Whole genome sequencing identifies recurring somatic mutations in the C-terminal domain of CXCR4, including a gain of function mutation in Waldenstrom’s Macroglobulinemia

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Abstract

Introduction: Waldenstrom’s Macroglobulinemia (WM) is an indolent non-Hodgkin’s lymphoma characterized by the accumulation of IgM-secreting lymphoplasmacytic cells (LPC) in the bone marrow. Using paired normal/WLymphoplasmacytic cell pair tissues and whole genome sequencing (WGS), we identified somatic mutations in the CXC chemokine receptor 4 (CXCR4) gene which were present in 19/60 (32%) WM patients. CXCR4 is a G-protein-coupled receptor, together with its ligand, the stromal cell-derived factor-1 (CXCL12/SDF-1), play an important role in leukocyte and lymphocyte hematopoiesis and trafficking. Upon SDF-1 stimulation, CXCR4 is phosphorylated and interacts with β-arrestins, which then trigger extracellular signal-regulated kinase (ERK) MAPKs and chemotaxis. CXCR4 signaling is then terminated through receptor internalization which is mediated via phosphorylation of its C-terminal tail.

Methods: Sanger sequencing was used to validate WGS results. To clarify the functional significance of one of the most common somatic mutation identified (C1013G), cloning by PCR was undertaken from CD19+ bone marrow cells from a WM patient with the C1013G-CXCR4 (C1013G-CXCR4) mutation. Wild type (WT) and C1013G-CXCR4 eDNA were subcloned into pLenti-IRE-GFP vector, and transduced using an optimized lentiviral based strategy for WM cells into BCWM-1 WM cells. Five days after transduction, GFP positive cells were sorted and used for functional studies. Surface expression of CXCR4 was determined by FACS using PE-conjugated anti-CXCR4 monoclonal antibody (12G5). CXCR4 internalization was studied by comparing CXCR4 surface expression before and after SDF-1 stimulation. Chemotaxis studies were performed using a transwell assay. The expression of phosphorylated ERK1/2 and total ERK2 was determined by western blot.

Results: All validated somatic mutations in CXCR4 were located in the C-terminal domain and included premature stop codons (C1013G, C1013A, C1000T), and frameshift mutations. These mutations are similar/identical to those reported in the germline of patients with WHIM (Warts, Hypogammaglobulinemia, Infections and Myelokathexis) Syndrome, a dominant autosomal genetic disorder caused by toxic CXCR4 activation. This activation is due to impaired CXCR4 internalization resulting in continued G-protein-dependent responses, and chemotaxis. Consistent with such a role, SDF-1 stimulation showed decreased internalization of CXCR4 in C1013G- and C1013G-CXCR4 eDNA versus WT CXCR4 WT transduced BCWM-1 WM cells. SDF-1e stimulated ERK1/2 phosphorylation was also more robust in C1013G-CXCR4 versus WT CXCR4 expressing cells. Lastly, C1013G-CXCR4 transduced cells displayed stronger migratory response toward SDF-1e versus WT CXCR4 expressing BCWM-1 cells.

Conclusions: C-terminal domain CXCR4 somatic mutations are common in WM and overlap with germline mutation identified in WHIM syndrome. Moreover, the most common of these mutations (C1013G) confers gain of function including decreased CXCR4 internalization and phosphorylation, and chemotaxis. These findings provide new insights into the pathogenesis of WM, and a framework for the study of CXCR4 inhibitors for WM therapy.

Summary

1. Through whole genome sequencing, we have identified novel, recurring somatic mutations in the C-terminal domain of CXCR4 gene in patients with WM. These mutations overlap with germline mutations identified in WHIM syndrome.

2. Our functional studies support that the most common of these mutations (C1013G) confers a gain of function, including decreased CXCR4 internalization, more robust ERK 1/2 phosphorylation, and chemotaxis.

3. CXCR4 signaling may therefore represent a novel strategy for targeted therapy in WM patients with activating mutations.

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