Tumor and Stem Cell Biology

LIN28B Promotes Colon Cancer Progression and Metastasis

Catrina E. King1,4, Miriam Cuatrecasas6, Antoni Castells6, Antonia R. Sepulveda2, Ju-Seog Lee7, and Anil K. Rustgi1,3,4

Abstract

LIN28B is a homologue of LIN28 that induces pluripotency when expressed in conjunction with OCT4, SOX2, and KLF4 in somatic fibroblasts. LIN28B represses biogenesis of let-7 microRNAs and is implicated in both development and tumorigenesis. We have determined that LIN28B overexpression occurs in colon tumors. We conducted a comprehensive analysis of LIN28B protein expression in human colon adenocarcinomas. We found that LIN28B overexpression correlates with reduced patient survival and increased probability of tumor recurrence. To elucidate tumorigenic functions of LIN28B, we constitutively expressed LIN28B in colon cancer cells and evaluated tumor formation in vivo. Tumors with constitutive LIN28B expression exhibit increased expression of colonic stem cell markers LGR5 and PROM1, mucinous differentiation, and metastasis. Together, our findings point to a function for LIN28B in promoting colon tumor pathogenesis, especially metastasis. Cancer Res; 71(12); 4260–8. ©2011 AACR.

Introduction

LIN28B is a homologue of LIN28 (1), a heterochronic gene initially described in Caenorhabditis elegans as a regulator of developmental timing (2–5). It induces pluripotency when expressed in somatic fibroblasts in conjunction with OCT4, SOX2, and KLF4 (6). Both LIN28 and LIN28B contain a cold shock domain and retroviral-type (CCHC) zinc fingers that confer RNA-binding ability (2, 7) and inhibit biogenesis of tumor-suppressive microRNAs of the let-7 family (8–11).

LIN28 and LIN28B are implicated in multiple developmental processes, largely as a consequence of their ability to repress let-7 biogenesis. In C. elegans, let-7 is critical to the larval fate transition and vulval specification (12); LIN28 mutants reiterate larval fates (2). In human embryonic stem cells, relief of let-7 suppression via LIN28 downregulation occurs during differentiation (13). During neuronal differentiation, LIN28 is also specifically downregulated and constitutive expression blocks gliogenesis in favor of neurogenesis (14). LIN28B mutations are associated with delayed onset of puberty in humans (15), which can be modeled via constitutive expression of LIN28 in mice (16). Induction of LIN28 overexpression in transgenic mice expands transit-amplifying cells in the intestinal crypt compartment causing gut pathology and death (16), thus suggesting potential roles for LIN28 and LIN28B in intestinal and colonic development and disease.

LIN28 and LIN28B are implicated in tumorigenesis in different cancers, but LIN28B overexpression in human cancers has been shown more frequently (17). For example, LIN28B is overexpressed in hepatocellular carcinomas (1).

In addition, an analysis of LIN28 and LIN28B expression in various cancers pointed to LIN28B as perhaps the more relevant homologue in tumorigenesis (17). This study implicates a role for LIN28B in Wilms’ tumors, where the c-myc binding site on LIN28B promoter is frequently demethylated, and chronic myeloid leukemia, where expression of LIN28B occurs more commonly in blast crisis. Moreover, LIN28B overexpression occurs in non–small-cell lung cancer, breast cancer, melanoma, whereas aberrations in LIN28 expression were not as readily identifiable (17).

LIN28B may be upregulated as a consequence of adenomatous polyposis coli (APC) mutation, which occurs in the vast majority of colon tumors, resulting in sustained β-catenin stabilization and upregulation of the Wnt pathway targets (18, 19). The oncogenic transcription factor c-myc is an established target of the Wnt pathway that functions in part via transcriptional activation of LIN28B (18, 20). Accordingly, increased LIN28B expression in colon tumors may occur as a consequence of elevated c-myc levels and Wnt pathway deregulation (2, 18, 20). Yet, the role of LIN28B in tumorigenesis in vivo remains to be determined.

We examined LIN28B expression in human colon tumors in tissue microarrays and found that its overexpression correlates with reduced patient survival and increased probability of tumor recurrence. To elucidate further roles of LIN28B in colon tumorigenesis, we constitutively expressed LIN28B in colon cancer cell lines and subsequently evaluated tumor-promoting properties of LIN28B-expressing cells.
xenografted nude athymic mice. Surprisingly, we observed increased differentiation with glandular formation in LIN28B-expressing tumors. However, although smaller than vector-only controls, the LIN28B-expressing tumors have a greater tendency to metastasize. In addition, we conducted gene expression profile analyses of colon cancer cell lines, primary xenograft tumors, and metastases constitutively expressing LIN28B. We found stem cell–related genes, including the colonic stem cell markers LGR5 and PROM1, to be upregulated as a function of constitutive LIN28B expression. Taken together, our findings support a role for LIN28B in the progression of colon cancer.

