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Article

Chemoenzymatic Labeling, Detection and Profiling of Core Fucosylation in Live Cells

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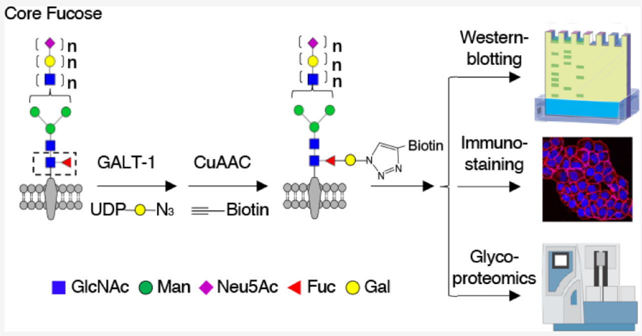
ABSTRACT: Core fucosylation, the attachment of an α-1,6-linked-fucose to the N-glycan core pentasaccharide, is an abundant protein modification that plays critical roles in various biological processes such as cell signaling, B cell development, antibody-dependent cellular cytotoxicity, and oncogenesis. However, the tools currently used to detect core fucosylation suffer from poor specificity, exhibiting cross-reactivity against all types of fucosylation. Herein we report the development of a new chemoenzymatic strategy for the rapid and selective detection of core fucosylated glycans. This approach employs a galactosyltransferase enzyme identified from*Caenorhabditis elegans*that specifically transfers an azido-appended galactose residue onto core fucose via a β-1,4 glycosidic linkage. We demonstrate that the approach exhibits superior specificity toward core fucose on a variety of complex N-glycans. The method enables detection of core fucosylated glycoproteins from complex cell lysates, as well as on live cell surfaces, and it can be integrated into a diagnostic platform to profile protein-specific core fucosylation levels. This chemoenzymatic labeling approach offers a new strategy for the identification of disease biomarkers and will allow researchers to further characterize the fundamental role of this important glycan in normal and disease physiology.



■ INTRODUCTION

Aberrant glycosylation is a hallmark of many human diseases, including autoimmune and inflammatory conditions, neurodegenerative diseases, and various cancers.[1−3] To study these phenomena, efforts have focused on elucidating the functional roles of glycans in both healthy and disease physiology. While lectins and antibodies are commonly used to detect specific glycan structures,[4−7] these methods often suffer from weak binding affinity and/or cross-reactivity toward other glycan structures.[8−10] Metabolic labeling with non-natural monosaccharides has also been widely utilized for the detection and study of glycoconjugates.[11−14] However, non-natural monosaccharides compete with natural substrates in the cell, leading to low metabolic incorporation and limiting detection sensitivity. Additionally, unique carbohydrate structures with defined monosaccharide connectivity such as α-1,2-fucose versus α-1,6-fucose, as well as higher-order structures such as di- or trisaccharide motifs, cannot be distinguished and thus are not selectively detected by metabolic labeling. Given the limitations of current methods and the large number of cellular glycans for which no selective detection strategy exists, there remains an urgent need for new technologies to label, visualize, and interrogate specific glycan structures.

Recently, chemoenzymatic labeling approaches have emerged to detect important glycans such as *O*-linked-β-*N*-acetylglucosamine (*O*-GlcNAc),[15] *N*-acetyllactosamine (LacNAc),[16] α-*N*-acetylgalactosamine (α-GalNAc or Tn antigen),[16] and fucose-α-1,2-galactose (Fucα(1,2)Gal).[17] These approaches exploit the acceptor substrate specificity of glycosyltransferases to install a non-natural sugar bearing a chemical handle onto the glycan structure of interest. The chemical handle is then used to covalently attach a reporter group via bioorthogonal chemistry to enable detection or affinity capture. Quantitative, highly selective, enzyme-directed labeling endows chemoenzymatic methods with superior specificity and sensitivity over most antibody, lectin, and metabolic labeling approaches. However, only a handful of chemoenzymatic labeling methods have been developed for glycan detection.

