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Inducible expression of microRNA-194 is regulated by HNF-1α during intestinal epithelial cell differentiation

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ABSTRACT

Maintenance of the intestinal epithelium is based on well-balanced molecular mechanisms that confer the stable and continuous supply of specialized epithelial cell lineages from multipotent progenitors. Lineage commitment decisions in the intestinal epithelium system involve multiple regulatory systems that interplay with each other to establish the cellular identities. Here, we demonstrate that the microRNA system could be involved in intestinal epithelial cell differentiation, and that microRNA-194 (miR-194) is highly induced during this process. To investigate this inducible expression mechanism, we identified the genomic structure of the *miR-194-2, -192* gene, one of the inducible class of miR-194 parental genes. Furthermore, we identified its transcriptional regulatory region that contains a consensus-binding motif for hepatocyte nuclear factor-1α (HNF-1α), which is well known as a transcription factor to regulate gene expression in intestinal epithelial cells. By chromatin immunoprecipitation assay and luciferase reporter analysis, we revealed that pri-miR-194-2 expression is controlled by HNF-1α, and its consensus binding region is required for the transcription of pri-miR-194-2 in vivo in an intestinal epithelial cell line, Caco-2. Our observations indicate that microRNA genes could be targets of lineage-specific transcription factors and that microRNAs are regulated by a tissue-specific manner in the intestinal epithelium. Therefore, our work suggests that induced expression of these microRNAs have important roles in intestinal epithelium maturation.

Keywords: microRNA; miR-194; Caco-2; differentiation; HNF-1α; intestine

INTRODUCTION

The intestinal epithelial system is a paradigm for the production of distinct cell lineages from multipotent progenitors (Crosnier et al. 2006). The molecular mechanism of balanced and continuous generation of intestinal epithelial cells has been extensively investigated, as it would be involved in the pathogenesis of gastrointestinal disorders such as intestinal epithelial tumors. Recent studies highlighted the critical roles of specific signaling pathways directing activation of certain transcription factors, such as Notch and Wnt pathways, in the development of intestinal epithelium, clearly indicating that its developmental program is under the control of dynamic gene regulatory networks (Sancho et al. 2004; Fre et al. 2005; Gregorieff and Clevers 2005; Stanger et al. 2005; Clarke 2006; Crosnier et al. 2006).

microRNAs (miRNAs) are 21–23-nucleotide (nt) noncoding RNAs that function as post-transcriptional regulators of gene expression in various species (Ambros 2004; Bartel 2004; Zamore and Haley 2005). miRNAs recognize their target(s) with the partially complementary sequences and repress their translation or modify their stability (Olsen and Ambros 1999; Jing et al. 2005). miRNAs play essential roles in diverse events, including control of developmental timing (Wightman et al. 1993), differentiation (Chen et al. 2004, 2006; Esau et al. 2004; Kim et al. 2006), apoptosis, cell proliferation (Brennecke et al. 2003; Cheng et al. 2005; Cimmino et al. 2005), and organ development (Giraldez et al. 2005).

miRNAs initially appear as relatively long transcripts called pri-miRNA, and it is now widely accepted that many

[3]These authors contributed equally to this work.

Abbreviations: miRNA, microRNA; SI, sucrase-isomaltase; kb, kilobases; LPH, lactase-phlorizin hydrolase; HNF-1α, hepatocyte nuclear factor-1 α; PCR, polymerase chain reaction; RT, reverse transcription; RACE, rapid analysis cDNA end; MEM, minimal essential medium; DMEM, Dulbecco's modified Eagle's Medium; PBS, phosphate buffered saline; FBS, fetal bovine serum.

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pri-miRNAs are transcribed by RNA polymerase II (Cai et al. 2004; Lee et al. 2004), suggesting that tissue- and time-specific expression of miRNAs is probably specified at the level of pri-miRNA transcription. Although hundreds of miRNAs were cloned in various species, the regulatory mechanisms of specific pri-miRNAs are still largely unknown. Studies regarding regulation of each pri-miRNA would therefore provide clues to the further understanding of miRNA biology in general or of particular functions in specific cell lineages.

In this study, we focused on the miRNA system as novel molecular machinery in intestinal epithelial cell differentiation. Our high-throughput miRNA expression profiling revealed a dynamic change of the miRNA expression pattern during Caco-2 differentiation, which is one of the most widely known intestinal epithelial differentiation models. In the model, we found that dozens of miRNAs were up- or down-regulated. Among such miRNAs, miR-194 was found to be highly up-regulated with tight tissue specificity. To know the precise regulatory mechanism, we determined the genomic structure of pri-miR-194-2. Given the genomic structure, we also identified a highly conserved genomic region close to the transcription start site of pri-miR-194-2 that contains a consensus binding motif for hepatocyte nuclear factor-1α (HNF-1α). Chromatin immunoprecipitation (ChIP) assay confirmed physical binding of HNF-1α to this conserved region in vivo, and other functional assays further showed the transcriptional role of HNF-1α for the pri-miR-194-2 transcription, suggesting that the conserved genomic region is the core promoter element for pri-miR-194-2 and that HNF-1α is the key transcription factor for the expression of this miRNA.

Because HNF-1α is one of the critical factors of intestinal epithelial gene expression during differentiation and maturation, miR-194 might constitute a certain part of its regulatory network, thereby contributing to the regulation of gene expression program in intestinal epithelial cells. Therefore, our work suggests that induced expression of miRNAs has an important role in intestinal epithelium maturation.

RESULTS

miR-194 is highly induced during intestinal epithelial cell differentiation

Adopting the differentiation system of intestinal epithelial cell line Caco-2, possible alteration of miRNA expression pattern during intestinal epithelial differentiation was examined. Induction of differentiation in Caco-2 cells was performed by conventional long-term confluent culture method. Following confirmation of maturation status by the differentiation markers such as lactase-phlorizin hydrolase (LPH) and sucrase-isomaltase (SI) (Fig. 1A), 156 mature miRNAs were quantified in differentiated and proliferative cells by the TaqMan-based high-throughput profiling method. As shown in Figure 1, B and C, several miRNAs were significantly up- or down-regulated during Caco-2 differentiation as judged by Ct values. Among such genes, expression of miR-133a and miR-133b has been previously detected in tissues other than intestine (Chen et al. 2006). Also, miR-146 has been reported to control Toll-like receptor and cytokine signaling (Taganov et al. 2006), whereas the miR-34 family has been shown to be involved in the p53 network (He et al. 2007). Inhibition of miR-148 and 210 increases the level of apoptosis, while inhibition of miR-152 decreases cell growth (Cheng et al. 2005). Consequently, differentially expressed miRNAs observed in the present experiment suggested that miRNA machineries that control general physiological events are involved in epithelial cell differentiation.

