Comprehensive proteomic analysis of Ibrutinib mediated changes on protein and PTM levels in human B cells.

Reinhild Rösler, Angelina Dangel, Sascha Endres, Sebastian Wiese
Core Unit Mass Spectrometry and Proteomics, Ulm University

Overview

- By targeting the Blyton's Tyrosin Kinase and thus disrupting the B cell receptor signalling cascade, Ibrutinib has proven to be an highly efficient drug in treatment of B cell malignancies.

- Using phosphoproteomics a comprehensive picture of effects induced by Ibrutinib has been created.

- Ibrutinib changes both protein and phosphorylation levels, altering cellular structures and translation events and thus reducing cell survival.

Introduction

The enhanced activation of the B cell receptor (BCR) signaling cascade is a crucial contribution in the pathogenesis, progression and/or maintenance of B cell leukemia, such as chronic lymphocytic leukemia (CLL). Recently, the orally bioavailable irreversible Blyton's tyrosine kinase (Btk) inhibitor ibrutinib has seen a remarkably success as a second-line treatment of patients with relapsed or refractory CLL or mantle cell lymphoma and as a first-line treatment of patients with CLL carrying a del(17p) or TP53 mutation. Despite this success, the underlying changes, such as modulation of various post-translational modifications (PTMs) and/or protein levels, induced by ibrutinib, are poorly understood.

Methods

Using SILAC-conditions, Jeko-1 cells, human B cells derived from a mantle cell lymphoma, were cultivated and exposed to 300 μM Ibrutinib over a course of three days. Samples were collected after 6, 24, 48 and 72 hours and subjected to analysis using SDS-PAGE based proteomics, samples collected after 72 hours were also subjected to SCX/TQ2-based phosphoproteomics. All samples were analysed on an Orbitrap-Velos Pro (Thermo Scientific, Bremen, Germany) online coupled to a RSLCnano (Thermo Scientific, Dreieich, Germany) using Multi-Stage Activation. Data analysis was performed using MaxQuant (MPI Martinsried, München).

Results

Figure 1: Characterization of time dependent protein changes upon Ibrutinib treatment. A) Heatmap showing expression level changes of the XX significantly regulated proteins upon drug treatment. B) Time dependent decline of the Tyrosine-protein phosphatase non receptor type 1 (SHP-1), a BTK-upstream component of the BCR-pathway. C) Peaks in the expression of the E3 ubiquitin-protein ligase UHRF1. D) Allo-guanine nucleotide exchange factor 2 (ARHGEF2) shows a constant increase in abundance over the monitored time frame.

Figure 3: GO-enrichment analysis of proteins and phosphosites regulated upon Ibrutinib treatment. A) Biological process (above) and Cellular compartment (below) GO-terms significantly enriched among the proteins differentially expressed upon treatment. B) GO-terms enriched among proteins showing altered phosphorylation levels under ibrutinib influence.

Ibrutinib significantly affects transcription, translation and cellular organisation.

Conclusion/Outlook

Ibrutinib affects critical pathways in malignant B cells and thus dramatically reduces the survival of these cells, causing the success of this high potential drug.

- Drugs, targeting other components of the BCR pathway, are in clinical use and their effects on proteomes will be characterized in order to find potential prospects for combined treatments.

- Despite its novelty, patients showing mutations leading to Ibrutinib resistance have been observed. The effects of these mutations will be analysed.

Contact

As a core facility of the Medical Faculty, Ulm University, the CUMP offers services in proteomic research, to initiate contact please e-mail Dr. Sebastian Wiese
sebastian.wiese@uni-ulm.de
http://fakultaet.medizin.uni-ulm.de/forschung/core-facilities/proteomics/

Funding

This work was in part funded by the DFG through SFB1074 "Experimental models and clinical translation in leukemia " (INST 40/514-1)