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Research Article

Development of Triantennary N-Acetylgalactosamine Conjugates as Degraders for Extracellular Proteins

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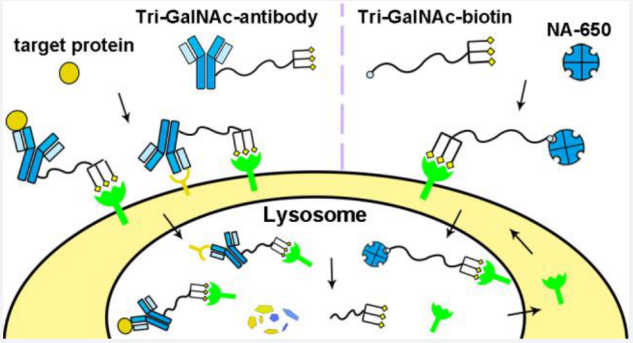
ABSTRACT: Targeted protein degradation (TPD) technology has drawn significant attention from researchers in both academia and industry. It is rapidly evolved as a new therapeutic modality and also a useful chemical tool in selectively depleting various protein targets. As most efforts focus on cytosolic proteins using PROteolysis TArgeting Chimera (PROTAC), LYsosome TArgeting Chimera (LYTAC) recently emerged as a promising technology to deliver extracellular protein targets to lysosome for degradation through the cation-independent mannose-6-phosphate receptor (CI-M6PR). In this study, we exploited the potential of the asialoglycoprotein receptor (ASGPR), a lysosomal targeting receptor specifically expressed on liver cells, for the degradation of extracellular proteins including membrane proteins. The ligand of ASGPR, triantennary *N*-acetylgalactosamine (tri-GalNAc), was conjugated to biotin, antibodies, or fragments of antibodies to generate a new class of degraders. We demonstrated that the extracellular protein targets could be successfully internalized and delivered into lysosome for degradation in liver cell lines specifically by these degraders. This work will add a new dimension to TPD with cell type specificity.



INTRODUCTION

Protein degradation is essential for maintaining cellular protein homeostasis. Most proteins in eukaryotic cells are degraded through the ubiquitin-proteasome system, where the E3 ubiquitin ligase recognizes a specific protein substrate and tags multiple ubiquitin motifs to it, leading to the subsequent proteolysis by the proteasome.[1,2] Lysosome is another major destination for protein degradation. Through autophagy and endocytosis, both intracellular and extracellular proteins enclosed in vesicles can be delivered into lysosomes for degradation.[3,4] Based on these mechanisms, targeted protein degradation by chimeric molecules emerged as a novel therapeutic modality. These chimeras are heterobifunctional molecules with one end binding to the protein of interest (POI) and the other end directing the ternary complex toward a certain degradation pathway. PROteolysis TArgeting Chimera (PROTAC) has received the most attention to date. PROTACs contain an E3 ligase ligand to route the targeted protein to the proteasome for degradation.[5,6] More recently, AUtophagy-TArgeting Chimera (AUTAC) was developed to degrade not only proteins but also organelles by using S-guanylation as the tag for autophagy.[7] However, these two types of chimeras are only capable of depleting cytoplasmic proteins or membrane proteins with a cytosolic binding domain. To broaden the scope of targets to include proteins without cytosolic binding domains, Bertozzi's group first developed LYsosome TArgeting Chimeras (LYTACs) by conjugating the ligand of the ubiquitously expressed cation-independent mannose-6-phosphate receptor (CI-M6PR) on the cell surface with a molecule that binds to the extracellular protein target.[8] The receptor–ligand interaction triggers the internalization of the extracellular proteins through receptor-mediated endocytosis, further inducing the degradation of the targets in the lysosome. CI-M6PR has been used to deliver therapeutic drugs conjugated with mannose-6-phosphate (M6P) derivatives for lysosomal enzyme replacement therapy and cancer treatment.[9,10] Various molecules, such as peptides, proteins, or liposome, were covalently linked to the modified M6P with enhanced affinity and stability to achieve targeted drug delivery.[11−14] To extend the usage of M6PR/M6P system to targeted protein degradation, LYTAC was constructed by conjugating a mixture of polyglycopeptides containing multiple M6P analogues per polymer to the antibody of POI. Different from the drug delivery process, which involves the internalization of a covalent linked M6P-protein target, LYTAC allows the trafficking of a complex formed by the noncovalent interaction between the protein target and LYTAC. It has been