Materials and Methods

Tumor tissue microarray analysis

A uniform cohort of 228 (133 men and 95 women) patients with colon carcinoma (88 in stage 2, and 140 in stage 3) diagnosed between November 1993 and October 2006 was selected. Rectal tumors were excluded. The age of the patients ranged from 35 to 88 years (mean: 66.4 years). The majority of the tumors (156) were located on the left colon; that is, 132 sigmoid, 13 splenic flexure, and 11 in the descendent colon, whereas 72 tumors were located on the right colon; that is, 26 cecum, 24 ascending, 14 transverse, and 8 in the hepatic flexure. Seven patients had synchronous tumors. Tumor size varied from 1.6 to 14 cm (mean: 3.8 cm). Six tumors were of grade 1, 212 of grade 2, and 10 of grade 3. Tissue samples were obtained from patients under Institutional Review Board approval. Two cores of normal mucosa and 2 cores of tumor tissue for each patient were paraffin embedded in ordered microarrays. Tumor microarrays were sectioned in preparation for immunohistochemical staining. LIN28B staining intensity was scored by a pathologist: 1, low LIN28B intensity; 2, intermediate intensity; and 3, high intensity. Log-rank tests (Mantel–Cox and Breslow) were conducted to compare survival and recurrence distributions of intensity groups (1–2 vs. 3). Correlation of staining intensity with survival and recurrence was determined via χ² analysis; 95% CIs were calculated for confirmation of statistical significance.

Immunohistochemistry

Paraffin-embedded tissue microarrays and xenograft tumors were incubated at 60°C prior to dewaxing and rehydration. Antigen retrieval was done by placing sections in 10 μmol/L citric acid (pH 6) and microwaving for 15 minutes. Endogenous peroxidases were quenched in 15-mL hydrogen peroxide and 185 mL of water. Tissues were washed with PBS prior to treatment with Avidin D and Biotin, using the Vectastain ABC Kit (Vector Labs). Samples were washed again prior to treatment with Avidin D and Biotin, using the Peroxidase Staining kit (Pierce) as per the manufacturer’s protocol. For detection, a 3,3′-diaminobenzidine (DAB) Peroxidase Detection Kit (Vector Labs) was used and color development was monitored using a light microscope. The development process was terminated by removing DAB and rinsing the sections with ddH₂O for 1 minute before counterstaining with hematoxylin.

Generation of constitutive LIN28B-expressing cell lines

DLD-1 and LoVo cells were obtained from American Type Culture Collection (ATCC; a recognized vendor) in 2000 and stored in liquid nitrogen prior to resuscitation in 2009 (cell lines were authenticated by ATCC via DNA fingerprinting and cytogenetic analyses). Stable LIN28B expression in DLD-1 and LoVo cells was achieved using MSCV-PIG-LIN28B and empty vector control plasmids (gifts from Dr. Joshua Mendell). We transfected Phoenix A cells with 30 μg plasmid DNA and monitored transfection efficiency via detection of GFP expression by light microscopy prior to virus collection. Viral containing supernatant was collected 48 hours posttransfection, filtered through a 0.45-μm membrane, immediately placed in liquid nitrogen, and stored at −80°C for later use. DLD-1 and LoVo cells were infected by applying virus-containing media plus polybrene (4 μg/mL) to cells and then subjecting them to centrifugation at 1,000 × g for 90 minutes. Inoculated cells were selected in puromycin, expanded, and subsequently sorted for high GFP intensity and corresponding LIN28B expression.

Xenograft tumors

CrTac-Ncr-Foxn1tm1 nude athymic mice (Taconic) were irradiated at 5 Gy 2 to 3 hours prior to injection of cells. Empty vector and LIN28B-expressing DLD-1 and LoVo cells were trypsinized and washed in PBS and resuspended at a concentration of 2 × 10⁶ cells/mL. Fifty microliters of cell suspensions was mixed with 50 μL of BD Matrigel Basement Membrane Matrix (BD Biosciences) to achieve a volume of 100 μL containing 1 × 10⁶ cells per injection. Cells were injected subcutaneously into the rear flanks of mice sedated with ketamine (100 mg/kg) and xylazine (10 mg/kg). Following injection, mice were monitored periodically and sacrificed after 6 weeks. All mice were cared for in accordance with University Laboratory Animal Resources (ULAR) requirements.

