Identification of the IFITM Family as a New Molecular Marker in Human Colorectal Tumors

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Abstract
We analyzed the expression profiles of intestinal adenomas from a new murine familial adenomatous polyposis model (ApcΔ14/+ ) using suppression subtractive hybridization to identify novel diagnostic markers of colorectal carcinogenesis. We identified 18 candidate genes having increased expression levels in the adenoma. Subsequent Northern blotting, real-time reverse transcription-PCR, and in situ hybridization analysis confirmed their induction in β-catenin-activated epithelial cells of murine adenomas. We showed that most of the genes also have altered expression levels in human colonic adenomas and carcinomas. We focused on the IFITM genes that encode IFN-inducible transmembrane proteins. Serial analyses of gene expression levels revealed high levels of expression in early and late intestinal neoplasm in both mice and humans. Using a conditional mouse model of Apc inactivation and a human colon carcinoma cell line, we showed that IFITM gene expression is rapidly induced after activation of the β-catenin signaling. Using a large-scale analysis of human tumors, we showed that IFITM gene expression is significantly up-regulated specifically in colorectal tumors and thus may be a useful diagnostic tool in these tumors. (Cancer Res 2006; 66[4]: 1949-55)

Introduction
Colon cancer is triggered by a series of point mutations and genetic alterations that progressively cause normal cells to transform into adenomas that could become progressively more dysplastic, resulting in carcinoma foci (1). The genetic alterations might occur in a preferred sequence and could determine the clinical characteristics of the colorectal tumor. Large-scale screening of gene expression profiles of colon cancers, using methods such as cDNA microarrays or serial analysis of gene expression reverse transcription-PCR, have identified many of these alterations (2–7). However, the screening of carcinomas cannot distinguish changes in gene expression that are critical to the initiation of tumorigenesis. Identification of genes induced early in the first step of tumorigenesis is important both for a better understanding of the biological processes leading to cancer development and for identifying novel molecular markers for diagnosis. It is well established that one of the initiating steps in colorectal carcinogenesis is a mutation in the APC tumor suppressor gene. APC mutations, which generally lead to a truncated protein, have been detected in ~80% of sporadic cancers and cause familial adenomatous polyposis (FAP; ref. 8). APC binds to β-catenin and regulates the activation of the Wnt/β-catenin pathway. Activation of the Wnt pathway leads to the association of β-catenin with the Tcf/lef transcription factors (9). This complex can activate the transcription of a variety of target genes. Loss of Apc function leads to an abnormal accumulation of β-catenin and dysregulated Wnt signaling.

Therefore, we have examined the expression of the deregulated genes in adenomas developed in a murine model of FAP that we have recently established, ApcΔ14/+, to identify targets critical for the initiation of cancer. These mice carry an Apc germ line mutation that leads to numerous adenomas developing in both the small and large intestine (10). The phenotypic similarity with human colon adenoma development makes this a useful mouse model system to investigate the molecular mechanisms leading to adenoma formation. We used a suppression subtractive hybridization (SSH) library approach to characterize the changes in gene expression that accompany the progression from normal murine intestinal epithelia to adenomas. We checked the relevance of the identified genes in human colon adenomas and carcinomas. Here, we report several candidate genes in which expression levels are altered in adenomatous polyps from both mice and humans. We focused on the IFN-inducible transmembrane gene family (IFITM). Using different mouse models of Apc inactivation and human colon tumor samples, we have established that expression of several members of the IFITM family was up-regulated in early and late intestinal neoplasms. We present evidence that the up-regulation of the IFITM genes seems to be an early event in β-catenin intestinal tumorigenesis. Finally, we have shown by analyzing various distinct types of human tumors and their corresponding non–tumor tissue that IFITM genes are specifically induced in colorectal tumors and thus may provide a new diagnostic marker in these tumors.

Materials and Methods
Animals and treatment. All experiments involving mice were carried out in accordance with French government regulations. The generation of ApcΔ14/+ and ApcΔ14/++;Vil-CreER2 mice has been previously described (10, 11). In ApcΔ14/++;Vil-CreER2 mice, the Cre recombinase was activated by a single injection of tamoxifen solution (1 mg; Sigma, St. Louis, MO). Four days after tamoxifen injection, the mice were killed and the intestines were collected.

