Characterization of endogenous CXCR4 inhibitory peptides to target Waldenström's Macroglobulinemia

Kaiser LM[1], Sauter D[2], Münch J[2], Buske C[1].

[1]Comprehensive Cancer Center Ulm, Institute of Experimental Cancer Research, University Hospital Ulm, Ulm, Germany;
[2]Institute of Molecular Virology, Ulm University Medical Center, Ulm, Germany

Waldenström's Macroglobulinemia (WM) is an indolent B cell lymphoma characterized by recurrent mutations of MYD88 and CXCR4. Recent data have shown that the mutational status of these two genes affects response to the BTK inhibitor ibrutinib with inferior outcome for patients carrying the CXCR4 mutation or for those patients without mutations in both genes. The surface protein CXCR4 plays a pivotal role in cell adhesion and migration of lymphoma cells. It is an evolutionarily highly conserved G protein-coupled receptor, binding to its chemokine ligand, CXCL12. Binding of CXCL12 triggers calcium influx via PLC and activate the NF-AT, NF-κB, and PI3K–AKT pathways as well as the mTOR and the MAPK cascades that regulate cell survival, proliferation, and chemotaxis. Based on the key role of CXCR4 we hypothesized that there are endogenous control mechanisms, regulating CXCR4 activity in normal and malignant lymphocytes. Based on screening a human blood-derived peptide library, we succeeded in identifying an as-yet-unknown, naturally occurring inhibitor of CXCR4 signaling. The 16-mer peptide termed EPI-X4 (Endogenous Peptide Inhibitor of CXCR4) was shown to specifically interfere in the cross-talk between CXCR4 positive hematopoietic stem cells and their environment. Importantly, in contrast to AMD3100, which is affecting mitochondria function, EPI-X4 was not immunogenic or cytotoxic. Plasma of WM patients showed elevated levels of the EPI-X4 peptide when compared to healthy controls, confirming that EPI-X4 is generated in WM patients. Treatment of WM cell lines with EPI-X4 lead to a marked deprivation in clonogenicity and migration regardless of the mutational status of CXCR4. To understand the molecular mechanisms behind EPI-X4 we performed gene expression analysis on the WM cell line BCWM.1 upon EPI-X4 treatment alone or in addition with CXCL12 by RNA-Seq. Our data showed significant impact of the peptide on genes affecting carbohydrate and lipid metabolism pathways as well as the RAF/RAS pathway and hypoxia associated signaling cascades, assigning EPI-X4 a role as a potential modulator of cancer metabolism. In addition to lipid metabolism pathways, differences in p53 and cell cycle regulators were significant. We started generating optimized derivatives of EPI-X4 by changing the amino acid structure of EPI-X4 with

longer half-life and enhanced antagonizing capability in the nanomolar range. The two derivatives JM#21 and WSC02 successfully blocked the 12G5 epitope of CXCR4 mutant cells. They efficiently induced apoptotic death (90% and 50% respectively compared to the control) with higher activity than AMD3100 in this assay. Migration of WM cells with or without activating CXCR4 mutations was inhibited by 95% compared to the control. In addition, WSC02 prolonged survival of NSG xenografts transplanted with WM cells by 26 days (median survival control 54,5 days versus 80,5 days for WSC02, respectively). Taken together, our data shed light on the role of a novel naturally occurring CXCR4 antagonist in WM, which as optimized derivative has the potential to be developed clinically.