Efficient Synergistic Immunotherapy for Inhibiting Tumor Postoperative Recurrence and Metastasis via Calcium Alginate Hydrogel Nanosystem

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Abstract
Immunotherapy is expected to become an promising strategy in inhibiting tumor postoperative recurrence and metastasis. However, the effect is still unsatisfactory because of lacking cooperativity between various therapeutic methods. In this study, we designed an efficient synergistic immunotherapy system as an all-around and multi-dimension method for inhibiting tumor postoperative recurrence and metastasis. The efficient synergy lay in enhancing immune effect and eliminating immune suppression in the meantime by took advantages of CpG oligodeoxynucleotides (CpG ODNs) and antiPDL1 antibody. We introduced nanomaterials based on the calcium alginate hydrogel which has an acknowledged biological safety to implement the above strategy and make the system multifunctional. This nanosystem have both therapy function and long-term monitoring capability in vivo to evaluate the recurrence and metastasis situation of postoperative residual tumor cells conveniently in real time. In vitro and vivo results proved that that this system could achieve a much better tumor inhibition efficiency and a good monitoring effect. This efficient synergistic immunotherapy nanosystem is expected to become a new promising strategy for postoperative immunotherapy of tumor.

Full-text
Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures
Figure 1

Schematic diagram of the efficient synergistic immunotherapy via calcium alginate hydrogel nanosystem for inhibiting tumor postoperative recurrence and metastasis in vivo. a) The preparation method of the injectable calcium alginate hydrogel loaded CpG and the CEA probe. b) The preparation method of the ICG@CANPs+antiPDL1 nanosystem. c) The therapeutic process of the efficient synergistic immunotherapy nanosystem. Step 1: Inject the nanosystem① in situ after surgery. The CpG loaded in the CA hydrogel can enhance the immune effect to kill the tumor cells. And the fluorescence signal of the probe which fixed in CA hydrogel could be detected to monitor the tumor recurrence by the probe in the real time. Step 2: Inject the nanosystem② into the vein of the tail. The antiPDL1 antibody on CANPs can eliminate the immune suppression and facilitate T cell to kill the tumor cells. And
the ICG loaded in CANPs can reveal whether there is a metastasis in the body through its fluorescence signal.
Figure 2

Calcium alginate hydrogel loads the CpG ODNs to enhance the immune effects through sustained release function. a) The preparation method of the injectable calcium alginate hydrogel loaded CpG. b) The treatment principle of CpG. c) The PH and coagulation time of the hydrogel with different percentage of CaCO3 and GDL. The percentage of calcium alginate was 2.5 % in all experiment. d) The CpG release rate of the CpG@CA hydrogel at different times in PBS. e) The schematic diagram of the therapeutic process. f) The cytokines concentration of different groups. f) The tumor growth curve of different groups. n=3 from in vivo experiments. Error bars denote s.e.m. P value: *, P < 0.05.
Figure 3

CEA probe fixed by hydrogel in situ for monitoring the tumor recurrence. a) Schematic diagram of the QDs-AuNPs probe we constructed based on the FRET theory. b) The monitoring principle of the CA hydrogel with CEA probe. c) The TEM picture and size column diagram of the QDs we prepared. d) The TEM picture of the AuNPs we prepared. e) The absorption spectrum of AuNPs (the purple curve) and the emission spectrum of QDs (the pink curve). f) The agarose gel electrophoresis picture of aptamer1, QDs-aptamer1, aptamer2 and AuNPs-aptamer2. All aptamers were labeled with the Cy3 dye. g) The detection standard curve of the QDs-AuNPs probe with CEA standards. Each sample was measured in triplicate. h) The detection standard curve of the QDs-AuNPs probe with used tumor cells culture medium. Each sample was measured in triplicate. i) The picture of CA hydrogel loaded probe in holes under ultraviolet rays. Different holes were added CEA solution with different concentration respectively.
CANPs carried antiPDL1 antibody to assist orthotopic therapy for inhibiting the tumor recurrence and metastasis to achieve efficient synergistic immunotherapy. a) The synthetic method of ICG@CANPs-antiPDL1. b) The treatment principle of our nanosystem. c) The TEM picture and size column diagram of the CANPs we prepared. d) The cell viability of CANPs under different concentrations. Each group has five samples. e) The pictures of tumor cells incubation experiment by inverted fluorescence microscope (Scale bar: 50 μm). The red fluorescence of ICG loaded in the nanoparticles indicated the location of nanosystems. f) The pictures of metastasis mouse models after injected nanosystems for different times by
live animal imager. The red fluorescence of ICG loaded in the nanoparticles indicated the location of nanosystems.

Figure 5

The efficient synergistic immunotherapy nanosystem used for inhibiting tumor recurrence and metastasis after surgical resection in vivo. a) The schematic diagram of treatment process in vivo. b) The flow cytometry result of CD3+CD8+ T cells in the lymph glands after different treatment of different groups. From left to right: Group 1, Group 2, Group 3, Group 4, Group 5, Group 6. c) Quantitative analyze of the CD8+ T cells content ratio in entire T cells. d) The immune cytokine content of each groups after treatment in different stages detected by ELISA. e) The primary tumor growth volume of each groups in 24 days after
f) The fluorescence alteration after treatment of the probe loaded in the CA hydrogel which was injected in situ. g) Pictures of lung tissue H&E stain sections of each groups. The blue is the nodules of the lung tumor. From left to right: Group 1, Group 2, Group 3, Group 4, Group 5, Group 6. n=5 from in vivo experiments. Error bars denote s.e.m.