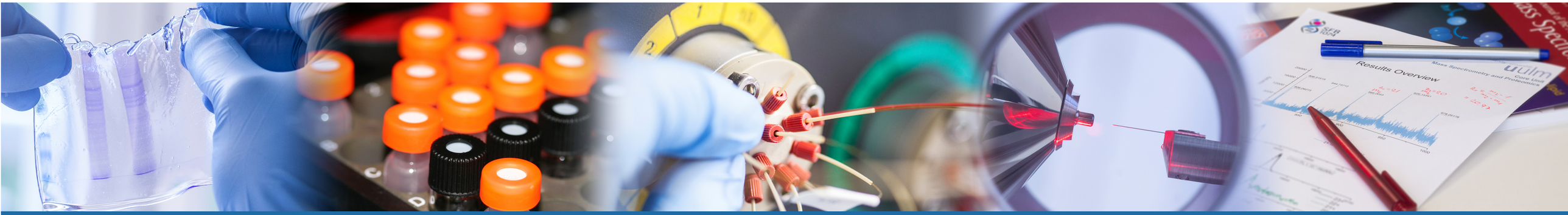

Comprehensive proteomic analysis of Ibrutinib mediated changes on proteins and PTMs in malignant human B cells

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Overview

- By targeting the Bruton's tyrosin kinase and thus disrupting the B cell receptor signalling cascade, Ibrutinib has proven to be an highly efficient drug in the treatment of B cell malignancies.
- Detailed studies in a reconstituted system indicate a high number of phosphorylation events on the PLCγ2 and the dependency on these for enzymatic activity within the B cell receptor pathway.
- Using global phosphoproteomics a comprehensive picture of effects induced by this potent drug 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) signalling cascade is a crucial contribution in the pathogenesis, progression and/or maintenance of B cell leukemia, such as chronic lymphocytic leukemia (CLL). Recently, the irreversible Bruton's tyrosine kinase (BTK) inhibitor Ibrutinib has seen a remarkable success as a first or second-line treatment of patients with various types of B cell malignancies. Despite these successes, the underlying changes, such as modulation of phosphorylation events on the BTK target PLCγ2 (1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2) as well as subsequent events in the BCR cascade like the modulation of various post-translational modifications (PTMs) and/or protein levels, induced by Ibrutinib, are poorly understood.

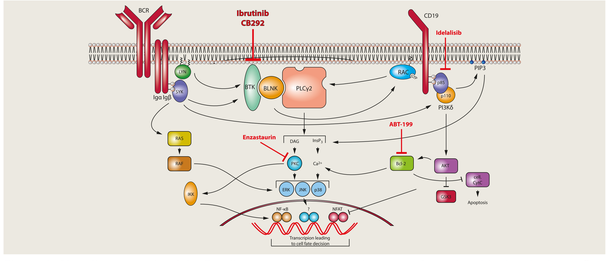

Figure 1: Signaling cascade of the B cell receptor pathway and targets of novel anti-leukemic drugs. Due to its importance in the development of leukemic disease, proteins of the BCR signalosome are targets of novel first and secondary treatments. An array of novel components has entered the clinic in the past years, Ibrutinib being one of the most promising among them.

Results

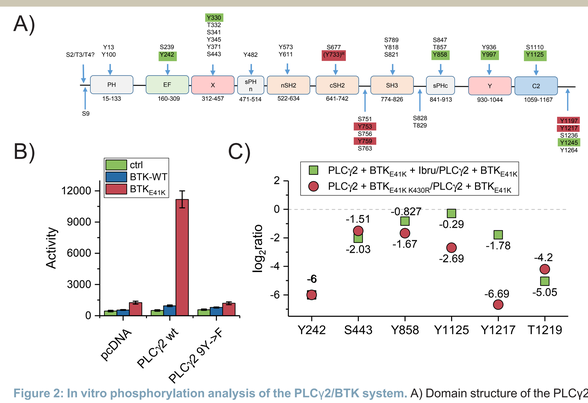

Figure 2: In vitro phosphorylation analysis of the PLCγ2/BTK system. A) Domain structure of the PLCγ2-protein and phosphorylation sites identified using LC/MS²-analyses. Phosphorylation sites highlighted (green, newly identified; red, previously known) were subsequently mutated to render the PLCγ2 inactive. B) Activity of the PCLγ2 in the presence of BTK or BTK_{E41K}, a constitutively active form. Only mutation of several phosphorylation sites inactivates PLCγ2, indicating a tightly controlled enzymatic function. C) Quantitative phosphorylation analyses using SILAC indicates a reduction of phosphorylation levels in PLCγ2 in the presence of Ibrutinib.

