Inhibition of Integrin αvβ8 in combination with low dose radiation induces antitumor effect in advanced immune checkpoint blockade refractory tumor model

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INTRODUCTION

Integrin $\alpha_v\beta_8$ activates TGFβ in immune cells. $\alpha_v\beta_8$ inhibitors have been shown to potentiate immune checkpoint blockade (ICB) in preclinical models [1]. In the tumor microenvironment (TME), $\alpha_v\beta_8$ is expressed across different cell types and can negatively regulate adaptive immunity to promote tolerance and immune homeostasis. Although $\alpha_v\beta_8$ is proposed to suppress the immune response by multiple mechanisms [2], our work showed the essential role of $\alpha_v\beta_8$ on antigen-presenting cells, specifically migratory dendritic cells (DC). We have identified that within the DC population, Itgb8 is exclusively expressed on subsets of DC sharing the transcriptional program of recently described mregDC/DC3, which functionally serve as a homeostatic mechanism to downregulate anti-tumor adaptive response [3,4]. $\alpha_v\beta_8$ inhibition in combination with ICB improved MHC class I antigen presentation and enhanced migratory and costimulatory gene signatures in mregDC to improve effector T cell function and suppress Tregs in tumors [1]. Radioimmunotherapy (RIT) induces immunogenic cell death and antigen presentation, however it concurrently activates immunosuppressive pathways. Interestingly, $\alpha_v\beta_8$ immunosuppressive activity was implicated in radiotherapy resistance [5]. We have explored whether antagonizing $\alpha_v\beta_8$ overcomes the suppressive effect of TGFβ and restores anti-tumor immunity in advanced ICB and RIT resistant tumors.

METHODS

Efficacy was evaluated after combination treatment with low dose radiation, $\alpha_v\beta_8$ (clone C6D4) and PD-1 (clone J43) mAb in an advanced CT26 colon cancer syngeneic mouse model. Mice were treated at tumor volume of >120 mm^3 and euthanized at 2,000 mm^3. Tumor volumes are presented as mean ± SEM. Statistics were performed by one-way ANOVA, unpaired two-tailed Student's t test or log-rank test. All animal work was approved by the site Institutional Animal Care and Use Committee and was performed in conformance with the *Guide for the Care and Use of Laboratory Animals* within an AAALAC-accredited program. Humane euthanasia criteria were predetermined on the basis of body weight and defined clinical observations. Bone marrow derived DC (BMdDC) cultures were generated by culturing bone marrow isolated from C57BL/6 mice in 2 ng/mL GM-CSF and 200 ng/mL FLT3 for 14 days. BMdDCs were stimulated with 0.2-5ug/mL of the TLR 3 agonist poly-IC or1:1 ratio of UV-irradiated MC38eGFP cells. Flow cytometry and transcriptomic analysis were used to assess the mechanism of action.

RESULTS

Complete response (CR) with improved survival when $\alpha_v\beta_8$ inhibition is added to RIT in CT26 syngeneic model of colorectal cancer in an advanced, ICB and RIT unresponsive stage.

A CT26 model was established to mimic the progression of late-stage tumors and was unresponsive to radiation, ICB and RIT. In CT26 implanted mice, $\alpha_v\beta_8$ is expressed on tumor stoma, and is not detectable on cancer cells. Addition of $\alpha_v\beta_8$ mAb to RIT markedly increased tumor regression (P=0.0067) and survival (P<0.0001). There were 8/10 complete responders with addition of $\alpha_v\beta_8$ mAb relative to 3/10 in RIT alone. Consistent with a recent report in a less advanced CT26 model [5], $\alpha_v\beta_8$ mAb + radiation resulted in similar efficacy as conventional RIT although the effect was modest in more advanced tumors (Figure 1, A, B).

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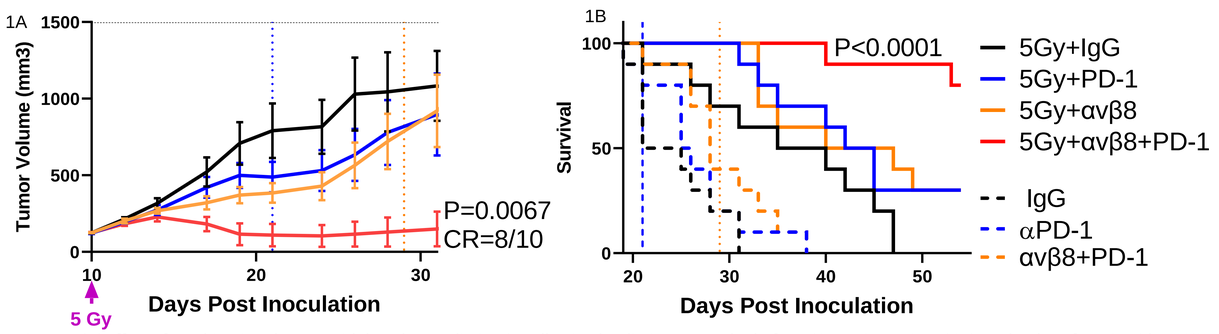

