The Role of Extracellular NAD⁺ on the Immune Microenvironment of Hepatocellular Carcinoma

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Abstract

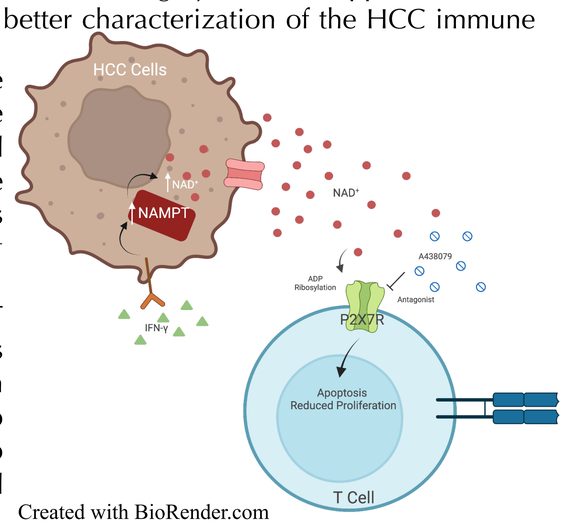
Due to its highly immunosuppressive tumor microenvironment (TME), the efficacy of immune checkpoint inhibitors (ICI), the most common form of immunotherapy, is limited to a subset of patients in hepatocellular carcinoma (HCC). Previously, NAD⁺ has been identified to induce immunosuppression in HCC by causing T cell suppression. A study was conducted to investigate whether inhibition of P2X7, an NAD⁺ receptor present on T cells, can revert NAD⁺ induced immunosuppression. *In vitro* studies using primary mouse T cells and *in vivo* studies using two mouse HCC models demonstrate that the application of A438079, a P2X7 receptor-specific small molecular inhibitor, can revert NAD⁺ induced T cell apoptosis, reduce tumor size and increase tumor-infiltrating immune cells. In conclusion, P2X7 receptor inhibition via A438079 is a promising method of reverting NAD⁺ induced immunosuppression to enhance anti-tumor immunity and is a potential target to be used in combination with ICI to enhance its efficacy in HCC.

Introduction

Immune checkpoint inhibitors (ICI) is the most common form of immunotherapy. However, its efficacy on HCC have been limited to a subset of patients, partially due to HCC's highly immunosuppressive TME. (Sangro et al., 2021) To improve the efficacy of ICI in HCC, a better characterization of the HCC immune environment is needed.

Previously, an upregulation of Nicotinamide Phosphoribosyltransferase (NAMPT), the rate limiting enzyme of NAD⁺ salvage pathway, has been identified in IFN-γ treated HCC cell lines. Thus, NAD⁺ has been identified as a candidate metabolite contributing to ICI resistance. Preliminary results have also identified the suppressive role of extracellular NAD⁺ on T cell.

The P2X7 receptor (P2X7R) is an NAD⁺ receptor present on T cells (Haag et al., 2007) Activation of P2X7R by NAD⁺ is known to induce apoptosis, dampen proliferation and result in overall immune suppression (Grassi, 2020). This study aimed to investigate whether the inhibition of P2X7R has the potential to upregulate anti-tumor immunity by preventing NAD⁺ induced T cell suppression



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Materials and Methods

A P2X7 receptor-specific small molecule inhibitor, A438079 was applied to both *in vitro* and *in vivo* models.

