High Frequency of LMAN1 Abnormalities in Colorectal Tumors with Microsatellite Instability

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Abstract

Glycosyl epitopes have been identified as tumor-specific markers in colorectal tumors and various lines of evidence indicate the significance of altered synthesis, transport, and secretion of glycoproteins in tumorigenesis. However, aberrant glycosylation has been largely ignored in microsatellite unstable (MSI-H) colorectal tumors. Therefore, we analyzed mutation frequencies of genes of the cellular glycosylation machinery in MSI-H tumors, focusing on frameshift mutations in coding MNRs (cMNRs). Among 28 candidate genes, LMAN1/ERGIC53, a mannose-specific lectin mediating endoplasmatic reticulum (ER)-to-Golgi transit of glycosylated proteins, showed high mutation frequency in MSI-H colorectal cancer cell lines (52%; 12 of 23), carcinomas (45%; 72 of 161), and adenomas (40%; 8 of 20). Biallelic mutations were observed in 17% (4 of 23) of MSI-H colorectal cancer cell lines. LMAN1 was found to be transcribed but truncated protein remained undetectable in these LMAN1-mutant cell lines. Immunohistochemical and molecular analysis of LMAN1-mutated carcinomas and adenomas revealed regional loss of LMAN1 expression due to biallelic LMAN1 cMNR frameshift mutations. In LMAN1-deficient colorectal cancer cell lines, secretion of the LMAN1 client protein α1-antitrypsin (A1AT), an inhibitor of angiogenesis and tumor growth, was significantly impaired but could be restored upon LMAN1 re-expression. These results suggest that LMAN1 mutational inactivation is a frequent and early event potentially contributing to MSI-H tumorigenesis. [Cancer Res 2009;69(1):292–9]

Introduction

Microsatellite instability is a hallmark of tumors associated with hereditary nonpolyposis colorectal cancer (HNPCC, Lynch Syndrome) but also occurs in ~15% of sporadic colorectal carcinomas. It is caused by loss of DNA mismatch repair (MMR) function due to germline or somatic mutations of four MMR genes (MLH1, MSH2, MSH6, and PMS2) in HNPCC patients or by epigenetic silencing (MLHI) in sporadic microsatellite unstable (MSI-H) colorectal tumors (1–4). Clinicopathologic characteristics of MSI-H colorectal cancer differ from their microsatellite stable (MSS) counterpart (5). For example MSI-H colorectal tumors occur at younger age, have better prognosis, are nearly diploid, show predominantly proximal localization, are characterized by strong lymphocytic infiltration and seem to be refractory to 5-fluorouracil chemotherapy.

MSI-H-carcinomas result from a distinctive oncogenic pathway and the detection of selective MSI-H target genes with relevance for carcinogenesis is essential for the understanding of MSI-H tumor development. MSI-H cancers accumulate a large number of mutations in mononucleotide repeats (MNR), and if located in coding regions (cMNR), these frameshift mutations can lead to truncated proteins with altered or complete loss of protein function, thereby affecting cell behavior. Several cMNR containing genes that potentially promote MSI-H tumor development and growth have been verified by functional studies, including TGFBR2, BAX, and ACVR2 (6–8). Woerner and colleagues (9) proposed a statistical model for predicting Selective Target Genes, which in combination with a human MNR mutation database (SelTarbase) can be applied to browse for putative target genes.

Glycosylation is the most common posttranslational modification of proteins, affecting their function in a variety of physiologic and pathologic events, including cell growth, migration, differentiation, tumor invasion, host-pathogen interactions, cell trafficking, and transmembrane signaling (10). A striking example is the regulation of the biological functions of integrins and cadherins by branched N-glycans in cell-cell and cell-extracellular matrix interactions (11). In the ER and in the early secretory pathway, glycan structures play a pivotal role in protein folding, oligomerization, quality control, sorting, transport, and secretion (12, 13).

