Targeting casein kinase 1 alpha (CK1 alpha) and transcriptional CDKs (CDK7/9) in human liposarcomas

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Introduction

Liposarcomas (LPS) are rare mesenchymal cell malignancies of adipocytic origin that are diagnosed in more than 3500 patients in the US each year. The two most common subtypes, well-differentiated LPS (WDLPS) and dedifferentiated LPS (DDLPS), are characterized by extrachromosomal DNA amplifications harboring the MDM2 (100%) and CDK4 (90+%) genes, generally with wild type TP53. Management of metastatic or surgically unresectable LPS remains purely palliative. Recent clinical trials targeting MDM2 or CDK4/6 with small-molecule inhibitors have shown promise but have been hampered by dose-limiting toxicities. The development of new therapeutics is greatly needed to improve outcomes for patients with LPS.

Abstract

To identify unique liposarcoma-specific vulnerabilities, we screened multiple human LPS cell lines for transcriptional CDK expression and found high levels of CDK7 and CDK9. We demonstrate that CDK9 inhibitors suppress LPS cell growth and induce apoptosis by decreasing MDM2 levels while inducing expression of p53. To enhance p53 activation in these cells, we screened for expression of known regulators of p53, including CK1α, whose inhibition has previously been shown to activate p53. We demonstrate that LPS cells express CK1α and that the cytotoxic effects of CDK9 inhibitors are enhanced upon CK1α depletion. These data led us to examine combined targeting of CK1α and CDK9 in LPS with the novel agent BTX-A51, which has previously been shown to inhibit CK1α, CDK9, as well as CDK7 with nanomolar efficacy in AML models. BTX-A51 potently reduces expression of MDM2 with marked induction of p53, resulting in profound apoptosis of LPS cells. Through CRISPR/Cas9-mediated p53 knockout, we establish that BTX-A51-mediated apoptosis is primarily p53-dependent. However, BTX-A51 also reduces expression of MCL1 and primes LPS cell lines and primary LPS cells for BIM-induced apoptosis, as demonstrated by BH3 profiling. Importantly, preliminary in vivo data in an LPS patient-derived xenograft model reveal that BTX-A51 is well-tolerated with tumor growth inhibition.

Figure 1. Mechanism of action of BTX-A51

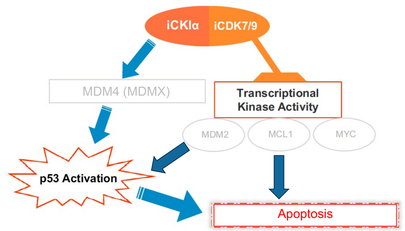


Figure 2. Selective CDK9 inhibitors are cytotoxic to LPS cells

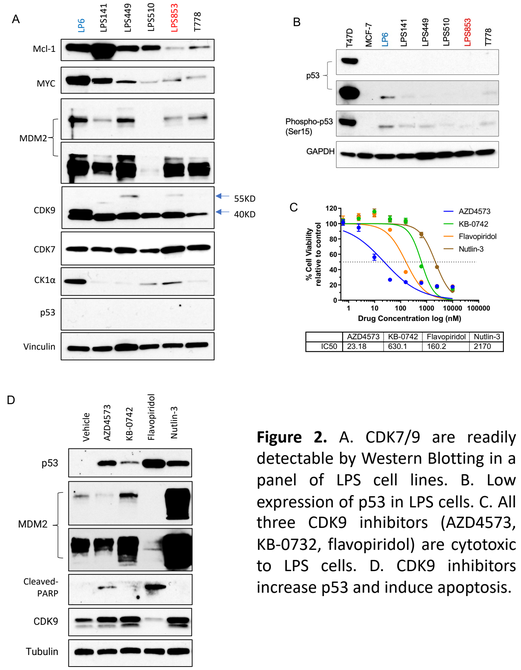


Figure 2. A. CDK7/9 are readily detectable by Western Blotting in a panel of LPS cell lines. B. Low expression of p53 in LPS cells. C. All three CDK9 inhibitors (AZD4573, KB-0732, flavopiridol) are cytotoxic to LPS cells. D. CDK9 inhibitors increase p53 and induce apoptosis.

