Abstracts

1370 DISTINCT ANTI-TUMOR RESPONSE TO LISTERIA-BASED VACCINES BETWEEN ORTHOTOPIC AND SUBCUTANEOUS SYNGENEIC MOUSE MODELS OF RENAL CELL CARCINOMA

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Methods For subcutaneous studies, 1 × 10⁶ Renca cells were implanted subcutaneously (s.c.) into the right hind flank and mice were subsequently vaccinated i.p. on day 3, 12, and 19 with Lm-based vaccines. For orthotopic studies, 5 × 10⁵ Renca cells expressing luciferase (Renca-luc) were implanted directly into the right kidney as previously described and mice were subsequently vaccinated i.p. on day 5, 12, and 17 with Lm-based vaccines. All mouse experiments were performed in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) at the TTUHSC. For flow cytometry analysis, tumor tissues were harvested, and minced, and single-cell suspensions prepared. Tumor cells were stimulated with Cell Activation Cocktail kit (423303, Biolegend) for 5-6 hours and processed as normal samples. (A, C). Tumor cells were stimulated with Cell Activation Cocktail kit (423303, Biolegend) for 5-6 hours and processed as normal samples. (A, C). Tumor cells were stimulated with Cell Activation Cocktail kit (423303, Biolegend) for 5-6 hours and processed as normal samples. (A, C). Tumor cells were stimulated with Cell Activation Cocktail kit (423303, Biolegend) for 5-6 hours and processed as normal samples. (A, C).

Conclusions Vaccination with Lm-LLO-ISG15 significantly controlled RCC tumor burden in both subcutaneous and orthotopic models, as compared to that of controlled Lm. Interestingly, treatment with Lm-LLO-ISG15 resulted in distinct anti-tumor responses in subcutaneous versus orthotopic RCC tumors.

Abstract 1370 Figure 1 Listeria-based vaccines is efficacious against RCC tumors Listeria-based vaccines is efficacious against both subcutaneous and orthotopic tumors. (A) Experimental schema for subcutaneous studies. Briefly, 1×10⁶ Renca cells in 100 μl PBS were implanted subcutaneously into the right hind flank and mice were subsequently vaccinated i.p. with Lm-based vaccines. (B) Tumor kinetic curve throughout the course of the experiment and (C) final tumor mass at the end of the experiment of both Lm-based vaccines. (D) Experimental schema for orthotopic studies. Briefly, 5×10⁴ Renca cells expressing luciferase (Renca-luc) were implanted directly into the left kidney and mice were subsequently vaccinated i.p. with Lm-based vaccines. D-luciferin (150mg/kg, Perkin Elmer) was injected i.p. to the mice and bioluminescence signals was detected by IVIS Imaging system at 10 min post-injection. (E) The tumor kinetic curve was plotted by using total flux (photon/sec) of the region of interest (ROI). (F) Final tumor mass at the end of the experiment of both Lm-based vaccines. All statistical analysis was done with Prism 8 GraphPad software version 8.3.0., using unpaired student t-test. *p<0.05, **p<0.01, ***p<0.001. Lm-LLO-OVA: Listeria-based vaccine targeting non-specific antigen, i.e., chicken ovalbumin. Lm-LLO-ISG15: Listeria-based vaccine targeting interferon-stimulated gene 15 (ISG15)

Abstract 1370 Figure 2 Distinct T cell response between s.c. and orthotopic tumors Distinct T cell response between subcutaneous and orthotopic tumors to Lm-LLO-ISG15. Data obtained from flow cytometry analysis for subcutaneous (A-D) and orthotopic studies (E-H). Briefly, tumor tissues were harvested, and minced, and single-cell suspensions prepared. Tumor cells were stimulated with Cell Activation Cocktail kit (423303, Biolegend) for 5-6 hours and processed as normal samples. Distribution of multi-cytokine produced by live CD8+ in subcutaneous tumors. Distribution of multi-cytokine produced by live CD8+ in orthotopic tumors. (F, H). Distribution of multi-cytokine produced by live CD8+ in orthotopic tumors. All statistical analysis was done with Prism 8 GraphPad software version 8.3.0., using unpaired student t-test. *p<0.05, **p<0.01, ***p<0.001. Lm-LLO-OVA: Listeria-based vaccine targeting non-specific antigen, i.e., chicken ovalbumin. Lm-LLO-ISG15: Listeria-based vaccine targeting interferon-stimulated gene 15 (ISG15), IFN-γ: interferon-gamma, IL-2: interleukin-2, TNF-α: tumor necrosis factor-alpha
Abstract 1370 Figure 3  Distinct MDSC response between s.c. and orthotopic tumors
Distinct myeloid-derived suppressor cells (MDSCs) response between subcutaneous and orthotopic tumors to Lm-LLO-ISG15. Data obtained from flow cytometry analysis for subcutaneous (A-C) and orthotopic studies (D-F). Briefly, tumor tissues were harvested to prepare single cell suspension, and stained with appropriate fluorochrome-conjugated anti-mouse monoclonal antibodies. Frequency of (A). total myeloid cells, (B). m-MDSCs, and (C). pmn-MDSCs from total viable cells in subcutaneous tumors. Frequency of (D). total myeloid cells, (E). m-MDSCs, and (F). pmn-MDSCs from total viable cells in orthotopic tumors. All statistical analysis was done with Prism 8 GraphPad software version 8.3.0., using unpaired student t-test. *p<0.05, **p<0.01, ***p<0.001. OVA: Listeria-based vaccine targeting non-specific antigen, i.e., chicken ovalbumin. ISG15: Listeria-based vaccine targeting interferon-stimulated gene 15 (ISG15), m-MDSCs: monocytic myeloid-derived suppressor cells, pmn-MDSCs: polymorphonuclear myeloid-derived suppressor cells