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

Overview

By targeting the Bruton’s tyrosin kinase and thus disrupting the B cell receptor signaling 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.

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 leukemias, 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 phosphatase 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.

Results

Figure 2: In vitro phosphorylation analysis of the PLCγ2/γ1γ2 system.

A) Domain structure of the PLCγ2 protein and phosphorylation sites identified using LC/MS-MS approach. Phosphorylation sites highlighted (gray, asterisk) determined; red, previously known were subsequently modulated to interfere the PLCγ2 reaction. B) Activity of the PLCγ2 in the presence of IB or IB+X in a constitutively active form. Only modulated reversible phosphorylation sites (red circle) indicating a high bacterial activity (B). C) Quantitative phosphoproteome analysis using SILAC indicates a reduction of phosphorylation levels in PLCγ2 in the presence ofibrutinib.

Figure 3: Effects of 34 facilein treatment on the global level of A) proteins and B) phosphophorylation in human B cells. Using SILAC-based quantitative phosphoproteomics, 415 proteins and 497 phosphorylation sites were quantified. Significantly regulated proteins and phosphophorylated sites indicate the influence ofibrutinib upon treatment.

Figure 4: Interaction network showing effects induced by ibrutinib treatment. Interaction network of proteins showing either significantly altered protein levels or phosphorylation levels changes and having at least 95% interactions as retained from the String Database (www.string-db.org) are shown. As shown in Figure 5, proteins involved in translation (green squares) 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 phosphophorylates regulated upon ibrutinib treatment.

A) Biological processes (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. Blue: altered transcription, translation and cellular organization transcriptionally.

Conclusion/Outlook

• Ibrutinib inactivates the BTK and thus prevents phosphorylation of the PLCγ2, subsequently affecting critical pathways in malignant B cells and 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 characterisation of these modifications by mass spectrometric means.

• Drugs targeting other components of the BCR pathway are in clinical use. Their effects on B cell proteins 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, CO5-7 cells were transfected as indicated with either empty vector (pcDNA3.1-) or vectors encoding PLCγ2, wild-type BTK, or BTK-I707A or a constitutively active form of BTK (BTK-I185). Forty-eight 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, 6x CO5-1 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 RSLi/nano (Thermo Scientific, Dreieich, Germany) using Multi-Stage Activation. Data analysis was performed using MaxQuant (MPI Martinsried, München).

Contact

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Poster

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