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RAPID COMMUNICATION

Inhibition of VEGFR2 overcomes venetoclax-resistance in diffuse large B-cell lymphoma cells

ABT-199 (venetoclax) induces cell apoptosis in lymphoid malignancies mainly through the mitochondrial apoptosis pathway. However, long-term use of ABT-199 causes secondary drug resistance, which limits its use. Here, we show that the acquired resistance to ABT-199 in diffuse large B-cell lymphoma (DLBCL) cells is related to the upregulation of phosphorylated vascular endothelial growth factor receptor 2 (p-VEGFR2), BCL-XL and MCL-1. To identify determinants of resistance, we conducted ABT-199-resistant cell lines. We found that VEGFR2 drove the ABT-199 resistance in addition to the known involvement of the B-cell lymphoma 2 (BCL-2) family members. Notably, we also found that the ABT-199 resistance was associated with the increased production of reactive oxygen species (ROS) in ABT-199 resistant cells. Thus, combinatorial therapy with Apatinib and ABT-199 may offer a new approach to address ABT-199 resistance.

BCL-2 family contains pro-apoptotic and anti-apoptotic proteins, which govern mitochondrial-dependent apoptosis. Evasion of apoptosis and improved tumor cell survival through the imbalance of BCL-2 family members is one of the main reasons for therapeutic resistance.[1] In B-cell tumors, the imbalance of BCL-2 is usually caused by genetic chromosomal abnormalities.[2] Thus, BCL-2 has been a promising therapeutic target in lymphoid cancers.

ABT-199, an inhibitor selectively targeting BCL-2, has been approved for the treatment of chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML).[3] However, disease progression on the basis of using ABT-199 is a great therapeutic challenge.[4] Here, we aimed to probe for the causes of ABT-199 resistance in DLBCL.

VEGFR2 is over expressed in DLBCL compared with normal controls and is an adverse factor affecting the prognosis of patients. In addition, our research also found that VEGFR2 was highly expressed in ABT-199-resistant cell line derived from OCI-Ly1 (OCI-Ly1-R). Compared to ABT-199 monotherapy, the combination of Apatinib and ABT-199 was even more potent in overcoming ABT-199 resistance. These results suggest the Apatinib/ABT-199 combination may be effective for DLBCL therapy.

To define the mechanisms that contribute to ABT-199 resistance in DLBCL, we constructed the ABT-199-resistant cell line. To validate the resistance of the OCI-Ly1 cell line, we evaluated the inhibitory effect of ABT-199 on cell viability using the cell counting kit-8 (CCK-8) assay. In contrast to the sensitive OCI-Ly1 cell line (OCI-Ly1-S), OCI-Ly1-R cells showed higher half-maximal inhibitory concentration (IC_{50}) values in response to ABT-199. When compared with the sensitive cell line, the IC_{50} was increased nearly 33-fold (Fig. 1A). Compared with their sensitive counterparts, the resistant cells expressed higher levels of p-VEGFR2, MCL-1 and BCL-XL (Fig. 1B; Fig. S1B). The resistant cells also showed higher levels of reactive oxygen species (ROS) (Fig. 1C). Next, we tested if the resistance to BCL-2 inhibition could be overcome by a combination therapy regimen. Cell apoptosis was significantly attenuated in OCI-Ly1-R cells after ABT-199 treatment. Apatinib alone has a killing effect on the OCI-ly1-R cell line, and the combination of Apatinib with ABT-199 markedly induced apoptosis (Fig. 1D, E; Fig. S1A). Our previous study found that homoharringtonine (HHT), a selective inhibitor of MCL-1, had a synergistic effect when combined with ABT-199 in AML.[5] We found that HHT and ABT-199 also had synergistic effects in DLBCL (Fig. 1F; Fig. S1C, and Table S1).

To test the findings *in vivo*, the DLBCL OCI-Ly1-S/R cell lines were subcutaneously injected into BALB/C nude mice. When the tumor volume reached ~75 mm^3 about 7 days after engraftment, the mice injected with OCI-Ly1-S cells were randomly assigned to two groups ($n = 5$/group) and treated with vehicle and ABT-199 (80 mg/kg) for 14 consecutive days, respectively. The mice injected with OCI-

