Graduate School of Creative Science and Engineering
Waseda University

博　士　論　文　概　要
Doctoral Dissertation Synopsis

論　文　題　目
Dissertation Title

Nanoparticles in the MnOx-SiO2 system for the applications to the combinational anticancer therapies of immunotherapy and other therapies

MnOx-SiO2系ナノ粒子の免疫療法および他療法との併用療法への応用

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Cancer has been the leading cause of death in many countries. The clinically used anticancer therapies are still surgery, radiotherapy, and chemotherapy. Due to the limited treatment effect of these clinically used anticancer therapies, many new anticancer therapies have been developed recently, including phototherapy, chemodynamic therapy, and so on. Although many good outcomes have been achieved in both clinical and preclinical practice, most single or combinational anticancer therapies are still faced with different limitations from the complex tumor microenvironment on the inhibitory effect on tumor growth and metastasis, especially the effect on inhibiting tumor metastasis, which is the main reason for death from cancers. Therefore, newly emerged immunotherapy, an anticancer therapy that focuses on activating immune responses, has attracted researchers' eyes and shown high potential for clinical practice. Whereas the effect of immunotherapy is still unsatisfactory because the tumor microenvironment is usually immunosuppressive. Therefore, it is urgent to improve the effectiveness of these exited anticancer therapies.

This thesis mainly focused on enhancing the anticancer effect of combinational anticancer therapies of immunotherapy and other exited anticancer therapies, including radiotherapy, chemotherapy, and phototherapy. Si-based and Mn-based nanomaterials were adopted to synthesize three different forms of Si-Mn-based nanoparticle systems. These three different forms of Si-Mn-based nanoparticle systems took effect through 2 aspects. Firstly, they can regulate the tumor microenvironment to a better status for anticancer therapies, including inducing oxygenation to relieve the hypoxic status of the tumor microenvironment, and breaking the redox balance to increase reactive oxygen species. Secondly, they possess the potential to activate immune responses to relieve the immunosuppressive status. Moreover, the in vivo anticancer effects were investigated using a bilateral animal model, which indicated that these Si-Mn-based nanoparticle systems have the potential to inhibit tumor growth and metastasis.

This thesis was divided into 5 chapters.

In chapter 1, The background of this study is carefully explained.

In chapter 2, Mn-covered-SiO_2 composite nanoparticles (SM NPs) were synthesized and applied to the combinational anticancer therapies of immunotherapy and Radiotherapy. The regulation functions to the tumor microenvironment, including the capability of inducing oxygenation, reactive oxygen species (ROS) generation, glutathione (GSH) depletion, and immune activation were carefully investigated. Moreover, the intracellular change after exposure to X-ray radiation, such as ROS levels, DNA damage, GPX-4, and LPO levels, was also analyzed.

Finally, in vivo anticancer effects were further analyzed to investigate the inhibitory effect of the combination of radiotherapy and SM NPs on both primary tumors and distant untreated tumors with or without the presence of the anti-CTLA-4 antibody utilizing a bilateral animal model. Moreover, the mechanisms of in vivo immune activation were also analyzed.

In chapter 3, Mn-doped mesoporous silica nanoparticles (MM NPs) were synthesized and applied to the combinational anticancer therapies of immunotherapy and chemotherapy. The influences of Mn-doping and Mn concentration doped into MSNs on the regulation functions to the tumor microenvironment were carefully analyzed from six aspects: GSH depletion, ROS generation, oxygenation, cell-killing effect, immune activation, and degradation promotion. Moreover, the in vivo anticancer effects of the combination of DOX, MM, and the anti-CTLA-4 on the inhibitory effect to primary tumors and distant untreated tumors were further analyzed utilizing a bilateral animal model, including the monitoring of tumor size and measurement of cytokine secretion from splenocytes.

In chapter 4, MM NPs, loaded with the photo agent of IR 780 and further covered by Mn were synthesized (IMM) and applied to the combinational anticancer therapy of immunotherapy and phototherapy. The photothermal capability and photodynamic capability induced by IMM were carefully investigated to compare with IR 780, including the investigation of the photostability and the influence of different power densities, IR 780 concentrations, and irradiation time periods. The regulation functions to the tumor microenvironment were also investigated through different aspects, including oxygenation, ROS generation, and GSH depletion. Moreover, the in vivo anticancer effects of the combination of IMM and an 808nm laser irradiation in inhibiting the primary tumors and distant untreated tumors were evaluated utilizing a bilateral animal model.

Observations from this thesis can be concluded as the following:

Due to the presence of Mn-based nanomaterials, three different Mn-Si-based nanoparticles both showed regulation functions to the tumor microenvironment, including the capabilities of inducing oxygenation, ROS generation, GSH depletion, and immune activation.

1) MnO_2 in the three different Mn-Si-based nanoparticles showed an obvious capability in inducing oxygenation. Because MnO_2 is a kind of redox-active transition-metal dioxide nanomaterial, which possesses the obvious catalytic ability, including the high reactivity toward H_2O_2 to produce O_2, due to the intermediate valence state of Mn^{4+}.

2) Mn^{2+} generated from the three different Mn-Si-based nanoparticles induced the

generation of the highly cytotoxic ROS of hydroxyl radical (•OH) due to the Mn^{2+}-based-Fenton-like reactions with H_2O_2 as shown in the following equation (a). MnO_2 can be reduced to Mn^{2+} due to the reaction with H_2O_2, H^+, and GSH as shown in the following equations (b) to (d), which are usually exiting with a higher concentration in the tumor microenvironment than in normal tissue.

a) $Mn^{2+} + H_2O_2 \rightarrow Mn^{3+} + \bullet OH + OH^-$

b) $MnO_2 + H_2O_2 + 2H^+ \rightarrow Mn^{2+} + 2H_2O + O_2$

c) $MnO_2 + 2H^+ \rightarrow Mn^{2+} + H_2O + 1/2\ O_2$

d) $MnO_2 + GSH \rightarrow Mn^{2+} + GSSG$

3) MnO_2 consumed GSH due to the reaction with GSH as shown in the above equation (d).

4) The Mn and Si in the three different Mn-Si-based nanoparticles showed an obvious inhibitory effect on distant untreated tumors and activated immune responses in a bilateral animal model. On the one hand, the three different Mn-Si-based nanoparticles regulated the special tumor microenvironment, which usually influenced the immune systems and induced an immunosuppressive environment in tumors. On the other hand, the synergistic effect of Mn-based and Si-based nanomaterials on immune activation contributed to converting the immunosuppressive environment to immune activated environment.

In chapter 5, General summary is described

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Academic papers	○"Tumor microenvironment-regulated nanoplatforms for the inhibition of tumor growth and metastasis in chemo-immunotherapy". Journal of Materials Chemistry B, March 2022, Volume 10, Issue 19, 3637-3647. Xueping Yu, Xiupeng Wang, Atsushi Yamazaki, Xia Li.
Lectures	"Tumor Microenvironment-Responsive Radiotherapy", The 43rd Annual Meeting of the Japanese Society for Biomaterials and the 8th Asian Biomaterials Congress,2021. Xueping Yu, Atsushi Yamazaki, Xiupeng Wang. [AO-2E22]
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Lectures	"Tumor microenvironment regulation-enhanced photo-immunotherapy", Tsukuba Biomedical Engineering Forum 2023, 2023. Xueping Yu, Xiupeng Wang, Atsushi Yamazaki.[O2-07]