Detection of GFP fluorescence by image analysis

Xenografted nude mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and fluorescence intensity was measured using a small animal imaging system (Kodak). Net tumor intensity was calculated over a fixed region of interest that encompasses the dimensions of the largest tumor. Statistical significance of comparisons between empty vector and LIN28B tumors was determined by applying Student’s t test, where P < 0.05 is statistically significant.

Gene expression analysis in xenograft tumors

Mice were euthanized in accordance with ULAR standards prior to excision of xenograft tumors. Extraneous tissues were dissected away and discarded, tumors were then immediately placed into RNAlater (Qiagen) and maintained on ice until
storage at −80°C. Tumor tissue was homogenized mechanically in RNAlater (Qiagen) and homogenized particulates were collected via centrifugation at 4°C. RNA was isolated from homogenized tissues by using a mirVana RNA isolation kit (Ambion). For detection of mature let-7a and let-7b microRNAs, TaqMan MicroRNA Assay kits (Applied Biosystems) were employed to synthesize probe-specific cDNA from 10 ng of total RNA per sample. Probe-specific cDNA was amplified using proprietary primers (Applied Biosystems) and TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems). PCR amplifications were conducted in triplicate and normalized to levels of endogenous U47.

Microarray analysis
RNA was isolated using a mirVana kit (Ambion) from empty vector and LIN28B-expressing DLD-1 and LoVo colon cancer cell lines, primary xenograft tumors, and LIN28B-expressing mesenteric metastases. Triplicate RNA isolates were submitted to the Penn Microarray Facility. Quality control tests of the total RNA samples were carried out using an Agilent bioanalyzer and a Nanodrop spectrophotometry. One hundred nanograms of total RNA was then converted to first-strand cDNA by using reverse transcriptase primed by a poly (T) oligomer that incorporated a synthetic RNA sequence. Second-strand cDNA synthesis was followed by Ribon-SPLA (Single Primer Isothermal Amplification; NuGEN Technologies Inc.) for linear amplification of each transcript. The resulting cDNA was fragmented, assessed by bioanalyzer, and biotinylated. cDNA yields were added to Affymetrix hybridization cocktails, heated at 99°C for 2 minutes, and hybridized for 16 hours at 45°C to Affymetrix Human Gene 1.0 ST Array GeneChips (Affymetrix Inc.). The microarrays were then washed at low (6× saline sodium phosphate EDTA) and high (100 mmol/L MES, 0.1 mol/L NaCl) stringency and stained with streptavidin-phycocerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycocerythrin stain. A confocal scanner was used to collect fluorescence signal after excitation at 570 nm. All protocols were conducted as described in the NuGEN Ovation manual and the Affymetrix GeneChip Expression Analysis Technical Manual.

Bioinformatics analysis
Three independent biological replicates for each condition were assayed on microarrays. Unsupervised hierarchical clustering by sample was conducted to confirm that replicates within each condition grouped with most similarity and to identify any outlier samples. Significance Analysis of Microarrays (SAM v3.0; www-stat.stanford.edu/~tibs/SAM/) was used to generate lists of statistically significant differentially expressed genes in pairwise comparisons of replicate averages between conditions. Constitutive LIN28B expression in vitro resulted in 13,156 genes with higher RNA abundance and 15,771 genes with lower RNA abundance when compared with empty vector controls. Candidate genes from the in vitro analysis were filtered by fold change (threshold = 5-fold), and the resulting gene lists were tested for overrepresentation. Constitutive LIN28B expression in vivo resulted in 11,242 genes with higher RNA abundance in LIN28B metastases than in control tumors and 17,579 genes with lower RNA abundance in LIN28B metastases than in control tumors. Candidate genes were further filtered by fold change (threshold = 4-fold), and the resulting gene lists were tested for overrepresentation of Gene Ontology annotation categories by using the DAVID Bioinformatics Resources (david.abcc.ncifcrf.gov).