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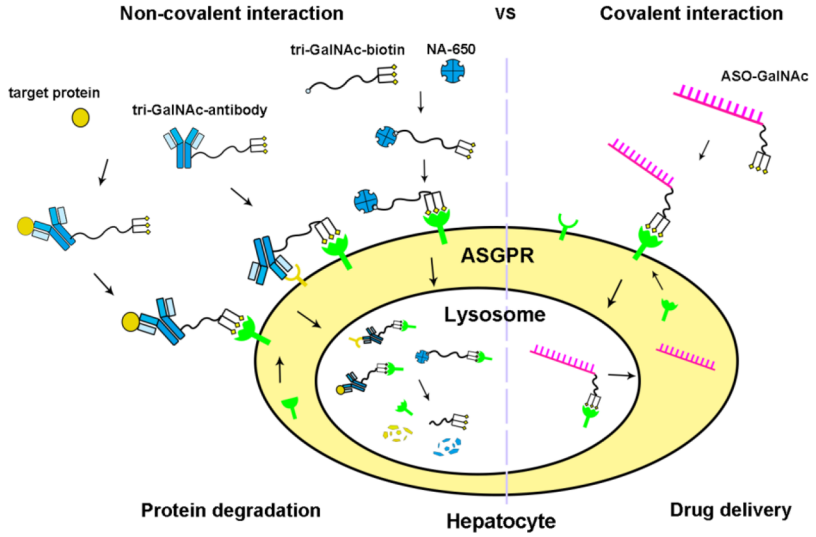

Figure 1. Comparison of the application of tri-GalNAc in targeted protein degradation and drug delivery. Small molecule- and antibody-based tri-GalNAc degraders noncovalently capture the protein targets and transport the targets to lysosome for degradation via the interaction with ASGPR. Oligonucleotides covalently linked to tri-GalNAc enable their internalization into the cell through ASGPR. After trafficking to lysosome, a small amount of the oligonucleotides can escape from the endosome or lysosome to block or induce degradation of RNA.

shown that LYTAC could successfully degrade both secreted and membrane proteins in the lysosome through CI-M6PR.[8]

Asialoglycoprotein receptor (ASGPR) is another well-defined lysosomal targeting receptor, responsible for clearing glycoproteins via clathrin-mediated endocytosis and lysosomal degradation. Unlike CI-M6PR, ASGPR is primarily and highly expressed in hepatocytes with 500 000 copies per cell.[15] The unique expression pattern together with rapid recycling rate (~15 min)[16] make ASGPR a promising candidate for liver-specific targeted protein degradation. It has been reported that ASGPR binds to galactose (Gal) and *N*-acetylgalactosamine (GalNAc), with a higher affinity to the latter than the former, in the presence of Ca^{2+} ions.[17,18] Studies using both cluster galactosides and synthetic oligosaccharides indicated that trivalent GalNAc ligand with a 15−20 Å spacing between each sugar exhibited the highest binding affinity and efficiency for endocytosis compared to mono- and bivalent GalNAc ligands.[19,20] Investigations on the triantennary ligand also suggest that cargo size below 70 nm is important for the proper receptor recognition and efficient endocytosis.[21] The comprehensive understanding of receptor−ligand interaction paved the way for the application of a ASGRP/triantennary GalNAc (tri-GalNAc) system in targeted drug delivery, especially for oligonucleotide therapy. Many tri-GalNAc-modified therapeutic nucleic acid agents, including siRNAs, anti-miRNAs, and antisense oligonucleotides (ASOs), are now in preclinical or clinical studies.[22,23] It has been shown that the conjugation of tri-GalNAc facilitates the uptake of the oligonucleotides and thus much lower dose is required compared to the free version.[24,25] Besides direct labeling of the therapeutic drugs, tri-GalNAc was also tagged to some drug carriers, such as lipid nanoparticle or poly(amidoamine) (PAMAM) dendrimer to achieve targeted delivery.[26,27] It was reported that modifying poly-γ-glutamic acids (PGA) with tri-GalNAc resulted in exclusive distribution in mice liver, while the nonmodified PGA were excreted into urine.[28] However, despite extensive use in drug delivery, the possibility of ASGPR-mediated targeted protein degradation has not been exploited prior to our study. During the preparation of this manuscript, research groups of Spiegel and Bertozzi independently reported their elegant design of chimeric molecules with tri-GalNAc for targeted protein degradation in liver cells.[29,30] While the former focused on small molecule-based lysosome targeting degraders,[29] the latter investigated antibody-based degraders for extracellular protein targets.[30] Inspired by Bertozzi's pioneering work on LYTACs based on CI-M6PR and due to our interest in both targeted protein degradation[31−34] and carbohydrate chemistry,[35,36] we initiated the investigation of ASGPR-mediated targeted protein degradation using chimeric molecules bearing a trivalent GalNAc ligand as liver cell-specific degraders for extracellular proteins including membrane proteins (Figure 1). Since liver is the major place for protein catabolism, selectively delivering undesired proteins to the liver for degradation can be potentially advantageous over ubiquitously delivery of protein targets to various types of cells unselectively for many therapeutic applications.