Core fucosylation, an abundant form of glycosylation catalyzed by fucosyltransferase 8 (FUT8), is the addition of L-

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fucose in an α-1,6 linkage to the innermost *N*-acetyl-D-glucosamine (GlcNAc) residue of N-linked glycans.[18] This modification plays many important roles in physiological processes, such as cell signaling, cell adhesion, long-term potentiation, antibody-dependent cellular cytotoxicity (ADCC) and tumorigenesis, and is a biomarker for human diseases.[19−31] For example, α-fetoprotein (AFP) is an FDA-approved biomarker for hepatocellular carcinoma (HCC), the primary form of liver malignancy and fourth leading cause of cancer death worldwide, but AFP levels also increase in other benign liver diseases such as chronic or acute viral hepatitis and liver cirrhosis.[23−25] Elevated core fucosylation of AFP is a signature of HCC, but not of benign liver diseases, and directly correlates with HCC pathogenesis, making it a more specific HCC biomarker.[25] Other reported core fucosylation-associated markers include the core fucosylated forms of prostate-specific antigen (PSA) and haptoglobin for aggressive prostate cancer and pancreatic cancer, respectively.[26−30] Core fucosylation of the constant region (Fc) of human immunoglobulin (Ig) G1 antibodies critically modulates the efficacy of ADCC.[21,31] Despite the importance of core fucosylation and its association with human pathologies, no selective method exists for the detection of core fucosylated glycans. Here, we report a strategy for the robust and selective detection of core fucosylation, thereby enabling the investigation of a broad range of physiologically important glycoproteins, therapeutic antibodies, and cancer biomarkers.

RESULTS AND DISCUSSION

GALT-1 Catalyzes Efficient Labeling Reactions on Core Fucose. A previous study by Aebi and coworkers identified the open reading frame M03F8.4 from the *Caenorhabditis elegans* genome as a galactosyltransferase (GALT-1) that could transfer a galactose (Gal) residue from uridine-5′-diphospho-galactose (UDP-Gal) to core fucose and form the β-1,4 glycosidic linkage.[32] We reasoned that GALT-1 might tolerate azido substitution of the C-6 hydroxyl of Gal, which would allow for selective tagging of core fucosylated glycans followed by copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) or strain-promoted azide−alkyne cycloaddition (SPAAC) (Figure 1A). To test the approach, bacterially expressed GALT-1 was purified, and the non-natural analogue UDP-6-deoxy-6-azido-galactose (UDP-Gal-6-N_3, **1**) was chemoenzymatically synthesized using 6-azido-6-deoxy-D-galactose, galactokinase from *Bifidobacterium infantis* (GalK),[33] and UDP-sugar pyrophosphorylase from *Arabidopsis thaliana* (AtUSP)[34] in the presence of ATP and UTP (75% isolated yield, Scheme S1). GALT-1 activity was first assessed using a dabsylated core fucosylated glycopeptide substrate **2**[32] in the presence of either UDP-Gal or UDP-Gal-6-N_3 **1** (Figure 1B). Complete conversion of **2** to the desired products **3** and **4**, respectively, was observed, as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Kinetic analysis of GALT-1 with UDP-Gal or **1** was then performed using the core fucosylated glycan N6000 and a turn-on fluorescent chemosensor for UDP (Figures 2A and S1).[35] Promisingly, we measured an apparent k_{cat}/K_m value of 9.94 × 10^2 M^{-1} min^{-1} for UDP-Gal-6-N_3, only 3-fold lower than the value of 3.20 × 10^3 M^{-1} min^{-1} obtained for the natural donor substrate, UDP-Gal. These results demonstrate that GALT-1 can efficiently transfer the non-natural sugar Gal-6-N_3 to core α-1,6-fucose residues for chemoenzymatic labeling.