Suppression subtractive hybridization. Male wild-type and ApcΔ14/+ mice were killed at 5 months old. We harvested 20 adenomas from the duodenum and colon of six ApcΔ14/+ mice. Normal duodenum and colon
tissues were harvested from wild-type mice. Total RNA and mRNA samples from murine adenomas and wild-type tissues were isolated with Trizol reagent (Invitrogen, San Diego, CA) and a poly(A) mRNA isolation kit (Ambion, Austin, TX) according to the manufacturer's protocol. Subtractive hybridization with mRNA samples from tumor-bearing intestine and normal intestine, and subsequent differential screening were carried out using the PCR-Select cDNA subtraction kit and the PCR-Select differential screening kit (Clontech, Palo Alto, CA), according to the manufacturer's protocols. For each subtracted cDNA library (duodenum and colon) ~4,000 clones were plated and 96 recombinant clones for each library were randomly picked and sequenced. BLAST search of the gene database was used to analyze sequence homologies.

Northern blotting. Experimental samples for Northern blotting were collected from Apc<sup>14/+</sup> mice and wild-type mice. Total RNA was isolated using Trizol (Invitrogen). For each sample, 10 µg of total RNA was electrophoresed through 1% agarose-6% formaldehyde gel. The RNAs were transferred to Hybond N+ membranes and hybridized with the corresponding 32P-labeled probes.

In situ hybridization. Immediately after killing the mouse, the entire gastrointestinal tract was removed, splayed open along its length, fixed in 10% formol and then rolled up from the proximal to distal end to form a Swiss roll. In situ hybridization was carried out on 7 µm slices of the paraffin-embedded Swiss rolls. SSH library clones were subcloned in PEGMT. Digoxigenin-labeled RNA probes were prepared by in vitro transcription with the digoxigenin RNA labeling kit (Roche, Meylan, France) using T7 or Sp6 RNA polymerases. Sections were incubated overnight at 68°C in the prehybridization buffer containing 200 ng/ml of digoxigenin-labeled RNA probe. Immunodetection of the hybridized probe was carried out using an anti-digoxigenin antibody (Roche, 1/4000).

Immunohistochemistry. Five-micrometer sections of the paraffin-embedded Swiss roll were cut and treated with 3% hydrogen peroxide. The antigen was revealed by treating the sections with citrate buffer (pH 6) in a microwave pressure cooker. We used polyclonal primary antibodies directed against Ifitm3 (1/50, provided by M.A. Surani, University of Cambridge, United Kingdom), and monoclonal primary antibodies directed against β-catenin (1/100, BD Biosciences).

Collection of patient samples. Five primary colorectal adenomas and corresponding normal tissue were collected at the Hotel-Dieu and Cochin Hospitals (Paris, France). Ten primary colorectal cancers and adjacent tissue were collected at the Laennec Hospital (Paris, France) and have been previously described (11).

Real-time quantitative reverse transcription-PCR analysis. For reverse transcription-PCR, 2 µg of total RNA was reverse-transcribed using Superscript II RNase H RT (Invitrogen) and random hexamers. Real-time quantitative reverse transcription-PCR was carried out with a LightCycler (Roche Diagnostics). Quantification was carried out in duplicate and expressed relative to 18s rRNA. For human samples, data are expressed as the ratio of gene expression levels in the adenoma or carcinoma to gene expression levels in the corresponding normal tissue. For mouse samples, gene expression levels were expressed relative to wild-type small intestine. For HT29 cells, we used the gene expression level in nontreated HT29-APC for calibration. The sequences of the primers are available in the supplemental data.

Cancer profiling arrays. A human cDNA probe which recognized IFITM1, IFITM2, and IFITM3 was obtained by reverse transcription-PCR (corresponding to 294–495 bp of the human <i>IFITM1</i> gene, National Center for Biotechnology Information accession no. NM_003641.2) and then subcloned. The cancer profiling array membrane (7847-1; Clontech) was hybridized with the 32P-labeled IFITM3 cDNA probe according to the manufacturer's protocol. For normalization, the filter was hybridized with a human ubiquitin probe (Clontech). For quantification, the filter was scanned and the spots were analyzed with Image J software.

