Targeting androgen receptor phosphorylation to overcome enzalutamide resistance in advance prostate cancer

Nur P. Damayanti[1], Justin A. Budka[1], Lumin Zhang[1], Pete Hollenhorst[2], Roberto Pili[1]

[1]Genitourinary Program, Division of Hematology & Oncology, Indiana University Melvin and Simon Bren Cancer Center, Indianapolis, Indiana
[2]Biochemistry & Molecular Biology, Indiana University School of Medicine; Indiana

Background: Androgen-deprivation therapy (ADT) is approved as standard care for advanced prostate cancer (PCa). However, the vast majority of patients develop castration resistant prostate cancer (CRPC) defined by disease progression despite ADT. The Food and Drug Administration (FDA) has approved anti-androgen drugs such as enzalutamide as treatment for CRCP patients. However, CRPC eventually fails to respond to anti-androgen treatment. To date, there is no effective treatment for anti-androgen resistant CRPC besides chemotherapy and radiopharmaceuticals. In our lab, we have previously demonstrated that a tyrosine kinase inhibitor (TKI), sunitinib, induces AR phosphorylation and confers differential association of AR with enzalutamide in renal cell carcinoma (RCC). We further demonstrated that combination of sunitinib and enzalutamide prevents AR translocation to nucleus and curtails cell proliferation of sunitinib resistant RCC cells. Therefore, the in this study we tested the hypothesis whether combination of TKIs, such as sunitinib, and enzalutamide may confer therapeutic benefit in enzalutamide resistant CRPC.
Methods: Combination benefit of kinase inhibitor and enzalutamide was tested in 2D culture and *in-vivo* setting. Western blot analysis was performed to study the protein expression and protein phosphorylation. Quantitative mRNA array was used to investigate the effect of combination therapy on differential expression of AR target genes. Immunofluorescence was applied to study differential AR phosphorylation in conjunction with western blot.
Results: In our preliminary study we observed, sunitinib, a TKI, re-sensitized both intrinsic (c22Rv1) and acquired (LNCaPR) resistant cells models of CRPC to enzalutamide treatment in synergistic fashion. Further, we observed that combination of sunitinib and enzalutamide curtails proliferation of c22Rv1 cells in 2D culture**.** Interestingly, we observed a similar inhibitory effect in *in vivo* setting, in which combination therapy of sunitinib and enzalutamide inhibited tumor growth compared to single agent and vehicle group ($P<0.0001$). Furthermore, Western blot analysis showed that the inhibitory effect of sunitinib and enzalutamide is AR dependent in c22Rv1 cells both in *in vitro* 2D culture model and *in vivo* model. Next, quantitative mRNA array showed that combination of sunitinib and enzalutamide decreased AR-FL and AR-V7 mRNA transcripts and induced differential expression of AR target genes compared to single agent enzalutamide in c22Rv1 cells in both *in-vitro* and *in-vivo* setting. Immunofluorescence study demonstrated differential phosphorylated AR expression in enzalutamide resistant cells, c22Rv1, and enzalutamide sensitive cells, LNCaP, with the the former showing more diffused expression pattern and higher expression of AR phospho- serine 81, AR phosphoserine 213, AR phosphotyrosine 534, global phosphotyrosine and phosphoserine and lower expression of AR phosphoserine 308 compared to the latter one. We also observed higher AR phosphoserine 81 expressions in tissue from a CRCP a patient compared to a hormone therapy sensitive patient. These results are corroborated by our Western blots showing the same trend of differential expression of multiple sites phosphorylated AR in enzalutamide resistant cells c22RV1 compared to enzalutamide sensitive cells LNCaP. Next, we noted that sunitinib reduced the expression of site specific AR phosphorylation at serine 81 in c22RV1 in both AR full length (AR-FL) and AR spliced variant (AR-V7).
Conclusions: Our preliminary results suggest a potential therapeutic benefit of modulating androgen receptor phosphorylation by tyrosine kinase inhibitors to overcome resistance to anti-androgen therapy.

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