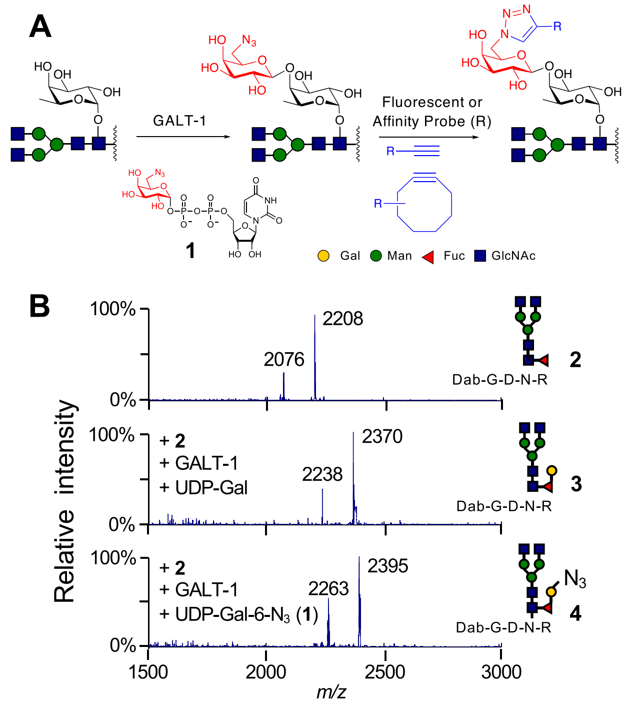
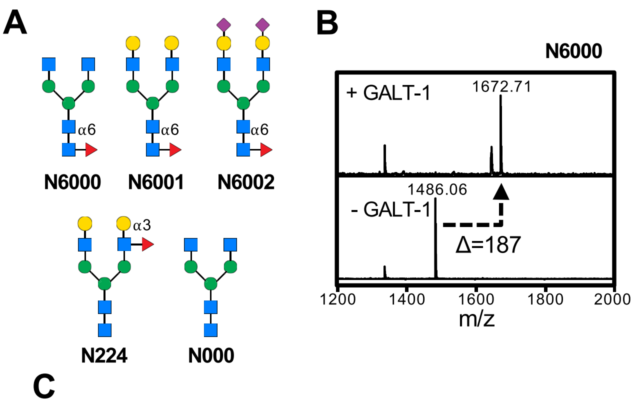
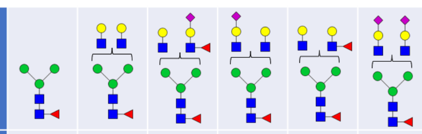


Figure 1. (A) Chemoenzymatic labeling strategy for the detection of core fucosylated glycoproteins. (B) MALDI-TOF MS analysis of GALT-1 reactions with a dabsylated glycopeptide acceptor substrate **2** (upper; m/z = 2208) and the natural UDP-Gal or non-natural UDP-Gal-6-N_3 **1** donor. Complete conversion of acceptor **2** to **3** (middle; m/z = 2370) or **4** (bottom; m/z = 2395) was observed. In each spectrum, the smaller peak (−132 Da) is due to loss of the dabsyl (Dab) group from the N-terminus of the peptide.





Core fucosylated glycans						
PSMs (galactosylated)	36	37	37	99	20	8
PSMs (total)	40	44	45	138	29	38
Modification percentage	90.0%	84.1%	82.2%	71.7%	69.0%	21.1%

Figure 2. (A) Chemoenzymatically synthesized N-glycan structures used to evaluate GALT-1 acceptor substrate specificity. N6000, N6001, and N6002 but not N224 and N000 were efficiently labeled by GALT-1. (B) MALDI-TOF analysis shows the specific labeling of core fucosylated N-glycan N6000 using GALT-1 and UDP-Gal-6-N_3 **1**. (C) GALT-1 modification efficiency toward various core fucosylated N-glycans in cells.

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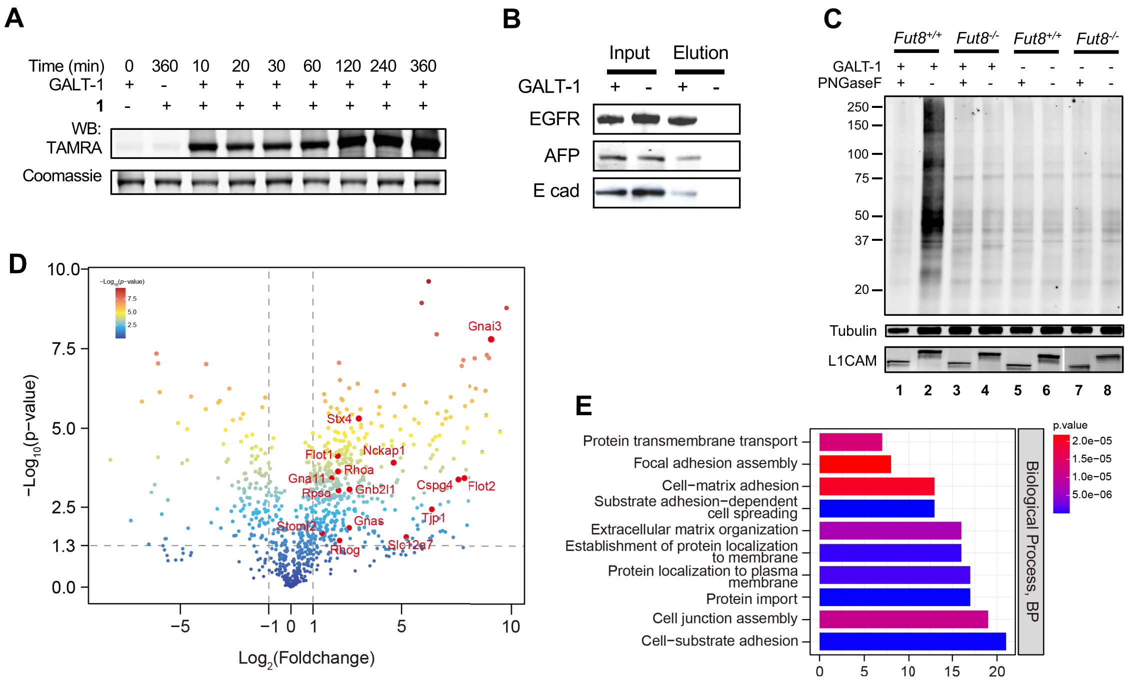

