MicroRNA-424 Predicts a Role for β-1,4 Branched Glycosylation in Cell Cycle Progression*

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MicroRNA regulation of protein expression plays an important role in mediating many cellular processes, from cell proliferation to cell death. The human microRNA miR-424 is up-regulated in response to anti-proliferative cytokines, such as transforming growth factor β (TGFB), and directly represses cell cycle progression. Our laboratory recently established that microRNA can be used as a proxy to identify biological roles of glycosylation enzymes (glycogenes). Herein we identify MGAT4A, OGT, and GALNT13 as targets of miR-424. We demonstrate that MGAT4A, an N-acetylglucosaminyltransferase that installs the β-1,4 branch of N-glycans, is directly regulated by miR-424 in multiple mammary epithelial cell lines and observe the loss of MGAT4A in response to TGFB, an inducer of miR-424. Knockdown of MGAT4A induces cell cycle arrest through decreasing CCND1 levels. MGAT4A does not affect miR-424. Knockdown of MGAT4A induces cell cycle arrest at high concentrations and leads to cell cycle arrest at high concentrations via attenuation of TGFB/SMAD signaling (18). This regulation is thought to occur in part through changes in the binding of galectin-3, an N-acetyllactosamine binding lectin that forms cell surface glycan/lectin lattices altering growth factor signaling (20). Changes in O-GlcNac levels also influence cell cycle progression (17). For example, O-GlcNAc transferase (OGT) is concentrated at the mitotic spindle and plays a role in chromatin dynamics (17, 21).

In this study we examine three predicted glycosylase targets of miR-424: MGAT4A, OGT, and GALNT13. MGAT4A (mannosyl(α-1,3)-glycoprotein-β-1,4-N-acetylglucosaminyltransferase, isozyme A) is responsible for installing the β-1,4 branch on complex N-glycans, whereas GALNT13 is one of 20 enzymes known to initiate canonical O-glycosylation. Although luciferase assays confirm all three as miR-424 targets, only MGAT4A is significantly altered in response to miR-424 in breast cell lines. Induction of miR-424 by the anti-proliferative cytokine TGFB correlated with loss of MGAT4A. Knockdown of MGAT4A inhibited cell proliferation and decreased CCND1 levels. No significant change in β-1,6 branching is observed in MGAT4A knockdown cells, suggesting that these effects on cell cycle progression are not due to a general loss of N-glycan branching but rather the manipulation of specific branching patterns.

The central dogma of molecular biology defines the major direction of information flow from DNA to RNA to protein (1). However, the last few decades of research has led us to realize that additional information is encoded in RNA-RNA interactions and posttranslational modifications (2, 3). MicroRNA (miRNA), small non-coding RNA sequences (~22 nucleotides), have been heralded as regulators of gene expression that control posttranscriptional mRNA stability or translation (4, 5). miRNAs tune out transcriptional noise, allowing tighter control over protein expression of critical proteins defining a cell (6). Much like miRNA, glycosylation has the ability to exert global regulatory effects within the cell and its surroundings, and dysregulation can lead to biological disorders (7). Recent work by our laboratory has shown that miRNAs are important regulators of glycosylation (8) and can be used as a proxy to define the biological functions of glycosylation enzymes (9).

The human miRNA hsa-miR-424-5p (miR-424), a member of the miR-15 family, plays a role in cell cycle regulation (10, 11). miR-424 is a critical component of mammary gland involu-
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**TABLE 1**

| Construct | Forward primer | Reverse primer |
|-----------|----------------|---------------|
| MGAT4A    | ATATGCTAGGGGCTGAGCTG | ATACTCGAGAGCCCTTTATTTTATAAGGAATTC |
| MGAT4A_M1 | GCTGATGACCTTGGTCCGTGTTAGT | AGAAATGCTGCTTCAAAGAAGATATTTCC |
| MGAT4A_M2 | GCGGGTTTATACACACGGTATTTCGGCC | AGAGATGCTGCTTCAAAGAAGATATTTCC |
| MGAT4A_M3 | CTGGGATCTTAAAGGCAAAGTTGCAGACGTTGAT | AGATGCTGCTTCAAAGAAGATATTTCC |
| OGT       | TCTGCTGACCATGTTAAGGCTTTAGGTTCAAT | AAGGATGCTGCTTCAAAGAAGATATTTCC |
| OGT_M     | GAGCTGGCCACCTCTAAGAAGCTCTTTG | AGAGATGCTGCTTCAAAGAAGATATTTCC |
| GALNT13   | ATATGCTAGGGGCTGAGCTG | ATACTCGAGAGCCCTTTATTTTATAAGGAATTC |