Although identification of sets of miRNA as regulators of general events is important, here, we rather sought to find some miRNAs that are regulated specifically in intestinal epithelial cells. Among differentially expressed miRNAs, miR-194 was one of the highly induced miRNAs during differentiation and showed the highest intensity in differentiated Caco-2 cells (Fig. 1D). Induction of mature miR-194 upon Caco-2 differentiation was also confirmed by Northern blot (Fig. 1E). Also, tissue distribution of mature miRNA-194 in an adult mouse was identified to be relatively specific in intestinal tracts (Fig. 1F), consistent with some previous results in other organisms such as zebrafish embryo (Wienholds et al. 2005).

These data collectively suggested that, among numerous regulated miRNAs, miRNA-194 is highly and specifically induced in intestinal epithelial cells during its differentiation.

Genomic structures of miR-194 primary transcripts

Given the high and specific expression of miR-194 in intestinal epithelial cells, we further sought to characterize this miRNA to know its regulation. According to miRBase, mature miR-194 can be derived from two separate loci on human genome that are registered as *miR-194-1* and *miR-194-2* (Fig. 2A). The miR-194-1 and miR-194-2 are encoded on human chromosomes 1 and 11, respectively. As like many other miRNAs, both miR-194-1 and miR-194-2 are so-called clustered miRNA and have cluster partners miR-215 and miR-192, respectively.

To examine whether either of the two loci can generate mature miR-194, we constructed miRNA expression vectors in which miR-194 loci were inserted downstream of the CMV promoter, and observed mature miR-194 generation using luciferase sensor reporter assay. As luciferase sensor mRNA have perfect complementary sequence against mature miR-194 in 3′UTR, if mature miR-194 is generated from the miR-194 loci, firefly sensor mRNA is cleaved by RNAi effect. Thus, miRNA generation could be observed as reduction of luciferase activity.

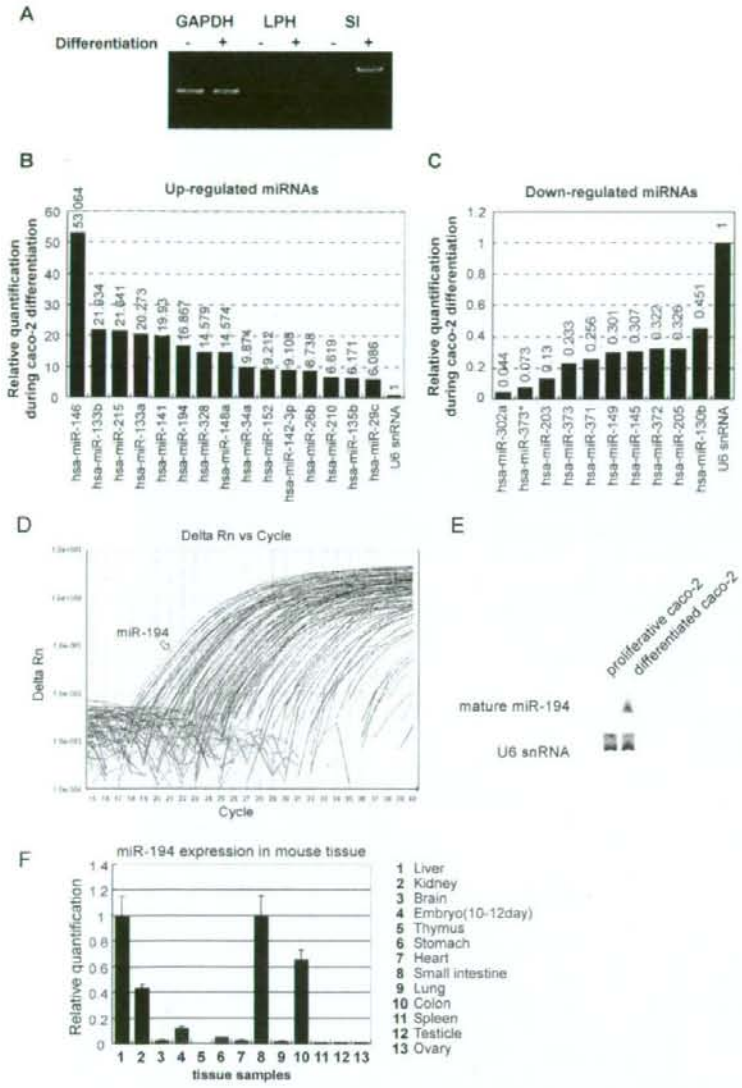

FIGURE 1. Altered expression profile of miRNA during Caco-2 differentiation. (*A*) Detection for differentiation markers by RT-PCR. RT-PCR was performed on total RNAs extracted from proliferative or differentiated Caco-2 cells. PCR products were separated by 5% acrylamide gel electrophoresis and stained by EtBr. (*B*) Up-regulated miRNAs during Caco-2 differentiation. A total of 156 miRNAs were quantified by TaqMan miRNA assays Human Panel–Early Access Kit. Relative Quantification (RQ) was normalized by U6 RNA endogenous control. Quantification results were arranged by RQ and cut-off detectors whose Ct values were more than 30 cycles in differentiated Caco-2 cells. (*C*) Down-regulated miRNAs during Caco-2 differentiation, observed in the quantification performed in *B*. (*D*) Amplification plot by TaqMan miRNA assay in differentiated Caco-2 cells. Arrow indicates amplification plot of miR-194. (*E*) Northern blot of mature miR-194. Total RNAs (30 μg) were separated in 8 M urea 12% PAGE and transferred to Nybond N+. After UV cross-linking, the membrane was hybridized with DIG-labeled RNA probe. Hybridized probes were detected by AP-conjugated anti-DIG antibody and visualized by CDP-star. (*F*) miR-194 expression in various mouse tissues. Expression of miR-194 was quantified by TaqMan miRNA assay. U6 snRNA was used as endogenous control. The expression level in small intestine was set to 1.

Forced expression of each locus showed marked decrease of the sensor luciferase activity in both Hela S3 and Caco-2 cells (Fig. 2B). As the results of this assay showed that both loci are able to generate mature miR-194, we sought to examine which locus is the origin of miR-194 that is induced upon differentiation of intestinal epithelial cells. Northern blot analysis of pre-miRNAs showed up-regulation of both pre-miRNAs (Fig. 2C), whereas TaqMan miRNA assay showed up-regulation of miR-194, miR-192, and miR-215 upon Caco-2 differentiation (Fig. 2D). Quantitative RT-PCR using a reverse-transcribed sample as a standard template also showed induction of pri-miRNA from both loci upon differentiation, but a relatively higher induction of pri-miR-194-2 was observed, compared with pri-miR-194-1 (Fig. 2E, left). Absolute quantification using genomic DNA as a standard template, which enables quantitative comparison between pri-miR-194-1 and pri-miR-194-2, also demonstrated that expression of pri-miR-194-2 was more abundant in differentiated Caco-2 cells, compared with pri-miR-194-1 (Fig. 2E, right).