Human colon cancer is associated with changes in protein glycosylation (14–17) and altered glycosylation has already been associated with tumorigenesis, tumor growth, and metastatic potential in colorectal cancer. Thus, epigenetic changes in glycosyltransferases have been found to contribute to the malignant phenotype of gastrointestinal cancer (18). Also O-glycosylation was shown to regulate apoptosis, proliferation, and metastatic potential of colorectal cancer cells (19, 20). Recent studies revealed that aberrant glycosylation on a secreted tissue inhibitor of the metalloproteinase-1 reinforces the invasive/metastatic potential of colon cancer (21). Moreover, a functional role of altered N-glycosylation patterns on colorectal cancer cell motility and invasiveness has been described (22).

Despite the obvious significance of altered glycoprotein synthesis, transport, and secretion in colorectal cancer, aberrant glycosylation and glycosylation pathways have not been investigated in MSI-H colorectal tumors. Focusing on this tumor subset, we examined cMNR frameshift mutations in genes encoding...
proteins of the cellular glycosylation machinery. Mutational inactivation and expression abnormalities of LMAN1, a carrier that mediates ER-to-Golgi transport of glycoproteins, was found to be a frequent and early event in MSI-H colorectal tumors. Because deficiency of LMAN1 in MSI-H cell lines caused impaired secretion of the antiangiogenic and growth-inhibiting protein \( \alpha \)-1-antitrypsin (A1AT), abnormalities of LMAN1 potentially contribute to MSI-H carcinogenesis.

Materials and Methods

**Database analysis.** Three databases and four filters were used for candidate gene selection. Information about ER/Golgi resident proteins was retrieved from the LOCATE database, whose XML source was downloaded (version human_v3.20070620) and prepared for local usage as a MySQL database. Protein subcellular localization in LOCATE v3 was defined by 463 GO terms, of which 30 GO terms were related to ER/Golgi subcellular localization (Filter 1; Supplementary Table S1). From this candidate subset, the MNR_ensembl database\(^5\) (version 45.36 g) allowed further specification of candidate genes by restriction of cMNR lengths to a minimum of seven repeat units (Filter 2). Subsequent exclusion of all cMNRs previously investigated revealed a list of 431 candidate genes (Filter 3; SelTarbase, 4th release 2007).\(^3\) Final selection by annotation of genes encoding proteins of the cellular glycosylation/deglycosylation and glyoprotein transport system led to 28 genes that were used for subsequent analyses (Filter 4). Automated primer design was performed by a perl script using primer3_core (Primer3 version 0.1)\(^6\) in combination with a self-constructed human mispriming repeat library (containing L1, L2, L3).

\(^4\)http://locate.imb.uq.edu.au/
\(^5\)http://www.seltarbase.org/?topic=MNR_ensembl
\(^6\)https://primer3.sourceforge.net/
ALU, etc. sequences (Supplementary Table S2). Our bioinformatics-based approach is outlined schematically in Fig. L4.

**cMNR frameshift mutation analysis.** Frameshift mutation analyses were performed as described previously (23). Primer design was set up to obtain short amplicons of ~100 bp (Supplementary Table S2), thus allowing robust amplification from archival tissues. PCR fragments were analyzed on an ABI3100 Genetic Analyzer (Applied Biosystems). Frameshift mutations were confirmed by DNA sequence analysis.