Figure 1 (A) Effect of combination therapy with low dose radiation (small animal radiation research platform (SARRP) at 5 Gray (Gy) on the day of staging (day 10)), PD-1 mAb (10 mg/kg twice weekly for 2 weeks) and $\alpha_v\beta_8$ mAb (7 mg/kg three times weekly for 3 weeks) measured by tumor burden. 5Gy+PD-1 and 5Gy+$\alpha_v\beta_8$ has a minimal effect on tumor growth inhibition showing slight improvement relative to radiation alone (5Gy+IgG). Addition of $\alpha_v\beta_8$ antagonism (5Gy+$\alpha_v\beta_8$+PD-1) improves anti-tumor responses leading to CR in 8 of 10 mice. (B) Kaplan-Meier Curve presenting time to progression. 5Gy+IgG improved survival over monotherapy with either $\alpha_v\beta_8$ or PD-1 mAb. 5Gy+$\alpha_v\beta_8$+PD-1 resulted in profound improvement of the survival over all other treatment conditions.

Durable complete response is established after αvβ8 antagonism

A subsequent study was conducted in the advanced CT26 model to assess the durability of the response to the whether triple combination. A late-stage CT26 model with moderate sensitivity to RIT was established.

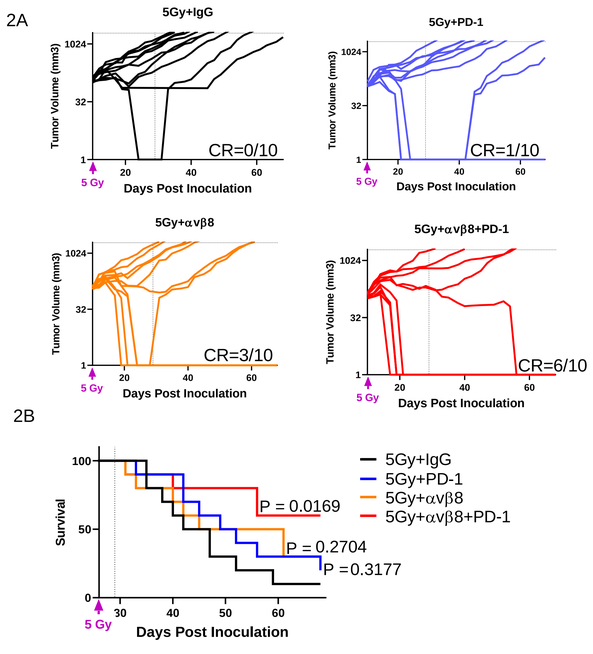

Figure 2 (A) Tumor volume (B) Kaplan-Meier Curve presenting time to progression. Radiation alone and RIT resulted in initial responses in 1/10 and 3/10 mice, respectively. However, most of initial complete responders eventually progressed, resulting in only 1/10 complete responder in RIT group (P=0.3177). Combination of radiation and $\alpha_v\beta_8$ mAb resulted in a better response rate than RIT and led to sustained complete responses in 3/10 mice (P=0.2704). Triple combination of radiation further enhanced the response resulting in 6/10 (P=0.0169). Overall, data showed $\alpha_v\beta_8$ inhibition sustained complete response rate while RIT or radiation alone were less or not effective, respectively.

MHCII hi DCs from bone marrow culture share mregDC/DC3 program and Itgb8 expression