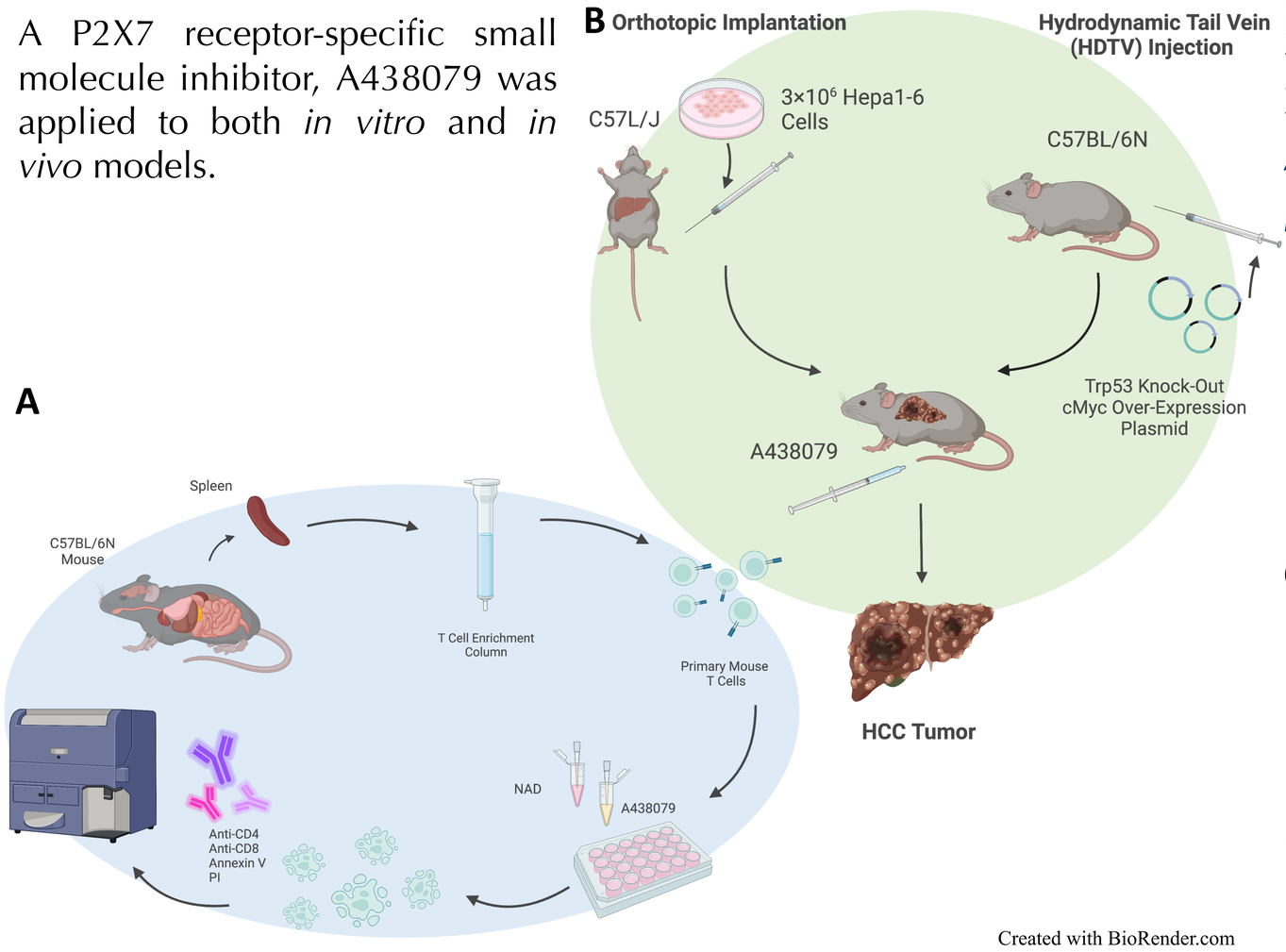

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Figure 1. Design of *In Vitro* and *In Vivo* Assays (A) Design of *in vitro* apoptosis assay using annexin V/propidium iodide double-labeled primary mouse T cell **(B)** Design of *in vivo* assay using orthotopic and hydrodynamic tail vein injection-induced mouse HCC models.

