Supporting information

Bioactive Polysaccharide Nanoparticles Improve Radiation-induced Abscopal Effect through Manipulation of Dendritic Cells

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Figure S1. Physicochemical characterization of polysaccharide nanoparticles. (A) Dynamic light scattering measurements and zeta potential of ANP and DNP. (B) TEM images of ANP and DNP. (C) FTIR spectra of APS and ANP.
Figure S2. Primary tumor volume of individual mouse at day 19 subjected with different treatments. Tumor volumes were compared by one-way ANOVA. Data represent mean ± SD. ***P<0.001.
Figure S3. The cytotoxicity of ANP against four cell lines. Different concentrations of ANP were incubated with normal (NIH3T3, HUVEC), tumor (4T1) and immune cells (BMDC) for 24 h at 37°C and the cell viability was measured by CCK8 viability kit.
Figure S4. ANP induced BMDC maturation was TLR dependent. ANP or LPS as the positive control was used to induce BMDC maturation, which can be inhibited by resatorvid (TLR4 antagonist, RES, 1 µg/mL) and chloroquine (TLR7/9 antagonist, CHQ, 5 µM). Expression of DC phenotypic markers CD80 (A), CD86 (B) and the percentage of CD80^+CD86^+ DC (C). Statistical significance was assessed using one-way ANOVA. Data represent mean ± SD. ***P<0.001.
Figure S5. ANP endocytosis was partially involved in BMDC maturation. Endocytic inhibitors including wortmannin (Phagocytosis blocker, Wort, 0.1 \( \mu \text{M} \)), chlorpromazine (Clathrin-induced endocytosis blocker, CPZ, 10 \( \mu \text{g/mL} \)) and genistein (Caveolae-induced endocytosis blocker, GEN 40 \( \mu \text{g/mL} \)) were used to investigate if the increased expression of DC maturation marker CD86 by ANP was interfered. Statistical significance was assessed using one-way ANOVA. Data represent mean ± SD. **\( P<0.01 \).
Figure S6. The effect of particle size of ANP on BMDC maturation. ANP with three particle size (110 nm, 170 nm, 250 nm) were incubated with BMDC for 24 h at 37°C for the determination of the effect of particle size on CD86 expression.
Figure S7. The percentage of CD80+ DC in irradiated tumors and TDLN. After each dose of radiation, ANP was injected (total 3 times) into the irradiated tumor. The day after the last radiation, tumor (A) and TDLN (B) were dissected for flow cytometry analysis. Statistical significance was assessed using one-way ANOVA. Data represent mean ± SD. *P<0.05, **P<0.01, ***P<0.001.
Figure S8. The percentage of TIL and T_{reg} cells in primary tumors. (A) Flow cytometry analysis of the abundance of TIL, CD4^+, CD8^+ and regulatory T cells (T_{reg}) in primary tumors. TIL were defined as CD45^+. T_{reg} cell was defined as CD4^+CD25^+Foxp3^+. Statistical significance was assessed using one-way ANOVA. Data represent mean ± SD. *P<0.05, **P<0.01.
Figure S9. T cell profiles in spleen. (A) Flow cytometry analysis of the abundance of CD4$^+$ and CD8$^+$ T cells in spleen. Statistical significance was assessed using one-way ANOVA. Data represent mean ± SD. *P<0.05, **P<0.01, ***P<0.001.