ALTERED PROTO-ONCOGENE EXPRESSION IN RESPONSE TO ELEVATED SUSTAINED HYDROSTATIC PRESSURE IN RENAL CELLS

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INTRODUCTION

Hydrostatic pressure has been shown to alter functions in many tissues and organs, including the kidneys [1]. For example, high blood pressure and diabetes mellitus are the two leading causes of chronic kidney failure; both are associated with elevated pressure in the kidneys. In fact, a correlation has been deduced in previous work equating an increase in renal interstitial pressure of 0.11 mmHg with a 1 mmHg increase in blood pressure [2].

It is our hypothesis that changes in the cellular mechanotransduction pathway associated with these elevated pressures lead to altered tissue/cell responses under such pathological kidney conditions. Once this signaling cascade is elucidated, an efficient rate-limiting step may be determined and used to design novel drug therapies to treat such pathologies.

This study will, therefore, aim to understand these complex signaling pathways by monitoring the molecular level responses of two important renal cells (specifically, tubular epithelial cells which are located in the renal cortex, and inner medullary cells which are located in the collecting duct of the medullary region of the kidneys) under a known pathological hydrostatic pressure (60 cmH_2O; [3]).

METHODS

Cell Culture

IMCD (cell line CRL-2123; ATCC) and LLC-PK1 (cell line CL-101; ATCC) cells were cultured under standard cell culture conditions (that is a sterile, humidified, 37°C, 5% CO_2/95% air environment) in Ham's F-12 and Medium 199, respectively, supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin (P/S). All cells were used between passage numbers 4 to 15 for the pressure experiments.

Pressure Experiments

Prior to the experiments, IMCD and LLC-PK1 cells were seeded (3,523 cells/cm^2) onto etched glass coverslips. After two days in culture, the standard culture media was removed and replaced with Ham's F-12 or Medium 199, respectively, supplemented with 5% FBS and 1% P/S. Kidney cells were then exposed to a sustained hydrostatic pressure of 60 cmH_2O for 6 hours, 12 hours, 24 hours, 3 days, or 7 days using a computer controlled pressure chamber. Control cells were maintained under 0.3 cmH_2O pressure for similar periods of time.

mRNA Expression

Following pressure exposure, kidney cells were lysed using Trizol (Invitrogen). Total RNA was extracted with chloroform and precipitated with isopropyl alcohol. Reverse transcriptase was performed with 2 μg of RNA. PCR was performed with primers specific to c-jun, c-fos, and GAPDH (the housekeeping gene). Agarose gel electrophoresis was conducted on the amplified products, and band intensities of the gene of interest with respect to the housekeeping gene were calculated for each sample. Each experiment was repeated four separate times. Statistical analysis was performed using student t-tests, with $p<0.1$ considered significant.

RESULTS

Results provided evidence that c-jun expression was altered in both medullary and tubular renal cells. Specifically, after 12 hours of pressure exposure c-jun was significantly upregulated by over 16% in the pressure exposed medullary cells ($p<0.1$). This upregulation continued through 7 days of pressure exposure. In contrast, no true trend was observed in the tubular renal cells; the only significant value seen in this set of data was at 7 days, when c-jun was upregulated by 15% in the pressure exposed tubular cells compared to the controls ($p<0.06$).

mRNA levels of c-fos were also affected by pathological levels of hydrostatic pressure. Specifically, significant c-fos upregulation in the pressure exposed medullary cells occurred at the 12 hour time period (by 16%; $p<0.1$) and at the 3 day time period (by 25%; $p<0.06$) compared to the controls. However, c-fos was significantly down-regulated (by 18%) after 7 days of pressure exposure ($p<0.05$). In contrast, in the tubular cells, c-fos expression remained slightly upregulated in pressure exposed cells over the entire length of the

pressure exposure; significant upregulation occurred at 6 hours (by 15%; $p<0.1$), at 3 days (by 10%; $p<0.025$), and at 7 days (by 20%; $p<0.01$).

DISCUSSION

Results from earlier proliferation studies provided the first evidence of increased renal tubular and medullary cell proliferation in response to a pathological hydrostatic pressure. These findings were intriguing and led us to question the mechanism involved in the functional changes observed in kidney cells following a pressure stimulus. In other studies [4], cytoskeletal elements have been implicated in this mechanotransduction process. Our fluorescence microscopy results confirmed these findings, and suggested that the microfilaments played a role in transmitting the mechanical (pressure) signal to the nucleus. In contrast, no reorganization occurred in the microtubules or intermediate filaments; therefore, these cytoskeletal elements did not appear to be a necessary component in the signaling cascade.

The present study confirmed that other chemical mediators (c-jun, and c-fos) are involved in this mechanotransduction pathway [5]. Results to date have shown a time-dependent upregulation in both c-fos and c-jun in tubular and medullary cells following exposure to 60 cmH_2O pressure. These results implicate MAPK cascade involvement in the pressure induced signaling cascade. Both c-fos and c-jun have specific and numerous precursors. For example, ERK, or extracellular signal regulated kinase, regulates the transcription of c-fos whereas JNK, or jun N-terminal kinase (also referred to as stress activated protein kinase, SAPK), regulates the transcription of c-jun [6]. In addition, and relevant to this study, both c-jun and c-fos have been implicated in tissue repair and/or apoptosis when transiently or consistently upregulated, respectively [7].

CONCLUSION

In conclusion, our research has begun to study the effects of elevated hydrostatic pressures (similar to those seen in kidney pathologies) on renal cell growth, cytoskeletal alignment, and altered gene expression.

Currently, short term (*i.e.* within minutes and up to 3 hours) studies of c-fos and c-jun gene expression are being conducted to determine which molecules are involved in the initial pressure-induced signaling cascade. Our preliminary results provide evidence that after one hour of pressure exposure, c-jun is up-regulated by 200% over the control. Future work in our lab will also focus on studying the involvement of the small G-protein class Rho as a link between actin rearrangement and the MAP kinase signaling pathway. Rho is involved in a variety of cell functions including cell growth [8], actin rearrangement [9], and MAP kinase activation [10]. Both long and short-term analysis of the localization and activation of these proteins will be performed with the hopes of correlating findings with both immediate and long-term pressure-related events.

This research project is versatile in that it applies to a wide variety of pathologies (diabetes, high blood pressure, bladder obstruction, etc) that currently exist in the clinical environment of the kidney. In the long run, the outcome of these studies may aid in the prevention and/or treatment of such kidney diseases.

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