For miRNA experiments, 1 × 10^5 cells were transfected with 50 nM concentrations of either mimics or inhibitors (miR-424 and scrambled controls; Dharmacon/GE Healthcare) in Lipofectamine 2000 (1 ml total volume, Life Technologies) and plated in single well of a 12-well tissue culture dish. After 48 h, cells were harvested with 0.25% trypsin-EDTA (200 µl, Corning) after which 1 ml of medium was added to quench the trypsin. Cells were pelleted (10,000 g, 4 °C) and washed 1 × in HBSS (Hank’s buffered saline solution), and the pellet was snap-frozen in liquid N2 for further analysis.

To generate shRNA knockdowns of MGAT4A, MISSION shRNA clones (TRCN000035813 (KD-1) and TRCN000035812 (KD-2)) or non-targeting control (SHC016, Sigma) were obtained as viral stocks from the shRNA Core at New York University Langone Medical Center. MCF-7 or MDA-MB-231 cells were obtained from the shRNA Core at New York University Langone Medical Center. MCF-7 or MDA-MB-231 were transfected with plasmid (250 ng) using Lipofectamine 2000 and plated in a well of a 96-well plate (100 µl total volume). After 24 h, the assay was performed as previously described (SwitchGear Genomics), and luminescence was read on a Biotek microplate reader. For each assay we calculated the average -fold change as a ratio miR-424/scramble relative to the treated empty vector for five replicate wells per condition. It should be noted that miR-424 decreases the signal of the empty vector, most likely due to its anti-proliferative effects; thus, we normalized our data to account for this effect. Data presented represent the average of three replicate assays.

**Western Blotting**—Cell pellets (treated or control) were thawed on ice in cold radioimmune precipitation assay buffer (~100 µl, Sigma) supplemented with protease inhibitors to lyse the cells. Lysates were quantified using the DC assay (Bio-Rad). Equal amounts of protein were resolved by 8% or 10% SDS-PAGE and transferred onto nitrocellulose membranes (Tris-Glycine buffer, 20% methanol, and either 1 h at 350 mA or 16 h at 90 mA). Blots were blocked in 5% (w/v) nonfat milk in TBST (TBS, pH 7.4, 0.05% Tween 20) for 1 h at room temperature. Blots were then incubated with the following primary antibodies for 16 h at 4 °C. Dilution buffers are noted: α-MGAT4A (clone 8C5, Abnova, 1:1000, 5% BSA in TBST); α-galectin-3-biOT (clone M3/38, eBioscience, 1:1000, 5% BSA in TBST); α-E-cadherin (clone EP700Y, Abcam, 1:5000, 5% BSA in TBST); α-actin (Thermo Scientific, 1:10,000, 5% milk in TBST). Blots were then washed 3 × 5 min in TBST after which the corresponding HRP-conjugated secondary antibodies (α-mouse IgG, α-rabbit IgG, Bio-Rad; α-biotin, Cell Signaling) were applied for 1 h at room temperature at a 1:5000 dilution in 5% milk in TBST. Blots were washed 3 × 5 min TBST and then developed using SuperSignal West Pico (Thermo Scientific).

**RNA Extraction and Quantitative Real-time PCR (qRT-PCR)**—Cell pellets (treated or control) were thawed, and total RNA was extracted (miRNAeasy mini kit; Qiagen). Only cell pellets frozen for 1 week or less were used for RNA extraction. RNA was quantified by using NanoDrop ND-1000 and reverse-transcribed (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) into cDNA using 750 ng of RNA input. Transcripts were quantified by qRT-PCR using PowerSYBR Green PCR Master mix (Applied Biosystems) in a LightCycler 480 (Roche Applied Science). Primers were designed with qPRIMERdepot (primerdepot.nci.nih.gov) and are listed in Table 2. Cycle threshold values were normalized to the β-actin gene ACTB as an internal control. Each run was performed in triplicate.

