Surface Hybridization of Macrophages with Dendrimer via Copper-Free Click Chemistry

Leyuan Xu[1], Olga Yu. Zolotarskaya[1], W. Andrew Yeudall[2,3], Hu Yang[1,3]

[1]Department of Biomedical Engineering, [2]Philips Institute of Oral and Craniofacial Molecular Biology, [3]Massey Cancer Center; Virginia Commonwealth University, Richmond, Virginia 23284

Statement of Purpose: Recruitment and congregation of monocytes/macrophages in response to hypoxia suggest that monocytes/macrophages can be used a vehicle to achieve targeted delivery of anticancer therapeutics to hypoxic tumor cells. Considering that anticancer drugs cannot be directly loaded into monocytes/macrophages due to their cytotoxicity, our group pioneered an innovative concept to use monocytes/macrophages for anticancer drug delivery by developing a cell-dendrimer hybrid vehicle in which highly branched dendrimers are covalently coupled to the cell surface, thus providing numerous sites for drug coupling (Holden CA. Int J Nanomedicine. 2010; 5:25-36). To retain cell viability and functions during surface chemistry, a fast and efficient chemistry is highly desirable. In this work, we explore a highly efficient and selective copper-free chemistry for cell-dendrimer hybridization.

Methods: Dibenzocyclooctyne (DIBO) was coupled to polyamidoamine (PAMAM) dendrimer generation 4 (G4)which was labeled with fluorescein isothiocyanate (FITC) to obtain G4-FITC-DIBO nanoparticles. The resulting nanoparticles were then immobilized to the N-azidoacetyl-mannosamine (Ac_4ManNAz) treated RAW264.7 macrophages via a copper-free bioorthogonal reactionas reported by Bertozzi group (Laughlin ST.Science. 2008;320: 664–667). The distribution of nanoparticles on the cell surface was visualized by using confocal microscopy. After cell surface modification, the cell viability was determined by WST-1 assay and the intracellular signaling pathways, AKT, p38 and NFκB were evaluated by Western blot analysis. In addition, the cell migration ability will beexamined by using in vitro scratch assay.

Results: RAW264.7 cells were incubated with 50 μMof Ac_4ManNAz for 48 hto incorporate azide groups into the cell surface. The azide-expressing cells or regular cells (negative control)were treated with 25 μMof G4-FITC-DIBO at room temperature. The bioorthogonal reaction wasconducted for 10 min, followed by washing in PBS and fixation for confocal microscopy imaging.Nuclei were stained with DAPI (blue). We observed a clear cell surface labeling in the Ac_4ManNAz-treated cells (Fig. 2). In the absence of Ac_4ManNAz on the cell surface, we observed FITC-labeled dendrimers inside the cell. These results indicate that the surface hybridization of macrophages with dendrimer is azide-dependent. In order to evaluate the drug deliverycapability of the hybridized macrophages, we examined the cytotoxicity and intracellular signaling pathways during the cell surface engineering. After incubation of Ac_4ManNAz, there was no significant decrease in cell viability and intracellular AKT and p38 inactivation. In addition, after surface hybridization of cells with G4-FITC-DIBO through strain-promoted azide–alkyne cycloadditionreaction, there was no significant decrease in cell viability observed(Fig. 3). These results indicate that cells remain healthy after hybridization. Next, we are going to evaluate cell migration ability after surface hybridization.

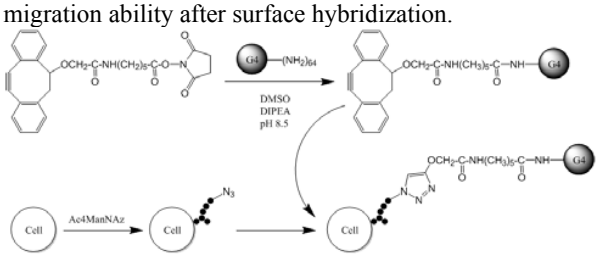

Figure 1. Scheme of macrophage hybridization with dendrimer through copper-free click chemistry.

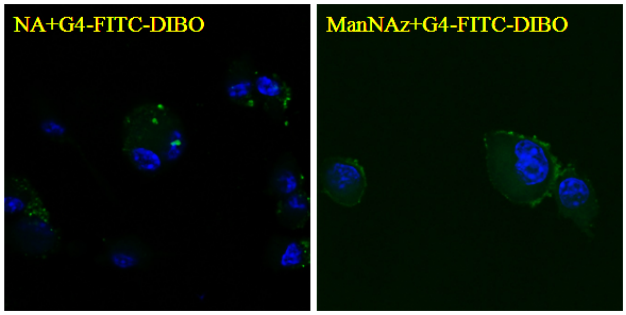

Figure 2. Confocal images(600×) of regular RAW264.7 cells (left) and azide-expressing RAW264.7 cells treated with G4-FITC-DIBO.

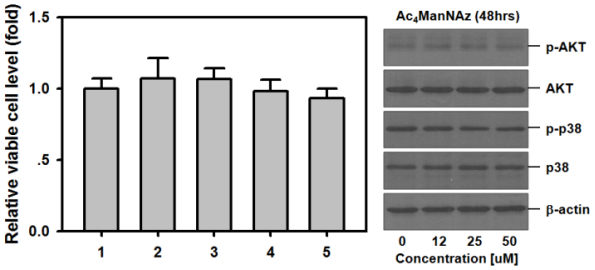

Figure 3. Relative cell viability after cell treatment (left) and intracellular AKT and p38 expression (right).1, NA; 2, Ac_4ManNAz; 3, G4-FITC-DIBO; 4, Ac_4ManNAz+G4-FITC; 5, Ac_4ManNAz+G4-FITC-DIBO.

Conclusions: FITC-labeled dendrimers coupled with DIBOwere efficiently immobilized onto the macrophage surface through a fast and selective bioorthogonal reaction. This new strategy avoids intensive chemical reactions to maintain cell viability and functions.

Acknowledgement: NSF CAREER Award CBET0954957.