RESULTS AND DISCUSSION

Tri-GalNAc-biotin Conjugate 1, a Small Molecule Lysosome Targeting Degrader, Can Facilitate the Uptake of NeutrAvidin through ASGPR in Liver Cells. As our initial study, we employed NeutrAvidin (NA) as the targeted protein. Commercially available tri-GalNAc-biotin conjugate **1** was used as the ligand of ASGPR to examine the uptake of NA (Figure 2A). HepG2 cells were treated with 2 μM of **1** and 500 nM of fluorescently labeled NA-650 concurrently for 4 h, and the fluorescence intensity inside the cells was measured by the plate reader to indicate the uptake of the NA-650. Our data showed that NA-650 was internalized into the cell in a time-dependent manner in the presence of **1**. No increase of the fluorescent signal was observed when the cells were treated with NA-650 alone or in the presence of negative control, tri-GalNAc-CO_2H **2** (Figure 2B). The treatment of increasing concentration of **1** showed reduced uptake of NA-650 at high doses, suggesting that the formation of binary complexes between **1** and receptor or **1** and NA-650 becomes dominant over the formation of ternary complex NA-650/**1**/receptor with excess degrader **1** (Figure S1A), which is

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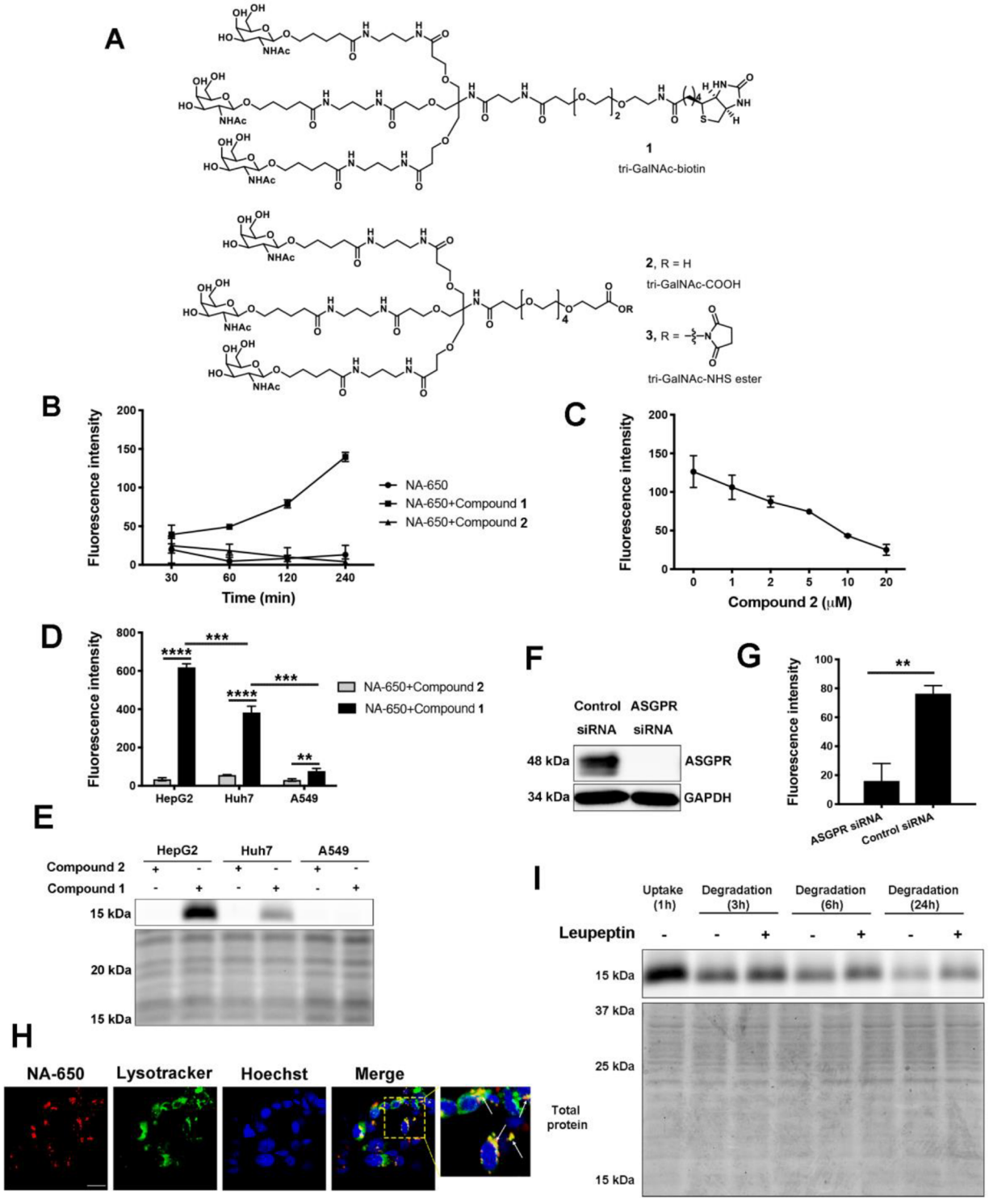

