XPO1 expression worsens the prognosis of unfavorable DLBCL that can be effectively targeted by selinexor in the absence of mutant p53

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Abstract
The XPO1 inhibitor selinexor was recently approved in relapsed/refractory DLBCL patients but only demonstrated modest anti-DLBCL efficacy, prompting us to investigate the prognostic effect of XPO1 in DLBCL patients and the rational combination therapies in high-risk DLBCL. High XPO1 expression (XPO1-high) showed significant adverse prognostic impact in 544 studied DLBCL patients, especially in those with BCL2 overexpression. Therapeutic study in 30 DLBCL cell lines with various molecular and genetic background found robust cytotoxicity of selinexor, especially in cells with BCL2-rearranged (BCL2-R+) DLBCL or high-grade B-cell lymphoma with MYC/BCL2 double-hit (HGBCL-DH). However, expression of mutant (Mut) p53 significantly reduced the cytotoxicity of selinexor in overall cell lines and the BCL2-R and HGBCL-DH subsets, consistent with the favorable impact of XPO1-high observed in Mut-p53-expressing patients. The therapeutic effect of selinexor in HGBCL-DH cells was significantly enhanced when combined with a BET inhibitor INCB057643, overcoming the drug resistance in Mut-p53-expressing cells. Collectively, these data suggest that XPO1 worsens the survival of DLBCL patients with unfavorable prognostic factors such as BCL2 overexpression and high-grade, in line with the higher efficacy of selinexor demonstrated in BCL2-R+ DLBCL and HGBCL-DH cell lines. Expression of Mut-p53 confers resistance to selinexor treatment, which can be overcome by combined INCB057643 treatment in HGBCL-DH cells. This study provides insight into the XPO1 significance and selinexor efficacy in DLBCL, important for developing combination therapy for relapsed/refractory DLBCL and HGBCL-DH.

Keywords: XPO1, DLBCL, HGBCL, TP53 mutation, Selinexor, MYC, BCL2

To the editor
XPO1 (exportin 1) is a well-characterized nuclear export protein responsible for the nuclear-cytoplasmic transport and cellular homeostasis of up to 220 cargoes, including the tumor suppressors p53 and IκB [1, 2]. Abnormal XPO1 expression correlates with worse prognoses in human malignancies. Targeting XPO1 is a promising therapeutic approach in cancer [1, 2]. The XPO1
inhibitor selinexor has received FDA approval recently to treat refractory/relapsed (R/R) diffuse large B-cell lymphoma (DLBCL) after at least 2 lines of systemic therapy, showing an overall response rate of 28% in the SADAL trial [3]. However, it remains largely unknown whether and how XPO1 interplays with other adverse predictors in DLBCL, how to predict selinexor effectiveness, and what combination therapy is optimal in R/R DLBCL patients. Here, we evaluated the prognostic significance of XPO1 expression in 544 well-characterized DLBCL cases, and investigated the therapeutic effect of selinexor in 30 DLBCL cell lines with variable genetic background.

Patients and Methods for this study are detailed in Additional file 1. XPO1 expression was observed in 217 of 544 (40%) DLBCL patients with a mean level of 24%.

High level of XPO1 expression (XPO1\textsuperscript{high}; > 30%) predicted significantly poor progressive-free survival (PFS) and overall survival (OS) in DLBCL patients (Fig. 1a). DLBCL is classified into prognostic favorable germinal center B-cell-like (GCB) and unfavorable activated B-cell-like (ABC) subtypes [4]. XPO1\textsuperscript{high} significantly shortened the PFS/OS in ABC-DLBCL but not GCB-DLBCL (Additional file 1: Figure S1A–B). XPO1\textsuperscript{high} showed significant association with p53 overexpression (p53\textsuperscript{+}) and dual p53\textsuperscript{+}MYC\textsuperscript{high} expression but not clinical features (Additional file 1: Table S1), unlike a previous study using a different scoring system for XPO1 expression in 131 DLBCL patients [5].

Whether XPO1\textsuperscript{high} interacts with other adverse prognostic factors and whether XPO1 is a potential

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therapeutic target in high-risk DLBCL patients were further examined. XPO1\textsuperscript{high} remarkably worsened the OS and PFS of DLBCL with BCL\textsubscript{2}\textsuperscript{high} or dual MYC\textsuperscript{high}BCL\textsubscript{2}\textsuperscript{high} expression (Fig. 1b,c), which is known as double-expressor lymphoma with unfavorable prognosis [6]. Trends of adverse impact were also observed on PFS in MYC-rearranged (R\textsuperscript{+}) patients (P = 0.097; Additional file 1: Figure SIC) and OS in patients with dual MYC-R\textsuperscript{+}BCL\textsubscript{2}-R\textsuperscript{+} (Fig. 1c) with dismal prognosis, defined as high-grade B-cell lymphoma with unfavorable prognosis [7]. In patients with TP53 mutation (Mut-TP53) [8], XPO1\textsuperscript{high} showed opposite prognostic effects in patients with and without Mut-p53 protein overexpression [9], suggesting the nuclear export may attenuate the oncogenic gain-of-function of Mut-p53. In contrast to the negative impact of XPO1\textsuperscript{high} in TP53/p53-negative patients (Fig. 1d) and in TP53-wild type (Wt-TP53) patients (Additional file 1: Figure S1D), a favorable effect was associated with XPO1\textsuperscript{high} in Mut-TP53/p53-positive patients, which was significant in the BCL\textsubscript{2}\textsuperscript{low} subset (Fig. 1e). Gene expression profiling [4] analysis identified a distinct gene expression signature for XPO1\textsuperscript{high} in patients with Mut-TP53 and MYC\textsuperscript{high} (Fig. 1f), including upregulation of SIRPA, which encodes SIRP\textalpha, a receptor for CD47 transmitting “do not eat me” signal in phagocytosis, and downregulation of several genes related to DNA repair, metabolism, splicing, or biosynthesis (Additional file 1: Table S2).