To stratify patients with colon cancer according to the similarity of their gene expression patterns to LIN28B-specific gene expression signature, we adopted a previously developed model (21–23). Briefly, we first identified genes whose expression was significantly associated with expression of LIN28B from cell culture. A highly stringent cutoff (P < 0.001) was applied to avoid inclusion of potential false-positive genes during training of prediction models. Expression data from selected genes (704 genes) were then combined to form a classifier according to compound covariate predictor algorithm, and the robustness of the classifier was estimated by misclassification rate determined during the leave-one-out cross-validation (LOOCV) in the training set (data from cell culture). When applied to gene expression data from the patients (n = 290; GSE14333), prognostic significance was estimated by Kaplan–Meier plots and log-rank tests between 2 predicted subgroups of patients. After LOOCV, sensitivity and specificity of prediction models were estimated by the fraction of samples correctly predicted.

Results
Abrupt LIN28B expression correlates with reduced patient survival
To determine potential roles of LIN28B in colon cancer pathogenesis, we examined LIN28B expression in human colon tumors and matched adjacent normal mucosa. Tumor and adjacent normal tissue samples resected from patients receiving adjuvant 5-fluorouracil were paraffin-embedded in duplicate on to tissue microarrays. Immunohistochemistry (IHC) was done for LIN28B, and staining intensity was scored by a pathologist blinded to clinical information. LIN28B staining in normal colon is faint; in contrast, intense LIN28B staining was observed in colon tumors (Fig. 1A–D; Supplementary Fig. S1). Colon tumor samples were further analyzed via log-rank tests (Mantel–Cox and Breslow) comparing overall survival and recurrence distributions of intensity groups. This revealed a correlation
between low-intensity LIN28B staining in stage I and II colon tumors with higher patient survival (Mantel–Cox, $P$ value = 0.046; Breslow, $P$ value = 0.013; Fig. 1E) and lower probability of tumor recurrence (Mantel–Cox, $P$ value = 0.036; Breslow, $P$ value = 0.108; Fig. 1F).

**LIN28B tumors exhibit reduced size and glandular differentiation**

Given that high LIN28B levels in colon tumors correlate with increased probability of tumor recurrence in patients, we hypothesized a biological role for LIN28B in colon cancer pathogenesis. To elucidate this further, we constitutively expressed LIN28B in DLD-1 and LoVo colon cancer cells. We then established xenograft tumors by injecting $1 \times 10^6$ cells subcutaneously into the rear flanks of nude athymic mice. We initially monitored tumor development via detection of GFP fluorescence (expressed from the MSCV-PIG retroviral vector) in a total of 83 xengografted mice: 41 empty vector controls and 42 LIN28B-overexpressing tumors. At 2 and 4 weeks postinjection, we found that LIN28B tumors emitted less fluorescence (Fig. 2A and B). This observation corresponds to a smaller tumor mass observed in LIN28B-expressing tumors (Fig. 2C and D). We next confirmed LIN28B overexpression in tumors via IHC (Fig. 3A and B). In addition, mature let-7 microRNAs, which are repressed by LIN28B, are reduced in the presence of constitutive LIN28B expression in xenograft tumor s (Supplementary Fig. S2). Surprisingly, LIN28B tumors exhibited increased areas of moderate differentiation, increased glandular formation, and increased mucin production, in contrast to empty vectors tumors that are poorly differentiated and rarely exhibit mucinous, gland-like structures (Fig. 3C–F). Viable tumor areas were scored for poor and moderate differentiation, as well as mucin production revealing statistically significant differences between empty vector and LIN28B-expressing xenograft tumors (Fig. 4). However, Ki-67 and caspase-3 staining did not reveal significant differences (data not shown).
**LIN28B tumors metastasize to multiple organs**

During necropsy, we observed mesenteric and liver metastases in mice xenografted with LIN28B-expressing cells upon gross examination (Fig. 5E), which prompted extensive examination of internal organs. Although we did not observe metastases in any mice in the empty vector control group, we did note liver, lung, perisplenic adipose, lymph node, and mesenteric metastases in mice bearing tumors with constitutive LIN28B expression (Fig. 5A and B). We confirmed that these metastases were derived from xenografted cells via immunohistochemical GFP detection, which is unique to xenografted cells (Fig. 5C and D). Overall, we identified metastases in approximately 17% of mice xenografted with LIN28B-expressing cells (7 of 42). Thus, constitutive LIN28B expression in colon cancer cells confers metastatic ability in at least a subset of tumors (Fisher’s exact one-tailed \( P = 0.0065 \)).