Cell cultures and treatment. The colon carcinoma cell line, HT29, containing a zinc-inducible APC gene (<i>HT29-APC</i>) and a control cell line containing an analogous inducible LacZ gene (HT29-LacZ; ref. 12) were cultivated in McCoy's 5A medium supplemented with 10% fetal bovine serum and 0.6 mg/ml hygromycin B solution at 37°C in a humidified 5% CO2 atmosphere. HT29-APC and HT29-LacZ cells were cultured with 120 mmol/L ZnCl<sub>2</sub> for 24 hours and total RNA was extracted from the lysates.

Results

Identification of genes overexpressed in adenomas from small and large intestine from Apc<sup>14/+</sup> mice. We have previously developed a new germ line Apc<sup>14/+</sup> mouse model in which the Apc exons 14 is deleted (10). Like other FAP mouse models, polypos develop after the wild-type Apc allele is lost (loss of heterozygosity). Unlike other Apc<sup>+/−</sup> mouse models in which tumors developed mainly in the small intestine, these mice developed many polyps in both the small intestine and the colon. Therefore, this mouse model has allowed us to determine the gene expression profile of adenomas in the small and the large intestine. We harvested 20 adenomas and normal tissue from both the small intestine and the distal colon. We used SSF to identify genes that were overexpressed in adenomas compared with normal adult tissue. We isolated 18 distinct genes in the adenomatous tissues (Table 1). We isolated a set of Paneth cell markers (Mmp7, Lyz3, Pla2g2a, and Defcr1 genes). In a previous study, we showed that Paneth cell markers are induced in murine and human intestinal neoplasia (11). These findings have been recently confirmed by another study (13). In the present study, we did not carry out any further analysis of these genes. The other 14 identified genes were then analyzed by either Northern blotting or real-time quantitative reverse transcription-PCR to confirm the differential expression between adenomas and normal mucosa (Fig. 1A and B). The RNA samples used for these experiments were from different adenomas than those used for the SSH libraries. Using two distinct methods, we repeatedly showed that the transcription levels of these genes were altered in adenomas. Of the 14 genes induced in adenomas, only three (S100a8, Miat, and Igk-C) were specifically induced in adenomas from either the small or the large intestine (Fig. 1A and B). These results suggest that the molecular mechanisms that lead to the formation of adenomas in both the small and large intestine are similar.

As intestinal adenomas contain many cells, including epithelial cells, fibroblasts and bloodborne cells, we used RNA in situ hybridization to determine the cellular location of the up-regulated genes. We obtained results for eight genes (Fig. 2). All genes were expressed in the dysplastic epithelial cells of the adenomas. Thus, analysis of bulk tissue may allow the isolation of specific epithelial markers of intestinal carcinogenesis. We observed that most of the genes tested in the adenomas, except slc38a4, were normally expressed in the proliferative compartment of normal mucosa, that is, the crypt compartment (Fig. 2). These results are consistent with studies on colorectal cancer cell lines, suggesting that the genetic program of intestinal adenoma cells is similar to that of the crypt progenitors (6).

Validation of the identified genes in human colorectal adenomas and adenocarcinomas. We evaluated the human homologues of the genes listed in Table 1 to determine whether their expressions were similarly altered in human intestinal tumors. We found two clones encoding for two families of genes, the ITITM and the matrix metalloproteinase (MMP) families. The cDNA sequence encoding Ifitm3 was highly homologous to the human ITITM1 (also called 9-27), IFITM2 (also named 1-8U) and IFITM3 (also named 1-8D) genes belonging to the IFN-inducible transmembrane family (14). Another murine cDNA clone was found to
be homologous to human MMP3 and MMP10. As these genes were highly homologous, we designed specific oligonucleotides primers to discriminate between each family member. However, we were not able to discriminate between IFITM2 and IFITM3 due to the high homology between them. *Exp* has no known human homologue and was excluded from further analyses. Tumor-specific changes in gene expression were validated using real-time quantitative reverse transcription-PCR analysis of cDNAs from a set of 5 human colorectal adenomas and 10 adenocarcinomas (Fig. 3A and B). Of the 13 analyzed genes, 8 were up-regulated by at least twice as much in human adenomas compared with normal tissue (**MMP3, LCN2, MMP10, IFITM1, TNFRS11B, S100A8, IFITM2, and MT4**). Of these eight, six remained overexpressed (**≥2-fold**) regardless of the tumor stage (**MMP3, LCN2, MMP10, IFITM1, TNFRS11B, and IFITM2/3**). We observed some variability in gene expression across different tumor and normal samples. This may be a real biological difference and/or an imbalance in tissue composition. Nevertheless, our results showed that most of the genes identified with our murine EAP model were also up-regulated in both early and late lesions in human colorectal carcinogenesis. They are thus potential biomarkers of colon cancer.