Having these observations, we focused on miR-194-2 cluster and further characterized this locus. Upon the database search, a registered cDNA, AK092802, was found as a putative 5′ part of the pri-miR-194-2 transcript (Fig. 3A). As shown by RT-PCR, the registered transcript AK092802 and the region encoding pre-miR-194-2 were transcribed primarily as a single RNA (Fig. 3B). Also, the 5′ region of the transcript including the transcription start site of pri-miR-194-2 was determined by 5′ RACE. Although 5′ RACE presented two fragments, sequence analysis revealed that these two fragments arise from the same transcription start site, while the difference in length was due to splicing (Fig. 3C). Furthermore, we performed 3′ RACE to identify the structure of pri-miR-194-2, -192 (Fig. 4A). The identified pri-miRNA shared the same 3′ terminal with that of another registered transcript, AW207381 (Fig. 4B), and induced

expression of the entire transcript was confirmed during Caco-2 cell differentiation (Fig. 4C). Therefore, we examined the genomic structure of pri-miR-194-2 that gives rise to a part of the inducible miR-194 during intestinal epithelial cell differentiation.

Identification of the core promoter element for pri-miR-194-2

To know the transcriptional mechanism, we then sought to analyze the promoter region of pri-miR-194-2. Upstream genomic region close to the transcription start site of pri-miR-194-2 contains several highly conserved regions among human, mouse, rat, and dog (from −162 to +21 with respect to the transcription start site of AK092802) (Fig. 5A). To identify the promoter region, we constructed reporter plasmids carrying various genomic sequences around the transcription start site of pri-miR-194-2 (Fig. 5B) and subjected them to luciferase assay. Results demonstrated that the region from −162 to +21 had a high promoter activity in differentiated Caco-2 cells, comparable to that of the longest region from −1003 to +358 (Fig. 5B). In contrast, deletion of the conserved region dramatically reduced promoter activity.

Since conserved regions in a gene promoter are expected to contain regulatory elements, we focused on such region found within the pri-miR-194-2 promoter. Luciferase assay using a mutated construct revealed that the region from −70 to −52 is critically required for the pri-miR-194-2 promoter activity that is driven by the identified conserved region in differentiated Caco-2 cells, indicating that the corresponding region is the core element of pri-miR-194-2 promoter (Fig. 5C).

HNF-1α regulates pri-miR-194-2 promoter

By a computational search for potential motifs of transcription factors, a putative binding site for HNF-1α was found in the conserved core element of pri-miR-194-2 promoter (Fig. 6A). HNF-1α is a member of a class of transcription factors that is distantly related to homeobox proteins, which contains a DNA binding domain. In addition, HNF-1α has been formerly described as a key transcriptional activator during Caco-2 differentiation (Wu et al. 1994; Mitchelmore et al. 1998; Boudreau et al. 2001, 2002; van Wering et al. 2002). Thus, we hypothesized that HNF-1α might bind to the core element of pri-miR-194-2 promoter and contribute to upregulate pri-miR-194-2 transcription. Indeed, HNF-1α was shown to physically interact to the core element of the pri-miR-194-2 promoter in Caco-2 cells, as judged by ChIP assay (Fig. 6B). Furthermore, forced expression of HNF-1α activated the promoter of both pri-miR-194-2 and SI in HeLa S3 cells, which usually do not express miR-194 (Fig. 6C). Forced expression of HNF-1α in Caco-2 cells also showed an additive effect on the promoter activity of pri-miR-194-2. Mutation in the HNF-1α binding site resulted

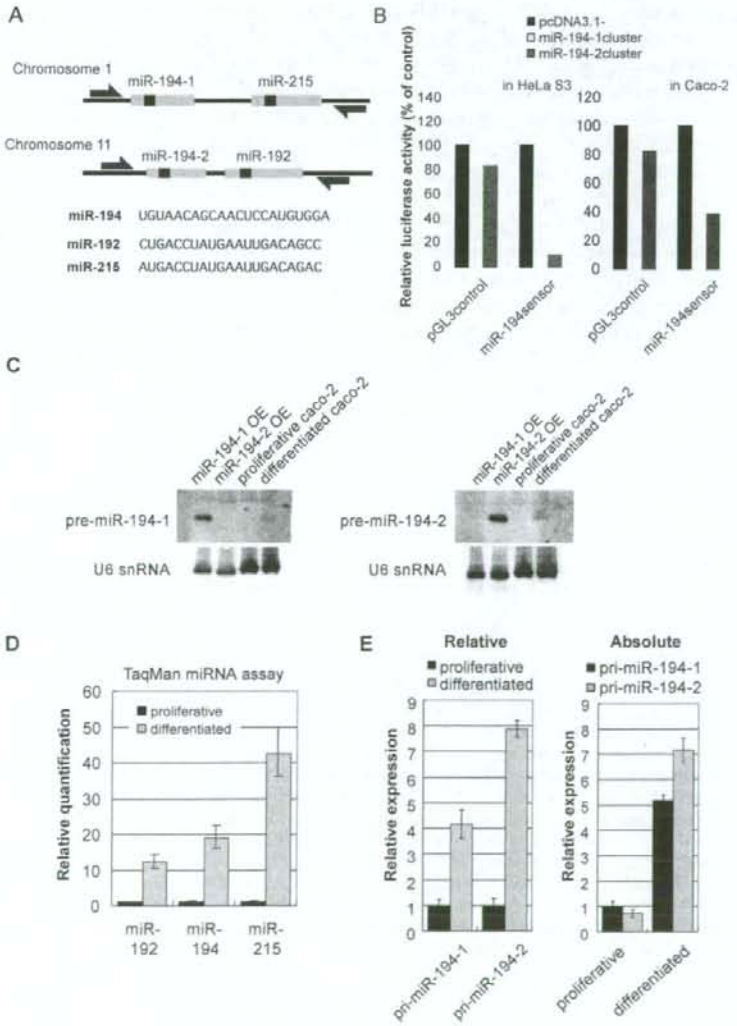

FIGURE 2. (Legend on next page)

in almost complete loss of the pri-miR-194-2 promoter activity, confirming the requirement of HNF-1α binding to the corresponding site for pri-miR-194-2 transcription. Taken together, these data suggest that HNF-1α is a key transcriptional regulator of pri-miR-194-2 and activates its promoter activity by physically binding to the core promoter element.