**Luciferase Reporter Assay**—The 3’-UTRs of MGAT4A (NM_001160154), OGT (NM_181672), and GALNT13 (NM_052917) were cloned from MCF-7 cDNA using Q5 Hotstart Polymerase (New England BioLabs) and inserted into the pLightSwitch_3’-UTR vector (SwitchGear Genomics) using the primers listed in Table 1. Plasmids were purified using Endo-Free Plasmid Maxi kit (Qiagen). HEK 293/T17 cells (7 × 10^5) were co-transfected with plasmid (250 ng) and microRNA mimics (60 nM) using Lipofectamine 2000 and plated in a well of a 96-well plate (100 µl total volume). After 24 h, the assay was performed as previously described (SwitchGear Genomics), and luminescence was read on a Biotek microplate reader. For each assay we calculated the average -fold change as a ratio miR-424/scramble relative to the treated empty vector for five replicate wells per condition. It should be noted that miR-424 decreases the signal of the empty vector, most likely due to its anti-proliferative effects; thus, we normalized our data to account for this effect. Data presented represent the average of three replicate assays.
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**Table 2**

| Gene symbol | Forward primer | Reverse primer |
|-------------|----------------|----------------|
| CCND1       | TCCCTCCTCCAAATCTCCAGA | GGCAGATGATGAAATGAACCTT |
| CDCA5A      | CACATTACCTGCTGAGAAGCCTC | AGAACCTGTGTCATTGGTCTT |
| GALNT13     | TCCCTGCATTTCTGTCTACCTCA | TGCATCTGACTGCTGCCTT |
| LGALS3      | GGGCGAGAGAAGACGAGAAGA | TGCAGACCTGGAAGCTGCTT |
| LGALS1      | AGGTTGTCCTGCTCTGCTC | CAAACCTGGAGAGTGCCTT |
| MGAT4A      | TCTTCAAGAGGACATACTACCA | GTGCTCTTCTGCGTATTAG |
| MGAT4B      | TCCCTGCATAAGTCTCTGCTT | TGCAGACCTGGAAGCTGCTT |
| MGAT5      | GTCAAGGCTACATGCACTAG | CAGCTGTCGGTACACGTG |
| OGT        | CACTGGAGGAGGCTGCTT | CAAGTCAAGATGAGTGGAG |
| SMAD3      | TGAGCAGAGGGCTTCAAC | CCGCGCCGCGGCTCAGCTACG |
| SMAD4      | GTCCCAGAGGAGGCTTCAAC | CCGCGCCGCGGCTCAGCTACG |
| TWBT1      | CACCGCGGCTCAGTAAGC | AGAATGACATCTAGGTCTCCGGCCC |

**Table 3**

| Name | miR5VR score | Alignment |
|------|--------------|-----------|
| MGAT4A | -1.25 | GUAUCUGAACAGACGAGA |
| GALNT13 | -1.25 | GUAUCUGAACAGACGAGA |
| OGT | -0.61 | UUAAUUGGACGAGACGAGA |

*mutation sites are underlined.*

Eclipse TE 2000-U (Nikon). Fluorescence images within an experiment were acquired under identical conditions.

**Results**

miR-424 Targeted the 3'-UTR of Predicted Glycosyltransferases—We identified MGAT4A, OGT, and GALNT13 as the top three predicted glycosyl gene targets of miR-424 using the summed miR5VR score on the miRanda database (Table 3) (25, 26). For OGT, the mRNA transcript containing the highest prediction score was a truncated product; thus, we examined the 3'-UTR of the full-length gene. To validate these interactions we used a luciferase assay in which the 3'-UTR of our glycosyl gene is appended to a luciferase reporter (Fig. 1A). In brief, HEK293/T17 cells were co-transfected with the reporter construct (3'-UTR, mutant or luciferase control) and an miR-424 or scrambled mimic. After 24 h, luminescence readings were obtained. Data are reported as -fold change of miR-424/scrambled treatments normalized to the luciferase controls (Fig. 1B). The results confirm that all three genes are targets of miR-424. We validated the predicted binding sites of MGAT4A and OGT by site-directed mutagenesis of the seed regions (Table 3, underlined residues). Mutation of all three predicted miR-424 binding sites in MGAT4A completely abolished miR-424 repression of luciferase. In contrast, mutation of the miR-424 binding site in OGT only partially diminished the repression of the luciferase signal, suggesting the presence of other miR-424 binding sites in the OGT 3'-UTR (Fig. 1B). Overall, our data demonstrate that miR-424 can regulate MGAT4A, OGT, and GALNT13 through binding to their 3'-UTRs.