Figure 2. Tri-GalNAc-biotin mediates ASGPR-dependent cellular uptake of NA-650 specifically in liver cells and transports NA-650 to lysosome for degradation. (A) Chemical structures of tri-GalNAc-biotin (compound **1**), tri-GalNAc-CO_2H (compound **2**), and tri-GalNAc-NHS ester (compound **3**). (B) Cellular uptake of NA-650 in HepG2 cells treated with NA-650 alone (500 nM) or NA-650 (500 nM) and compound **1** (2 μM) or **2** (2 μM). (C) Inhibition of the internalization of NA-650 (500 nM) mediated by **1** (2 μM) in HepG2 cells by compound **2**. (D, E) Comparison of the internalization of NA-650 (500 nM) mediated by **1** (2 μM) among HepG2, Huh7, and A549 cells incubated with NA-650 and compounds **1** or **2** for 16 (D) or 6 h (E). Data presented as mean ± SD, $n = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (F) Knockdown of ASGPR by siRNA. (G) Uptake of NA-650 (500 nM) in the presence of **1** (2 μM) within 4 h in HepG2 cells treated with control or ASGPR siRNA. (H) Confocal microscopy images of HepG2 cells treated with NA-650 (500 nM) and compound **1** (2 μM) for 18 h. Legend: internalized NA-650 (red); lysosome stained by Lysotracker (green); nuclei stained by Hoechst 33342 (blue); merged area (yellow). White arrows indicate the colocalization of NA-650 and the lysosome; scale bar: 20 μm. (I) In gel fluorescence analysis of NA-650 (500 nM) internalization and degradation in HepG2 cells by compound **1** (2 μM) in the presence or absence of leupeptin (0.1 mg/mL).

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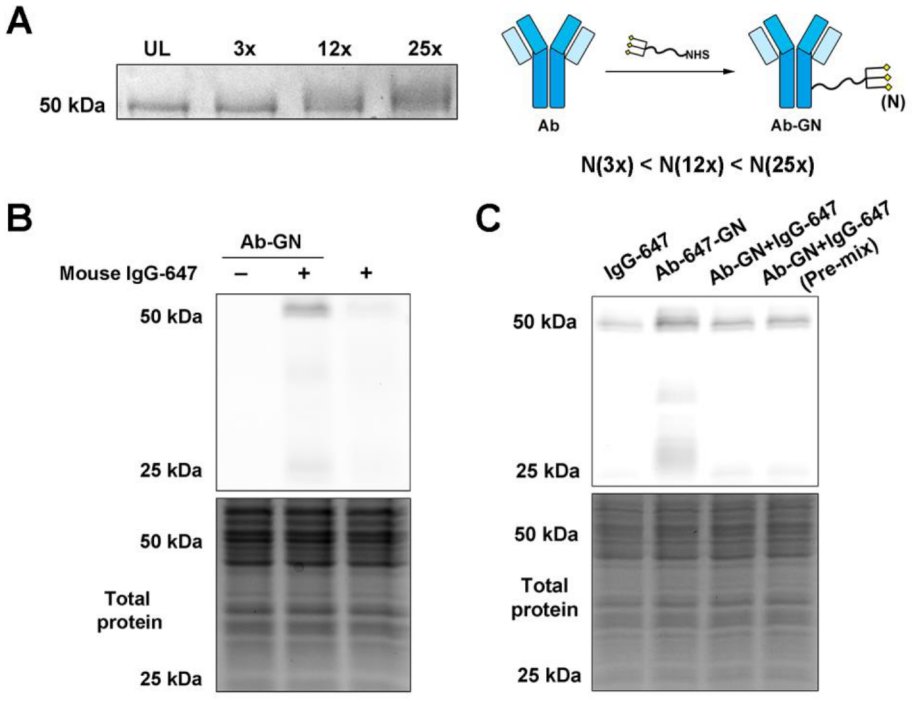