DISCUSSION

In the present study, we identified the structure of pri-miR-194-2 and determined both transcription start site and 3′ end using 5′ RACE and 3′ RACE, respectively. As pri-miRNAs could be either a protein-coding RNA or a noncoding RNA, we searched in silico for a candidate open reading frame (ORF) within a pri-miR-194-2, -192 transcript and did not find a single protein-coding ORF. In addition, conserved genomic region of pri-miR-194-2, -192 was restricted to the region encoding miRNA hairpin structures. Therefore, we suggest that pri-miR-194-2, -192 is a noncoding RNA.

We also identified that miR-194-2 and miR-192 are encoded within the intron but not in the exon (Fig. 4B). To assure this, we have shown that mature miR-194 surely arises from the miR-194-2, -192 cluster region, a partial sequence of the second intron (Fig. 4B), by forced expression of the corresponding region (Fig. 2B). This means that these miRNAs are processed by splicing and cropping of the pri-miRNA in the nucleus. Indeed, although the PCR product shown in Figure 3B contained the second intron, the PCR product shown in Figure 4C lacked the corresponding intron sequence. This difference may be attributed to the relatively short extension time (1.5 min) used for the PCR in Figure 4C, for detection of the transcript containing the intron (4.8 kb), but also suggests that the second intron containing the miR-194-2, -192 cluster is primarily transcripted along with the three exons but is rapidly spliced out from the pri-miR-194-2.

On the other hand, concerning these processing steps, a model of intronic miRNA processing was recently suggested (Kim and Kim 2007). According to their model, miRNA-harboring intron is detained while other introns are rapidly spliced out. In our study, RT-PCR analysis in Figure 3B showed 2.4-kbp and 1.8-kbp products, which might represent the nascent transcript and the partially spliced transcript for pri-miR-194-2, respectively. As the reverse primer used in the present RT-PCR is placed within the second intron, the result may indicate that the first intron is processed more rapidly, compared with the miRNA-harboring second intron. Therefore, pri-miR-194-2 might be processed through the proposed model.

Also in our study, we demonstrated that miR-194 is highly expressed in differentiated intestinal epithelial cells. Induced expression of miR-194 could be accounted by the regulatory mechanism of *miR-194-2* gene in that a tissue-associated transcription factor, HNF-1α, plays a central role in its transcription. Although HNF-1α is well known for its regulatory roles of various genes specific for the intestine, its expression is not tightly restricted to the intestine but is also found in the liver or the kidney (Mendel and Crabtree 1991). However, mature miR-194 has been reported to appear not only in the intestine but also in the liver and in the kidney (Lagos-Quintana et al. 2003; Krutzfeldt et al. 2005; Wienholds et al. 2005; Kato et al. 2007). Our TaqMan miRNA analyses have also confirmed expression of miR-194 in these tissues (Fig. 1F). These findings are consistent with our present study describing the regulation of miR-194 expression by HNF-1α. Recent studies, however, have highlighted the regulation of mature miRNA generation at the post-transcriptional processing level (Thomson et al. 2006; Viswanathan et al. 2008) Although our data show consistent increase of mature miR-194 upon increase of its pri-miRNAs, there remains a possibility that expression of mature miR-194 might also be regulated at the processing level.

Our promoter analyses revealed that the consensus motif for HNF-1α found within the conserved region of the pri-miR-194-2 promoter plays a critical role in induction of *miR-194-2* gene upon Caco-2 cell differentiation. HNF proteins are known to interact with other transcription factors to regulate the expression of various intestine-specific genes. For example, different members of the HNF family that are expressed in the intestine, such as HNF-1β and

FIGURE 2. Expression of miR-194-2, -192 cluster was induced during Caco-2 differentiation. (*A*) Genomic organization of miR-194 clusters. miR-194-1 and miR-215 are located within an ~400-bp region in chromosome 1. miR-194-2 and miR-192 are located within an ~300-bp region in chromosome 11. Open arrows represent pri-miRNA detection primers used in *E*. (*B*) Both miR-194 loci potentially express mature miR-194. miR-194 expression vector and pGL3miR-194sensor were cotransfected into HeLa S3 cells (*left*) or Caco-2 cells (*right*). As pGL3miR-194sensor contained a sequence completely complementary to mature miR-194 in 3′UTR of luciferase, expression of mature miR-194 is detected by reduced firefly luciferase activity by RNAi. Firefly luciferase reporter activities were normalized by *Renilla* luciferase. The mean value of cells cotransfected with pcDNA3.1 mock vector was set to 100. (*C*) Northern blot of the pre-miR-194s, each arising from distinct loci. Total RNA (30 μg) extracted from proliferative or differentiated Caco-2 cells that were pretreated with Dicer siRNA was analyzed. Positive control (10 μg total RNA) was obtained from Dicer knockdowned 293 cells transfected with miR-194 cluster expression vectors (miR-194-1 OE and miR-194-2, OE, respectively). Probes were designed to hybridize around the loop sequence of each transcript. (*D*) Inducible expression of miRNAs from miR-194 clusters during Caco-2 differentiation. Expression of miR-192, miR-194, and miR-215 were quantified by TaqMan miRNA assay. U6 snRNA was used as endogenous control to normalize expression of miRNAs. The expression level of each miRNA in proliferative Caco-2 cells was set to 1. (*E*) Two distinct miR-194 loci contribute to its induced expression during Caco-2 cell differentiation. RT-PCR was performed with primers indicated in *A*, and GAPDH was used as endogenous control. For relative quantification, expression of each pri-miR-194 within proliferative Caco-2 was used as a standard (*left*). For absolute quantification, genomic DNA was used as a standard. Bar graph is drawn so that pri-miR-194-1 in proliferative Caco-2 cells is set to 1 (*right*).