Figure 3. (A) Time-course analysis of the labeling of the core fucosylated protein bovine thyroglobulin using GALT-1 and UDP-Gal-6-N_3 **1**. Core fucose labeling was followed by CuAAC using TAMRA-alkyne **5** and was detected by Western blotting (WB) with an anti-TAMRA antibody. Coomassie staining confirmed the presence of protein in unlabeled reactions lacking GALT-1 or **1**. (B) Known core fucosylated proteins were chemoenzymatically labeled and detected from human MDA-MB-231 breast cancer (EGF receptor; EFGR), human HepG2 liver cancer (AFP), and human MCF-7 breast cancer cell lines (E cadherin; E cad). Cell lysates were chemoenzymatically labeled with GALT-1 and UDP-Gal-6-N_3 **1**, followed by CuAAC using TAMRA-alkyne **5**. The TAMRA-labeled proteins were immunoprecipitated using an anti-TAMRA antibody, and EGFR, AFP and E cad were detected in the elution by WB. (C) Specific labeling of core fucosylated proteins in cell lysates. Brain lysates from *Fut8*$^{+/+}$ and *Fut8*$^{-/-}$ mice were treated with and without PNGaseF prior to chemoenzymatic labeling of core fucosylated glycans with GALT-1 and **1**. Following CuAAC using biotin-alkyne **6**, the biotinylated glycoproteins were subjected to SDS-PAGE and detected by blotting with streptavidin conjugated to Alexa Fluor 680. FUT8 is the sole enzyme responsible for core fucosylation, and PNGaseF removes N-glycans from proteins. The N-glycosylated protein L1CAM was used as a positive control for PNGaseF. (D) Volcano plot displaying the log2 fold-change (x axis) and −log10 P-values (y axis) for core fucosylated protein identification enriched from CHO-K1 cells. Fold-change refers to the intensity ratio of the released peptides by dimethyl labeling relative to no chemoenzymatic labeling (without GALT-1). (E) Gene ontology analysis of identified glycoproteins categorized by biological processes (GOBP).

We next investigated the acceptor substrate specificity of GALT-1 toward various core fucosylated glycans. A small library of representative N-glycans was chemoenzymatically prepared (Figure 2A).[36] We found that GALT-1 efficiently labeled the core fucosylated N-glycans N6000, N6001, and N6002, as determined by MALDI-TOF MS (Figures 2B, S1 and S2). Kinetic analyses revealed a decreasing trend in apparent k_{cat}/K_m as the glycan antennae were elaborated (9.43×10^2 M^{-1} min^{-1}, 4.83×10^2 M^{-1} min^{-1}, and 3.43×10^2 M^{-1} min^{-1} for N6000, N6001, and N6002, respectively, Figure S1). Importantly, GALT-1 did not label the outer-arm α,1−3 fucose of N224 or the structure N000, which lacks fucose (Figure S2).