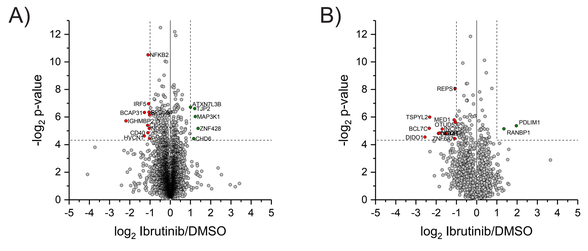

Figure 3: Effects of 3d Ibrutinib treatment on the global levels of A) proteins and B) phosphorylations in human B cells. Using SILAC based quantitative phosphoproteomics, 6410 proteins and 4447 phosphosites were quantified. Significantly regulated proteins and phosphorylations indicate the influence of Ibrutinib upon treatment.

Poster

If you are interested in our work feel free to follow the QR-code to a digital copy of this and other posters illustrating our current research activities.

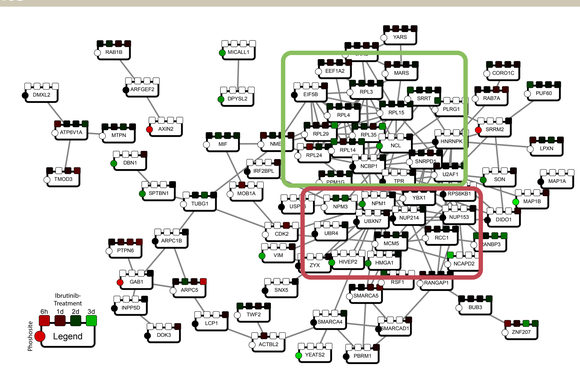

Figure 4: Interaction network showing effects induced by Ibrutinib treatment. Interaction network of proteins showing either significantly altered protein levels or phosphorylation level changes and having at least binary interactions as retrieved from the String Database (www.string-DB.org) are shown. As shown in Figure 3, proteins involved in translation (green square) are affected by Ibrutinib. In addition, proteins known to have important roles in B cell malignancies, such as NPM1 (red square), are altered in either protein or phosphorylation levels.

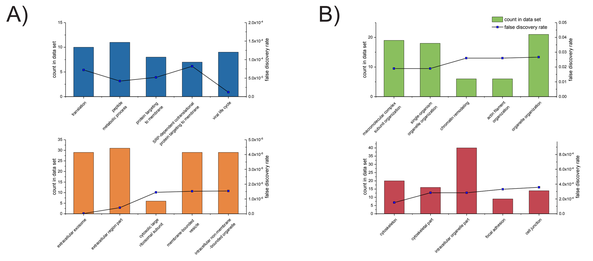

Figure 5: GO-enrichment analysis of proteins and phosphosites regulated upon Ibrutinib treatment. A) Biological process (above) and Cellular compartment (below) GO-terms significantly enriched among the proteins differentially expressed upon treatment. B) GO-terms enriched among proteins showing altered phosphorylation levels under Ibrutinib influence. Ibrutinib alters transcription, translation and cellular organization dramatically.

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Conclusion/Outlook

- Ibrutinib inactivates the BTK and thus prevents phosphorylation of the PLCγ2, subsequently affecting critical pathways in malign B cells and thus dramatically reducing the survival of these cells, causing the success of this high potential drug.
- Global proteomic analyses indicate an involvement of various other PTMs as an indirect effect of Ibrutinib treatment. This necessitates characterisatin of these modifications by mass spectrometric means.
- Drugs targeting other components of the BCR pathway are in clinical use. Their effects on B cell 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 subjects of further research efforts.

Methods

For the analyses of PLCγ2 phosphorylation events, COS-7 cells were transfected as indicated with either empty vector (pcDNA3.1) or vectors encoding PLCγ2, wild-type BTK (BTK-WT) or a constitutive active form of BTK (BTK_{E41K}). Twenty-four hours after transfection, the cells were incubated for 18 h with myo-[2-3H] inositol, and the enzymatic activity was then measured by means of the inositol phosphate formation.

Using SILAC-conditions, Jeko-1 cells, human B cells derived from mantle cell lymphoma, were cultivated and exposed to 300 nM or 500 nM Ibrutinib over a course of three days. Samples were collected after 6, 24, 48 and 72 hours and subjected to analyses using SDS-PAGE based proteomics, samples collected after 72 hours were also subjected to SCX/TiO2-based phosphoproteomics. All samples were analyzed on an Orbitrap-Velos Pro (Thermo Scientific, Bremen, Germany) online coupled to an RSLCnano (Thermo Scientific, Dreieich, Germany) using Multi-Stage Activation. Data analysis was performed using MaxQuant (MPI Martinsried, München).

Contact

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