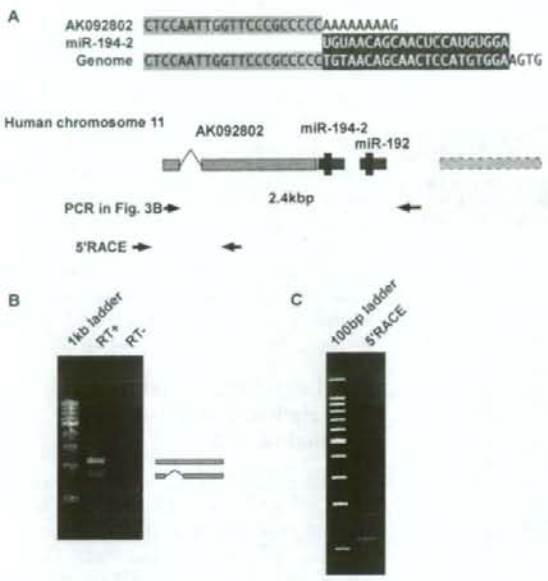

FIGURE 3. Identification of the transcription start site of pri-miR-194-2. (*A*) Genomic sequence and EST information surrounding miR-194-2. 3′ End of AK092802 is adjacent to 5′end of miR-194. Arrow bars represent predicted PCR products. (*B*) AK092802 and miR-194-2, -192 cluster are linked in a single transcript. RT-PCR was performed by total RNA extracted from differentiated Caco-2 cells, using primers indicated in *A*. PCR products were separated by 1% agarose gel electrophoresis and stained by EtBr. Sequence analysis of the amplified products revealed that the 2.4-kbp fragment contained an intronic sequence, while the intronic sequence was spliced out in the 1.8-kbp fragment. (*C*) 5′ RACE analysis of pri-miR-194-2. 5′ RACE was performed using random primers instead of oligo dT primer. PCR was performed by primers indicated in *A*. PCR products were separated by 5% polyacrylamide gel electrophoresis and stained by EtBr. Consistent with *B*, two fragments were observed, and sequence analysis of these fragments revealed that difference was due to an intronic sequence. Both fragments had the same transcription start site.

HNF-4, coordinately enhance target gene expression (Wu et al. 1994; Hu and Perlmutter 1999, 2002; Boudreau et al. 2001). Furthermore, GATA family and caudal related homeobox protein Cdx2 also coordinately enhance expression of intestine-specific genes (Krasinski et al. 2001; Boudreau et al. 2002; Wang et al. 2004). It is therefore speculated that the consensus motif for HNF-1α found within the core promoter region of pri-miR-194-2 may play a pivotal role in different aspects of regulation for this miRNA. Evolutional conservation of this consensus site among phylogenetically distant species further indicates a strong functional link between HNF-1α and miR-194-2 expression and also its significance in essential physiological events.

From the view of environmental regulation of miRNA, a recent study reported that miR-192 ectopically appears in diabetic renal glomeruli and that TGF-β is involved in the induction of this miRNA (Kato et al. 2007). As we have determined in the present study that miR-192 is the clustering partner of miR-194-2, it is possible that TGF-β may also regulate expression of miR-194. Interestingly, it is reported that TGF-β1 can modulate the differentiation process of Caco-2 cells in certain environments (Schroder et al. 1999). Therefore, it is of interest to examine the role of TGF-β upon regulation of miR-194 expression, as this cytokine shows diverse effects on intestinal physiology.

In conclusion, miR-194 is highly induced during intestinal epithelium differentiation, and pri-miR-194-2 expression

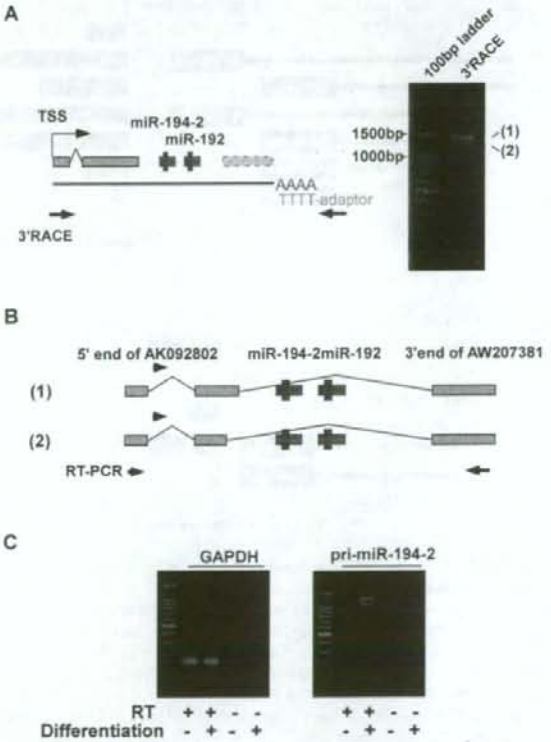

FIGURE 4. Identification of pri-miR-194-2 structure. (*A*) 3′ RACE analysis of pri-miR-194-2. Schematic representation of the primers used in the analysis is shown (*left*). PCR products were separated by 2% agarose gel electrophoresis and stained by EtBr (*right*). Two fragments are shown, designated as 1 and 2. (*B*) Schematic representation of pri-miR-194-2 structures. Sequence analysis revealed that difference in fragments 1 and 2 observed in *A* comes from the difference in length of the second exon. Both fragments had the same 3′ end, which also coincided with the 3′ end of AW207381. (*C*) Induced expression of pri-miR-194-2 during Caco-2 cell differentiation. To confirm the increased expression of the identified pri-miR-194-2 transcript during Caco-2 differentiation, RT-PCR was performed using primers represented in *B*. PCR products were separated by 2% agarose gel electrophoresis, and stained by EtBr.

