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The annual Congress of the European Hematology Association is the most important European conference regarding latest advances in hematology. Over 2300 abstracts have been submitted for inclusion in the program of 19th EHA congress, and only 200 abstracts were selected for a presentation in one of the 40 oral sessions covering all fields of hematology. During the meeting three posters where I was a senior author were presented by members of my team and I participated in the preparation of all of them. The annual EHA Congress is an excellent opportunity to meet cooperating international researchers as well as scientists working in a the field of our studies. It is also a unique opportunity to hear new lectures on the most up-to-date issues in hematooncology.

Poster list:

(1) SK053 – an allosteric protein disulphide isomerase inhibitor induces differentiation of human acute myeloid leukemia cells
(2) HDAC6 inhibition augments the efficacy of anti-CD20 monoclonal antibodies by up-regulating CD20 level in malignant B-cells
(3) Src inhibitors downregulate CD20 and modulate the activity of the CD20 promoter
SK053 – an allostERIC PROTEIN DISULphIDE ISOMERASE INHIBITOR INDUCES DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Background: Introduction of differentiation-inducing agents including all-trans retinoic acid (ATRA) and arsenic trioxide to the treatment of acute promyelocytic leukemia (APL) was a remarkable therapeutic breakthrough resulting in cure rates exceeding 80%. However, there is no such significant progress in the treatment of other acute myeloid leukemia (AML) types. Thus search for new agents exerting anti-leukemic effects by targeting novel and unique cellular mechanisms is of utmost clinical importance. Numerous human proteins involved in tumor formation contain allosteric disulfide bonds that are cleaved by oxoreductases or by thiol-disulfide exchange. Such disulfide modifications participate in post-translational protein control and affect protein function. Targeting of allostere disulfide bonds is a novel promising strategy in cancer therapy (Hopp J. Nat Rev Cancer 2013). We have recently developed SK053, a small molecule inhibitor of thiodoxin/thioredoxin reductase system, that showed anti-tumor effects both in vitro and in murine tumor models (Klossowski S, Muchowicz A et al., J Med Chem 2012). Our ongoing studies revealed that SK053 is not a target-specific, but mechanism-selective inhibitor of enzymes involved in allostere disulfide bonds formation such as protein disulfide isomerase (PDI).

The aim of the studies was to determine whether targeting the formation of allostere disulfide bonds with SK053 can induce antitumor effects in acute myeloid leukemia.

Fig. 1. SK053 binds to PDI and inhibits its enzymatic activity. 
(A) binding of biotinylated active SK053 analog - SK321 and biotinylated inactive compound - SK053 to mammalian PDI protein. (B) SK053 inhibits the enzymatic activity of PDI in a turbidimetric assay of thiol-disulfide redox balance serving as a positive control. (C) postylation of biotinylated proteins using maleimide-reactive biotin from the lysates of HEK293 cells preconcentrated for 1 with 100 μM SK321, solution with 1 μM PDI and 5 μM biotin or 1 μM PDI and 5 μM biotin + 5 μM PDI (Cu). The presence of PDI and biotin was detected with Western blots. (D) The presence of PDI and biotin was detected with Western blots. (E) The presence of PDI and biotin was detected with Western blots. (F) The presence of PDI and biotin was detected with Western blots. (G) The presence of PDI and biotin was detected with Western blots. (H) The presence of PDI and biotin was detected with Western blots. (I) The presence of PDI and biotin was detected with Western blots.

In summary, SK053 targets PDI and thiodoxin/thioredoxin reductase system, has significant anti-leukemic activity and induces differentiation of various types of human AML cells. Thus, targeting of enzymes involved in allostere disulfide bonds formation with small molecule inhibitors presents a novel and promising therapeutic strategy in acute myeloid leukemia.

All the authors declared no relevant financial relationship to disclose as well as no off-label drug usage to be included into presented data.

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SRC inhibitors downregulate CD20 and modulate the activity of the CD20 promoter

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INTRODUCTION

Anti-CD20 monoclonal antibodies have made a breakthrough in the treatment of non-Hodgkin’s lymphoma and chronic lymphocytic leukemia. They trigger indirect effector mechanisms of the immune system, namely complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and immunophagocytosis. Although for many years CD20 has been described as a stable antigen, accumulating evidence indicates that CD20 can be modulated at both transcriptional and posttranscriptional levels. Down-regulation of surface CD20 levels has been linked with tumor resistance to rituximab. Here, we demonstrate that inhibition of Src family kinases (SFKs) results in increased resistance of tumor cells to antitumor activity of anti-CD20 mAbs. Our observations strongly imply that CD20 down-regulation roles on transcriptional mechanisms and highlight the role of AKT in SFKs-dependent transcriptional regulation of CD20.

OBJECTIVES

The aim of this study was to investigate in more detail the molecular basis of Src family tyrosine kinases-dependent regulation of CD20 levels and the influence of Src family kinases inhibitors on antitumor activity of anti-CD20 monoclonal antibodies possible of anti-CD20-positive B cell malignancies.

RESULTS

Fig.1 SFKs inhibitors downregulate surface CD20 levels and affect CDC in Raji cells

Fig.2 SFKs inhibitors impair NK cell cytotoxicity in ADCC assay

Fig.3 Dasatinib impairs antitumor activity of rituximab in in vivo model

CONCLUSIONS

Our studies indicate for the first time that Src family tyrosine kinases are involved in the transcriptional regulation of CD20 levels in lymphoma cells. SFKs inhibitors downregulate macromolecules in the CD20 monoclonal antibodies, both in vitro and in vivo.

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All authors declared no relevant financial relationship to disclose as well as no off-label drug usage to be included in presented data.
HDAC6 inhibition augments the efficacy of anti-CD20 monoclonal antibodies by up-regulating CD20 level in malignant B-cells

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BACKGROUND

CD20, an integral membrane protein expressed on the surface of normal and malignant B-cells, is widely used as a molecular target for monoclonal antibodies (mAbs) in the therapy of NHL and CLL. Unfortunately, about 50% of patients do not respond to anti-CD20 mAbs. One of the reasons of this resistance is low CD20 level. Accumulating evidence indicates that CD20 can be modulated at several levels, both transcriptional and posttranscriptional, and its up-regulation results in increased efficacy of anti-CD20 mAbs. CD20 expression is reported to be regulated epigenetically e.g. by histone deacetylases (HDACs).

OBJECTIVE

The aim of our project was to check if specific inhibitors of HDACs can influence CD20 level and improve the efficacy of anti-CD20 mAbs.

Fig. 1. Pan-HDAC inhibitors and specific HDAC6 inhibitors up-regulate CD20 level and improve R-CDC

Fig. 3. CD20 up-regulation by HDAC6 inhibitors does not rely on regulation of transcription

Fig. 2. Inhibition of HDAC6 up-regulates CD20 level in lymphoma cell lines, EBV transformed B-cells and primary CLL cells

Fig. 4. HDAC6 silencing augments CD20 and improves R-CDC while HDAC6 overexpression does not affect it

Fig. 5. HDAC6 is implicated in CD20 trafficking

CONCLUSIONS

The results of our study strongly suggest that combining HDACi with anti-CD20 antibodies can be a successful therapeutic modality for patients suffering from B-cell malignancies. The use of isoform-selective inhibitors of HDAC6 may be an effective strategy in enhancing the efficacy of anti-CD20 mAbs. Potentially these compounds would have less adverse effects than HDAC pan-inhibitors. However, their use in the therapy requires further investigation.