Degradation of hyaluronan (HA) by PEGPH20 promotes anti-tumor immunity and enhances the effect of checkpoint blockade in an HA-accumulating mouse syngeneic tumor model

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INTRODUCTION

The glycosaminoglycan hyaluronan (HA) is abundant in many solid tumors, and its accumulation is often associated with poor patient outcomes. Degradation of HA by intravenously administered PEGylated recombinant human hyaluronidase PH20 (PEGPH20) remodels the tumor stroma, reduces intra-tumoral pressure, decompresses tumor blood vessels, and facilitates drug delivery. Recently, PEGPH20 was shown to enhance tumor growth inhibition induced by the immune checkpoint inhibitor anti-PD-L1 in HA-accumulating pancreatic and orthotopic breast syngeneic tumor models, and to enhance tumor T-cell infiltration. HA is a component of commonly used syngeneic mouse tumor models, and MC38 (Charles River Laboratories, CRL) was identified to further evaluate the combinatorial effect of PEGPH20 and anti-PD-L1 in HA-accumulating tumors.

METHODS

HA ELISA: Tumors were digested with protease K and supernatants assayed for HA using Hyaluronan Duo Set ELISA (R&D Systems).

Quantitative PCR: Tumor segments were lysed using FastPrep. Total RNA was isolated using RNeasy Lipid Tissue Mini Kits (QIAGEN) and reverse transcribed using Taqman Reverse Transcription Kits (ThermoFisher). PCR was performed by ViA7 Real-time PCR (TaqMisher) using a panel of Taqman primer/probes that included immune surface markers, cytokines, chemokines, immune checkpoints, and immunomodulatory enzymes. Data were analyzed using the 2-DDCT method, and normalized to β-actin expression.

Immunohistochemistry (IHC): Formalin fixed tissue sections were analyzed using Halozyme’s HA exploratory prototype assay using a biotinylated HA affinity probe (HTI-601) and visualized with DAB. Sections were counterstained with hematoxylin. HA accumulation was quantified by positive pixel count using digital image analysis.

Tumor studies: All tumor growth inhibition and immunophenotyping studies were performed in the MC38 colon tumor model at CRL. Tumors were implanted subcutaneously, and PEGPH20 (37.5 μg/kg) was administered intravenously 24 h prior to anti-PD-L1 (10F.9G2, 5 mg/kg i.p.) using a biweekly dosing regimen. Treatments were initiated when tumors reached 80-120 mm3.

RESULTS

MC38-CRL Tumors Accumulate HA and Contain Immune Activity

Figure 1. Screening of mouse syngeneic tumor models for HA content and immune activity. (A) Indicated mouse cell lines were implanted into syngeneic mice of either sex and assayed for total HA content by ELISA. MC38-CRL accumulated the most HA among the models tested. Data depict the mean HA content of individual tumors ± SEM. (B) The same tumor models as in (A) were screened by qPCR for the expression of genes associated with immune activity and regulation. Rows depict relative expression levels of individual genes, grouped by unsupervised clustering. Red indicates higher expression; green indicates lower expression. Columns depict individual tumors. Due to high levels of HA accumulation and relatively high expression of immune-related genes, the MC38-CRL model was selected for further studies with PEGPH20 and anti-PD-L1.

Tumor HA Degradation by PEGPH20

Figure 2. PEGPH20 decreased HA content in the tumor microenvironment. Representative images of HA+ CRL tumors treated with (A) vehicle or (B) PEGPH20 at 37.5 μg/kg and stained for HA content by IHC 24 h after a single treatment. Each image depicts an individual tumor. (C) Tumors were analyzed by IHC 24 h after 1, 2, or 3 doses of PEGPH20; data depict mean positive pixel count ± SEM of 3 individual tumors for each condition.

PEGPH20 Enhanced Tumor Growth Inhibition Induced by anti-PD-L1 and Extended Survival in 2 of 3 Studies

Figure 3. Combination treatment with PEGPH20 and anti-PD-L1 significantly enhanced tumor growth inhibition and survival versus either agent alone in 2 of 3 studies. (A) Growth curves of MC38-CRL tumors treated with the indicated agents from 3 independent studies. Each measurement time point depicts the mean tumor volume ± SEM for that group. Data were analyzed using repeated measures ANOVA. N = 10 animals per group. Where indicated (*), 1-3 tumors were excluded from analysis due to ulceration. (B) Kaplan-Meier survival analysis from the same 3 studies shown in (A). Data were analyzed using the Logrank test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS = not significant. In Studies 1 and 3, PEGPH20 significantly enhanced the tumor growth inhibition induced by anti-PD-L1, in the same two studies, combination treatment with PEGPH20 and anti-PD-L1 significantly prolonged survival compared to either treatment alone.

PEGP20 Increased Tumor Accumulation of T and NK Cells, and Decreased TAM Frequency

Figure 4. Immunophenotyping analysis of tumors treated with PEGPH20 alone and in combination with anti-PD-L1. (A-D) Absolute numbers of CD8+ TIL (A), CD4+ TIL (B), NK cells (C), and TAM (D) per gram of tumor tissue. (E-F) Percentages of CD8+ and DC (F) among total live CD45+ cells. Data were analyzed using one-way ANOVA with Sidak’s post-hoc test. When necessary, data were log transformed to achieve equal variance across groups. N = 6 animals per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS = not significant. As a monotherapy, PEGPH20 increased the absolute numbers of CD8+ TIL, CD4+ TIL, and NK cells compared to control-treated tumors. Likewise, PEGPH20 in combination with anti-PD-L1 increased the absolute numbers of the same 3 populations compared to anti-PD-L1 alone. Among total CD45+ cells, PEGPH20 decreased TAM frequency and increased DC frequency.

SUMMARY

- MC38-CRL tumors, which are immunologically active and sensitive to PD-1 blockade as previously described, contain high levels of HA relative to other syngeneic models.
- Degradation of HA by PEGPH20 enhances the effect of anti-PD-L1 on MC38-CRL tumor growth in 2 of 3 studies.
- Combination of PEGPH20 with anti-PD-L1 prolonged survival in 2 of 3 studies.
- PEGPH20, alone, and in combination with anti-PD-L1, reshaped the tumor immune milieu by increasing accumulation of effector cells and lowering the percentage of tumor-associated macrophages.

REFERENCES

1Shepard et al. Front. Oncol. 5:392, 2015
3Provenzano et al. AACR #461, 2017
4CIR et al. AACR #41, 2017
5Thompson et al. Mol Cancer Res. 10:2444-54, 2012

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