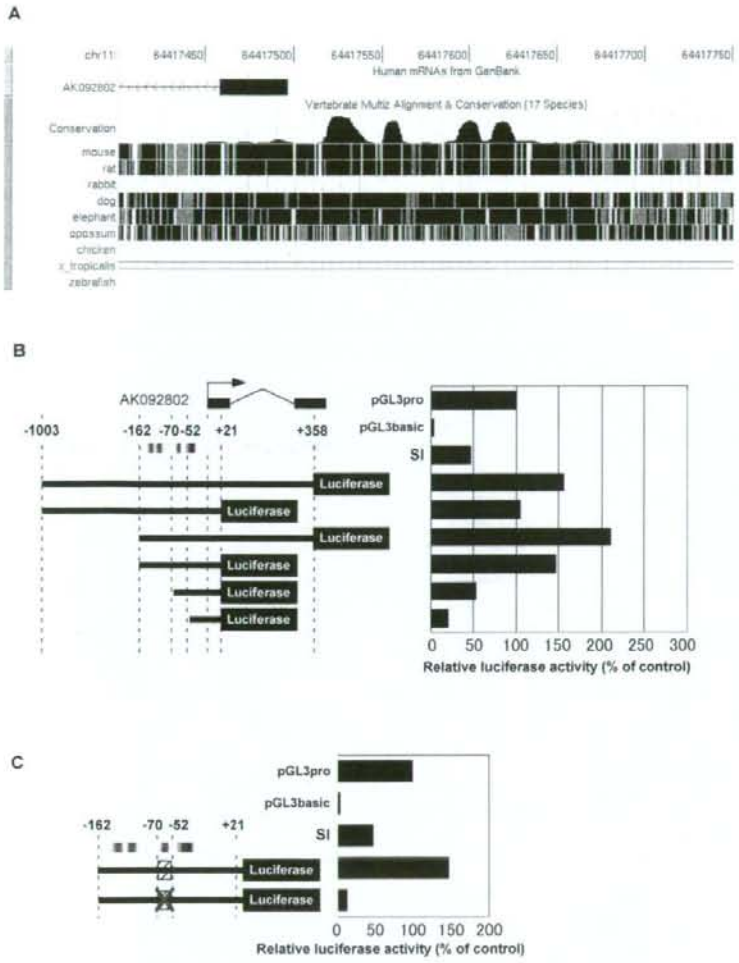

FIGURE 5. Potential HNF-1α binding element is located in the pri-miR-194-2 promoter. (*A*) Conserved sequences found within the pri-miR-194 promoter region. This figure is derived from the UCSC genome browser. (*B*) Schematic representation of human miR-194-2 promoter reporter constructs (*left*) and analysis of their promoter activity (*right*). Firefly luciferase reporter plasmids were constructed containing various regions of the putative pri-miR-194-2 promoter between positions −1003 to +358, designated with respect to the 5′end of AK092802. Arrow indicates transcription start site of pri-miR-194-2. pGL3 promoter (pGL3 pro) contains SV40 promoter, while pGL3basic has no promoter sequence upstream of the luciferase coding region. (*C*) Mutation of the region from −70 to −52 dramatically reduced pri-miR-194-2 promoter activity in Caco-2 cells. A scrambled sequence was introduced in the mutant pri-miR-194-2 promoter. Luciferase activities were normalized by *Renilla* luciferase activities.

is regulated by HNF-1α. As HNF-1α is one of the critical regulators of intestinal epithelial gene expression, control of miR-194 by this transcription factor adds miRNAs to the regulatory network of gene expression in intestinal epithelial cells. Therefore, the present work suggests that induced expression of miRNAs by tissue-specific transcription factors has an important role in intestinal epithelium maturation.

MATERIALS AND METHODS

Cell culture

Caco-2 cells were cultured in Minimal Essential Medium (Sigma) supplemented with nonessential amino acids and 10% heat-inactivated fetal bovine serum. For the differentiation assay, cells were seeded onto collagen-coated plate, and growth medium was changed every 3 d. HeLa S3 and 293 cells were cultured in Dulbecco's modified Eagle medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum.

RNA extraction, reverse transcription, and real-time quantitative PCR

Total RNA extraction was performed using miReasy mini kit with DNase I treatment (Qiagen). For detection of differentiation markers, LPH and SI, total RNAs were reverse transcribed by QuantiTect Reverse Transcription Kit (Qiagen). Semi-quantitative PCR was performed using LA Taq (Takara) with specific primer sets as follows:

LPH primer F 5′-TTTCTGTACGGACGGT TTCC-3′ and
LPH primer R 5′-AGAAAACGTGTCCCA AATGC-3′;
SI primer F 5′-AATCAGATGGCACAGGG TTC-3′ and
SI primer R 5′-TTCCTTTCCCCCATACAT GA-3′;
GAPDH primer F 5′-GAAGGTCGGAGTC AACGGATTT-3′ and
GAPDH primer R 5′-ATGGGTGGAATCA TATTGGAA-3′.

Quantification of mature miRNAs was performed by TaqMan miRNA assays Human Panel–Early Access Kit (Applied Biosystems) according to the manufacturer's instruction. As the kit did not contain probes for miR-192, data for miR-192 are not included in Figure 1B. Quantification of individual miRNAs (Fig. 2D) was performed by a TaqMan miRNA assay kit (Applied Biosystems).

Northern blot

To synthesize DIG-labeled RNA probe, vectors were constructed by inserting the following oligonucleotides into pcDNA3:

For mature miR-194 detection, 5′-TCCACATGGAGTTGCTGT TACA-3′;
For pre-miR-194-1 detection, 5′-AACTCCATGTGGACTGTG TACCAATTTCCAGTGGAGATGC-3′; and