To corroborate these data, we examined the substrate specificity of GALT-1 in cells. As fucosylated glycans are biosynthesized through the secretory pathway in the Golgi, the Golgi apparatus contains a comprehensive inventory of core fucosylated glycan structures. We reasoned that if we could achieve targeted expression of GALT-1 in the Golgi, GALT-1-catalyzed galactosyl transfer reactions on core fucose structures should occur. By isolating and analyzing the glycans from the cell, we could then obtain structural information regarding the core fucosylated cellular substrates of GALT-1. A Golgi-targeting sequence was genetically inserted upstream of GALT-1 to generate a fusion protein. Ectopic expression of this fusion protein in SMMC-7721 cells (a hepatocellular carcinoma cell line) showed that it was largely localized in the Golgi apparatus, as determined by immunostaining analysis (Figure S3). After expression for 48 h, cells were collected, and N-linked glycopeptides were enriched and analyzed by LC−MS/MS, followed by structural interpretation of site-specific N-glycans using StrucGP software[37] (Table S1). The false discovery rate (FDR) was set as ≤1% for both peptides and glycans.

A total of 3296 PSMs (peptide spectrum matches) was identified, among which 670 PSMs were core fucosylated. 511 PSMs were modified with galactose at the core fucose, yielding an overall modification percentage of 76.2%. Representative spectra are shown in Figure S4. To investigate the glycan substrate preference of GALT-1, we compared the modification percentage of the top-ranking core fucosylated glycan structures (Figure 2C). Consistent with our in vitro data, the most efficient substrates were glycans with only the core pentasaccharide structure (90%), followed by glycans with two LacNAc branches (84.1%, resembling N6000, N6001) and biantennary glycans with one fucose and/or *N*-acetylneuraminic acid (Neu5Ac; 82.2% and 71.7%, respectively, resembling N6002). Core fucosylated biantennary glycans possessing two Neu5Ac had a lower modification efficiency of 21.1%. Taken together, these results demonstrate the excellent selectivity of GALT-1 for core fucose and its ability to efficiently label a range of core fucosylated N-glycan structures.

Chemoenzymatic Labeling and Profiling Core Fucosylated Proteins in Cells. We next sought to chemoenzymatically label proteins bearing core fucosylated glycans. Bovine thyroglobulin, a known core fucosylated glycoprotein, was incubated with GALT-1 and UDP-Gal-6-N_3 **1**. At specific time points, the reaction was subjected to CuAAC with tetramethylrhodamine (TAMRA)-alkyne **5** (Figure S5). A time-dependent increase in TAMRA labeling of thyroglobulin was observed by in-gel fluorescence (Figure 3A). Moreover, we detected robust chemoenzymatic labeling of proteins secreted from cultured HepG2 liver cancer cells (Figure S6). In both cases, minimal TAMRA signal was observed in the absence of GALT-1, UDP-Gal-6-N_3, or the alkyne, indicating that enzyme-specific labeling had occurred. To investigate the scope of our approach, MDA-MB-231, MCF-7, and HepG2 cell lysates were all subjected to chemoenzymatic TAMRA labeling. Following immunoprecipitation with an anti-TAMRA antibody, the labeled lysates were immunoblotted for known core fucosylated glycoproteins. Notably, specific labeling of core fucosylated epidermal growth factor receptor (EGFR), α-fetoprotein (AFP), and E-cadherin (E cad) was detected (Figure 3B).

To further confirm the specificity of the GALT-1-mediated chemoenzymatic reactions, we labeled core fucose in tissue from mice lacking FUT8, the sole enzyme responsible for core fucosylation. Brain lysates from *Fut8* knockout ($Fut8^{-/-}$) and wild-type ($Fut8^{+/+}$) mice[19] were treated with or without PNGase F, which removes N-linked glycans, and then subjected to chemoenzymatic labeling and Western blot detection (Figure 3C). Only $Fut8^{+/+}$ samples treated with GALT-1 in the absence of PNGase F (lane 2) exhibited specific labeling. PNGase F removal of N-linked glycans (lane 1) and $Fut8^{-/-}$ samples (lane 4) incubated under identical conditions were not labeled over background. We attribute the background labeling observed in all lanes to the CuAAC reaction with biotin-alkyne **6** and endogenously biotinylated proteins (Figure S7). Together, these findings provide strong evidence that α1−6 core fucose sugars on N-glycans are the only substrates modified by GALT-1. The high specificity of GALT-1 toward core fucose is significant given that many lectins commonly used to detect core fucose, such as *Lens culinaris* agglutinin (LCA), *Aleuria aurantia* lectin (AAL), and *Aspergillus oryzae* lectin (AOL), exhibit cross-reactivity with other fucosylated glycan motifs (Figure S8).[6−10] Thus, our findings establish the superior specificity of this chemoenzymatic approach for core fucose detection relative to existing methods.

