltoweissy has completed her PhD through a scholarship and cooperation work between Faculty of Science, Alexandria University, Egypt and Rheinische Friedrich-Wilhelms-University Medical Center Bonn, Institute for Physiology II, Germany. She achieved Post-doctoral studies at the Gastroenterology and Endocrinology Department, Georg-August University Medical Center, Göttingen, Germany. She received the Doctor of Natural Sciences (Dr. rer. nat.) degree at the Nephrology and Rheumatology Department, Georg-August University Medical Center, Göttingen, Germany. Currently, she works as a Major Scientific Researcher at the later department and as an Assistant Professor of Physiology at the Zoology Department, Alexandria University, Egypt. She has published more than 15 papers in reputed journals and serving as a reviewer for privileged journals.

eltoweissymar@yahoo.com

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