**Human tissues and cancer cell lines.** Human tissues were obtained from the local tissue bank established within the German Collaborative Group on HNPCC. Informed consent was obtained from all patients and the study protocol was approved by the local Ethics Committee. The MSI-H status of all colorectal tumor tissues used in the present study (n = 161) has been determined previously using the National Cancer Institute/IGC-HNPC reference marker panel (3) and one additional mononucleotide marker CAT25 (24). Colorectal cancer cell lines have been described previously (23, 25). All cell lines were grown under standard conditions in RPMI 1640 (Invitrogen, Life Technologies, Inc.) supplemented with 10% FCS, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.1 mmol/L CaCl2, and 0.01 mmol/L MgCl2, sonicated (Bandelin-Sonopuls, Bandelin electronic GmbH & Co. KG), and subsequently ultracentrifuged at 100,000 × g for 15 min at 4°C (Beckmann TLA 100.2 rotor). Protein concentration was determined by the Lowry method (26). Sixty-five micrograms of protein was separated on a 4% to 12% Bis-Tris Gel (NuPAGE Invitrogen Life Technologies, Inc.) followed by Western Blot analysis using primary antibody directed against LMAN1 (1:1,000, rabbit-monomospecific Sigma Prestige Antibodies; HPA002320) or anti-actin antibody (1:10,000, monoclonal, C4, MP Biomedicals) as control. Horseradish peroxidase (HRP)- conjugated antibodies were used as secondary antibodies (Anti-Rabbit IgG, HRP Conjugate (Promega); Anti-Mouse IgG, peroxidase-linked whole antibody (GE Healthcare Europe)). Visualization was performed with Western Lighting Chemiluminescence Reagent Plus (Perkin-Elmer LAS, Inc.) on Kodak BioMax light films (Sigma-Aldrich).

**Isolation of genomic DNA and RNA.** Genomic DNA was isolated from cancer cells and paraffin-embedded archival specimens after manual microdissection of tumor and corresponding normal tissues using the DNeasy Tissue kit (Qiagen). RNA was isolated using the RNeasy Mini kit (Qiagen).

**Transient transfection.** Colo60H cells (n = 105) were transiently transfected by electroporation using the Amaxa Nucleofector (Amaxa; A1AT ELISA. Cells were grown in T25-flasks to ~80% confluence (representing 7 μg DNA), and conditioned cell culture medium was harvested after 24 h. For the ELISA 100 μL undiluted conditioned medium was used.

**Reverse transcription-PCR.** One microgram of total cellular RNA was reverse transcribed using Superscript II Reverse Transcriptase according to the manufacturer’s instructions (Invitrogen, Life Technologies, Inc.). Primers for the LMAN1 gene were designed to flank the A9 cMNR (Sense primer, 5'-Fluorescein-GCTCTCGATCTCCTACACTCTCA-3'; antisense primer, 5'-GGAGGATTGAGCAGCTTCTCA-3'). LMAN1-specific cDNA primers were designed to span exon 8 and exon 9. PCR products were visualized on ethidium bromide–stained 2% agarose gels. All cell lines were examined by frameshift mutation analysis on cDNA for comparison with genomic DNA mutation data. Mutations were confirmed by DNA sequence analysis. As a control for loading and integrity of mRNA, reverse transcription-PCR (RT-PCR) analysis of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sense primer, 5'-CCACCCAGAAGACTGTGGAT-3'; antisense primer, 5'-TT-CAGCTCAGGGATGACCTT-3') was performed.

**Western blot analysis.** Cell pellets were lysed in radioimmunoprecipitation assay buffer (50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.1 mmol/L CaCl2, and 0.01 mmol/L MgCl2, sonicated (Bandelin-Sonopuls, Bandelin electronic GmbH & Co. KG), and subsequently ultracentrifuged at 100,000 × g for 15 min at 4°C (Beckmann TLA 100.2 rotor). Protein concentration was determined by the Lowry method (26). Sixty-five micrograms of protein was separated on a 4% to 12% Bis-Tris Gel (NuPAGE Invitrogen Life Technologies, Inc.) followed by Western Blot analysis using primary antibody directed against LMAN1 (1:1,000, rabbit-monomospecific Sigma Prestige Antibodies; HPA002320) or anti-actin antibody (1:10,000, monoclonal, C4, MP Biomedicals) as control. Horseradish peroxidase (HRP)- conjugated antibodies were used as secondary antibodies (Anti-Rabbit IgG, HRP Conjugate (Promega); Anti-Mouse IgG, peroxidase-linked whole antibody (GE Healthcare Europe)). Visualization was performed with Western Lighting Chemiluminescence Reagent Plus (Perkin-Elmer LAS, Inc.) on Kodak BioMax light films (Sigma-Aldrich).