miR-424 Targeted Endogenous MGAT4A in Breast Cell Lines—Next, we examined the effects of miR-424 transfection on the expression of endogenous MGAT4A, OGT, and GALNT13 in the mammary epithelial cancer cell line MCF-7. In brief, MCF-7 cells were transfected with either miR-424 or scrambled mimic (50 nM), and cells were analyzed 48 h post-transfection. Of the three glycosylation enzymes, only MGAT4A showed a significant decrease in mRNA levels by qRT-PCR upon miR-424 treatment (p < 0.001, Fig. 1, C and F). Concomitant with this finding, the protein level of MGAT4A, but not OGT, is seen to decrease by Western blot analysis in miR-424-treated MCF-7 cells (Fig. 1, D and E). Thus we focused our studies on MGAT4A.
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We analyzed the impact of miR-424 on MGAT4A levels in three different cell lines: MCF-7, HMLE, a transformed normal breast cell line; A549, a human metastatic lung cell line (Fig. 1F). CDC25A, a validated miR-424 target, was used as a positive control. Transfection of miR-424 mimics inhibited MGAT4A expression in both breast cell lines but had no impact in A549. In contrast, CDC25A was inhibited by miR-424 in all cell lines.

We next performed flow cytometry analysis on synchronized MCF-7-MGAT4A-KD-1 and control cells to examine more closely changes in cell cycle progression. In brief, cells were starved for 24 h to synchronize their cell cycles. Media containing serum was then added, and cells were analyzed at 0 and 20 h by the method of Crissman and Steinkamp (28). Analysis showed an arrest in the 2N phase for the MGAT4A-KD-1 cells, were then analyzed for expression of MGAT4A, CDC25A, and miR-424. Levels of endogenous miR-424 increased, in agreement with the previous work (12, 13). In line with these findings, expression of both MGAT4A and CDC25A decreased after 48 h of treatment (Fig. 2). This suggests that MGAT4A levels are controlled by endogenous miR-424 in response to TGFβ.

Loss of MGAT4A Led to Decreased Cell Proliferation and Cell Cycle Arrest—To study whether loss of MGAT4A mimics the effects of miR-424 we generated MGAT4A knockdown cell lines (MGAT4A-KD) in two different breast cancer cells, MCF-7 and MDA-MB-231. In brief, cells were treated with lentiviral vectors containing either MGAT4A shRNA or a non-targeting control (NTC) and selected with puromycin. For MCF-7, we generated knockdowns with two different shRNAs (KD-1, KD-2). The knockdown of MGAT4A in both cell lines was confirmed by qRT-PCR analysis (Fig. 3A and D). KD-1 and KD-2 gave similar levels of knockdown of MGAT4A by qRT-PCR (~70%), but KD-1 showed a stronger effect by Western blot analysis (Fig. 3B). Although these cells could be cultured, proliferation rates of the MGAT4A-KD cell lines were significantly lower than those of NTC cells for both MCF-7 and MDA-MB-231 (Fig. 3, C and E). MGAT4A-KD cell lines showed decreased CCND1 expression (MCF-7-KD-1, 30%; MCF-7-KD-2, 50%; MDA-MB-231-KD-1, 42%, compared with NTC, p < 0.01; Fig. 3, A and D). CDC25A expression decreased in MCF-7-MGAT4A-KDs (KD-1, 31%; KD-2, 21%, compared with NTC, p < 0.01) but not MDA-MB-231-MGAT4A-KD. These results imply a concomitant change in cell cycle progression.

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FIGURE 1. Validation of predicted glycogenes as miR-424 targets. A, schematic depiction of the 3’-UTR luciferase reporter assay and miR-424 binding site mutants. B, luciferase assays show MGAT4A, OGT, and GALNT13 are significantly targeted by miR-424. The graph displays average normalized -fold change (mimic/scramble). **, p < 0.01. Student’s t test versus empty vector. The gray bars represent mutants of appropriate miRNA binding sites (see Table 3). C, MCF-7 cells treated with miR-424 mimic did not alter OGT or GALNT13 transcript levels by qRT-PCR. The graph shows -fold change of mRNA (2-ΔΔCt) versus scramble (mir-scr). D and E, representative Western blot analysis of cells treated as in C and probed for OGT and MGAT4A protein. F, cell lines (MCF-7, HMLE, A549) were treated with miR-424 mimic or anti-miR, and changes in MGAT4A (top) and CDC25A (bottom) transcript levels were analyzed by qRT-PCR as previously described. **, p < 0.01; *, p < 0.05. Student’s t test versus appropriate scrambled control. All experiments were done in biological triplicates. Error bars represent the S.D.