**Stem cell-like gene expression profile induced by constitutive LIN28B expression**

We next conducted microarray analysis on total RNA isolated from empty vector and LIN28B-expressing cell lines and primary tumors, as well as LIN28B-LoVo metastases. We identified more than 184 transcripts (164 upregulated; 20 downregulated) that are more than 5-fold changed with constitutive LIN28B expression in vitro (accession numbers GSM646687 and GSM646692). Some transcripts upregulated by LIN28B, including the hematopoietic stem cell marker KIT (also called CD117 or c-KIT) and the intestinal stem cell markers LGR5 (leucine-rich repeat-containing G-protein-coupled receptor 5) and PROM1 (prominin 1), have reported roles in stem cell biology (Table 1). Of note, LGR5 is a transcriptional target of canonical Wnt signaling (24, 25). Additional Wnt targets including DKK1 (Dickkopf homolog 1;...
refs. 26, 27) and CCND2 (cyclin D2; refs. 28, 29) are upregulated by LIN28B overexpression as well (Table 1). These results were verified by qRT-PCR (data not shown).

In addition, we identified more than 22 genes that are more than 4-fold upregulated and 57 genes that are more than 4-fold downregulated by constitutive LIN28B expression in vivo (accession numbers GSM646700 and GSM646708). Many genes that are upregulated with constitutive LIN28B expression in vitro are also increased by constitutive LIN28B expression in vivo, including the stem cell markers LGR5 (24) and PROM1 (30) and the let-7 targets HMGA2 (high-mobility group AT-hook 2; refs. 31, 32) and IGF2BP1 (insulin-like growth factor 2 mRNA-binding protein 1; ref. 33); these results were verified by qRT-PCR analysis (data not shown). Overall, the expression profile of empty vector tumors varies dramatically from that of LIN28B-expressing primary tumors (Supplementary Fig. S3A). Notably, the expression profile of LIN28B metastases also differs

Figure 4. Differentiation and mucin production in LIN28B tumors. A, poor differentiation in xenograft tumors. Area exhibiting poor differentiation scored as a percentage of viable tumors. LIN28B-expressing tumors exhibit fewer areas of poor differentiation. B, moderate differentiation in xenograft tumors. Area exhibiting moderate differentiation scored as a percentage of viable tumors. Moderate differentiation is increased in LIN28B-expressing tumors. C, mucin positivity in poorly differentiated tumor areas. Mucin was detected via mucicarmine staining. Mucin positivity in poorly differentiated area scored as a percentage of viable tumor. D, mucin positivity in moderately differentiated tumor areas. Mucin positivity in poorly differentiated area scored as a percentage of viable tumor. Differentiation is increased in LIN28B-expressing tumors. Mucin production is increased in LIN28B-expressing tumors.

Figure 5. A subset of LIN28B-expressing primary tumors metastasize. A, lung metastasis from a LIN28B-DLD-1 subcutaneous tumor. H&E staining. B, liver metastasis from a LIN28B-LoVo subcutaneous tumor. H&E staining; note invasion of metastasis into normal liver. C and D) metastases are GFP-positive. IHC for GFP expressed from MSCV-PIG retroviral vector confirms that these tissues originated from xenograft injections. E, metastases visible upon gross examination. Arrows indicate liver and mesenteric metastases from LIN28B-LoVo tumors. Magnification: 100× in A and B; 200× in C and D.
from that of LIN28B primary xenograft tumors (Supplementary Fig. S3A). Some genes, such as TNS4 (tensin 4), CHI3L1 (chitinase 3-like 1), and KLK6 (kallikrein-related peptidase 6), specifically modulated in LIN28B metastases versus LIN28B primary tumors have described roles in migration, invasion, and metastasis (refs. 34–36; Table 1).

Because we observed metastases with constitutive LIN28B expression in cells, we sought to determine whether the gene expression profile induced by LIN28B resembled that of naturally occurring metastatic human colon tumors. Therefore, we cross-compared the LIN28B-specific gene expression signature with that of patients with colon adenocarcinomas. For this analysis, we used publicly available gene expression data from 290 patients with colon adenocarcinomas (37). When patients were stratified according to the LIN28B-specific gene expression signature (Supplementary Fig. S3B), disease-free survival of patients with tumors bearing gene expression patterns similar to the LIN28B signature is significantly shorter ($P = 0.02$, by log-rank test, Supplementary Fig. S3B), indicating that LIN28B in colon cancer may influence clinical outcome in patients with colon cancer by promoting early metastasis. Specificity and sensitivity for correctly predicting LIN28B overexpression during LOOCV were both 1.0.