Figure 3. Tri-GalNAc labeled full length antibody goat anti-mouse IgG (Ab-GN) delivers target protein mouse anti-biotin IgG-647 into the cells. (A) Goat anti-mouse full length antibody labeling with various amounts of tri-GalNAc: UL = unlabeled; 3x = 3 mol equiv; 12x = 12 mol equiv; 25x = 25 mol equiv. N = the number of tri-GalNAc labeled on the antibody. (B) Uptake of mouse anti-biotin IgG-647 (50 nM) in the HepG2 cells treated with or without Ab-GN (25 nM) for 6 h. (C) Mouse anti-biotin IgG-647 (50 nM) uptake mediated by Ab-GN (25 nM) with or without 1 h premix before treatment for 6 h. The uptake of anti-biotin IgG-647 (50 nM) and Ab-647-GN (25 nM) were measured for comparison.

often termed as the hook effect.[37] We next extended the incubation time for **1** and NA-650 in HepG2 cells to 24 h. A continuous increase of the fluorescent signal was detected in the early phase while the signal gradually reached the plateau after 16−20 h (Figure S1B).

To verify that the internalization of NA-650 was mediated through ASGPR, various concentrations of **2** were added to compete for the receptor with the **1**/NA-650 complex. The results showed that the uptake of NA-650 negatively correlated with the amount of **2**, suggesting that the internalization of NA-650 required the interaction between **1**/NA-650 complex and ASGPR (Figure 2C). We then compared the uptake of NA-650 into HepG2, Huh7, or A549 cells with various ASGPR expression levels (Figure S1C) and found that the amount of NA-650 accumulated in the cells significantly reduced with the decrease of ASGPR level. Similar to Figure 2B, compound **2** without the biotin moiety failed to deliver NA-650 to all of these cell lines (Figure 2D, E). Moreover, the knockdown of ASGPR by siRNA dramatically impeded the internalization of NA-650 into HepG2 cells (Figure 2F, G; Figure S1D). All of these data confirmed the involvement of ASGPR in the transportation of NA-650 and indicated that the biotinylated ligand containing tri-GalNAc specifically delivered the targeted protein into liver cells.

Tri-GalNAc-biotin Conjugate 1 Delivers NeutrAvidin to Lysosome for Degradation. Next, we investigated whether NA-650 was delivered into lysosomes and degraded after being endocytosed into the cell. Confocal images showed the distribution of NA-650 in the cytoplasm and colocalization with the lysosome indicated by Lysotracker. This confirmed the ASGPR-mediated uptake and trafficking of the protein target to the lysosome (Figure 2H). To evaluate the degradation of NA-650, HepG2 cells were incubated with NA-650 and **1** for 1 h, followed by the replacement of fresh media to allow further degradation. Compared to the amount of NA-650 enriched in the cell within 1 h incubation, decreasing amounts of NA-650 were detected at 3, 6, and 24 h postmedia change. The addition of known lysosome inhibitor leupeptin moderately reduced the degradation of NA-650 at each time point (Figure 2I). These data indicated that the degradation of NA-650 occurred after it was transported into the lysosome.

A tri-GalNAc Labeled Full Length Antibody (Goat Anti-mouse IgG) Facilitates the Uptake of Its Protein Target (Mouse Anti-biotin IgG-647). Given the successful internalization and degradation of NA by **1** in the model system, we hypothesized that an antibody conjugated with tri-GalNAc can function similarly as **1** tested above—capturing the extracellular targeted protein and delivering it into the lysosome for degradation. To validate the feasibility of our hypothesis, we first functionalized an antibody with tri-GalNAc to generate an antibody-based degrader (tri-GalNAc-antibody). Tri-GalNAc-CO_2H **2** was converted to its active *N*-hydroxysuccinimide (NHS) ester **3** under standard conditions. The antibody was then conjugated with NHS ester **3** by reacting with the lysine residues on the antibody. After testing different molar ratios for the antibody conjugation, we found that the best labeling efficiency was achieved by using 25 equiv of NHS ester **3** (Figure 3A). Moreover, comparing the internalization of antibodies coupled with various equivalents of tri-GalNAc revealed that a higher degree of tri-GalNAc labeling on the antibody resulted in a greater internalization capacity (Figure S2).