**Immunohistochemistry.** For immunohistochemical staining, 2-μm sections were deparaffinized and rehydrated. For antigen retrieval, slides were boiled in 10 mmol/L citrate buffer (pH 6.0) for 5 × 5 min and endogenous peroxidase activity was blocked with hydrogen peroxide (0.6%) for 20 min. Slides were rinsed with deionized water and then washed in PBS/0.1% Tween 20 (PBS-T) for 5 min. The slides were then stained by 2-h incubation at room temperature with anti-LMAN1 primary antibody (1:200, rabbit-monomospecific Sigma) in 1% horse serum/PBS-T. A biotinylated anti-mouse/anti-rabbit antibody (1:50; Vector Laboratories, Inc.) for 30 min at room temperature. Immunoreactions were visualized with 3,3'-diaminobenzidine (Dako), followed by counterstaining with hematoxylin. Tumor-infiltrating lymphocytes, muscle cells, and normal colon tissue served as positive controls.

**Figure 2.** Candidate gene expression in colorectal cancer cell lines. A, RT-PCR analysis of LMAN1 expression in colorectal cancer cell lines with homozygous wild-type (+/+), homozygous mutant (−/−), or heterozygous mutant (+/−) cMNR alleles. The expected LMAN1 PCR fragment of 122 bp could be amplified from cDNA of normal human colon, 1 MSS cell line (SW948), and all MSI-H colorectal cancer cell lines. As a housekeeping gene, GAPDH was amplified. B, Western Blot analysis of MSI-H colorectal cancer cell lines. A single band of 53 kDa corresponding to the LMAN1 protein was detected in MSI-H and MSS colorectal cancer cell lines harboring heterozygous or homozygous wild-type cMNR alleles in MSI-H and MSS cell lines. MSI-H colorectal cancer cell lines with biallelic cMNR frameshift mutations lacked LMAN1 protein expression. No mutated LMAN1 protein (expected size, ~36 kDa) was visible in heterozygously and homozygotously mutated cell lines. Molecular weight marker bands are depicted. Probing with an antiactin antibody served as loading control.
were used. ELISA was established according to manufacturer's instructions (ImmunDiagnostik).

Results

Selection of candidate genes. A bioinformatics-based approach was used to identify cMNR-harboring candidate genes encoding proteins of the cellular glycosylation machinery. Database analysis and specific selection criteria such as subcellular location, cMNR length, and involvement in glycan synthesis, trimming, or export were applied to preselect candidate genes (see Materials and Methods). Based on this search strategy, 28 genes, encoding for proteins of the glycosylation machinery in the ER or Golgi, were selected for further investigation (Fig. 1A).

Frameshift mutation analysis. As a first step, we determined the cMNR frameshift mutation profiles for these 28 genes (32 cMNR) on a panel of 48 colorectal cancer cell lines (MSS, n = 25; MSI-H, n = 23; Fig. 1B). A wide spectrum of mutation frequencies was observed ranging from 0% to 52%, whereas no frameshift mutations in any of these genes were detectable in MSS colorectal cancer cell lines. LMAN1, harboring an A9 repeat in its coding region, showed the highest frameshift mutation frequency (52%; 12 of 23) in MSI-H colorectal cancer cell lines (Fig. 1B). In most cases, frameshift mutations affected only one allele. However, in some MSI-H colorectal cancer cell lines, biallelic cMNR mutations occurred in LMAN1 (17%; 4 of 23) and/or XYLT2 (8%; 2 of 23).