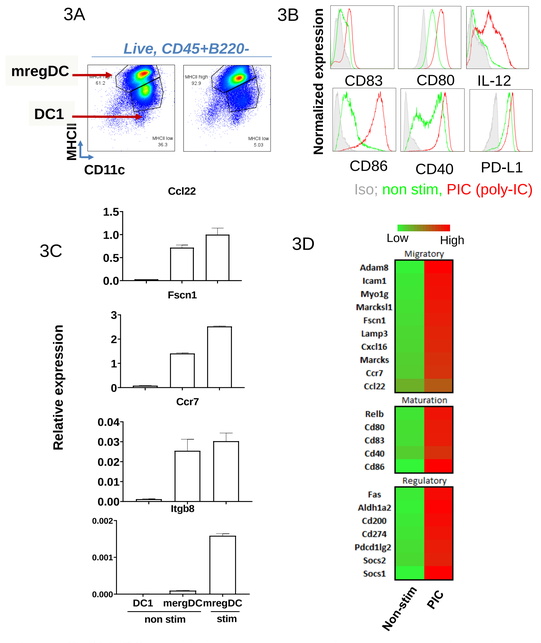

Figure 3. BMdDC established in ex-vivo culture, expressed a subpopulation of MHCII high cells after stimulation with TLR agonists, including poly-IC (Fig. 3A). Sorting, followed by flow cytometry (Fig. 3B) and transcriptomic analysis (Fig 3C) of MHCII high DCs revealed enrichment in maturation (CD40, CD80, CD83, CD86, IL-12) and immunoregulatory markers (PD-L1, Pdcd1lg, Fas, Aldh1a2), indicating that those cells acquire the molecular state described as mregDC. Complete mregDC gene signature was further confirmed using bulk RNA-seq cell-type deconvolution analysis (Fig.1D). Culture-derived mregDC recapitulated the expression of integrin $\alpha_v\beta_8$, similar to previous observations in animal models [1]. Since Itgb8 was induced exclusively on MHC II high DC after antigen stimulation, data suggested that integrin expression was acquired after the mregDC program was established (further confirmed by trajectory-based differential expression analysis, data not shown).

Irradiated cells induce mregDC/DC3 state and Itgb8 expression

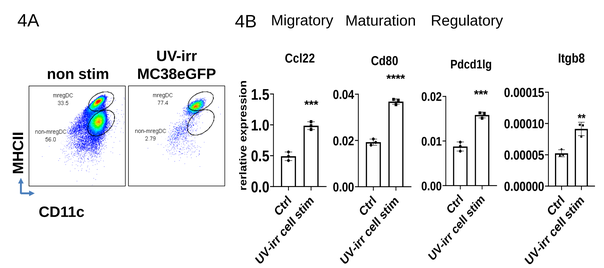

Figure 4. Cell death, including radiation-induced apoptosis, was equally efficient at inducing the immunoregulatory and maturation program of mregDC. Data suggests that apoptotic cells and antigen uptake may drive DCs towards mregDC state (Fig.4A, flow cytometry; Fig.4B-qPCR). Either irradiated-cells or TLR agonist stimulation upregulated expression of Itgb8, suggesting a role of this integrin in inducing an immunosuppressive phenotype.

CONCLUSION

Inhibition of $\alpha_v\beta_8$ in combination with RIT eradicated an advanced tumor, unresponsive to the respective monotherapies or conventional RIT. Previously, our work has shown that $\alpha_v\beta_8$ drives antitumor effect by improvement of DC function and reduced tumor tolerance, leading to increased CD8 T cell infiltrates [1]. Superior efficacy after radiation-mediated antigen release further support this mechanism is driven by improvement of antigen cross-presentation and T cell cross-priming. mregDC can take on either immunoregulatory or immunogenic programs, however driving factors are still elusive. Consistent with the previous findings [3], we have shown that antigen uptake from dying cells induces mregDC program, suggesting radiation may be a mechanism that induces the mregDC state. In conclusion, our data propose that mechanistically αvβ8 inhibition can overcome radiation-induced immunosuppressive effect on antigen presentation and is a promising therapeutic approach to durable clinical response in patients with relapsed/refractory tumors.

REFERENCES

1.Reszka-Blanco NJ, Yadav V, Krumpoch M, Cappellucci L, Cui D, Dowling JE, et al., Inhibition of integrin αvβ8 enhances immune checkpoint induced anti-tumor immunity by acting across immunologic synapse in syngeneic models of breast cancer. AACR; Cancer Res 2021;81(13_Suppl):Abstract nr 1559

2. Dodagatta-Marri E, Ma H-Y, Liang B, Li J, Meyer DS, Chen S-Y, et al., Integrin αvβ8 on T cells suppresses anti-tumor immunity in multiple models and is a promising target for tumor immunotherapy. Cell Report. 2021; 36(1): 109309

3.Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, et al., A conserved dendritic-cell regulatory program limits antitumour immunity. Nature. 2020; 580 (7802): 257-262

4. Garris CS, Arlauckas SP, Kohler RH, Trefny MP, Garren S, Piot C, Engblom C, et al., Successful anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-γ and IL-12. Immunity. 2018; 49(6): 1148-1161

5. Jin S, Lee WC, Aust D, Pilarsky C, Cordes N, β8 integrin mediates pancreatic cancer cell radiochemoresistance. Mol Cancer Res. 2019; 17(10): 2126-2138