Poly(I:C) Treatment in a Spontaneous Hepatocellular Carcinoma Mouse Model

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Introduction: Hepatocellular carcinoma (HCC) is the most common type of liver cancer, and it is the 6th most common cancer worldwide. Prognosis for HCC is poor since most HCC diagnoses occur in the late stage. As a result, it has the 3rd highest mortality rate among cancers worldwide with a 5 year survival rate of only 15%. A good prognosis in HCC is directly linked to proinflammatory genes within the tumor microenvironment. One key proinflammatory gene, toll-like receptor 3 (TLR3), is expressed in several subsets of immune cells and is involved in antiviral responses. Synthetic TLR3 ligands, such as polyinosinic:polycytidylic acid (poly(I:C)), activate natural killer cells and is cytotoxic to HCC cells [1]. This study evaluates the effectiveness of poly(I:C) in delaying tumor growth by tracking tumor growth after treatment using Magnetic Resonance Imaging (MRI).

Materials and Methods: A total of eleven 8-week-old C57BL/6 mice were used to generate a spontaneous HCC model. HCC was induced through the injection of a mixture of 3 plasmids (pT2/Caggs-V12NRas: Activated Ras as a transposon; pT2/Caggs-shp53/GFP: shRNA for p53 as a transposon; and pPGK-SB13: Sleeping Beauty transposase) in a lactated Ringer's solution. Injection volume was 10% of body weight via the tail vein and was performed in less than 10 seconds with mice immobilized in a restrainer. Induced tumors were detectable 6-8 weeks after injection, and treatment started 8 weeks after the injection. The mice were treated via injection intraperitoneally with either poly(I:C) (5 µg per gram of body weight, n = 5) or an equivalent volume of phosphate buffered saline (PBS, n = 6). Both the poly(I:C)-treated group and the vehicle (PBS) group were injected 3 times the first week on day 0, 2, and 4, and once a week thereafter. Tumor size was determined using a T1-weighted 3D MPRAGE sequence on a Bruker Clinscan 7T MRI (TE = 2.64 ms, TR = 1130 ms, TI = 1000 ms, flip angle = 20°, number of slices = 40, slice thickness = 0.5 mm, pixel size = 0.16 x 0.16 mm). Scans were performed on the day of first treatment and each subsequent week up to 4 weeks after the initial treatment. The mice were euthanized after the last MRI scan and the tumor volume was verified ex vivo.

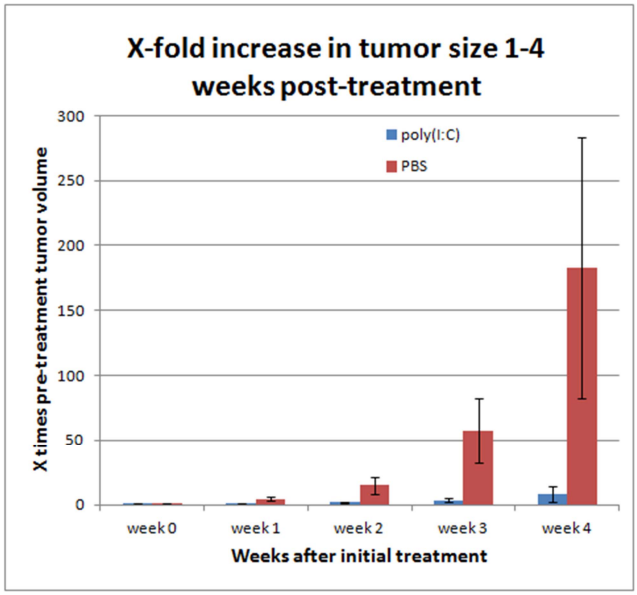

Figure 2: Total tumor volume increase in poly(I:C) and PBS groups compared to initial tumor size (week 0)

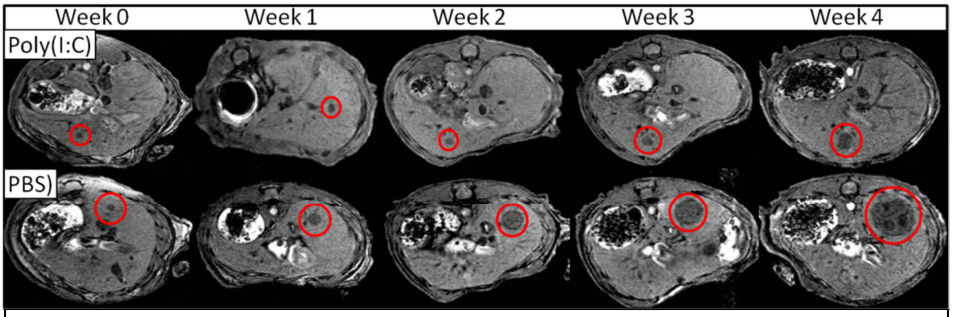

Figure 1: Poly(I:C) and PBS-treated mice weekly tumor growth

Results and Discussion: Tumor size increased in all 11 mice with reduced growth in the poly(I:C)-treated group as shown in Figure 1. Figure 2 shows an example of a poly(I:C)-treated and PBS-treated mouse in the same cohort, where tumor volume increased 104 times in the PBS group and only 8 times in the poly(I:C) group. The mice showed an increase in total tumor volume 4 weeks after treatment of 94-318 times and 3-18 times the pre-treatment volume for the PBS and poly(I:C) groups respectively. The difference was significant with a p-value of 0.01, thus the treatment has been shown to be effective in delaying tumor growth. Moreover, 3 of 5 mice in the poly(I:C) group showed reduced tumor volumes after the first week mainly due to the initially high frequency treatment of 3 times a week compared to 1 time a week thereafter.

Conclusion: It is clear after tracking tumor growth for 4 weeks in treated and vehicle groups that weekly treatment with poly(I:C) is effective in significantly delaying tumor growth in spontaneous HCC models. This shows promise for the use of TLR3 ligands as a targeted therapy to treat HCC.

References:

[1] Chew V, Tow C, Huang C, etc. Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. JNCI 2012;104:1796-1807.