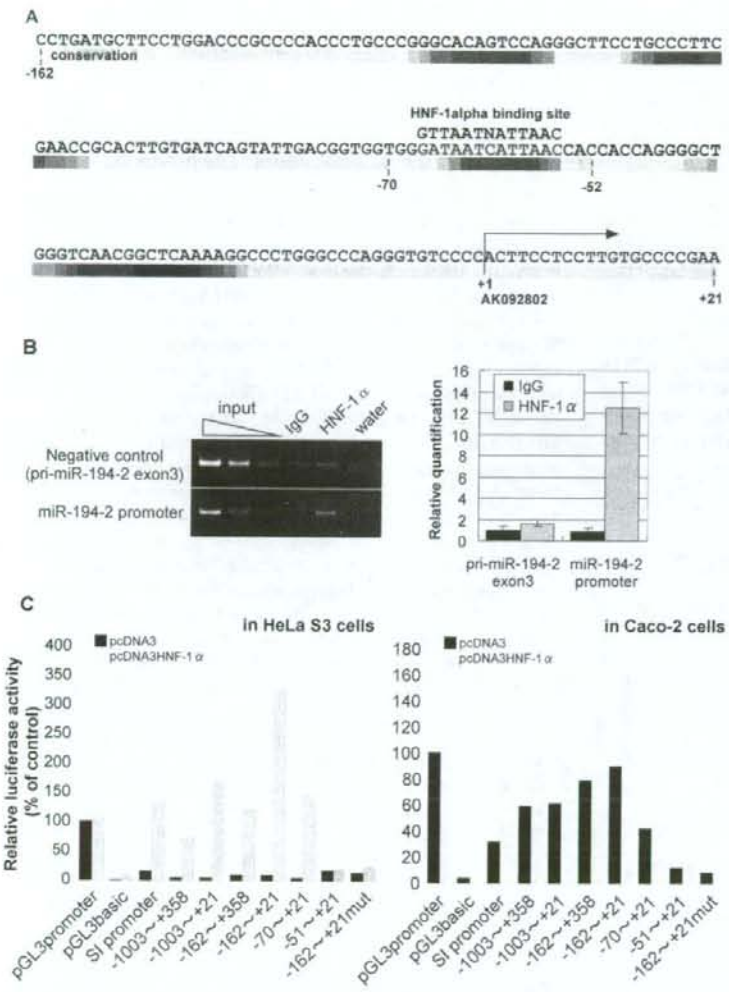

FIGURE 6. HNF-1α binds and regulates the miR-194-2 promoter in Caco-2 cells. (*A*) Sequence of pri-miR-194-2 promoter between −162 and +21. The region between −162 and +21 is partially conserved among human, mouse, and dog. Conserved sequences are indicated as density map (higher conservation becomes black). The transcription start site is indicated by an arrow. Consensus sequence of HNF-1α binding site is located at region between −67 and −55. (*B*) Chromatin immunoprecipitation (ChIP) analysis showing binding of HNF-1α to the miR-194-2 promoter in vivo in Caco-2 cells. Fixed chromatin from differentiated Caco-2 cells was prepared and immunoprecipitated either by anti HNF-1α antibody or normal goat IgG. ChIP primers were designed to amplify the region containing putative HNF-1α binding site in the pri-miR-194-2 promoter. Negative control primers were designed to amplify exon 3 of the pri-miR-194-2. PCR products were separated by acrylamide gel electrophoresis and stained by EtBr (*left*). Results of the quantitative PCR are shown as the relative amount of precipitated chromatin, in which chromatin precipitated by goat-IgG is set to 1. (*C*) Human pri-miR-194-2 promoter activity is up-regulated by forced expression of HNF-1α. Various luciferase reporter plasmids were cotransfected with either pcDNA3 mock vector (black bar) or HNF-1α expression vector (open bar) into HeLa S3 cells (*left*), or Caco-2 cells (*right*). SI-promoter contains promoter sequence of the sucrase-isomaltase (SI) gene, which is formerly reported to be up regulated by HNF-1α. Luciferase activities were normalized by *Renilla* luciferase activities.

For pre-miR-194-2 detection, 5′-AACTCCA TGTGGAAGTGCCCACTGGTTCCAGT GGGGCTGC-3′.

DIG-labeled RNA probes were synthesized by DIG RNA labeling kit (Roche). For pre-miRNA detection, knockdown of Dicer was performed before RNA extraction, using siRNA. The Dicer siRNA was transfected into cells by LipofectAmine2000 (Invitrogen). The sequence of the Dicer siRNA was as follows:

Dicer siRNA sense, 5′-UGCUUGAAGCAG CUCUGGAdTdT; and
Dicer siRNA anti-sense, 5′-UCCAGAGCU GCUUCAAGCAdTdT.

The letters "dT" represent deoxythymidine. Thirty micrograms of total RNA was separated by 8 M urea PAGE and transferred to Nybond N+ (GE Healthcare). After UV cross-linking, the membrane was hybridized with DIG-labeled probes in hybridization buffer (50% formamide, 5× SSC, 0.1% SDS, 2× Denhardt's solution, and salmon sperm DNA). Detections were achieved by AP-conjugated anti-DIG antibody (Roche) and CDP-star (GE Healthcare).

RT-PCR for pri-miRNA

Pri-miRNA cluster detection PCR was performed using Quantitect SYBR PCR kit (Qiagen) with specific sets of primers as follows:

pri-miR-194-1 F, 5′-AGCGTTTCAAATCT ACCAGT-3′;
pri-miR-194-1 R, 5′-TATCTTCTGTGTACC TGCCA-3′;
pri-miR-194-2 F, 5′-ATGATAAGAAGCCT CGGTGA-3′; and
pri-miR-194-2 R, 5′-GTGGGACCATGAGT GCTGCA-3′.

AK092802 and miRNA cluster RT-PCR (Fig. 3B) was performed with the following primers, and the sequence of the detected PCR products were confirmed by direct sequencing. Forward primer downstream of the transcription start site (DTSS F) was 5′-TTCCTCCTTGTGCCCCGAAG-3′, and pri-miR-194-2 R was used as the reverse primer (this primer is placed within the second intron). Template cDNA was prepared by reverse-transcription by SuperScript II (Invitrogen) using oligo dT primer.

RT-PCR for pri-miR-194-2 entire transcript (Fig. 4C) was performed with the following primers, Forward primer was DTSS F, and reverse primer was; 5′-CATCCCAGCCA CAGAGCATC-3′.

RACE analysis

5′ RACE was performed using GeneRacer kit (Invitrogen) according to manufacturer's protocol, except for the use of random primer in reverse transcription. PCR amplification of 5′ end of pri-miR-194-2 was performed by touchdown PCR by LA Taq (Takara) using GeneRacer 5′ primer and reverse primer 5′-CAGCAGGCA TTTTGGGAGAC-3′. Amplified 5′ RACE fragments were cloned into pGEM T-Easy (Promega) for sequence analysis. 3′ RACE was also performed using GeneRacer kit (Invitrogen). PCR Amplification of the 3′ end of pri-miR-194-2 was also performed using the gene-specific forward primer 5′-TTCCTCCTTGTGCCCCGAAG-3′ and GeneRacer 3′ primer. Amplified 3′ RACE fragments were also cloned into pGEM T-Easy (Promega) for sequence analysis.

Vector constructions

miRNA expression vectors were constructed by cloning miRNA coding region into pcDNA3.1(−) (Invitrogen). miRNA coding regions were amplified by Phusion DNA polymerase (New England Biolab) from Caco-2 genomic DNA using primers as follows:

miR-194-1cluster F, 5′-ATACTCGAGTAGAACATGAATAAATC GAGAC-3′;
miR-194-1cluster R, 5′-TATGAATTCTTACTCAATACATTTA CATGGTAG-3′;
miR-194-2cluster F, 5′-ATACTCGAGCCTGGGGCCACGAAGAC TGG-3′; and
miR-194-2cluster R, 5′-ATAGGATCCGGGGAATGAGACAGAG GGAGG-3′.

miR-194-2 promoter deletion variants were amplified by the following primers and cloned into pGL3basic between XhoI-MluI sites. Cloning primers were as follows:

−1003 primer F, 5′-ATGCACGCGTATGTCACCACCAGGGGT CGC-3′;
−162 primer F, 5′-ATGCACGCGTCCTGATGCTTCCTGGACCCG-3′;
−70 primer F, 5′-ATGCACGCGTTGGGATAATCATTAACCACC-3′;
−51 primer F, 5′-ATGCACGCGTCACCAGGGGCTGGGTCAACG-3′;
+21 primer R, 5′-TCGACTCGAGTTCGGGGCACAAGGAGGAA G-3′; and
+358 primer R, 5′-TCGACTCGAGACTCAGCCTGGCGGCCCTTC-3′.

Scrambled mutation was induced using Scrambled F 5′-GATAC TAACGTAAGCCACCAGGGGCTGGGT-3′ and Scrambled R 5′-ACGTTAGTATCATAGCCACCGTCAATACTG-3′. HNF-1α expression vector was constructed by cloning HNF-1α cDNA into pcDNA3 (Invitrogen). HNF-1α primers were 5′-ATG CAAGCTTGCCACCATGGTTTCTAAACTGAGCCAGC-3′ and 5′-TAATGAATTCTTACTGGGAGGAAGAGGCCA-3′.