The selective labeling of core fucosylation enables the enrichment and system-wide profiling of core fucosylated proteins in cells. Cell lysates of CHO-K1 (Chinese hamster ovary) cells were enzymatically reacted with UDP-Gal-6-N_3 **1**, conjugated with biotin-alkyne **6**, and subsequently captured on streptavidin beads. The bound glycoproteins were subjected to stringent washing and on-resin proteolytic digestion. The released peptides were further isotopically derivatized by dimethyl labeling of amino groups via reductive amination and quantified by mass spectrometry. Only proteins with a fold-change >2, *P*-value <0.05 (Student's *t*-test) relative to control samples carried without GALT-1 and at least two identified unique peptides were considered as core fucosylated proteins. In total, we identified 413 putative core fucosylated proteins (Figure 3D, Table S2). To assess the reproducibility of our labeling approach, we conducted three replicates and found that ~60% of glycoproteins were identified in all three replicates. Cellular localization analysis showed that the majority of the proteins were located on the endoplasmic reticulum (ER) lumen, membranes, and mitochondrion (Figure S9A). Gene ontology analysis categorized by biological processes (GOBP) showed that these identified glycoproteins were involved in cell junction, cell−matrix adhesion, focal adhesion assembly and substrate adhesion (Figure 3E). Gene ontology analysis categorized by cellular components (GOCC) further showed that a large number of the identified glycoproteins were closely related to cell membrane function (Figure S9B).

Chemoenzymatic Labeling of Core Fucosylated Glycans on Cell Surfaces. Next, we applied this strategy to label core fucosylated glycans on live cell surfaces. CHO-K1 cells were incubated with GALT-1 and UDP-Gal-6-N_3 **1** at 37 °C for 2 h, while the control groups were carried out without GALT-1. The cells were then biotinylated using dibenzocyclooctyne (DBCO)-biotin **7** (Figure S5), followed by staining with Alexa Fluor 488-conjugated streptavidin and flow cytometry. Stronger fluorescence signals were readily observed in the experimental group compared to the control group, where minimal background was observed (Figure 4A). Depletion of *Fut8* using shRNA largely abolished the fluorescence signal. Reconstituted expression of FUT8 in *Fut8*-depleted cells effectively rescued the

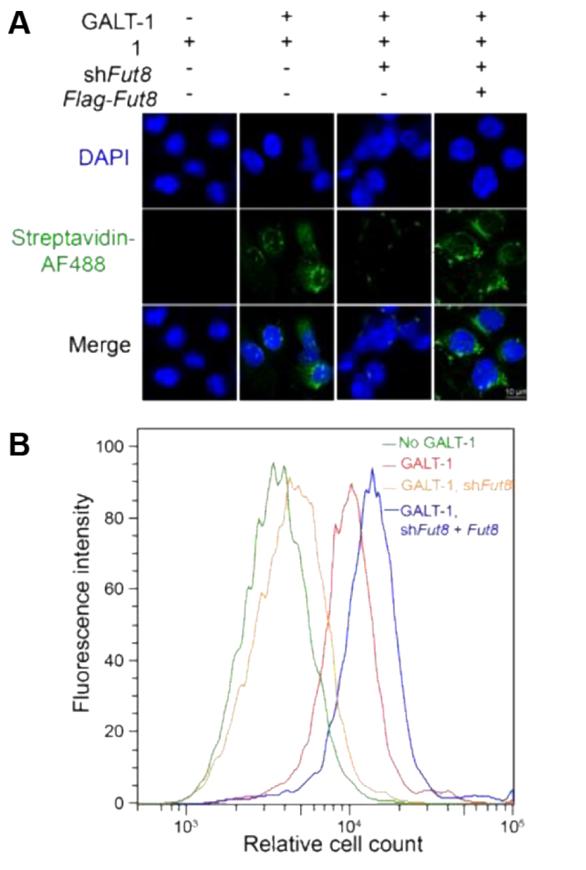

Figure 4. (A) Immunofluorescence imaging and (B) flow cytometry analysis of core fucosylation on CHO-K1 cells stably expressing scramble shRNA (no GALT-1, GALT-1), *fut8*-targeting shRNA (sh*Fut8*; GALT-1, sh*FUT8*) or shFUT8 with reconstituted shRNA-resistant Flag-tagged *Fut8* expression (*Flag-Fut8*; GALT-1, sh*Fut8* + *Fut8*). CHO-K1 cells were treated with UDP-Gal-6-N_3 **1** in the presence or absence of GALT-1 as indicated, followed by CuAAC using DBCO-biotin **7**. Biotinylated cells were then probed with streptavidin-Alexa Fluor 488 and subjected to immunofluorescence or flow cytometry analysis. DAPI was used to stain nuclei.

