Synthetic Glycocalyx on Cell Surfaces for Enhancing Anti-tumor Responses of Immune Cells

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Statement of Purpose: Cell surface saccharides play an essential role in regulating many important processes, ranging from cell migration and tissue programming to the disease progression, immune response and cell apoptosis.[1] Specifically, cell-surface glycans mediate a variety of biology in immune system, including leukocyte trafficking, endocytosis of microbes and modulation of cell signaling, through their interactions with glycan-binding proteins on different immune cell surfaces.[2] Nevertheless, the complex and heterogeneous structures of natural glycan have hampered the efforts to study the influence of saccharides with specific structures on cell functions. As such, it is particularly critical to generate the well-defined glycans at the cell surface for achieving the desired cellular phenotypic outcomes or elucidating the mechanisms of glycan-mediated signaling events at a molecular level. Herein, we designed a chem-bio strategy by the combination of HaloTag protein (HTP) fusion technique and reversible addition-fragmentation chain transfer (RAFT) polymerization for introducing synthetic well-defined glycopolymers onto cancer cell surfaces to regulate the immune response of tumor-associated macrophages (TAMs).

Methods: HTP fused to the platelet-derived growth factor receptor (PDGFR) transmembrane domain was stably expressed in HeLa cell membranes. We then synthesized a new key molecule, the chloroalkane-conjugated chain transfer agent (Cl-CTA), 5-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-2-cyano-5-oxopentan-2-yl benzodithioate, *via* the reaction between N-hydroxysuccinimide group and primary amine. By utilizing the synthetic Cl-CTA, the RAFT polymerization of two sugar monomers, 2-methacrylamide glucopyranose (MAG) and 2-methacrylamide mannose (MAM), were carried out to generate well-defined glycopolymers containing chloroalkane linker (pMAG and pMAM) for conjugation with membrane-bound HTP. HeLa cells stably expressing HTP were incubated with pMAG or pMAM at 37°C for one hour to achieve pMAG-HeLa or pMAM-HeLa. To test whether the glycopolymers-engineered HeLa cells could enhance the immune responses, we performed the co-culture of human monocytic U937 cells with natural HeLa and glycopolymers-engineered HeLa, respectively. Then the supernatants were collected for detecting the secretion levels of IL-10, IL-12p70 and TNF-α by enzyme linked immunosorbent assay (ELISA).

Results: The results showed that macrophages in all three groups secreted extremely low level of IL-10 (a typical immunomodulatory/M2 phenotype cytokine) (Figure 1(a)). Moreover, the concentrations of IL-10 in the pMAG/MAM-HeLa-treated groups are even nearly close to ~0 pg/mL. As shown in Figure 1(b), the levels of IL-12p70 (a typical immunostimulatory/M1 phenotype cytokine) were significantly increased after pMAG/pMAM-HeLa stimulation and the concentrations of IL-12p70 in the two groups were ~3.4 and ~3.2 times higher compared with that in the natural HeLa-treated group, respectively. These data (IL-12p70^{high}, IL-10^{low}) demonstrated that glycopolymers-engineered HeLa stimulation successfully induced macrophages to M1 polarization and enhanced the secretion of IL-12p70. It is proved that the secretion level of TNF-α were also significantly improved after stimulation with pMAG/pMAM-HeLa cells (Figure 1(c)), exhibiting ~16.3 and ~9.4-fold increase compared to that in natural HeLa-treated group, respectively. Besides, M1 polarization of macrophages also leads to secretion of the reactive oxygen molecule, nitric oxide (NO), which promotes tumor cells destruction. As shown in Figure 1(d), similar to the results of TNF-α secretion levels, significantly increased release of NO in the pMAG/pMAM-HeLa and U937 cells co-culture supernatants was found compared with the natural HeLa-treated group. All data showed that the glycopolymers-engineered HeLa up-regulated pro-inflammatory cytokines secretion, indicating the synthetic glycopolymers anchored on the HeLa cells could enhance immune responses of macrophages.

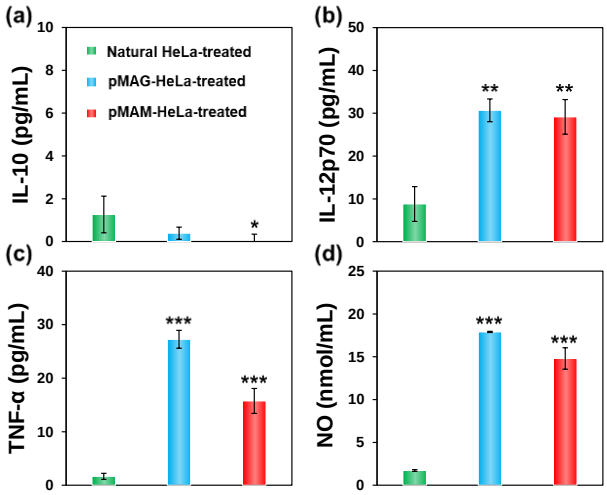

Figure 1. Glycopolymers-engineered HeLa cells altered the cytokine secretion of macrophages (IL-10 (a), IL-12p70 (b), TNF-α (c) and NO release (d)). Natural HeLa-treated group was used as control. Data are expressed as the mean ± SD of three independent experiments (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Conclusions: We proved that the glycopolymers-engineered HeLa cells with HTP anchors promoted the activation of macrophages, induced macrophages to M1 polarization and up-regulated pro-inflammatory cytokines secretion. This study demonstrates a promising potential of glycopolymers-engineered tumor cells in cancer immunotherapy.

References:

[1] Griffin ME. Cell Chem. Biol., 2016;23:108-121.

[2] Collins BE. Curr. Opin. Chem. Biol., 2004;8:617-625.