Metabolic Labeling and Targeted Modulation of Adipocytes

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Statement of Purpose: Adipocytes are central to energy storage and endocrine signaling, influencing diseases like cancer and diabetes. Here we present a metabolic glycan labeling method for adipocytes, enabling targeted modulation via click chemistry. By tagging adipocytes with azido groups and conjugating DBCO-cargos, we could modulate their interactions with macrophages (BMDM). This platform could improve understanding of adipocytes with immune cells and advance adipocyte-based therapies and in vivo.

Methods: Materials: D-Mannosamine hydrochloride, DBCO-Cy5, sodium azide, chloroacetic anhydride, acetic anhydride, cisplatin, DBCO-sulfo-amine, L-luciferin potassium, octyl isocyanate, MTT, CCK-8 kit, DBCO-Sulfo-NHS, DMSO, Nile Red, 3T3 differentiation kit, Calreticulin, Adiponectin, MBL-2, MEG-E8

Methods: Stability of Cell-Surface Azido Groups: 3T3-L1 adipocytes were treated with Ac4ManNAz (50 μM) for 72 hours, followed by PBS washes and culture in fresh media for 24, 48, and 96 hours. Cells were then treated with DBCO-Cy5 (20 μM) for 30 minutes, trypsinized, and analyzed via flow cytometry. In Vivo Targeting of 3T3-L1 Adipocytes: Azido-labeled adipocytes were injected into the left fat pad of mice, with unlabeled cells on the right as controls. After 48 hours, DBCO-Cy5 was administered, and fluorescence was imaged using IVIS to quantify targeting. Coculture of Adipocytes and BMDMs: After conjugating signaling molecules to adipocytes, they were cocultured with BMDMs at a 1:1 ratio for 24 hours. Excess adipocytes were removed, and macrophages were collected and antibody-stained for flow cytometry.

Results: First we validated the in vitro metabolic glycan labeling of adipocytes. Compared to control, adipocytes treated with Ac4ManNAz showed significantly enhanced Cy5 signal, indicating the successful metabolic labeling with azido groups, which is also confirmed by flow cytometry analysis (Fig. 1).

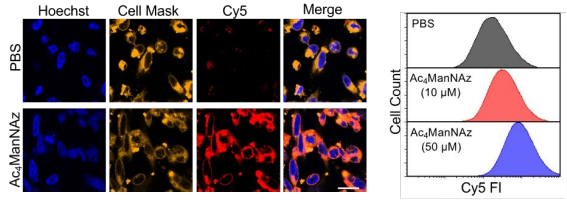

Figure 1. In vitro metabolic labeling of adipocytes

Next, we investigated the stability of azido tags on cell membrane. After labeling, adipocytes were placed in fresh media for up to 96 hours, then labeled with DBCO-Cy5 to detect azido groups. Surface azido levels decreased initially but remained stable from 24 to 96 hours (Fig. 2), supporting that low-proliferative adipocytes retain azido expression longer than faster-dividing cells.

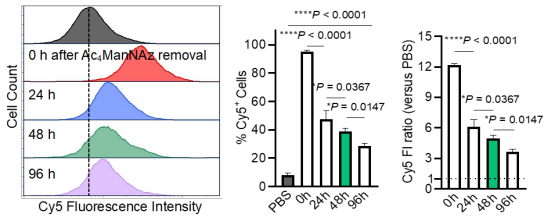

Figure 2. Stability of azido tag on adipocytes

Then, we studied if azido-labeled adipocytes facilitate DBCO-cargo conjugation in vivo. Azido-labeled adipocytes, were inoculated into the left mammary fat pad of mice, and control into the right. After 48 hours, DBCO-Cy5 was injected. Imaging showed higher Cy5 accumulation in Ac4ManNAz-pretreated adipocytes, with a ~3-fold increase, confirmed by flow cytometry, demonstrating successful in vivo conjugation via click chemistry (Fig. 3).

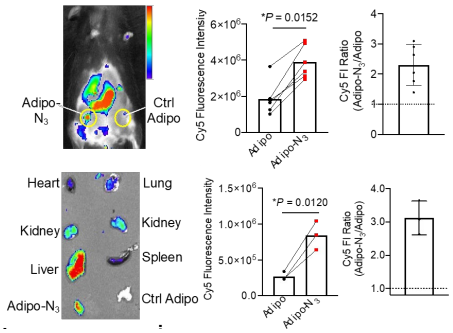

Figure 3. Azido-labeled adipocytes mediate conjugation of DBCO-cargo in vivo

Finally, we explored whether signaling molecules can be conjugated onto azido-labeled adipocytes to manipulate adipocyte-macrophage interactions. We found that ACRP, CRT and MFG-E8 conjugation enhanced phagocytosis, while MBL-2 did not (Fig 4.). These findings suggest that conjugating signaling molecules can modulate adipocyte-macrophage interactions.

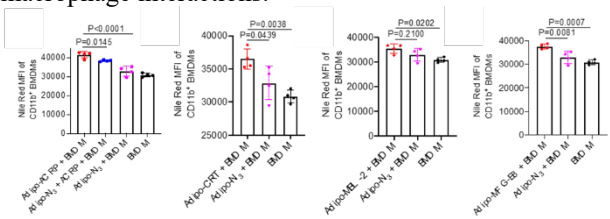

Figure 4. Modulation of macrophage-mediated phagocytosis of adipocytes.

Conclusions: In summary, we demonstrated the successful metabolic glycan labeling of adipocytes, characterized the labeling kinetics. We also validated in vivo targeting via click chemistry and orchestrated adipocyte-macrophage interactions through surface conjugation of signaling molecules. This technology advances adipocyte-based cell therapies and enables further in-depth study of adipocyte interactions with immune system in cancer, diabetes, and other diseases.