Abstract

Chemotherapy is still one of the most widely used anticancer therapies. It is mostly about inhibiting the proliferation of rapidly dividing cells, so it is not selective for tumor cells. As a result, many undesirable side effects are associated with chemotherapy. The disadvantageous properties of chemotherapeutics can be largely eliminated by using conjugates of polymers with low molecular weight drugs. An example of such a conjugate is a doxorubicin-linked HPMA polymer. In addition to the properties obtained by polymer binding, such as achieving solubility in aqueous solutions, reducing systemic toxicity, increasing the maximum tolerated dose, or passive targeting by the EPR effect, the fact that doxorubicin induces immunogenic cell death is used in therapy with this drug.

It has already been shown that after complete cure of the experimental mice with polymeric conjugates of HPMA with doxorubicin, some mice develop long-term resistance to re-inoculation with a lethal dose of tumor cells. Resistance is specific to the particular line of tumor cells from which the mouse was cured, and $CD8^+$ cytotoxic T cells and IFNγ play an important role.

In this work, we monitored changes in the proportion of immune populations and their activation markers after treatment with HPMA-based polymers with doxorubicin and free doxorubicin compared to untreated controls. The results suggest that the immune response against the tumor is more potentiated by polymer conjugate therapy. Activation of the immune system is manifested mainly by changes in the expression of the monitored markers for activated T lymphocytes (CD25), exhausted (PD-1) and chronically activated (CTLA-4) T lymphocytes and in an increased proportion of effector memory cytotoxic T lymphocytes ($CD44^+CD62L^-$). We observed these changes in tumor infiltrating lymphocyte population (TIL) and also in the periphery, in the spleen. Activation of the immune system was further confirmed by elevated plasma levels of IFNγ in mice treated with the polymer conjugate.

It was further investigated whether HPMA-based polymeric conjugates with doxorubicin may be suitable for combination tumor therapy with checkpoint inhibitors. Based on the data obtained, we can say that polymer conjugate therapy increases the proportion of CTLA-4 and PD-1 expressing lymphocytes compared to free drug therapy even compared to untreated controls. Therefore, it would be appropriate to use checkpoint inhibitor therapy to block these molecules and prolong the functional activity of lymphocytes.

Key words

Targeted tumor therapy, HPMA-based polymer conjugates, doxorubicin, antitumor immune response, cytotoxic T lymphocytes, CTLA-4, PD-1