



Article

Lysophosphatidic Acid Is a Proinflammatory Stimulus of Renal Tubular Epithelial Cells

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In this report, we have stimulated human proximal tubular epithelial cells (HKC-8) with LPA and 175 other possibly pathological stimuli, and simultaneously detected the levels of 27 intracellular phosphoproteins and 32 extracellular secreted molecules with multiplex ELISA. This quantification revealed a large amount of information concerning the signaling and the physiology of HKC-8 cells that can be extrapolated to other proximal tubular epithelial cells. LPA responses clustered with pro-inflammatory stimuli such as TNF and IL-1, promoting the phosphorylation of important inflammatory signaling hubs, including CREB1, ERK1, JUN, I_B_, and MEK1, as well as the secretion of inflammatory factors of clinical relevance, including CCL2, CCL3, CXCL10, ICAM1, IL-6, and IL-8, most of them shown for the first time in proximal tubular epithelial cells.

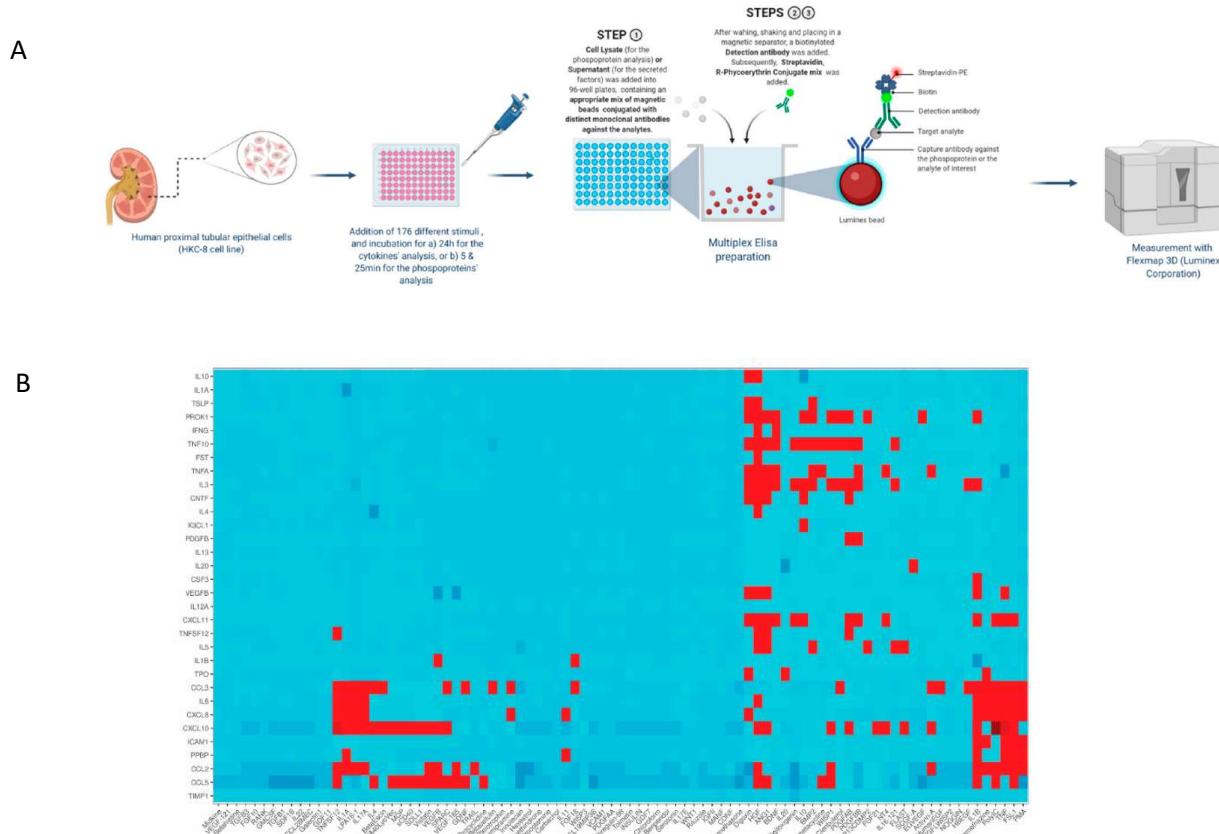


Figure . A. Multiplex ELISA for the detection of secreted factors and signaling molecules phosphorylation. Human kidney proximal tubular epithelial cells, HKC-8, were stimulated with 176 stimuli. Supernatants were collected at 24 h and cell lysates at 5 and 25 min post stimulation. Supernatants or cell lysates were added to a mix containing magnetic beads internally dyed with precise proportions of red and infrared fluorophores, thus, rendering unique spectral signature microspheres. Each unique microsphere-bead was conjugated with a distinct monoclonal antibody against a secreted factor or a phosphoprotein. Biotinylated detection antibodies were added to the mix, followed by a streptavidin-R-Phycoerythrin complex. This process allows the simultaneous recognition of 32 secreted factors or 27 phosphoproteins in one sample. **B.** Heatmap showing the production of cytokines upon the immunological stimuli, LPA being among them.