Figure 3. CK1α depletion sensitizes LPS cells to CDK9 inhibition

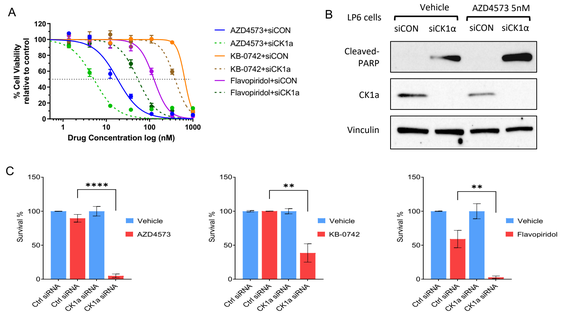


Figure 3. A. CK1α depletion reduces the IC50 of CDK9 inhibitors. B&C. CK1α depletion enhances apoptosis induced by AZD4573 and further suppresses colony formation by CDK9 inhibitors.

Figure 4. Co-targeting of transcriptional CDKs and CK1α with BTX-A51 in MDM2-amplified liposarcoma (LPS) Cell Lines

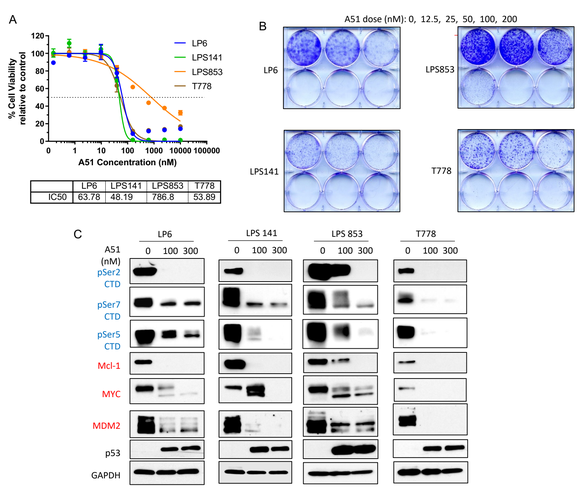


Figure 4. A. A51 quite effectively reduced cell viability of 3 LPS cell lines. B. A51 reduced colony formation. C. A51 altered the levels of CDK7 and CDK9 activities as well as expression of target genes regulated by CDK9.

Figure 5. A51 induces apoptosis in Liposarcoma LP6 cells

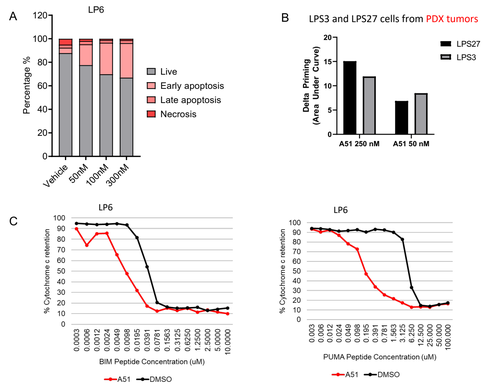


Figure 5. A. A51 induced apoptosis in a dose-dependent manner as reflected by Annexin V staining. B. BH3 profiling of LPS3 and LPS27 cells from PDX tumors demonstrating enhanced Delta-priming by A51. C. In the presence of A51, a lower concentration of BIM or PUMA is required to achieve Cytochrome C release.

Figure 6. CRISPR/Cas9-mediated p53 KO partially reduces the effect of A51 on LPS cell apoptosis and viability

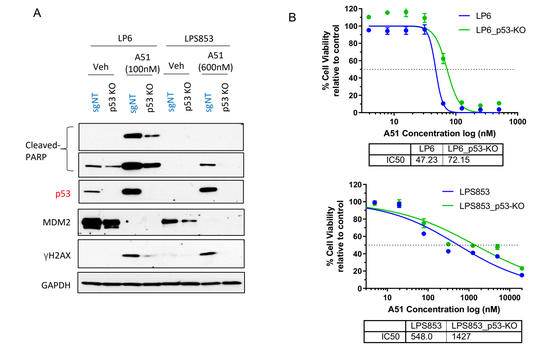
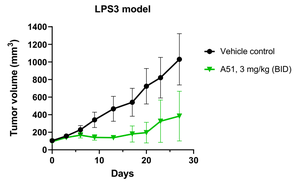


Figure 6. A. The induction of PARP cleavage by A51 was attenuated upon deletion of p53. B. Deletion of p53 increased the IC50 of A51.

Figure 7. A51 is well-tolerated and effective in liposarcoma PDX LPS3



Conclusions

Our results suggest that BTX-A51 has potent preclinical efficacy in treating LPS, primarily through inhibition of CK1α and CDK9. Future mechanistic studies will further clarify mechanisms of BTX-A51-mediated apoptosis, as well as the contribution of CDK7 inhibition to anti-tumor activity. Our data justify a planned clinical trial that will evaluate the efficacy of BTX-A51 in patients with advanced WDLPS or DDLPS.

Acknowledgment

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References

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3. Minzel et al., Cell (2018)