A438079 Reverts NAD⁺ Induced T Cell Apoptosis in Primary Mouse T Cells

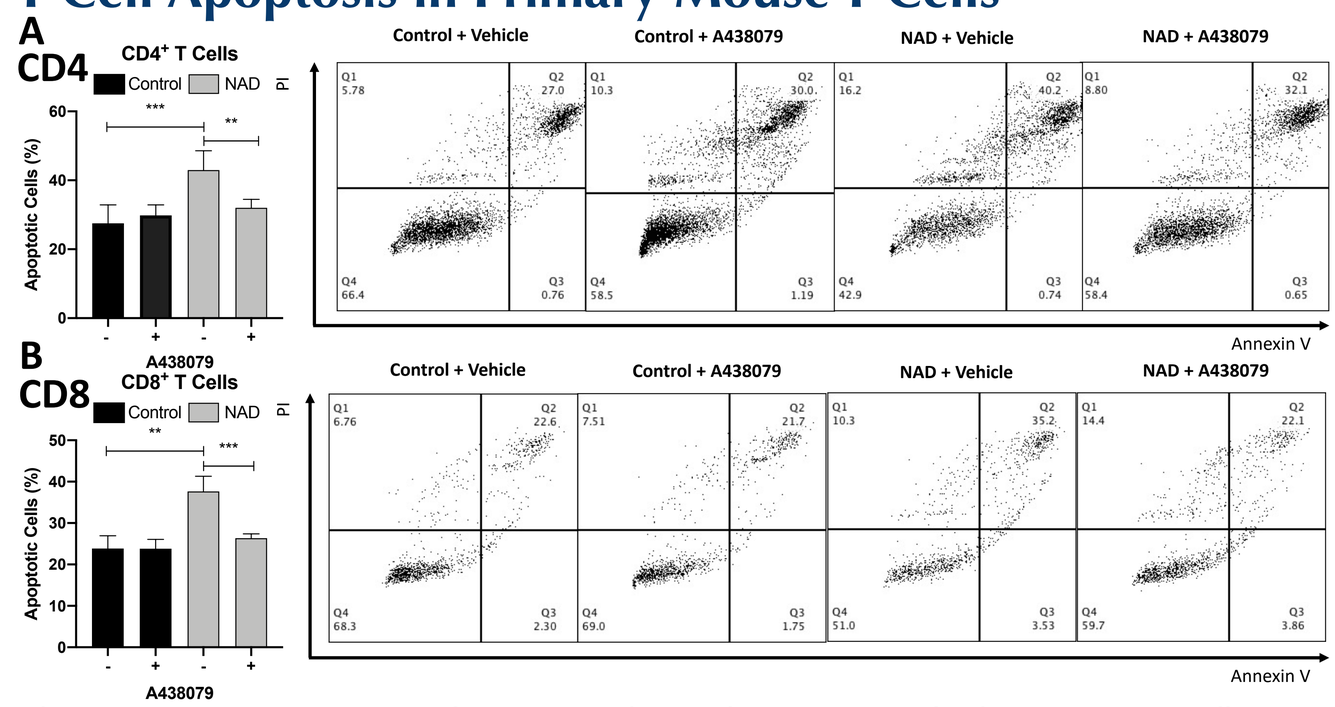

Figure 2. A438079 Treatment Reduces Apoptosis Rate in NAD⁺ Treated Primary Mouse T Cell (A) (L) Proportion of apoptotic CD4⁺ T cells in each condition. (R) Representative flow cytometry results for CD4⁺ T cells in each condition. **(B)** (L) Proportion of apoptotic CD8⁺ T cells in each condition. (R) Representative flow cytometry results for CD8⁺ T cells in each condition. Error bar: mean ± S.D. Two-tailed unpaired t-test: ** P≤0.01, *** P≤0.001