FIGURE 2. TGFβ induces miR-424 and decreases MGAT4A and CDC25A in HMLE. A, levels of MGAT4A and CDC25A decrease, whereas mature miR-424 increases in HMLE cells treated with 5 ng/ml TGFβ for 48 h by qRT-PCR. The graph shows -fold change of mRNA (2-ΔΔCt) versus vehicle-treated cells. **, p < 0.01 versus vehicle-treated control. B, representative Western blot analysis of MGAT4A in HMLE cells treated as described (top). The graph (bottom) represents average -fold change of MGAT4A band density for treated versus vehicle-treated control normalized to Ponceau staining in three independent replicates. *, p < 0.05. All experiments were done in triplicate. Error bars represent the S.D.
indicating a reduction in cells transitioning from G1 to S phase by \( \sim 30\% \) (Fig. 4). These results are consistent with previous observations for miR-424 (14) and imply that MGAT4A levels affect cell cycle progression.

**Impact of MGAT4A Levels on the Cell Cycle Are Not Due to the Loss of \( \beta-1,6 \) Branched Glycans**—The elaboration of biantennary branched N-glycans to triantennary requires the addition of GlcNAc to either the C-4 position on the 1,3 mannosyl arm (MGAT4A/B) or to the C-6 position on the 1,6 mannosyl arm (MGAT5) (29). Branching to the tetraantennary then requires the action of the other MGAT enzyme (Fig. 5A) (16, 30). Increased N-glycan branching has been shown to drive responsiveness to TGF\( \beta \) signaling leading to growth arrest (18). In the previous study responsiveness correlated with binding by the lectin PHA-L, a known binder of \( \beta-1,6 \) branched N-glycans (31). Analysis of glycan microarray data from the Consortium for Functional Glycomics shows that it has no recognition of \( \beta-1,4 \) branched triantennary structures (Fig. 5B). To test whether loss of MGAT4A alters \( \beta-1,6 \) branched N-glycan production through MGAT5, we examined the transcript levels of all three branching enzymes in MCF-7-MGAT4A-KD-1. As previously discussed, we observed a \( \sim 70\% \) decrease in MGAT4A levels; however, no significant compensatory changes in either MGAT4B or MGAT5 were observed (Fig. 5C). It should be noted that MGAT4A/\(-/-\) knock-out mice retain only 2–20\% \( \beta-1,4 \) GlcNAc transferase activity (32), and MGAT4B has a lower affinity for both UDP-GlcNAc and substrates (33), arguing that it cannot compensate for loss of MGAT4A activity in our knockdown cells. Staining of MGAT4A-KD cells with PHA-L showed no noticeable changes in PHA-L binding (Fig. 5D). Taken together, our data argues that MGAT4A exerts its effects on cell cycle progression independent of \( \beta-1,6 \) branched glycans and implies a specific role for the \( \beta-1,4 \) branch in cell cycle regulation.

**Galectin-3 Expression Increases in Response to miR-424 and Loss of MGAT4A in MCF-7**—Previous work established galectin-3 as a key mediator between N-glycan branching patterns and changes in growth factor signaling that result in altered proliferation (18, 20). Galectin-3 has also been shown to have glycan-independent effects on the cell cycle, halting cell cycle progression at the G1 phase by regulating transcription factor binding (36). We analyzed the cellular localization of galectin-3 in MGAT4A-KD cells by fluorescence microscopy (Fig. 6E).

Localization of galectin-3 dictates its role, with cell surface galectin-3 causing sensitization of cytokine receptors (20) and nuclear galectin-3 playing a direct role in transcription factor binding (36). We analyzed the cellular localization of galectin-3 in MGAT4A-KD cells by fluorescence microscopy (Fig. 6E). We could not observe galectin-3 in NTC cells. Galectin-3 in
MGAT4A-KD-1 cells was localized to the nucleus, with little cell surface binding observed (Fig. 6E, red channel). For comparison, we examined the expression of E-cadherin, a noted cell surface marker. Neither the expression (Fig. 6D) nor the membrane levels of E-cadherin (Fig. 6F) were altered. Although it is unclear whether the change in galectin-3 is important to the cell cycle arrest observed in miR-424 and MGAT4A-KD cells, our data suggest that nuclear galectin-3 may cooperate with miR-424 and MGAT4A in controlling cell cycle progression.