We then examined the uptake of the targeted protein by cotreating HepG2 cells with tri-GalNAc-modified goat anti-mouse IgG (Ab-GN) and fluorescent protein target mouse

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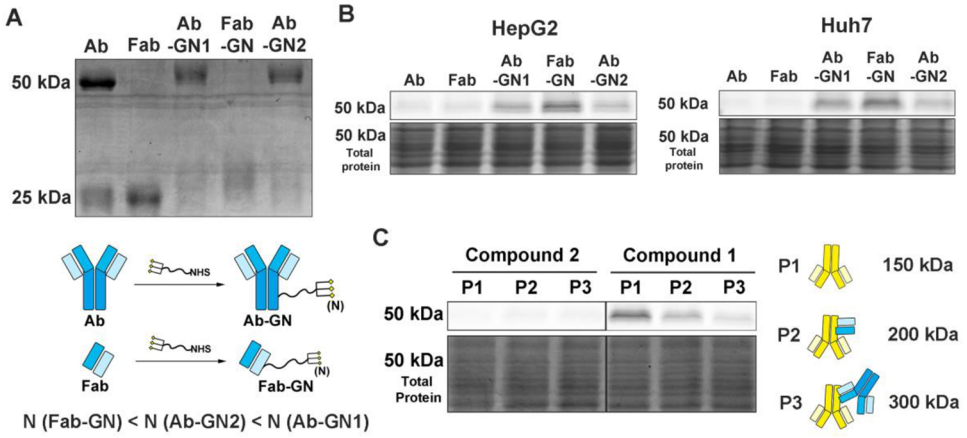

Figure 4. Uptake of mouse IgG-647 mediated by tri-GalNAc-labeled antibodies and compound 1. (A) Antibodies labeled with tri-GalNAc (25 mol equiv). (B) Comparison of the 6 h uptake of mouse anti-rabbit IgG-647 (50 nM) mediated by 25 nM of the goat anti-mouse IgG and goat anti-mouse IgG Fab with or without tri-GalNAc (GN) labeling. (C) Cellular uptake of mouse anti-biotin IgG-647 (P1, 50 nM), premixed mouse anti-biotin IgG-647 (50 nM)/goat anti-mouse IgG Fab (200 nM) complex (P2), and premixed mouse anti-biotin IgG-647 (50 nM)/goat anti-mouse IgG (200 nM) complex (P3) in the presence of compounds **1** (200 nM) or **2** (200 nM) for 6 h.

anti-biotin IgG-647 for 6 h. The addition of Ab-GN increased the uptake of mouse anti-biotin IgG-647 compared to the cells treated with mouse anti-biotin IgG-647 alone, but the efficiency was relatively low (Figure 3B). To identify the factors that gave rise to the low uptake efficiency, fluorescent goat anti-mouse IgG-647 was directly labeled with NHS ester **3** (Ab-647-GN). Greater fluorescent intensity was observed in the cells treated with Ab-647-GN alone than the cells cotreated with Ab-GN and mouse IgG-647 (Figure 3C), suggesting that the low uptake of the targeted protein was not restrained by the internalization efficiency of tri-GalNAc-Ab itself. We then preincubated mouse anti-biotin IgG-647 and Ab-GN to allow the complex formed prior to the treatment. The premixing did not enhance the amount of internalized mouse IgG-647 (Figure 3C), suggesting that the complex formation is not the rate-limiting step for the tri-GalNAc-Ab mediated uptake.

Comparison of the Uptake Efficiency of the Protein Targets (Mouse Anti-biotin IgG-647 and Mouse Anti-rabbit IgG-647) Mediated by Fab Fragment and Full Length Antibodies Labeled with Different Numbers of tri-GalNAc. It has been reported that the size of the complex may play a role in the recognition and processing by the ASGPR. Efficient uptake of liposome could only be achieved when their sizes are less than 70 nm.[21] To compare the internalization efficiency of the protein target by degraders with different sizes, in addition to the full-size goat anti-mouse IgG (MW = 150 kDa), we labeled goat anti-mouse IgG Fab monomer (MW = 50 kDa) with NHS ester **3** to yield a smaller degrader, Fab-GN (Figure 4A). Apart from size, the number of ligands labeled on each antibody may influence the accessibility of the degrader to the receptor and contribute to the difference in the target protein uptake efficiency. By adjusting the initial antibody concentration, we were able to produce two types of full-length antibody degraders with high (Ab-GN1) or low (Ab-GN2) tri-GalNAc labeling numbers. MALDI-MS indicated that Ab-GN1 was labeled with 5.7 tri-GalNAc residues in average per antibody, while Ab-GN2 had a lower average labeling number around 4.7 (Figure 4A, S3). All three antibodies (Ab-GN1, Ab-GN2, and Fab-GN) should bind to mouse anti-biotin IgG-647 with similar affinity. We then cotreated HepG2 and Huh7 cells with 50 nM of the protein target (mouse antirabbit IgG-647) together with 25 nM of goat anti-mouse IgG (Ab) and goat anti-mouse IgG Fab (Fab) with or without tri-GalNAc (GN) labeling. The amount of florescent mouse anti-rabbit IgG-647 inside the cells was monitored 6 h post-treatment. In gel fluorescence analysis showed that all three types of tri-GalNAc-antibodies were able to enhance the internalization of mouse anti-rabbit IgG-647 compared to the cells treated with nonmodified antibodies. Among them, Ab-GN1, with only one more labeled tri-GalNAc residues than Ab-GN2 in average, exhibited higher uptake efficiency, suggesting that even slightly higher number of ligands on the degrader could facilitate the target protein internalization (Figure 4B). Interestingly, Fab-GN significantly boosted the uptake of the mouse IgG-647 compared to both of Ab-GNs, despite that fewer tri-GalNAc residues (~3.2) were modified on the antibody. We then decided to confirm this trend by studying three tri-GalNAc-antibody-mediated uptake of another protein target, fluorescent mouse anti-biotin IgG-647. Our results again showed that the highest target uptake was achieved by Fab-GN (Figure S4). Fab-GN, the tri-GalNAc conjugate with the lowest molecular weight and size, can promote the most efficient uptake of two different protein targets among the three antibody-based degraders, suggesting that the size of the tri-GalNAc-Ab may affect the endocytosis process mediated by ASGPR.

