Synergizing sunitinib and radiofrequency ablation to treat hepatocellular cancer by triggering the antitumor immune response

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Supplementary data

Figure legends

Supplementary Figure 1. Treatment of HCC-bearing mice with Sunitinib-RFA caused an increase in the number of tumor-infiltrating CD8$^+$ T cells, but not CD4$^+$ T cells. Mice with size-matched tumors were randomly exposed to no treatment (control), SU, RFA, or both. Two weeks post RFA, tumor infiltrating leukocytes (TILs) were isolated and stained for CD4 and CD8. The absolute number of CD4$^+$ and CD8$^+$ T cells were calculated by flow cytometric assay. The accumulated results were shown. n=4, ***P<0.001, error bars represent means ± S.D. Statistic analysis was performed by Student t-test.

Supplementary Figure 2. Treatment of HCC-bearing mice with Sunitinib-RFA induced an increase in the frequency of CD8$^+$ T cells and the decrease in the frequency of FoxP3$^+$Tregs in spleen. Mice with size-matched tumors were randomly exposed to no treatment (control), SU, RFA, or both. Two weeks post RFA, splenocytes were isolated from each mouse and analyzed by flow cytometry. (A) Representative flow cytometry to show the frequency of CD8$^+$T cells in spleen in the mice with different treatments. (B) Accumulative frequency of CD8$^+$T cells in the mice with different treatments. n=4, *P<0.05, **P<0.01, error bars represent means ± S.D. (C) Representative flow cytometry to show the frequency of FoxP3$^+$Tregs in the mice with different treatments. (D) Accumulative frequency of FoxP3$^+$Tregs

in the mice with different treatments. n=4, ***P*<0.01, ****P*<0.001, error bars represent means ± S.D. Statistic analysis was performed by Student t-test.

Supplementary Figure 3. Sunitinib in combination with RFA activated TSA effector CD8$^+$ T cells in spleen. Mice with size-matched tumors were randomly exposed to no treatment (control), SU, RFA, or both. Two weeks post RFA, the spleen in each mouse was harvested and used to isolate splenocytes. The cells received TSA stimulation with epitope I and IV. The intracellular production of IFN-γ and TNF-α in CD8$^+$ T cells was measured by flow cytometry. (A) Representative flow cytometry to show the frequency of IFN-γ-producing CD8$^+$ T cells in splenocytes in the mice with different treatments. (B) Accumulative frequency of IFN-γ-producing CD8$^+$ T cells in splenocytes in the mice with different treatments. n=4, **P*<0.05, ***P*<0.01, error bars represent means ± S.D. (C) Representative flow cytometry to show the frequency of TNF-α-producing CD8$^+$ T cells in splenocytes in the mice with different treatments. (D) Accumulative frequency of TNF-α-producing CD8$^+$ T cells in splenocytes in the mice with different treatments. n=4, ****P*<0.001, error bars represent means ± S.D. Statistics was performed by Student t-test.

Supplementary Figure 4. Sunitinib suppressed RFA-induced PD-1 up-regulation in spleen. Mice with size-matched tumors were randomly exposed to no treatment (control), SU, RFA, or both. Two weeks post RFA, spleen in each mouse was harvested to isolate splenocytes for flow cytometric assay. (A-B) Representative and cumulated proportion of CD8$^+$ T cells expressing PD-1 in spleen. (C-D) Representative and cumulated proportion of CD4$^+$ T cells expressing PD-1 in spleen. The results showed that RFA promoted PD-1 production in both CD4$^+$ and CD8$^+$ T cells which can be suppressed by SU. n=4, ****P*<0.001, error bars represent means ± S.D. Statistics was performed by Student t-test.

Supplementary Table 1. Sequences of real-time PCR primers

Target	Forward primer (5'-3')	Reverse primer (5'-3')
IFN-γ	GAAAGCCTAGAAAGTCTGAATAACT	ATCAGCAGCGACTCCTTTTCCGCTT
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL-2	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
PD-1	CAGGTACCCTGGTCATTCAC	CATTTGCTCCCTCTGACACT
PD-L1	CATTTGCTCCCTCTGACACT	TGAGTCCTGTTCTGTGGAGG
HGF	ATGTGGGGGACCAAACTTCTG	GGATGGCGACATGAAGCAG
c-Met	GTGAACATGAAGTATCAGCTCCC	TGTAGTTTGTGGCTCCGAGAT
HIF-1α	ACCTTCATCGGAAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
VEGFA	CAGGCTGCTGTAACGATGAA	CTATGTGCTGGCTTTGGTGA
VEGFR1	CTCAGGGTCGAAGTTAAAAGTGC	TTGCCTGTTATCCCTCCCACA
VEGFR2	TTTGGCAAATACAACCCTTCAGA	GCTCCAGTATCATTTCCAACCA
18S	AAGTCCCTGCCCTTTGTACACA	GCCTCACTAAACCATCCAATCG