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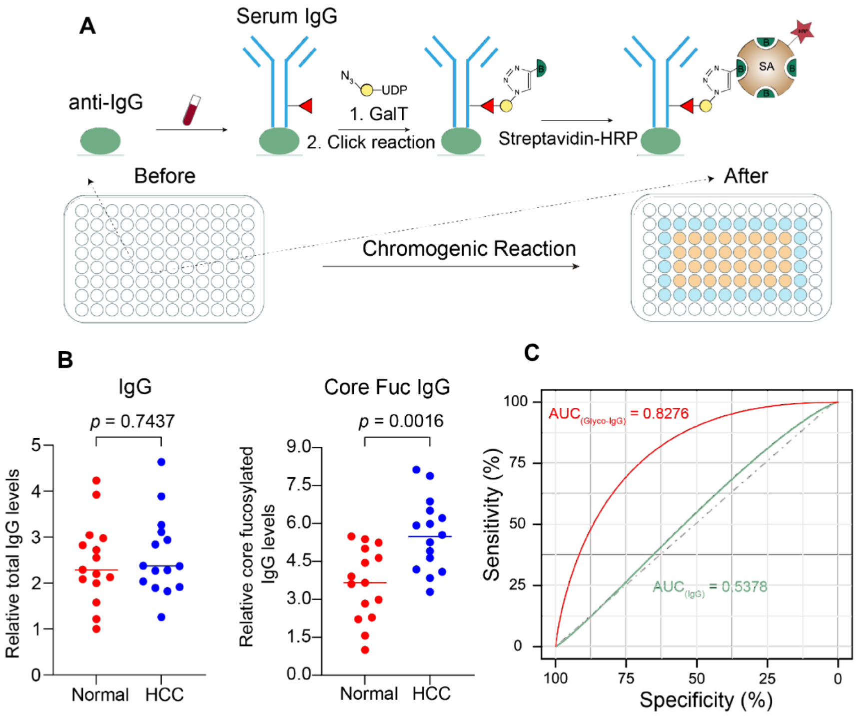

Figure 5. Capture and detection of core fucosylated IgG from clinical serum samples. (A) Schematic depiction of the method. Global IgG from the serum samples was captured in ELISA wells using an anti-human IgG antibody. Core fucosylated IgG was labeled with UDP-Gal-6-N_3 **1** using GALT-1, followed by CuAAC using biotin-alkyne **6**. Signal was detected after probing with streptavidin (SA) conjugated to horseradish peroxidase (HRP). (B) Analysis of total IgG (left) and core fucosylated IgG (Core Fuc IgG; right) from serum samples collected from 15 hepatocellular carcinoma (HCC) patients and 15 healthy donors (normal) by ELISA assay. (C) Receiver operating characteristic (ROC) curve for total IgG and core fucosylated IgG is indicative of good diagnostic performance.

labeling signal, confirming the on-target specificity of the shRNA and GALT-1 labeling selectivity (Figures 4A and S10). Flow cytometry analysis gave similar results (Figure 4B). Furthermore, the fluorescence signals increased with increasing concentrations of GALT-1 or UDP-Gal-6-N_3, in a dose-dependent manner (Figure S11). Interestingly, LCA lectin labeling signals did not show significant differences between control cells and *Fut8*-depleted cells (Figure S12), thus highlighting the high selectivity of our chemoenzymatic method compared to existing approaches. To further confirm the specificity of the GALT-1-mediated labeling, we employed specific glycosidases: PNGase F, α1,2-fucosidase for the cleavage of α1,2-linked fucose residues, and α1,3/4-fucosidase for the cleavage of nonreducing α1,3- and α1,4-linked fucose residues. As expected, treatment with PNGase F nearly abolished the labeling signal, while treatment with α1,2-fucosidase or α1,3/4-fucosidase had no impact on the labeling signal (Figure S13). Taken together, these data demonstrate that chemoenzymatic labeling with GALT-1 is highly specific for core fucosylated N-glycans on cell surfaces.