**IFITM gene expression is controlled by the Wnt/β-catenin signaling in mouse and human intestinal epithelium.** We focused on the IFITM family. The precise function of these genes is not known. In mouse embryos, Ifitm3 has been described as a key player for the specification of germ cell fate, and Ifitm1 seems to regulate epithelialization of the somites (15, 16). Interestingly, Ifitm1 has been recently described as a potential target gene of the Wnt pathway during gastrulation (15). We first analyzed the temporal expression pattern of IFITM genes at early and late stages of murine intestinal tumorigenesis. We used a probe to detect all the family members. Using **in situ** hybridization, we detected a strong induction of IFITM expression in all early aberrant crypt foci, in microadenomas and adenomas from both the small intestine and colon from Apc**Δ14/+** mice (Figs. 2A and 3A, a-d). These results were confirmed by immunohistochemistry (Fig. 4A, e-h). We confirmed **IFITM** induction in the early stages of tumoral progression by examining its expression in an intestinal conditional knockout mouse model of Apc that we have recently established (11). This knockout model is based on the Cre/loxP system and uses an inducible tamoxifen (Tam) Cre recombinase (Cre ERT2), which is driven by the villin promoter. After 2 days, there is a disruption of the Apc gene in the epithelium cells along the entire small intestine. Four days after a single injection of Tam, the Apc**lox/lox**/vil-CreER**T2** mice presented an enlargement of the crypt foci, in microadenomas and adenomas from both the small intestine and colon from Apc**Δ14/+** mice. (Figs. 2B and 4B, a and b). This was associated with an abnormal activation of the Wnt/β-catenin signaling, as seen by a cytosolic and nuclear localization of β-catenin (Fig. 4B, c and d). We observed an up-regulation of the **IFITM** expression in the dysplastic region of the Apc**lox/lox**/vil-CreER**T2** mice (Fig. 4B, e and f). These results strongly suggest that expression of the **IFITM** family members was induced and that their transcription is dependent on activation of β-catenin signaling in the intestinal epithelium. Next, using real-time quantitative reverse transcription-PCR, we analyzed the level of expression of each member of the murine **IFITM** family (Ifitm1, Ifitm2, Ifitm3, Ifitm5, and Ifitm6) both in the early dysplastic lesions of the conditional Apc mouse model (Apc**lox/lox**/vil-CreER**T2**, 4 days after tamoxifen injection) and in murine adenomas from the small intestine and colon of Apc**Δ14/+** mice. We observed a strong induction of Ifitm1, Ifitm2, and Ifitm3 regardless of the tumor stages analyzed. By contrast, Ifitm6 was