## Discussion

We found that increased LIN28B staining intensity in non-metastatic colon tumors correlates with reduced overall survival and increased probability of tumor recurrence in patients receiving adjuvant 5-fluorouracil. We then examined tumor-promoting properties of LIN28B-expressing colon cancer cells via tumorigenesis studies in nude athymic mice. Primary xenograft tumors that constitutively express LIN28B display decreased size and increased differentiation characterized by glandular formation relative to empty vector controls. Yet, LIN28B-expressing xenograft tumors are capable of metastasis, which is not observed with empty vector xenograft tumors do not.

We have shown increased LIN28B expression in primary colon tumors, which may be the result of increased LIN28B transcriptional activity mediated by c-myc. Because c-myc is a transcriptional target of canonical Wnt signaling, it is possible that LIN28B is upregulated in colon tumors as a consequence of APC mutation (or other changes that deregulate Wnt signaling), which occurs in the majority of sporadic colon tumors. The ability of c-myc to transactivate LIN28B has not been described and may account for differential properties of LIN28B and LIN28B in colon cancer. However, it is important to note that distinct functions have not been described for either homologue to date and it is possible that the 2 are completely redundant.

Although our findings reveal potential roles for LIN28B in colon cancer metastasis, it is surprising for genes that promote tumorigenesis and/or metastasis to simultaneously enhance differentiation. Thus, our finding that primary tumors constitutively expressing LIN28B are smaller and more differentiated than their empty vector counterparts is intriguing. Given that constitutive LIN28B expression in undifferentiated cells blocks gliogenesis in favor of neurogenesis (14), it is possible that LIN28B overexpression in colonic epithelial cells results in restriction of cell fate as observed previously in neuronal cells. Similar phenotypes have previously been described in the intestine. For example, loss of the Math1 (also called Atoh1) transcription factor leads to deletion of goblet, enteroendocrine, and Paneth cells, while permitting enterocyte differentiation in the intestinal epithelium (38).

### Table 1. Transcripts upregulated by LIN28B

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Fold change&lt;sup&gt;b&lt;/sup&gt; (vector vs. LIN28B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGR5</td>
<td>Leucine-rich repeat-containing G-protein-coupled receptor 5</td>
<td>7.2</td>
</tr>
<tr>
<td>PROM1</td>
<td>Prominin 1</td>
<td>22.6</td>
</tr>
<tr>
<td>KIT</td>
<td>v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog</td>
<td>36.6</td>
</tr>
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<td>HMGA2</td>
<td>High mobility group AT-hook 2</td>
<td>2.6</td>
</tr>
<tr>
<td>IGF2BP1</td>
<td>Insulin-like growth factor 2 mRNA-binding protein 1</td>
<td>9.4</td>
</tr>
<tr>
<td>CCND2</td>
<td>Cyclin D2</td>
<td>6.6</td>
</tr>
<tr>
<td>DKK1</td>
<td>Dickkopf homolog 1</td>
<td>37.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Selected transcripts upregulated by constitutive LIN28B expression in colon cancer cells in vitro.

<sup>b</sup>Fold changes indicated are the mean of 3 experimental replicates.

*Table 1. Transcripts upregulated by LIN28B*
Furthermore, LIN28B upregulates the colonic epithelial stem cell markers LGR5 and PROM1. In the intestine, expression of the cell surface protein PROM1 is restricted to the crypt and adjacent epithelial cells (30) whereas expression of the orphan receptor LGR5 occurs exclusively in cycling columnar cells within the crypt base (24). Because LGR5 and PROM1 mark intestinal and colonic epithelial stem cells, upregulation of these factors by LIN28B suggests possible roles for LIN28B in intestinal stem cells.

Our finding that high LIN28B staining intensity in primary human colon tumors correlates with reduced patient survival and increased probability of tumor recurrence is especially intriguing, given that LIN28B-expressing xenograft tumors metastasize in the context of a more differentiated primary tumor. While low tumor grades usually correspond to early stage and better prognosis, metastasis and poor prognosis are observed in a subset of low-grade tumors (39, 40). Given that we find both differentiation and metastasis with constitutive LIN28B expression in primary tumors, LIN28B overexpression may mark low-grade tumors with poor prognostic potential. Thus, LIN28B may serve as a potential diagnostic marker that may prove to be an invaluable tool in colon cancer wherein early diagnosis is critical to survival.

References


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