Chemoenzymatic Labeling and Detection of Core Fucosylated Biomarkers in Patient Serum. There is strong interest in developing improved diagnostic methods to detect early stage HCC.[24,25] Increased core fucosylation of serum glycoproteins is associated with HCC pathogenesis, but not with other benign liver diseases.[25] Thus, we sought to explore the translational potential of our approach for the detection of core fucosylated biomarkers. Serum from healthy or HCC patients were depleted of (i) serum albumin and antibodies or (ii) serum albumin only. Following chemoenzymatic labeling with UDP-Gal-6-N_3 **1** and biotin-alkyne **6**, samples were subjected to streptavidin blot detection. Robust labeling of core fucosylated serum glycoproteins was observed in both depletion cases (Figure S14). In the serum albumin-depleted samples, strong signal was detected at ~50 kDa where the Ig heavy chain is expected to appear, consistent with high levels of core fucosylated antibodies. Notably, stronger core fucosylation signals were observed overall and on specific protein bands in HCC samples compared to healthy controls (Figure S14). These findings agree with the elevated core fucose levels reported on serum glycoproteins in HCC patients.[25]

Among the variety of serum glycoproteins, core fucosylated IgG antibodies and AFP have been described as highly sensitive diagnostic and prognostic markers for hepatitis B virus-related HCC.[23−25] Thus, we employed our GALT-1 labeling method to capture and detect these two core fucosylated proteins from patient sera to assess its clinical diagnostic potential. We collected 15 serum samples each from HCC patients and healthy donors, respectively. The serum IgG proteins were captured on precoated anti-human IgG antibody ELISA plates. Core fucosylated IgG proteins were then subjected to the GALT-1-mediated biotinylation reaction and quantified using streptavidin-horseradish peroxidase (HRP) (Figure 5A). Correspondingly, the total amount of IgG proteins was also determined using an HRP-conjugated anti-human IgG antibody. Notably, the relative levels of core fucosylated IgG from cancer patients were significantly higher than those from healthy donors (two-tailed *t*-test, $p = 0.0016$), while no statistical difference was observed in terms of total IgG levels ($p = 0.74$) (Figure 5B). To determine the diagnostic performance of the selected cohort, a receiver-operating characteristic (ROC) curve was constructed (Figure 5C). The area under the ROC curve (AUC) value was

0.827, indicative of good diagnostic performance. We also compared the relative levels of total and core fucosylated AFP from healthy donors and patients with HCC, hepatitis C, or cirrhosis. To our delight, a significant increase in the average level of core fucosylated AFP was observed in sera from HCC patients compared to healthy donors (Figure S15). Encouragingly, no significant change in core fucosylated AFP was detected between healthy donors and patients with hepatitis C or cirrhosis, two other liver conditions associated with elevated AFP, but not with elevated core fucosylated AFP. Together, these results suggest that our selective core fucosylation labeling method may have important clinical applications.

■ CONCLUSIONS

In summary, we have developed a chemoenzymatic strategy for the facile and highly selective labeling, enrichment, and profiling of core fucosylated glycans. We took advantage of the excellent substrate specificity of a galactosyltransferase from *C. elegans* to transfer an azido-functionalized galactose onto the core fucose residue of N-glycans, followed by installation of a reporter group via click chemistry. This enabled the fluorescent labeling and imaging of core fucosylated glycans on live cell surfaces. Furthermore, biotinylation of core fucose allowed for the selective enrichment, detection, and glycoproteomic identification of core fucosylated glycoproteins. Notably, we demonstrated that this method could also be integrated into an ELISA format to track changes in core fucosylation levels on IgG and other serum proteins from clinical samples, distinguishing differences in the core fucosylation levels of HCC patients compared to healthy donors and patients with hepatitis C or cirrhosis. The strategy reported herein further expands the tool kit available for selective glycan structure detection and editing. We envision that this approach will be valuable not only for advancing an understanding of the physiological functions of core fucosylation, but also for evaluating therapeutic antibodies, developing clinical diagnostics, and discovering new disease biomarkers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.4c09303.

- Experimental procedures, supplementary schemes and figures, and NMR spectra (PDF)
- Intact glycopeptides identified from control raw file and GalT-catalyzed raw file using StrucGP (XLSX)
- Core fucosylated proteins based on GalT labeling method (XLSX)

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Author Contributions

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Notes

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