To clarify whether the observed genetic alterations that arise in cultured cell lines also occur in tumors, we performed similar analyses on 50 MSI-H colorectal tumors. For this analysis, we chose those candidate genes, which were most frequently mutated in MSI-H colorectal cancer cell lines. Most of the analyzed genes also showed frameshift mutations in these primary tumors and the observed frequencies were similar to those found for MSI-H colorectal cancer cell lines (Fig. 1C). In particular, in primary MSI-H colorectal cancers, highest cMNR mutation frequencies occurred in 3 genes including LMAN1 (45%), XYLT2 (25%), and B4GALNT4 (23%; Fig. 1C), a finding that was corroborated by extended analysis of these 3 genes on additional MSI-H tumor panels (LMAN1, n = 111; XYLT2, n = 41; B4GALNT4, n = 43). Based on the high mutation frequency of LMAN1 in MSI-H colorectal carcinomas, we asked whether such alterations could also be found in preneoplastic lesions. When we analyzed a subset of 20 MSI-H colorectal adenomas, a significant fraction of these tumors (40%; 8 of 20) showed cMNR frameshift mutations in the LMAN1 gene (data not shown). From these results, we conclude that LMAN1 mutations occur frequently and early in MSI-H tumors.

Figure 3. Immunohistochemical detection of LMAN1 protein on MSI-H colorectal tumors. Representative immunohistochemical staining patterns of MSI-H colorectal tumors using an LMAN1-specific antibody. LMAN1 staining was visualized by using 3,3′-diaminobenzidine (brown). Tumor sections were counterstained with hematoxylin (blue). In all tumor sections, positive LMAN1 staining of stromal cells and tumor-infiltrating lymphocytes was used as control. Scale bars, 100 μm in all panels. A, four carcinomas are shown that display either complete or partial loss of LMAN1 expression, sometimes even comprising areas with a heterogeneous speckled pattern. B, in two MSI-H colorectal adenomas, complete loss (left) as well as focally restricted loss of LMAN1 expression was observed (right). Arrow, crypt with mixed staining.
Wild-type and mutant LMAN1 transcript and protein expression in MSI-H colorectal cancer cell lines. LMAN1 mutations would be of importance only if the affected gene is expressed. Hence, we analyzed LMAN1 expression, both at the transcript and protein level. LMAN1 mRNA was found to be expressed in normal human colon mucosa as well as in all tested cell lines, including those harboring monoallelic or biallelic LMAN1 frameshift mutations (Fig. 2). When LMAN1 protein was traced by Western Blot analysis, a band of 53 kDa corresponding to the expected size of wild-type LMAN1 protein was detectable in all 19 heterozygously mutated cell lines. However, this band was missing in all four cell lines Colo60H, LoVo, HDC9, and Vaco6, harboring biallelic LMAN1 mutations (Fig. 2B). Moreover, no truncated LMAN1 protein (expected size of 36 kDa) was detected in cell lines with monoallelic or biallelic LMAN1 frameshift mutations although the LMAN1-antibody recognizes an NH2-terminal epitope.

These results indicate that LMAN1 frameshift mutant alleles are stably expressed at the transcript level but—in contrast to the wild-type alleles—remain undetectable at the protein level. The identification of LMAN1-deficient cell lines provides a valuable tool for studying the functional consequences of LMAN1 inactivation.

LMAN1 shows loss of expression in MSI-H colorectal carcinomas and adenomas. In cultured cells, biallelic mutation of LMAN1 caused loss of expression at the protein level. To investigate the expression of LMAN1 in primary tissues, we performed immunohistochemical analysis on a set of 50 MSI-H colorectal carcinomas. A significant fraction of these carcinomas showed either local (38%; 19 of 50) or complete loss (6%; 3 of 50) of LMAN1 expression. Representative examples of this heterogeneous staining pattern are outlined in Fig. 3A, including complete loss or local loss of LMAN1 protein expression. Moreover, when a small number of MSI-H colorectal adenomas was subjected to immunohistochemical staining, a similar pattern (Fig. 3B) was observed. We hypothesized that this regional loss of LMAN1 expression might be attributable to biallelic mutational inactivation of the LMAN1 gene in specific tumor areas. To address this issue, we isolated DNA from areas with or without LMAN1 expression by regional microdissection on a set of 50 MSI-H carcinomas (n = 4) and MSI-H adenomas (n = 3; Fig. 4A). DNA fragment analysis revealed biallelic cMNR frameshift mutations in the LMAN1 gene (Fig. 4Ai) and concomitant loss of LMAN1 protein expression in these tumor areas. Areas with decreased LMAN1 expression compared with normal tissue (Fig. 4Aii) showed heterogeneous mutations for LMAN1 (Fig. 4Aiii). Correlation between protein expression and frameshift mutation pattern was also observed in adenomas (Fig. 4B).

