P792 CRISPR/CAS9-BASED MODEL OF HETEROZYGOUS CXCR4 WT/R334X MUTATION TO STUDY CELLULAR PHENOTYPES IN WHIM SYNDROME

Topic: 11. Bone marrow failure syndromes incl. PNH - Biology & Translational Research

Katarina Zmajkovicova¹, Sumit Pawar¹, Sabine Maier-Munsa¹, Adriana Badarau², Arthur G. Taveras³

¹ X4 Pharmaceuticals (Austria) GmbH, Vienna, Austria; ² Former Employee at X4 Pharmaceuticals (Austria) GmbH, Vienna, Austria; ³ X4 Pharmaceuticals, Boston, United States

Background: WHIM syndrome is a phenotypically heterogeneous primary immunodeficiency characterized by Warts, Hypogammaglobulinemia, Infections, Myelokathexis, neutropenia and lymphopenia. WHIM syndrome pathogenesis is causally linked to heterozygous gain-of-function (GOF) mutations in the C-terminus of the chemokine receptor CXCR4, a master regulator of immune cell trafficking and homeostasis, causing desensitization defects and hyperactivation of downstream signaling. The most frequently reported mutation in patients with WHIM syndrome is c.1000C>T, which results in a C-terminal truncation of the receptor at position R334 (R334X).

Aims: In vitro assays using cell lines with exogeneous overexpression of CXCR4WHIM variants can model the GOF cellular phenotypes of WHIM syndrome but may not entirely mimic the condition in patients, who typically have one wildtype (WT) CXCR4 allele. We aimed to characterize a cellular model of homozygous and heterozygous CXCR4R334X in the endogenous locus, to better understand the pathogenic impact of harboring mutations in one or both alleles.

Methods: CRISPR-Cas9 platform was used to establish a model of heterozygous mutations found in patients with WHIM syndrome. Jurkat cell line (with endogenous expression of WT CXCR4) was edited to harbor the c.1000C>T/R334X mutation in a single allele (WT/RX) or in both alleles (RX/RX). Unedited parental Jurkat cell line and Jurkat cells with edited silent mutations (WT/WT) were used as controls.

Results: Upon stimulation with C-X-C chemokine ligand 12 (CXCL12), RX/RX cell lines displayed an internalization defect (65% of CXCR4 receptors remaining on the cell surface at 100 nM CXCL12) compared to the parental (24%) and WT lines (20%). The RX/WT-expressing cells had an intermediate phenotype (43%). We analyzed the signaling responses downstream of activated CXCR4, for which GOF phenotypes had been reported in R334X patient cells. Calcium mobilization in response to CXCL12 was enhanced in cells harboring the R334X mutation, reaching a 2.5- to 3-fold higher maximum effect (Emax) compared to cells with WT CXCR4. ERK activation downstream of CXCR4 reached a higher amplitude (8-fold increase over baseline) and duration after stimulation with CXCL12 in all lines expressing R334X compared to the parental and WT/WT cell lines (6-fold increase). Presence of a single mutant allele seemed to confer the full GOF phenotype in both signaling readouts in this cell line. Migration of cells toward CXCL12 was significantly enhanced in R334X-expressing cells. Mavorixafor, a CXCR4 antagonist currently in clinical trials for the treatment of patients with WHIM syndrome, was active in an unbiased manner on a spectrum of CXCR4-related functions (ligand binding inhibition, calcium mobilization, ERK activation and chemotaxis) with comparable biological activity and potency in cells expressing the WT and R334X CXCR4 receptor.

Summary/Conclusion: We established the first model recapitulating the heterozygous CXCR4WT/R334X mutations found in patients with WHIM syndrome using the CRISPR/Cas9 platform. This cellular model recapitulates the functional defects found in immune cells from patients with WHIM syndrome. When stimulated with CXCL12, WT/RX-expressing cells appeared to display full GOF phenotype in downstream signaling assays compared to RX/RX-expressing cells. This observation is consistent with the dominant inheritance pattern of WHIM syndrome. The present study brings several novel insights in CXCR4WHIM biology and enriches the toolbox of models available for studying WHIM syndrome.

Disclaimer: Articles published in the journal HemaSphere exclusively reflect the opinions of the authors. The authors are responsible for all content in their abstracts including accuracy of the facts, statements, citing resources, etc.