**Table 1. Genes up-regulated in murine intestinal tumors**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Full name</th>
<th>Accession no.</th>
<th>GO ID</th>
<th>SSH library</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igk-C</td>
<td>immunoglobulin κ chain, constant region</td>
<td>X02816</td>
<td>GO:0006959</td>
<td>duodenum</td>
</tr>
<tr>
<td>Pdx1</td>
<td>peroxiredoxin 1</td>
<td>NM_011034</td>
<td>GO:0006979</td>
<td>duodenum</td>
</tr>
<tr>
<td>Stc38a4</td>
<td>amino acid transport system A3 solute carrier family 38, member 4</td>
<td>NM_027052</td>
<td>GO:0006865</td>
<td>duodenum</td>
</tr>
<tr>
<td>Stc2</td>
<td>stanniocalcin 2</td>
<td>NM_011491</td>
<td>unknown</td>
<td>duodenum</td>
</tr>
<tr>
<td>ExpI</td>
<td>extracellular proteinase inhibitor</td>
<td>NM_007969</td>
<td>unknown</td>
<td>duodenum</td>
</tr>
<tr>
<td>Plat</td>
<td>plasminogen activator, tissue</td>
<td>NM_008872</td>
<td>GO:0006508</td>
<td>duodenum</td>
</tr>
<tr>
<td>Ifitm3</td>
<td>IFN-induced transmembrane protein 3 mil-1</td>
<td>NM_025378</td>
<td>GO:0007275</td>
<td>colon</td>
</tr>
<tr>
<td>Lcn2</td>
<td>lipocalin 2</td>
<td>NM_008491</td>
<td>GO:000680</td>
<td>colon</td>
</tr>
<tr>
<td>Mmp3*</td>
<td>matrix metalloproteinase 3 stromelysin1</td>
<td>NM_010809</td>
<td>GO:0006508</td>
<td>colon</td>
</tr>
<tr>
<td>Mmp10*</td>
<td>matrix metalloproteinase 10 stromelysin 2</td>
<td>NM_019471</td>
<td>GO:0006508</td>
<td>colon</td>
</tr>
<tr>
<td>Mt4</td>
<td>metalloprotein 4</td>
<td>NM_008631</td>
<td>GO:0006875</td>
<td>colon</td>
</tr>
<tr>
<td>S100A8</td>
<td>S100 calcium binding protein A8 (calgranulin A)</td>
<td>NM_013650</td>
<td>GO:0006935</td>
<td>colon</td>
</tr>
<tr>
<td>TNFRS11B</td>
<td>tumor necrosis factor receptor superfamily, member 11B (osteoprotegerin)</td>
<td>NM_008764</td>
<td>GO:0007165</td>
<td>colon</td>
</tr>
<tr>
<td>Vamp8</td>
<td>vesicle-associated membrane protein 8</td>
<td>NM_016794</td>
<td>GO:0016192</td>
<td>colon</td>
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<tr>
<td>Defcr1</td>
<td>defensin-related cryptdin peptide 1</td>
<td>M33225</td>
<td>GO:0006952</td>
<td>colon</td>
</tr>
<tr>
<td>Lys</td>
<td>lysozyme</td>
<td>NM_017372</td>
<td>GO:0004274</td>
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<tr>
<td>Mmp7</td>
<td>matrix metalloproteinase 7</td>
<td>NM_010810</td>
<td>GO:0006508</td>
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<tr>
<td>Pla2Gq*</td>
<td>phospholipase A2, group II (platelets, synovial fluid)</td>
<td>NM_011108</td>
<td>GO:0016042</td>
<td>colon</td>
</tr>
</tbody>
</table>

NOTE: Gene ontology (GO ID) was obtained from the Mouse Genome Informatics database of the Jackson Laboratories (http://www.informatics.jax.org/).

*Clone obtained in the SSH library revealed an identity with the Mmp3 and Mmp10 genes.

†Distinct clones revealed identity with several cryptdin genes.
induced only in adenomas and we observed no induction of Ifitm5 (Supplemental Fig. S1B). This suggested that the three neighboring genes, Ifitm1, Ifitm2, and Ifitm3 may share regulatory elements that are present within the cluster, leading to a common mechanism of transcriptional regulation (Supplemental Fig. S1A).

We then tested if the expression of human IFITM genes is also rapidly altered after deregulation of the β-catenin signaling. We analyzed the expression level of the IFITM1, IFITM2, and IFITM3 genes in the human colon carcinoma cell line, HT29, containing a zinc-inducible APC gene. In HT29 cells (HT29-Apc and HT29-βgal cells), the APC gene is mutated, which results in a constitutive activation of the Wnt/β-catenin signaling. Induction of wild-type APC upon zinc treatment induced a strong reduction in β-catenin signaling, returning to basal levels (12). Using real-time reverse transcription-PCR analysis, we observed that HT29 cells contained high endogenous levels of IFITM1, IFITM2, and IFITM3 and that induction of wild-type APC causes a net reduction on their level of expression within 24 hours (Fig. 4C). This indicated that IFITM gene expression is regulated by the β-catenin signaling. Altogether, our results strongly suggest that the IFITM family seems to be a good candidate target gene of the Wnt pathway in colorectal cancer.

