Beata Pyrzynska, PhD
Department of Immunology
Medical University of Warsaw
Banacha 1a, 02-097 Warsaw, Poland

Report from active participation in 55th American Society of Hematology Annual Meeting and Exposition, New Orleans, LO, USA, December 6-10, 2013 – Beata Pyrzynska

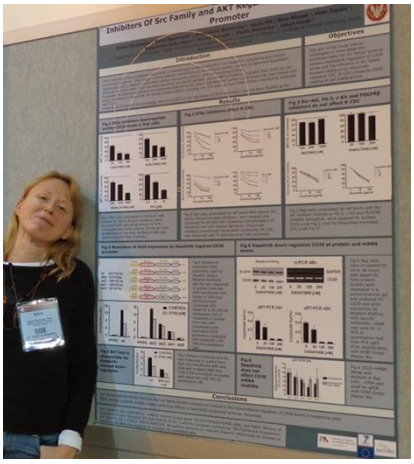
The 55th ASH Annual Meeting gathered international participants who attended the education and scientific sessions and presented their work during the oral or poster sessions. Additionally, the participants in training had opportunity to attend the Trainee Day (Dec. 6th). Beata Pyrzynska participated in the ASH Meeting as an author of a poster presented during the session "Lymphoma: Pre-Clinical – Chemotherapy and Biologic Agents" held on Dec. 7th.

Inhibitors of SRC family and AKT regulate the activity of CD20 promoter.

Pyrzynska B (presenting author), Bojarczuk K, Winiarska M, Bil J, Miazek N, Zapala P, Bobrowicz M, Dwojak M, Siernicka M, Golab J

Background

One of the major mechanisms responsible for the resistance to anti-CD20 therapy seems to be the reduced level of CD20 antigen on the surface of tumor B-cells. We have previously discovered that CD20 expression is strictly dependent on the activity of Src family tyrosine kinases and AKT kinase. We have noticed that treatment of B cells with Src family inhibitors or AKT inhibitors leads to a dose-dependent reduction of CD20 at both transcript and protein level. On the other hand the overexpression of constitutively active AKT (CA-AKT) leads to a significant increase in CD20 mRNA level. To uncover the transcriptional mechanisms governing the CD20 expression we employed the construct encoding the promoter region of CD20 cloned upstream of the firefly luciferase gene. The truncated and mutated versions of the CD20 promoter were used in the luciferase assays to elucidate the role of particular transcription factors binding sites in the regulation of CD20 expression.



Results

Src family inhibitor (dasatinib) and AKT kinase inhibitor (MK-2206) strongly decreased the activity of CD20 promoter in luciferase assays, while the overexpression of CA-AKT partially blocked the inhibition caused by dasatinib. Using the truncated versions of the CD20 promoter we found that lack of the region (-313/-198) made the promoter insensitive to dasatinib treatment. Since this particular region is known to contain a putative Octamer

transcription factor binding site (BAT-box, Thevenin et al., 1993), we introduced mutations in the BAT-box sequence. Although basal

promoter activity was indeed decreased, dasatinib was equally effective in reducing the activity of both wild-type and mutated CD20 promoter. Collectively, our results indicate that Octamer transcription factor is an important regulator of basal CD20 expression, but it is not the major mediator of the effects caused by Src family inhibitors.

Conclusion

Our studies indicate that the Src family tyrosine kinases and AKT kinase are involved in the transcriptional regulation of CD20 antigen in lymphoma cells. The activity of CD20 promoter is significantly reduced upon treatment with Src family inhibitors, namely dasatinib. The particular region of CD20 promoter (-313/-198) was identified as the major region sensitive to dasatinib treatment. The transcriptional machinery responsible for the reduction of CD20 expression by dasatinib needs further investigation since the expected Octamer transcription factor does not mediate the effects caused by dasatinib.

The poster presented by Dr Pyrzynska was visited by several participants and provided the opportunity to exchange information about our research results with laboratories of Dr. Adrian Wiestner (Bethesda, MD, USA), Dr. Thorsten Zenz (Heidelberg, Germany) and Dr. John Byrd (Columbus, OH, USA).