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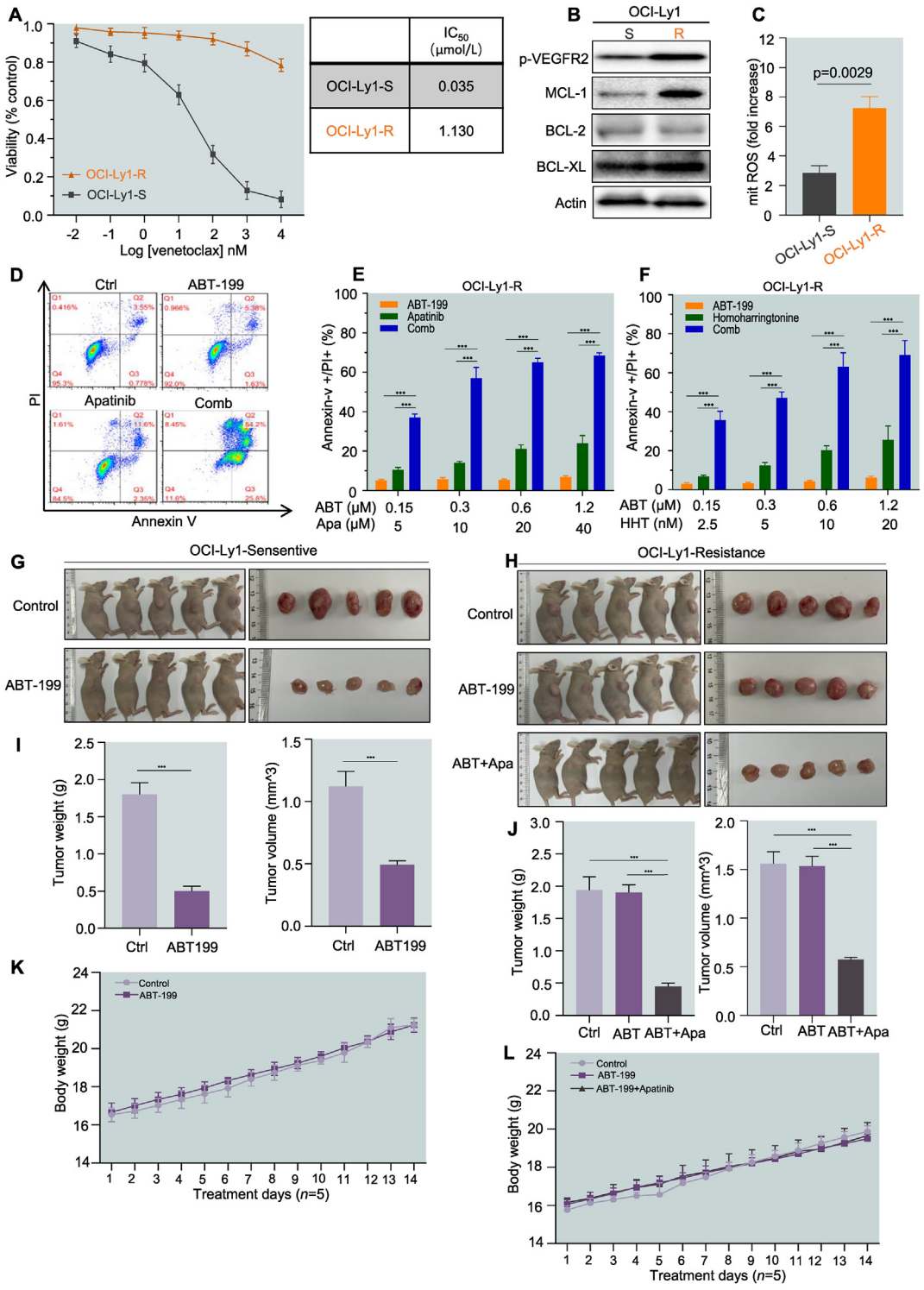

Figure 1 Inhibition of VEGFR2 overcomes ABT-199-resistance in OCI-Ly1 cells. **(A)** Dose—response curve of the drug-resistant OCI-Ly1-R and the drug-sensitive OCI-Ly1-S cell lines. **(B)** Western-blot showed MCL-1, VEGFR2, BCL-XL and BCL-2 protein levels in OCI-Ly1-S and OCI-Ly1-R cells. **(C)** The production level of reactive oxygen species in OCI-Ly1-S and OCI-Ly1-R cells. **(D, E)** OCI-Ly1-R cells were treated with the indicated concentrations of ABT-199 and Apatinib for 24 h, after which the percentage of Annexin-V$^+$ apoptotic cells were determined by flow cytometry after Annexin-V and PI double staining. **(F)** The percentage of Annexin-V$^+$ apoptotic OCI-Ly1-R cells at 24 h after exposure to ABT-199, homoharringtonine (HHT) and both drugs (and DMSO as control). **(G—L)** BALB/C nude mice were subcutaneously injected with OCI-Ly1-S/R cell lines, treated with vehicle, ABT-199 (80 mg/kg), or ABT-199 combined with Apatinib (100 mg/kg) as indicated. Tumor size (G, H), and tumor weight and volume (I, J) were measured. During the treatment, the body weight of the mice was monitored daily (K, L).

Ly1-R cells were randomly divided into three groups ($n = 5$/group) and treated with vehicle, ABT-199 (80 mg/kg) and ABT-199 combined with Apatinib (100 mg/kg) for 14 consecutive days, respectively. Interestingly, in the OCI-Ly1-S group, ABT-199 treatment can effectively reduce the tumor burden, reflected by the decreased volume and weight of the tumor (Fig. 1G, I, K). In the OCI-Ly1-R group, the ABT-199 treatment did not reduce the tumor volume compared with the control, while the tumor burden was significantly reduced in the combined group (Fig. 1H, J, L).

VEGFR2 is considered to play an important role in ABT-199 resistance. To test this notion, we knocked down the *VEGFR2* gene, and Western Blot was used to verify the knockdown effect of VEGFR2 (Fig. S1D). Compared with the scramble, the expression of the VEGFR2 significantly decreased. The cell counts and the colony-formation also decreased after shRNA interference (Fig. S1E, 1G). The flow cytometry results demonstrated that the proportion of apoptosis of OCI-Ly1-R cells after interference was remarkably increased as shown in Figure S1F.

In conclusion, our findings show that targeting VEGFR2 with emerging inhibitors would be a potential and effective way to counteract ABT-199 resistance. Given the relationship between higher VEGFR2 status with resistance, this combination therapy regimen can be exploited to overcome ABT-199 resistance. Together with the *in vitro* and *in vivo* efficacy, our results strongly support future research and clinical trials testing the combinations of Apatinib with ABT-199.

Author contributions

Conception and design were performed by WZX. Research performance was performed by YFS and YX. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.07.012.

Abbreviations

AML	Acute myeloid leukemia
BCL-2	B-cell lymphoma 2
CCK8	Cell counting Kit-8
CLL	Chronic lymphocytic leukemia
DLBCL	Diffuse large B-cell lymphoma
HHT	Homoharringtonine
IC_{50}	Inhibitory concentration
MCL-1	Myeloid cell leukemia-1
ROS	Reactive oxygen species
VEGFR2	Vascular endothelial growth factor receptor 2

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