The IFITM family is a potential new marker for human colorectal tumors. We confirmed that IFITM genes were markers...
of human colon cancer by analyzing their expressions in a large number of different human tumors and normal tissue. We used a cancer profiling array (Clontech) which contained normalized cdNA from 154 tumors and the corresponding normal tissue from individual patients. The array consisting of cdNA from 5 bladder, 10 breast, 10 cervix, 10 colon, 10 kidney, 3 liver, 10 lung, 10 ovary, 7 pancreas, 4 prostate, 10 rectum, 10 skin, 7 small intestine, 10 stomach, 10 testis, 10 thyroid, 3 trachea, 10 uterus, and 5 vulva tumor samples. For hybridization of this array, we used a human IFITM cdNA probe which detects all the family members (IFITM1, IFITM2, and IFITM3; Fig. 5A). After normalization with an ubiquitin probe, we found a highly statistically significant increase in IFITM mRNA levels in colon and rectal carcinomas ($P < 0.001$; Fig. 5B). IFITM expression was also elevated in stomach and lung carcinomas albeit to a lower significance ($P < 0.05$). We observed no statistically significant difference between normal and tumor tissue in the other tumor types. These data suggest that the up-regulation of IFITM gene expression is highly specific to human colorectal carcinogenesis and may be of clinical relevance for the diagnosis of these tumors.

Discussion

The identification of useful markers for the detection and diagnosis of human colon cancer is a major goal in cancer research, which also provides valuable information in the development of cancer. We used a murine FAP model to generate candidate markers and then tested them on the more genetically and environmentally heterogeneous human neoplasia. We used PCR-based subtractive hybridization to detect murine adenoma–associated genes in both the small and the large intestine from Apc$^{−/−}$ mice. Gene validation showed very similar gene expression profiles regardless of the location of the adenomas in the intestine. Of the 18 genes identified, we observed a large number of genes showing similar expression induction in mice and human intestinal neoplasia. This suggested a common biological process in the progression of mice and human intestinal tumors. Some of the genes we identified have already been reported as being overexpressed in human colon cancers, thus validating our approach (17, 18). However, we have also identified several members of the IFITM family as being new targets of Wnt/β-catenin signaling in the intestinal epithelium, which may prove useful as potential biomarkers for the detection of colorectal lesions.

Ifitm3 was first isolated from a genetic screen aimed at identifying the genes involved in the acquisition of germ cell competence (16). It was proposed that the epiblast cells having the highest expression of ifitm3 initiated germ cell specification and that homotypic association discriminated germ cells from somatic neighbors. Ifitm3 was then shown to belong to a murine gene family of five members (Ifitm1, Ifitm2, Ifitm3, Ifitm5, and Ifitm6) that are clustered within a 68 kbp genomic region on chromosome 7 (19). Several homologues exist in humans, cows, and rats and they are all evolutionarily conserved in mammals. The human homologues (IFITM1, IFITM2, and IFITM3) are also clustered on chromosome 11 within an 18 kbp genomic sequence (14, 19). These genes respond to type I and II IFNs and encode for IFN-induced transmembrane protein thought to be involved in the homotypic cell adhesion functions of IFN (14, 20). Although IFN signaling in colon cancer remains to be shown, it is known that IFN plays key roles in modulating the immune response and thus may control tumor development. It has been proposed that IFITM1 modulates the susceptibility to natural killer cells and the invasiveness of gastric cancer cells (21). Recent studies have shown that during embryogenesis, BMP4 and Wnt/β-catenin signaling were able to control the level of expression of these genes (15, 16). Our data showed that they are also induced after activation of the Wnt/β-catenin signaling during intestinal tumorigenesis. It has been recently shown that BrG-1, a component of the chromatin-remodeling complex SWI/SNF, is required for IFITM1 expression, suggesting that it plays an important role in regulating these genes (22). BrG-1 is also known to interact with β-catenin to promote target gene expression (23). Thus, the regulation of IFITM gene expression seems to be complex.