To further verify the potential role of molecule size in ASGPR mediated cellular uptake, we next compared the uptake efficiency of proteins in different sizes using the same small molecule-based degrader, **1**. Incubating mouse anti-biotin IgG-647 with goat anti-mouse IgG or anti-mouse IgG Fab fragment could enable the formation of protein complexes with increased molecular weight and sizes. HepG2 cells were treated with mouse anti-biotin IgG-647 (P1), premixed mouse anti-biotin IgG-647/goat anti-mouse IgG Fab (P2), and premixed mouse anti-biotin IgG-647/goat anti-mouse IgG (P3) in the presence of **2** or **1** for 6 h. The results showed that the uptake efficiency of protein targets decreased as the size of the complex increased when cells cotreated with the same amount of **1** (Figure 4C). Acid **2** did not induce the internalization of protein targets at all. Consistent with the results of tri-GalNAc-antibodies, **1** also displayed a higher

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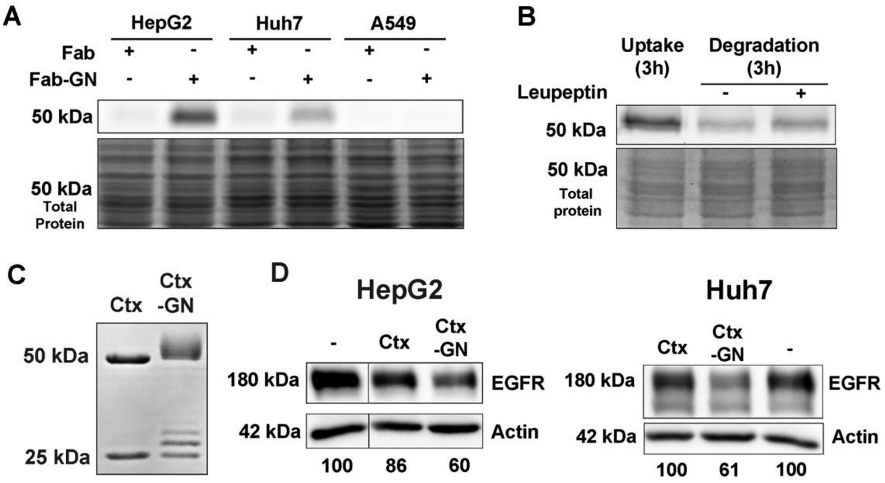

Figure 5. Tri-GalNAc-antibody mediates the uptake and degradation of mouse anti-biotin IgG-647 and EGFR in liver cells. (A) Internalization of mouse anti-biotin IgG-647 in cells incubated with mouse anti-biotin IgG-647 (50 nM) and 25 nM of goat anti-mouse IgG Fab with or without tri-GalNAc (GN) labeling for 6 h. (B) Mouse anti-biotin IgG-647 (50 nM) endocytosis and degradation in HepG2 cells in the presence or absence of leupeptin (0.1 mg/mL) for 6 h. (C) Ctx labeling with tri-GalNAc (25 mol equiv). (D) EGFR degradation in the presence of 30 nM Ctx-GN in HepG2 and Huh7 cells after 48 h treatment.

uptake efficiency for the smaller degrader-target complex. The affinity of **1** to mouse anti-biotin IgG-647 (P1), premixed mouse anti-biotin IgG-647/goat anti-mouse IgG Fab (P2), and premixed mouse anti-biotin IgG-647/goat anti-mouse IgG (P3) should be very similar. The decreased uptake efficiency from P1 to P2 and from P2 to P3 appears to correlate with the increased size of the target complexes. Our data indicate that the internalization driven by a small molecule tri-GalNAc-conjugate through ASGPR is also more efficient for smaller degrader−protein target complexes.

