Abstract

6. Data presented here support the rationale for the current clinical investigation of axitinib treatment. 

5. Axitinib inhibited the activity of p53 in RCC xenografts as measured by p21 expression, which is known to directly repress CXCL12 through the up regulation of p53. MDSC trafficking into tumor tissue is implicated in the development of resistance to other anticancer therapies. Further, we observed that both the influx of MDSC and resistance to VEGF-targeted therapies could be prevented by concurrent administration of an HDM2 antagonist, a drug whose biological effects are mediated primarily through the up regulation of p53. MDSC trafficking into tumor tissue is regulated by chemokines, many of which (e.g. SDF-1/CXCL12) are produced in response to HIF-2 expression. p53 is known to directly repress CXCL12 transcription, and we have shown that HDM2 blockade suppresses HIF-2 expression, suggesting that the drug has both direct and indirect effects on CXCL12 expression. Western blot analysis of tumor lysates confirmed that HDM2 antagonism mediates its effects on MDSC through the suppression of chemokine production, including CXCL12. These findings suggested that the ability of HDM2 antagonism to prevent sunitinib resistance might be due, at least in part, to the suppression of CXCL12 production and MDSC recruitment. Consequently we hypothesized that agents that block CXCL12/CXCR4 signaling directly would duplicate the effects of HDM2 blockade on MDSC trafficking and prevent resistance to VEGF-targeted therapies.

Methods

Mice were inoculated with 786-0 and A498 RCC xenografts, the tumors permitted to grow to ~300 mm3, and then treatment initiated with the CXCR4 inhibitor X4P-001, axitinib, both agents in combination, or saline (control). Tumors were treated and measured daily for 22 days. Tumors were removed and either snap frozen in liquid nitrogen for western blot analysis or fixed in formalin for immunohistochemistry (Ki67). IHC and IF analysis was quantitated using ImageJ software.

Results

1. The combination treatment of X4P-001 and axitinib demonstrated significantly more potent anti-tumor activity than either single agent alone in two renal xenograft models.

2. The addition of axitinib to RCC xenografts results in a significant increase in proliferation of tumors as measured by Ki67 staining. The increase of Ki67 staining is suppressed with the addition of X4P-001 to axitinib.

3. X4P-001 suppressed the increased MDSC tumor infiltration caused by axitinib treatment.

4. Activities of several key signaling molecules including p-STAT3 and p-AKT were inhibited, consistent with the observed suppression of MDSC infiltration and tumor cell survival.

5. Axitinib inhibited the activity of p53 in RCC xenografts as measured by p21 activity, which was unaffected by the addition of X4P-001.

6. Data presented here support the rationale for the current clinical investigation of X4P-001 in RCC where suppressive TME is driven by hypoxia induced MDSCs (NCT02667886).

Conclusion

The combination of axitinib and X4P-001 retards tumor growth to a greater extent than either drug alone.

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The addition of axitinib to RCC xenografts results in a significant increase in tumor MDSC infiltration as measured by cd11b and Gr-1 staining. The infiltration of MDSCs is suppressed with the addition of X4P-001 to axitinib.