From these results we conclude that primary MSI-H colorectal carcinomas and adenomas frequently show heterogeneous LMAN1 expression abnormalities caused by regional biallelic cMNR frameshift mutations in MSI-H tumor cells.

LMAN1 deficiency causes decreased A1AT secretion. Because LMAN1 is a mannose specific lectin involved in ER/Golgi transport, LMAN1-deficiency could vastly influence protein distribution or secretion and, hence, cell homeostasis. One well-known client...
protein of LMAN1 is A1AT, an inhibitor of several serine proteases (27) that is involved in local neoangiogenesis and growth inhibition. Notably, a strong correlation between lower local levels of A1AT and enhanced tumor growth has been described (28). We therefore chose to compare A1AT-secretion in LMAN1-deficient versus LMAN1-proficient cell lines. When we examined conditioned medium of these colorectal cell lines by using an A1AT-specific ELISA, significantly decreased levels of A1AT were measured in LMAN1-deficient (~5 µg/L) compared with LMAN1-proficient cell lines (~15 µg/L; Fig. 5A). These lower medium levels are not due to lower expression levels of A1AT as RT-PCR analysis revealed similar A1AT transcript levels in all cell lines (data not shown). A moderate but nonsignificant decline in A1AT concentration was also observed in MSI-H colorectal cancer cell lines harboring heterozygous LMAN1 mutations (data not shown). To test whether LMAN1 reconstitution can overcome this secretion defect, LMAN1-deficient Colo60H cells were transiently transfected with LMAN1 cDNA. Indeed, there was a 10-fold increase in A1AT concentration in the medium of Colo60H cells overexpressing LMAN1 compared with the vector control (Fig. 5B). No effect of LMAN1 transfection on A1AT transcript levels was observed (data not shown).

In conclusion, the decreased A1AT secretion in LMAN1-deficient cell lines and the increased A1AT secretion after transient expression of LMAN1 in a deficient background indicate a potential role of LMAN1-mediated A1AT secretion in tumor progression.

Discussion

Our screening approach for frameshift mutations in cMNRs of genes that encode proteins of the cellular glycosylation machinery identified LMAN1/ERGIC53 as a major mutational target in MSI-H colorectal cancer cell lines and tumors. LMAN1 is a marker for the ER/Golgi intermediate compartment, a mobile membrane structure that carries secretory proteins along microtubules from the ER to the Golgi (29). LMAN1 binds as a mannose-specific lectin to its substrates by a luminal carbohydrate-recognition domain in the neutral and Ca2+-rich ER (30) and Man9 seems to be the preferred glycan structure for binding (31). LMAN1 can also act as a transport receptor for the serine protease inhibitor A1AT (27) and is involved in the ER-export of the lysosomal glycoproteins cathepsin C and Z as well as the blood coagulation factors V and VIII (27, 32–34). Genetic alterations in LMAN1 are associated with a human disease termed combined deficiency of coagulation factors V and VIII (F5/F8D).

Our results suggest that genetic alterations in LMAN1 also might be involved in human tumorigenesis. Several lines of evidence support this hypothesis.