A438079 Reduces Tumor Size in Mouse HCC Tumor Models

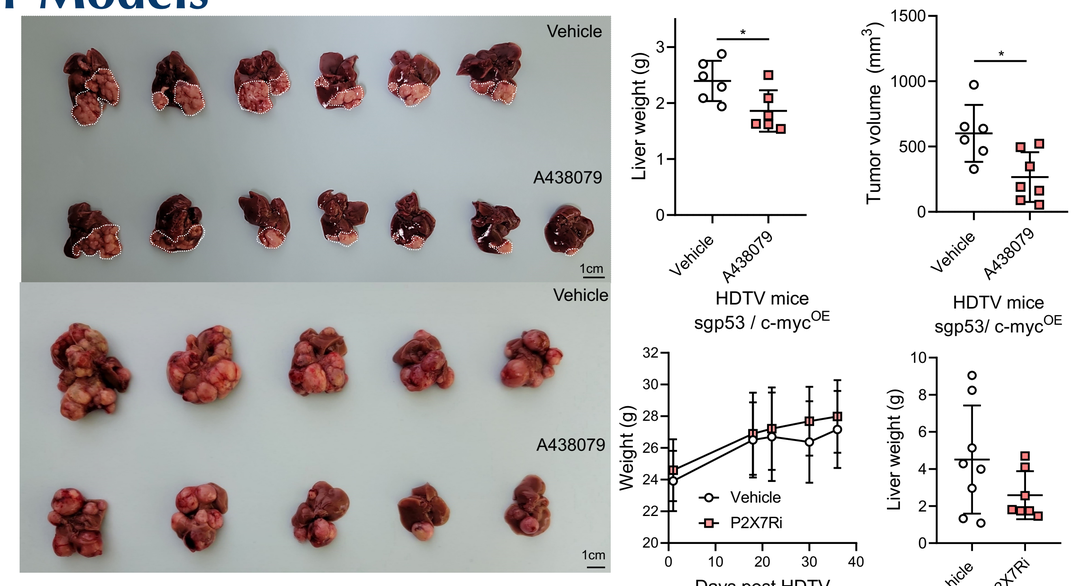

Figure 3. A438079 Treatment Reduces Tumor Size in Mouse HCC Tumor Models (A) Orthotopic model (L) the size and composition of the tumors. The tumor region is indicated in white. (M) The weight of the total liver. (R) The volume of the resected tumor. **(B)** HDTV injection-induced model. (L) The size and composition of the tumors. (M) Bodyweight change of the mice throughout treatment. (R) The weight of the total liver. Error bar: mean ± S.D. Two-tailed unpaired t-test: * P≤0.05