Discussion

Cell cycle progression is controlled by external growth and arrest signals from growth factors and cytokines (Fig. 7). Glycosylation can play a role in mediating such signals through altering the trafficking and sensitivity of cell surface cytokine and growth factor receptors (18, 37–39). MicroRNA, which shapes the identity of a cell through fine-tuned regulation of gene expression, allows us to predict the biological role of specific glycosylation enzymes and the glycans they synthesize (miRNA proxy approach) (8, 9). Herein we demonstrate that miR-424, a cell cycle regulator, targets the 3′-UTR of the glycosylation-related genes MGAT4A, OGT, and GALNT13, suggesting these enzymes may play a role in cell cycle regulation. miRNA can display cell type-dependent effects due to differences in both endogenous miRNA cohorts and gene transcript levels (40). In keeping with this, only MGAT4A, the major enzyme controlling the β-1,4 branch of multiantennary N-glycans (32), was targeted by miR-424 in the breast cancer line MCF-7. Regulation of MGAT4A is more pronounced in breast cell lines than in the lung cell line A549 (Fig. 1). Although OGT was not regulated by miR-424 in MCF-7, the miRNA-dependent regulation of OGT is of special interest. Previous analysis of the 3′-UTR of OGT revealed that it is one of the most highly
regulated glycogenes (41). O-GlcNAc, the epitope controlled by this enzyme, is involved in cell proliferation (42, 43), consistent with targeting by miR-424. Thus, further exploration of the interaction between miR-424 and OGT in alternate biological systems is warranted.

miR-424 is up-regulated in normal mammary epithelia in response to TGFβ/H925, a potent inducer of cell cycle arrest (13, 27). Subsequently, miR-424 targets and suppresses a number of cell cycle regulators such as cyclin D1 (CCND1) and CDC25A, resulting in G1-S phase arrest and slower proliferation (13, 14). Treatment of the normal breast epithelial cell line HMLE with TGFβ/H925 caused up-regulation of miR-424 and concomitant down-regulation of MGAT4A and CDC25A (Fig. 2), suggesting that MGAT4A plays a role in this signaling network.

Knockdown of MGAT4A had a profound effect on cell proliferation in multiple breast cell lines (Fig. 3). We observed a decrease in G1-S progression and a loss of cyclin D1 expression, suggesting arrest of cell cycle progression (Fig. 4). Glycan branching has been previously shown to directly impact cell proliferation mediated by galectin-3 binding to cell surface receptors (44). However, these studies focused on alterations in β-1,6 branched glycans, increases in which first stimulate and then arrest growth rates (18). Knocking down MGAT4A, which biosynthesizes β-1,4 branched glycans, did not affect β-1,6 branched glycans, which are biosynthesized by MGAT5 (Fig. 5). A role for MGAT4A in cell proliferation is supported by work showing MGAT4A is targeted by let-7, a known tumor suppressor (45). This suggests that specific branching patterns (β-1,4 versus β-1,6) may play distinct roles, imposing an additional level of control on cell signaling through modulation of glycoproteins such as cytokine and growth factor receptors (Fig. 7).

Both elevated miR-424 and loss of MGAT4A increased expression of galectin-3 (Fig. 6). MCF-7-MGAT4A-KD cells showed a pronounced increase in nuclear galectin-3 by fluorescence microscopy. Nuclear galectin-3 plays a role in late-G1 cell cycle arrest and suppression of anoikis, the programmed cell death in response to loss of cell adhesion (34, 35). Anoikis suppression is observed in migrating metastatic cancer cells and correlates with decreased proliferation (48). Although the specific signaling pathways between β-1,4-branched glycans, cell cycle arrest, and nuclear galectin-3
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FIGURE 7. Scheme of the proposed signaling network involving miR-424 and MGAT4A. MGAT4A synthesizes β-1,4 branched N-glycans on glycoproteins (e.g. receptors, adhesion molecules). Inhibition of MGAT4A reduces pro-growth signaling through glycans-dependent alteration of these glycoproteins. MGAT4A and the direct cell cycle regulators CDC25A and CCND1 are modulated by miR-424, a regulator of cell proliferation.

 have not yet been mapped out, our data sheds light on a connection between cell cycle regulation and anoikis suppression of breast epithelial cells in response to changes in specific N-glycan branching patterns.

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