First, the high mutation frequency observed in tumor tissues predicts LMAN1 as a MSI-H target gene whose mutational inactivation seems to be positively selected during MSI-H carcinogenesis. Second, biallelic mutations occurred frequently in MSI-H colorectal adenomas and carcinomas and, although regionally restricted, were associated with complete loss of LMAN1 protein expression. Such biallelic mutational inactivation usually points to a tumor suppressor function of the affected gene. Our results on cell lines with biallelic LMAN1 mutations also provide some clue about the mechanism that leads to loss of LMAN1 protein expression. LMAN1 mutant transcripts harbor a premature termination codon and, hence, are predicted to be NMD-sensitive i.e., they should be recognized and degraded by the nonsense-mediated mRNA decay (NMD) system (35). However, LMAN1 transcripts were easily detectable by RT-PCR analysis in cell lines with biallelic frameshift mutations and hence seemed NMD resistant. Because no truncated protein band of ~36 kDa could be detected by Western blotting, mutant LMAN1 transcript is probably an additional member of a recently described new class of NMD-escape transcripts affected by nonsense-mediated translational repression (35).

Third, LMAN1-deficient cell lines show loss of LMAN1 transport function as shown by impaired A1AT secretion. Our data on A1AT secretion support the results of Nyfeler and colleagues (27) on mouse embryonic fibroblasts (MEF) derived from LMAN1 (−/−) knockout mice. LMAN1-deficient MEFs secreted only ~25% A1AT in a given time period compared with LMAN1 (+/+) MEFs. Our results show a significant decrease of A1AT secreted into the cell culture medium reaching ~20% of the level secreted by the LMAN1-proficient control cell line Caco2.
How might LMAN1 deficiency contribute to MSI-H carcinogenesis? One possible answer to this question might come from the biological function. As a protein shuttle, LMAN1 mediates intracellular transport of several client proteins from the ER to the Golgi. One of its recently identified client proteins is A1AT, a serine protease inhibitor in human plasma. A1AT expression is found in many different tissues including the human colon (36, 37). A1AT not only exerts antiproteolytic function but also confers angioinhibitory activity. Studies on tumor cell xenografts in nude mice have shown that systemic administration of A1AT delayed tumor progression and reduced microvessel density (28). Comparative cDNA microarray expression analyses of human normal and tumor tissues revealed that lower levels of A1AT transcripts correlated with larger tumor size (28) and higher levels of noncirculating A1AT within tumors tend to be associated with better prognosis (38). Interestingly, it was hypothesized that A1AT is protective against MSI-H colorectal cancer development (39). The work by Huang and colleagues (28) also shows significant variations in local A1AT levels in human tumor tissues. Our observation of intratumoral heterogeneity of local LMAN1 deficiency might provide a molecular explanation for this finding (27).

Apart from A1AT secretion defects, alternative explanations may be considered. For example, LMAN1 deficiency might promote MSI-H colorectal tumorigenesis by affecting the secretion of other known client proteins such as cathepsin C and Z or by interference with interacting proteins such as WHAMM and MCFD2 (40, 41). Moreover, it is reasonable to assume that additional and yet unknown LMAN1 cargo and binding proteins exist, which might be affected by a loss of LMAN1 function. Although only secreted proteins have been identified as LMAN1 client proteins, one might speculate that membrane-bound glycoproteins are also transported by this mechanism. Support for this hypothesis is gained from our preliminary observation that changes in cell surface glycoprotein pattern occur in LMAN1-deficient cell lines upon reconstituted LMAN1 expression.7

Overall, our combined bioinformatics/molecular biology approach has identified a member of the cellular glycoprotein transport machinery as a putative novel MSI-H target gene frequently mutated in MMR-deficient colorectal tumors. Our work also points to genetic alterations in other genes of the cellular glycosylation machinery such as XL1T2 and B4GALT4. Mutational inactivation of these genes and their encoded proteins is expected to directly change glycosylation of substrate proteins at or beyond the surface of affected MSI-H tumor cells, thereby offering novel diagnostic and therapeutic strategies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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