Next, selinexor was assessed in 30 DLBCL cell lines, which resulted in significantly reduced cell viability with varying IC\textsubscript{50} values (Fig. 2a). ABC-DLBCL and GCB-DLBCL cells were similarly vulnerable to selinexor.
Limited efficacy of selinexor in HGBCL with Mut-TP53/p53+ calls for combination strategy. Previous studies showed the synergy between selinexor and venetoclax in DLBCL and double-hit lymphoma [10, 11]. However, in the SADAL trial [3], patients with MYChigh (but not BCL2high) expression had a lower overall response rate than those without. MYC expression can be inhibited by targeting the bromodomain and extra-terminal domain (BET) proteins [12]. We therefore combined selinexor with a novel BET inhibitor INCB057643. Synergistic effect was observed in DLBCL/HGBCL cells, especially in HGBCL-DH cells with Mut-TP53/p53+ (Fig. 2d), supporting INCB057643/selinexor combination as a therapeutic option for HGBCL-DH patients.

In summary, this study demonstrates that XPO1high is a valuable biomarker in DLBCL with unfavorable prognostic factors, predictive of significantly poorer outcomes in ABC-DLBCL, BCL2high DLBCL and double-expres-sor lymphoma but not Mut-p53-expressing DLBCL. Targeting XPO1 with selinexor is similarly effective in GCB-DLBCL and ABC-DLBCL cells, and remarkably effective in BCL2-R+ DLBCL and HGBCL cells without Mut-TP53/p53-positivity. In DLBCL/HGBCL cells, Mut-TP53/p53-positive expression predicts resistance to selinexor. INCB057643 synergizes with selinexor in HGBCL-DH cells, overcoming resistance in Mut-TP53/p53-positive HGBCL-DH. These findings warrant future investigation on the role of XPO1, selinexor, and combined BET inhibition in R/R DLBCL and HGBCL-DH.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13045-020-00982-3.

Additional file 1: Table S1: Clinicopathologic and molecular character-istics of DLBCL patients with high or low XPO1 expression. Table S2: Significantly differentially expressed genes between XPO1high and XPO1low DLBCL patients with concurrent TP53 mutation and high MYC expression. Figure S1: Biomarker study for XPO1 and selinexor (A–B) XPO1high expression but not the GCB subtype of DLBCL. (C) XPO1high expression showed a trend of unfavorable prognostic effect on PFS in MYC-rearranged (MYC-R+) DLBCL. (D) XPO1high expression was associated with significantly poorer survival in DLBCL patients with wild type (Wt) TP53. (E) ABC-DLBCL and GCB-DLBCL cells showed similar sensitivity to the cytotoxicity of selinexor. (F) TP53 mutation (Mut-TP53) significantly reduced the anti-lymphoma efficacy of selinexor in HGBCL-DH cells. IC50 values were calculated by GraphPad Prism 8 based on the cell viability data after 72-hour treatment.

Abbreviations

DBLCL: Diffuse large B-cell lymphoma; R/R: Relapsed or refractory; GCB: Germi-nal center B-cell-like; ABC: Activated B-cell-like; PFS: Progressive-free survival; OS: Overall survival; MYC-R: MYC rearrangement; BCL2-R: BCL2 rearrangement; HGBCL-DH: High-grade B-cell lymphoma with MYC and BCL2 double-hit; Wt: Wild type; Mut: Mutant or mutated; BET: Bromodomain and extra-terminal domain.

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Authors’ contributions

Conception and design were performed by MD, ZYXM, BX, and KHY. Research performance was performed by MD, MZ, ZYXM, LVP, BX, and KHY. Provision of study thought, materials, key reagents and technology were performed by MD, MZ, ZYXM, LVP, AT, CV, XF, GB, FZ, KD, AC, WT, YZ, EDH, WWLC, JH, MP, AJMF, MBM, BMP, JHvK, MAP, JNW, FH, LA, YL, MA, BX, and KHY. Collection and assembly of data under approved IRB and Material Transfer Agreement were done by MD, MZ, ZYXM, LVP, AT, CV, XF, GB, FZ, KD, AC, WT, YZ, EDH, WWLC, JH, MP, AJMF, MBM, BMP, JHvK, MAP, JNW, FH, LA, YL, MA, BX, and KHY. Data analy-sis and interpretation were performed by MD, MZ, ZYXM, LVP, BX, and KHY. Manuscript writing was performed by MD, ZYXM, BX, and KHY. Final approval of manuscript was performed by all authors who read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this study are included in the figures and additional files.

Ethics approval and consent to participate

The study was approved by as being of minimal to no risk or as exempt by the institutional review board of each participating institution.

Consent for publication

Not applicable.

Competing interests

All authors declare no conflicts of interest.

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