The Cloning and Functional Characterisation of Murine Phosphatidylinositol 3-kinase gamma

By

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ABSTRACT

The family of PI3-kinases are an important group of lipid kinases involved in cellular signalling. All members of the family are responsible for phosphorylating the 3-hydroxyl groups of inositol phospholipids. Their structural conformation and substrate specificity separates members into three distinct classes, these being the Class I, II and III PI3-kinases. The Class I family is the best-characterised of all the members and can be further subdivided into 2 groups, the Class IA and Class IB PI3-kinases. The Class IA PI3-kinases are activated through the downstream recruitment of protein tyrosine kinases. In contrast, the Class IB PI3-kinases are dependent on heterotrimeric G proteins for their activation. This study focuses on the only known member of the Class IB PI3-kinases, PI3kγ.

The chemokine gene superfamily is a group of chemotactic cytokines originally identified on their ability to directly recruit distinct and overlapping subsets of leukocytes, and therefore has a critical role in the maintenance, activation and regulation of the immune system. To date, all chemokine receptors are G protein-coupled receptors. Activation of chemotactic receptors results in a transient increase in the level of 3-phosphorylated phospholipids, products of the PI3kinase family. More recently, this increase has been attributed to PI3kγ.

The present study addresses the role of PI3kγ in lymphocyte migration *in vitro* and *in vivo*, in response to chemotactic factors. A novel isoform of PI3kγ, MmPI3kγ1111, was isolated from a murine macrophage cDNA library. Comparison of the sequence of MmPI3kγ1111 with the previously cloned human and porcine

orthologues demonstrated above 90% identity. Consistent with the human and porcine PI3kγs, MmPI3kγ1111 possesses all four conserved domains common to the Class IB subfamily. Uniquely, MmPI3kγ1111 contained an additional 11 amino acids in the catalytic domain. During the latter stages of this study a murine orthologue of PI3kγ, MmPI3kγ1100, was independently cloned. MmPI3kγ1100 did not contain the additional 11 amino acids found in MmPI3kγ1111.

MmPI3kγ1111 was over-expressed in HEK 293 cells and examined for lipid and protein kinase activity. Consistent with MmPI3kγ1100, MmPI3kγ1111 produced PI 3 P *in vitro*, hence lipid kinase activity. MmPI3kγ1111 also demonstrated autophosphorylation, a characteristic common to all known Class IB PI3-kinases. Furthermore, phosphorylation of MmPI3kγ1111 downregulated the ability of MmPI3kγ1111 to act as a lipid kinase.

Mutation of the lysine residue (position 833) critical for the activation of all Class I PI3-kinases resulted in a loss of both the lipid and protein kinase activities of MmPI3kγ1111. The catalytically-inactive form of MmPI3kγ1111 was stably transfected into a B lymphocyte cell line (B300.19) expressing the CCR6 chemokine receptor. B300.19-huCCR6 cells expressing the catalytically-inactive form of MmPI3kγ1111, MmPI3kγKR, demonstrated a marked decrease in the ability to migrate towards huMIP-3α, the ligand for huCCR6, in an *in vitro* chemotaxis assay. Subcutaneous air pouches were used to examine the role of MmPI3kγ1111 in *in vivo* B lymphocyte migration. However, in contrast to the *in vitro* data obtained, the *in vivo* model did not provide clear support for a role for MmPI3kγ1111 in lymphocyte

migration. The biological significance of these data, and future research directions are addressed in this thesis.

CHAPTER 5: THE ROLE OF MmPI3kγ1111 IN LYMPHOCYTE MIGRATION *IN VITRO* AND *IN VIVO*

CHAPTER 6: GENERAL DISCUSSION