Tri-GalNAc-antibody Mediates the Uptake and Degradation of Both Exogenous and Endogenous Protein Targets through ASGPR in Liver Cells. Different uptake efficiencies were observed for two different protein targets (mouse anti-biotin IgG-647 and mouse anti-rabbit IgG-647) using three different antibody-based degraders: full size antibody with high or low tri-GalNAc labeling, as well as tri-GalNAc-labeled Fab fragment. Owing to the highest uptake efficiency mediated by Fab-GN, we next compared the uptake of mouse anti-biotin IgG-647 in HepG2, Huh7, and A549 cell lines in the presence of Fab-GN. Similar to the small molecule-based degrader, the amount of target protein internalized into cells was highly dependent on the ASGPR expression in different cell lines, meaning that the highest uptake was observed in HepG2 cells followed by Huh7 cells. No detection of the mouse IgG-647 in A549 cells indicated that the protein target can only be efficiently transported into ASGPR-expressing cell with the assistance of tri-GalNAc-modified antibody (Figure 5A). The degradation of internalized mouse IgG-647 was detected after the removal of Fab-GN and mouse IgG-647 from the media for 3 h. The addition of leupeptin moderately inhibited the degradation (Figure 5B). Moreover, we found that continuous treatment of known lysosome inhibitors chloroquine or leupeptin for 6 h increased the accumulation of mouse IgG-647 in both HepG2 and Huh7 cells (Figure S5). These results indicated that the protein was depleted through lysosome degradation pathway.

We next explored the application of the lysosome targeting degraders for endogenous proteins. We generated an antibody-based degrader targeting the cellular membrane protein, epidermal growth factor receptor (EGFR), which is commonly overexpressed and mutated in human tumors.[38−41] Cetuximab (Ctx), a monoclonal antibody against EGFR approved by the Food and Drug Administration (FDA), was conjugated with tri-GalNAc following the same procedure as secondary antibody used previously to generate the degrader Ctx-GN (Figure 5C). MALDI analysis revealed that each Ctx was labeled with 6.0 tri-GalNAc motifs in average (Figure S6). To explore Ctx-GN-mediated EGFR degradation, HepG2 and Huh7 cells were treated with 30 nM Ctx-GN for 48 h, and a nearly 40% downregulation of EGFR was observed in Ctx-GN-treated cells compared to the cells incubated with nonmodified Ctx or without treatment (Figure 5D). This result demonstrated the feasibility of our degraders on degrading endogenous proteins.

CONCLUSION

The emergence of chimeric molecules that are capable of depleting pathogenic proteins through native degradation pathways have the potential to overcome a major limitation of traditional therapeutic strategies, which generally need to bind to the protein target and alter its function. Targeted protein degradation using chimeric molecules only needs a binder to the protein target. Depletion of the entire pathogenic protein also offers unique advantages over functional inhibition in many cases. However, the most developed targeted protein degradation strategy, namely PROTAC, is restricted to degrading intracellular targets. The M6P analogue-based LYTAC opened up a new direction of research area for targeted protein degradation by expanding the scope of targets to extracellular proteins. Similar to proteasome targeting degraders, where only handful of E3 ubiquitin ligase ligands are available, more lysosome targeting ligands need to be explored for expanded utilities of lysosome targeting degraders. We described our initial proof-of-concept studies using a tri-GalNAc-biotin small molecule and tri-GalNAc labeled antibodies to deliver the extracellular protein targets into the lysosome for degradation. We have shown that the internalization and lysosomal degradation of the protein targets through ASGPR are possible by both small molecule- and

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antibody-based lysosome targeting degraders. We also observed that smaller complexes[42] exhibited higher uptake efficiency in several cases. In addition to the well-known factors, such as the length of linker, the type of linker, polyvalency, binding affinities to the receptor and protein target, and expression levels of receptor and protein target, the size of the complex may be an additional parameter for the optimization of triantennary GalNAc conjugate-mediated lysosomal degradation of extracellular proteins. Overall, our studies demonstrated the feasibility of ASGPR-mediated liver cell-specific targeted protein degradation strategy and uncovered a potential new therapeutic application of triantennary GalNAc in addition to its well-known utilities in liver-specific delivery of oligonucleotides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.1c00146.

Materials and methods and supplemental figures (PDF)

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Author Contributions

Y.Z. and W.T. conceived the project. Y.Z. carried out all biological experiments and interpreted the data. P.T. prepared compound **3** from **2** and provided insights to the project. N.T.M. and X.L. conducted the MALDI-MS characterization and analysis. Y.Z. and W.T. wrote the manuscript with input from all authors.

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Notes

The authors declare no competing financial interest.

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