A438079 Increases Immune Cell Infiltration in Mouse HCC Tumors Models

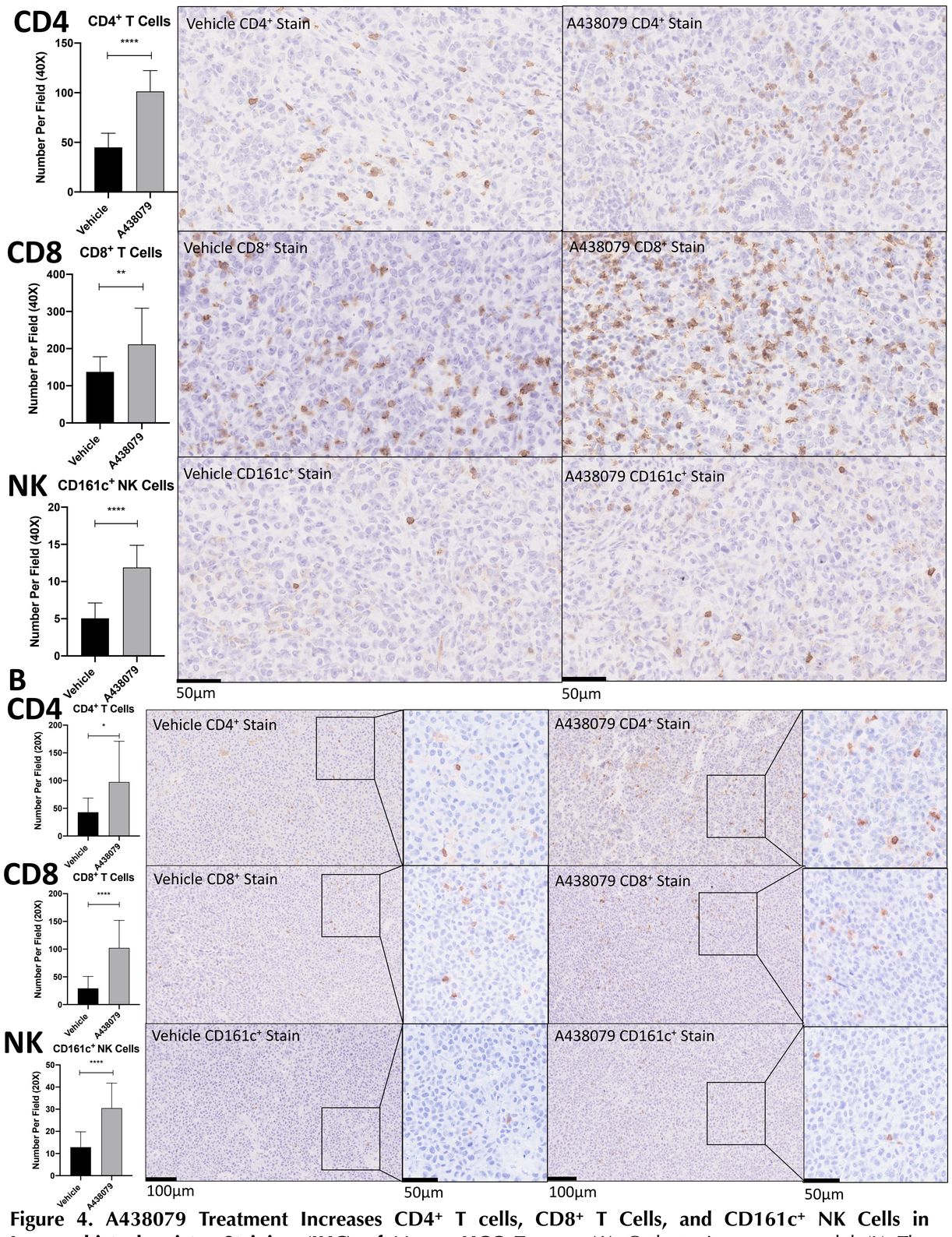

Figure 4. A438079 Treatment Increases CD4⁺ T cells, CD8⁺ T Cells, and CD161c⁺ NK Cells in Immunohistochemistry Staining (IHC) of Mouse HCC Tumors (A) Orthotopic mouse model (L) The average number of CD4⁺, CD8⁺, and CD161c⁺ cells present in each section. (R) Representative sections for CD4, CD8, and CD161c stained tumors. **(B)** HDTV injection-induced model. (L) The average number of CD4⁺, CD8⁺, and CD161c⁺ cells present in each section. (R) Representative sections for CD4, CD8, and CD161c stained tumors. Error bar: mean ± S.D. Two-tailed unpaired t-test: * P≤0.05 ** P≤0.01 *** P≤0.001 ****P≤0.0001

Conclusion and Discussion

The *in vitro* study demonstrates a reduction in apoptosis rate of NAD⁺ treated T cells upon A438079 treatment. The *in vivo* studies show a reduction in tumor size upon A438079 treatment in both models. The immunohistochemistry staining of the harvested tumors indicate an increase in the number of tumor-infiltrating immune cells upon A438079 treatment.

Taken together, P2X7 receptor inhibition via A438079 is a promising method for reverting NAD⁺ induced immune suppression, enhancing anti-tumor immunity and reducing tumor burden in HCC. Further investigation can involve using P2X7R inhibition in combination with ICI to see whether there are synergistic effects.

References

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