

P042 The Role of PI3K Isoforms in Cytokine Signalling and Wound Healing in A549 Lung Epithelial Cells

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Parenchymal lung cell functions are controlled by surface receptors for cytokines, chemokines, and other ligands. A shared feature of these receptors is the activation of members of the phosphoinositide 3-kinases (PI3K) family.

We used A549 cells to evaluate the role of PI3K isoforms in cytokine functions. Using lipofectamine delivery, we used siRNA to knockdown individual PI3K catalytic subunits. Optimal knockdown was obtained 72h post-transfection. To assess the contribution of each PI3K isoform to recognized PI3K-dependent signaling, we monitored the outcome of siRNA targeting on phosphorylation of PKB/Akt, S6 and ERK, following cytokine stimulation. The class I p110 β PI3K isoform was essential for the activation of kinases downstream of PI3K after 15m incubation period with IL-1 β (1ng/ml) or TNF α (100ng/ml), but not insulin(100pg/ml) nor IL-13(30ng/ml).

In wound healing assays, a pipette tip was used to score a line in the cell monolayer, and migration was monitored. Wound closure occurred at 48h post-wounding. These studies demonstrated the importance of both class I p110 β and class II β PI3K in migration, as wound closure was delayed in monolayers treated with siRNA against either of these isoforms.

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