# PHOSPHORYLATION OF USP1 BY BCR-ABL LEADS TO DEREGULATION OF ITS FUNCTIONS AND PROGRESSION OF CHRONIC MYELOID LEUKEMIA

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## BACKGROUND

The development of chronic myeloid leukemia (CML) is associated with the appearance of the Bcr-Abl oncoprotein, which is formed as a result of reciprocal translocation between chromosomes 9 and 22. Due to the constitutive tyrosine kinase activity Bcr-Abl uncontrollably phosphorylates its protein partners. USP1 was identified as a potential partner for interaction with Bcr-Abl by mass spectrometric analysis. USP1 is a deubiquitinating protein that prevents proteosomal degradation of proteins, its' dysfunction is typical for malignant cells. We believe that during the formation of the protein complex, Bcr-Abl kinase activates USP1 by phosphorylation, which promotes the accumulation of cancer protein in cells and the progression of CML.

## AIM

To study the molecular mechanisms of Bcr-Abl/USP1 protein complex formation, features of USP1 phosphorylation and the effect of Bcr-Abl tyrosikinase on its activity in CML cells.

## MATERIALS AND METHODS

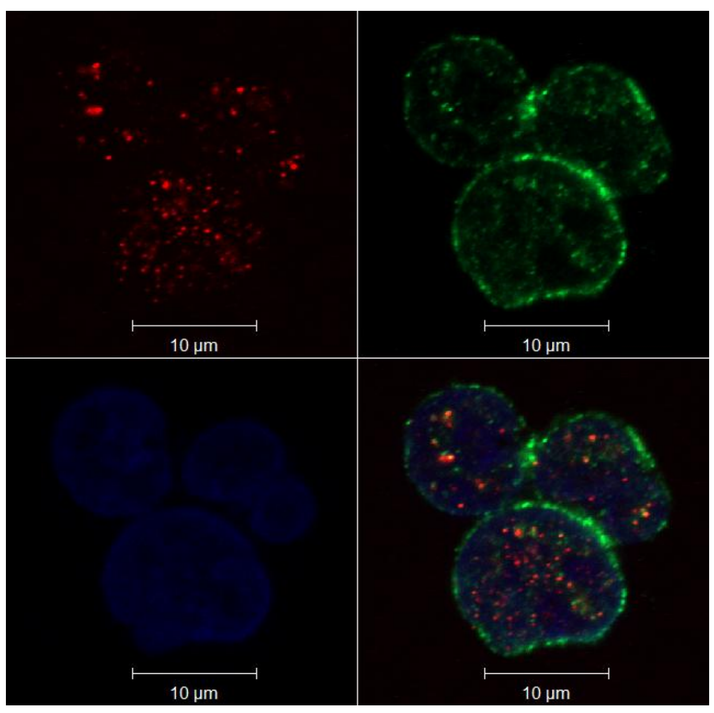
Confocal K562 cells were grown in RPMI 1640 medium with the addition of 10% FBS at + 37°C, 95% relative humidity and 5% CO2. To inhibit tyrosine kinase activity, 2.5 mM imatinib was added to the cells and incubated for 24 hours. The study of the Bcr-Abl/USP1 protein complex and phosphorylated forms of USP1 was performed using co-immunoprecipitation, Western blot, immunofluorescence analysis, confocal microscopy and quantitative analysis. Bioinformatic analysis of phosphorylation sites was performed using KinasePhos, NetPhos 2.0 Server and PhosphoPICK software.

## RESULTS

The interaction and nuclear colocalization of Bcr-Abl and USP1 in K562 cells were shown, which became a prerequisite for studying phosphorylated forms of USP1. Bioinformatic analysis provided Y504, Y678, Y681 phosphorylation sites for the USP1. Experimentally, by co-immunoprecipitation and immunofluorescence analysis, the presence of tyrosine-phosphorylated forms of the USP1 in the nuclei of K562 cells was confirmed. It has been found that under the effect of a tyrosine kinase inhibitor, the USP1 changes its nuclear localization to cytoplasmic, which is known to be one of the signs of loss of enzyme activity


***Fig 1**. Colocalization of USP1 (green) and Bcr-Abl (red) proteins in K562 cell. Merge localization signals USP1 and Bcr-Abl proteins (yellow).*


***Fig 2**. Precipitation of endogenous proteins from lysate of K562 cells, using antibodies against Bcr-Abl.*



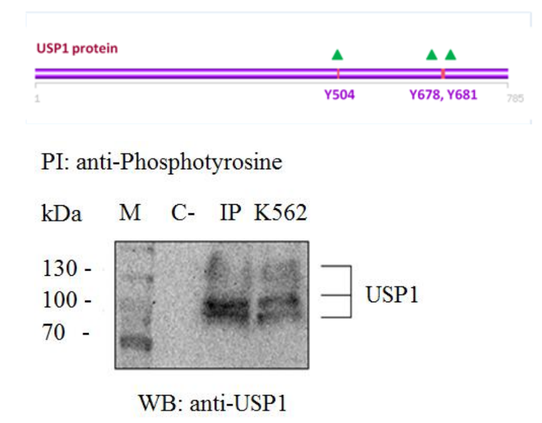




***Fig 3**. Analysis of phosphorylated forms of USP1 in K562 cells: immunofluorescent staining, location of tyrosine sites on USP1, results of bioinformatics analysis and precipitation of proteins using antibodies against phosphotyrosine.*

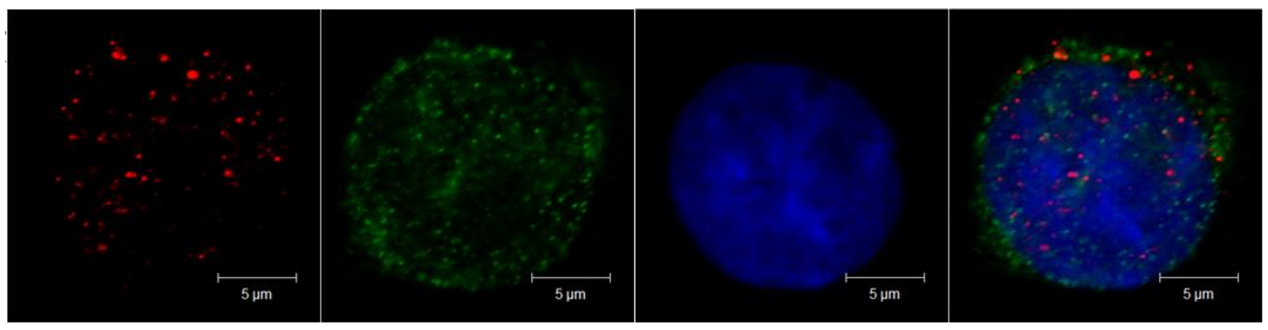



***Fig 4.** Immunofluorescence analysis in K562 cell after incubation with 2,5 mM imatinib for 24 hours. The cell nuclei are stained with DAPI (blue),USP1 protein (red) and Bcr-Abl (green) oncoprotein. Merge localization USP1 and Bcr-Abl proteins (yellow).*

## CONCLUSION

A new protein complex Bcr-Abl/USP1 was detected in CML cells, the formation of which may result in uncontrolled phosphorylation of the USP1. The relationship between Bcr-Abl tyrosine kinase activity and partial disruption of nuclear exports for the USP1, which is a sign of an inactive form of the protein, has been shown. We believe that uncontrolled phosphorylation of the USP1 by Bcr-Abl kinase deregulates its functions and by deubiquitination prevents proteosomal degradation of the oncoprotein, which contributes to its accumulation and progression of CML.

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