The identification of the IFITM family members as potential targets of β-catenin signaling in intestinal epithelium is particularly important as it provides new insights into the biology of human colorectal carcinogenesis and may prove to have clinically useful applications. IFITM family members are expressed in murine pluripotent embryonic stem cells (15) and in germ cell precursors (16). Our results showed that they are also expressed in the stem cell compartment of the intestinal tract. Using conditional Apc mutant mice, we showed that they were rapidly induced after deletion of Apc alleles which leads to the formation of adenomas. We also observed that their levels of expression remained high during the tumorigenic process. The tumorigenic process in the
intestinal epithelium is now known to be linked to a constitutive activation of the Wnt pathway leading to an abnormal process in the self-renewal of the stem and progenitor cells (8, 24). Therefore, it is tempting to propose that one function of this gene family is linked to maintenance and propagation of the pluripotent state. A recent study reported that Ifitm1 knock-down mice mimic the Wnt mutant phenotypes, suggesting that Ifitm1 was a key mediator of the Wnt response during gastrulation (15).

In our large-scale analysis of IFITM expression in human tumors, we found highly significant up-regulation in colorectal tumors. We also found that IFITM1, IFITM2, and IFITM3 were up-regulated in colorectal adenomas. Then this gene family could be a new potential relevant clinical colorectal tumor marker. The most widely used tumor marker for colon cancer is the carcinoembryonic antigen (CEA) tumor marker. As CEA is significantly less sensitive for diagnosing the earlier stage disease, it was abandoned as a marker.

Figure 4. IFITM gene expression correlates with adenoma formation and activation of β-catenin signaling. A, expression pattern of IFITM genes in tumor progression from Apc^1611^ mice. In situ hybridization (a-d) and immunohistochemical analysis (a-h) revealed basal levels of expression in the crypt of wild-type mice (a and e) and a high level of expression in abnormal crypt foci (A.C.F; b and f), microadenomas (c and g), and adenomas (d and h).

B, early induction of IFITM expression following a conditional invalidation of the Apc gene in the intestine. Representative H&E stained sections of jejunum from Apc^lox/lox^/vill-CreERT^2^ mice (a) and Apc^lox/lox^/vill-CreERT^2^ mice (b) 4 days after tamoxifen treatment. β-catenin (c and d) and IFITM (e and f) immunostainings of jejunum sections from Apc^lox/lox^/vill-CreERT^2^ mice (c and e) and Apc^lox/lox^/vill-CreERT^2^ mice (d and f) on day 4.

C, repression of IFITM gene expression after APC induction. Levels of IFITM and APC transcripts were analyzed by real-time quantitative reverse transcription-PCR at 24 hours after ZnCl2 treatment.
screening marker for early cancer and it has been used only for the detection of tumor recurrence after colorectal surgery. Therefore, it is important to identify new tumor markers with better diagnostic capabilities than CEA. IFITM genes seem to be a suitable histologic marker for human intestinal tumors and a potential indicator for the diagnosis of human colorectal cancer. The elevated production of IFITM may be detected in serum if secreted from tumor cells or from the exfoliated tumor cells in stools. Therefore, whether IFITMs can be used as part of a set of indices for early detection of human colorectal cancer needs to be investigated.

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References

3. Kitahara O, Furukawa Y, Tanaka T, et al. Alterations of IFITM genes in human tumors and normal tissues. A, cDNA probe which recognizes all the IFITM family members were hybridized on a cancer-profiling array (Clontech) containing cDNA from 154 human tumors (T) and the corresponding normal (N) tissue. B, results from (A) were normalized to the hybridization signals of an ubiquitin probe (data not shown) and were quantified by Image J software. Columns, mean of IFITM gene expression in one set of tumor tissue compared with the normal tissue; *, P < 0.001, significant differences between tumor and normal tissue.

Figure 5. Expression analysis of IFITM genes in human tumors and normal tissues. A, a cDNA probe which recognizes all the IFITM family members were hybridized on a cancer-profiling array (Clontech) containing cDNA from 154 human tumors (T) and the corresponding normal (N) tissue. B, results from (A) were normalized to the hybridization signals of an ubiquitin probe (data not shown) and were quantified by Image J software. Columns, mean of IFITM gene expression in one set of tumor tissue compared with the normal tissue; *, P < 0.001, significant differences between tumor and normal tissue.

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