Precise gene editing reveals role of FES tyrosine kinase in neutrophil phagocytosis via SYK activation

Tom van der Wel[1], Riet Hilhorst[2], Hans den Dulk[1], Tim van den Hooven[2], Nienke M. Prins[1], Joost A.P.M. Wijnakker[1], Bogdan I. Florea[3], Eelke B. Lenselink[4], Gerard J.P. van Westen[4], Rob Ruijtenbeek[2], Herman S. Overkleeft[3], Allard Kaptein[5], Tjeerd Barf[5] & Mario van der Stelt[1*]

[1]Dept. of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, Leiden. [2]PamGene International BV, 's-Hertogenbosch. [3]Dept. of Bio-organic Synthesis, Leiden Institute of Chemistry, Leiden University, Leiden. [4]Dept. of Drug Discovery & Safety, Leiden Academic Centre for Drug Research, Leiden University, Leiden. [5]Covalution Biosciences BV, Ravenstein.

Introduction

The successful development of new kinase-targeting drugs strongly depends on our understanding of underlying molecular and cellular mechanisms of action, i.e. preclinical target validation.[1] A key step in this process is to obtain proof of target engagement, which correlates the exposure at the site of action to a pharmacological and phenotypic readout. Information about kinase engagement is essential for determining the dose required for full target occupancy without inducing undesired off-target activity.[2]

Objectives

The aim of this study was to develop chemical tools that report on target engagement of endogenously expressed protein kinases by small molecules in human cells.

Methods

We describe a chemical genetics strategy that allows the study of non-receptor tyrosine kinase FES, a promising therapeutic target for cancer and immune disorders. CRISPR/Cas9-mediated gene editing was used in combination with a rationally designed, complementary fluorescent probe to visualize endogenous FES activity in HL-60 cells. We replaced a single oxygen atom by a sulphur in a serine residue at the DFG-1 position of the ATP-binding pocket in an endogenously expressed kinase, thereby sensitizing the engineered protein towards covalent labeling and inactivation by a fluorescent probe.

Results/Conclusions

Our results reveal that FES plays a key role in the phagocytosis of bacteria by activation of SYK kinase, a central regulator of immune function in neutrophils. Moreover, we demonstrate that our chemical genetics strategy is not limited to FES and can also be applied to multiple other kinases. The selectivity acquired by combining gene editing and a complementary probe brings the advantages of acute, pharmacological inhibition without the need for extensive hit optimization programs to identify compounds of adequate potency and selectivity. We thus envision that the presented methodology could provide powerful tools to study the function of poorly characterized kinases and aid in their validation as therapeutic targets.

References

1. Nomura, D. K., Dix, M. M. & Cravatt, B. F. Activity-based protein profiling for biochemical pathway discovery in cancer. *Nat. Rev. Cancer* **10**, 630–638 (2010).
2. Simon, G. M., Niphakis, M. J. & Cravatt, B. F. Determining target engagement in living systems. *Nat. Chem. Biol.* **9**, 200–205 (2013).