Transfection and luciferase assay

Transfections were performed using LipofectAmine2000 (Invitrogen) according to the manufacturer's protocol. For examination of miRNA generation, miRNA expression vector, luciferase miRNA-sensor vector, and pRL-TK were mixed (10:9:1), and cotransfected into cells were cultured in 96-well plate. For examination of miRNA promoter activity, the HNF-1α expression vector, luciferase miRNA promoter vector, and pRL-TK were mixed (20:19:1) and cotransfected into cells cultured in 96-well plate. Luciferase activity was measured by the Dual luciferase assay kit (Promega).

ChIP assay

To cross-link chromatin, differentiated Caco-2 cells were treated with 1% formaldehyde for 10 min at room temperature. Cross-linking was stopped by addition of 0.125 M glycine. After being washed twice with ice-cold PBS, cells were resuspended in NP-40 nuclear extraction buffer (10 mM HEPES at pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40, and protease inhibitor) and centrifuged at 3000 rpm for 10 min. Crude nuclei were resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl at pH 8.0, and protease inhibitor), and extensively sonicated by Bioruptor. Sonicated chromatin was centrifuged at 15,000 rpm, and the supernatant was collected (input control). The supernatant was mixed with 9 vol of ChIP dilution buffer (11 mM Tris/HCl at pH 8.0, 154 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1.1% Triton X-100, 0.11% sodium deoxycholate, and protease inhibitors) and precleared with preblocked Protein G–Sepharose (GE Healthcare). Precleared chromatin was immunoprecipitated with 5 μg of anti-HNF-1α antibody (Santa Cruz, sc-6547) or 5 μg of normal goat IgG (Vector Laboratory, I-5000), and the immuno complexes were collected by preblocked Protein G–Sepharose. The beads were washed sequentially by RIPA, RIPA containing 500 mM NaCl, LiCl wash buffer, and twice by TE. Collected chromatin were eluted in ChIP elution buffer (1% SDS, 100 mM $NaHCO_3$), adjusted to 200 mM NaCl, and incubated for at least 6 h at 65°C to reverse cross-link. After treatment with RNase A (Nippongene) and Proteinase K (Roche), DNA fragments were extracted by phenol/chloroform and ethanol precipitation. Quantitative PCR was performed with Quantitect SYBR PCR kit (Qiagen) using the following primers: miR-194-2 promoter ChIP primer F, 5′-TGATCAGTATTGACGGTGGTG-3′; primer R, 5′-AAGGAGGAAGTGGGGACAC-3′. Also used were negative control (exon3 of pri-miR-194-2) primer F, 5′-CCCACTGAC CTGTGTCCTTT-3′; primer R, 5′-AGAGGGGTTGGAGGTGAG AC-3′. Detected PCR products were sequenced after cloning into pGEM T-Easy vector (Promega).

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クローン病の皆さんへ

知っておきたい
治療に必要な基礎知識

難治性炎症性腸管障害に関する調査研究班（渡辺班）

2009年2月作成

〈目　次〉

1. 主任研究者からのメッセージ

炎症性腸疾患と呼ばれる、潰瘍性大腸炎とクローン病は、「難病」として厚生労働省の特定疾患に指定されています。どちらの病気も、以前は欧米に比べて病気にかかる率が低く、1/5～1/10程度と考えられていましたが、ここ10年、両疾患ともに患者数は増加の一途をたどっており、これからも増えていくことが予想されています。

潰瘍性大腸炎とクローン病は、いまだ原因が解明されておらず「難病」と言われていますが、適切に治療が行われれば決して命を脅かす病気ではなく、多くの患者さんでは適切な治療により、普通の生活を送ることができる「寛解」に導くことが可能です。

「潰瘍性大腸炎・クローン病は治らない病気」と悲観する患者さんもおられますが、その他のほとんどの病気や生活習慣病も炎症性腸疾患と同じように原因不明で、慢性かつ根本的治療法がありません。さらに「適切な治療が行われれば普通に生活ができ、適切な治療が行われなければ大変」なのは炎症性腸疾患もその他の病気も同じなのです。

したがって、炎症性腸疾患を特殊な病気と考えることなく、自分の病気とその治療をしっかりと理解し、適切な治療をきちんと継続していくことが、「病気とうまく付き合う」ために最も重要なことになります。

今回、皆さんの病気と治療法の理解に役立てていただけるように小冊子を作成しました。この小冊子が、皆さんのより良い生活を送るための一助になることを切に願っています。

厚生労働科学研究費補助金 難治性疾患克服研究事業
難治性炎症性腸管障害に関する調査研究班
主任研究者　渡辺　守
(東京医科歯科大学 消化器内科)

2. クローン病とは

ニューヨークのマウントサイナイ病院の医師であるクローンらが1932年に最初に報告したことからクローン病と呼ばれている病気です。

クローン病は口から肛門まで消化管のどの部位にも炎症が生じる可能性があります。炎症が生じた部位では粘膜が傷ついてはがれて(潰瘍)、腹痛や頻回の下痢、血便などの症状が現れます。

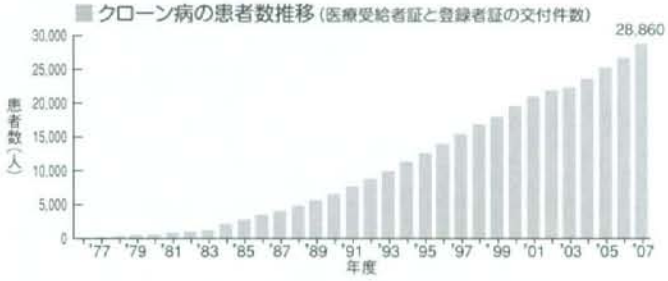
病気の原因は、遺伝的な要因に腸内細菌や食餌など様々な環境因子が重なり、通常は身体を防御するために機能している免疫に異常をきたすことで、この病気が生じると考えられています。

クローン病は、さまざまな症状がある状態を活動期、治療により症状が治まった状態を寛解期と言いますが、この活動期と寛解期を繰り返すことがこの病気の特徴です。さらに、経過の中で腸管が硬く狭くなったり(狭窄)、腸管に孔が開いて腸管と腸管あるいは腸管と皮膚がつながったり(瘻孔)することがあり、薬による治療で症状が抑えられない場合は手術を行う場合も稀ではない病気です。

したがって、治療により一旦、寛解期に入っても、再び消化管に炎症が生じたり(再燃)、新たな部位に炎症が生じること(再発)を予防するために長期にわたる治療が必要になります。

1)増え続けているクローン病

この病気は、以前は稀な疾患とされていましたが、その後増加し続け、2007年度末には約2万9千人の患者さんが登録されています。男女比は2対1と男性に多く、発症は20歳代がピークです。



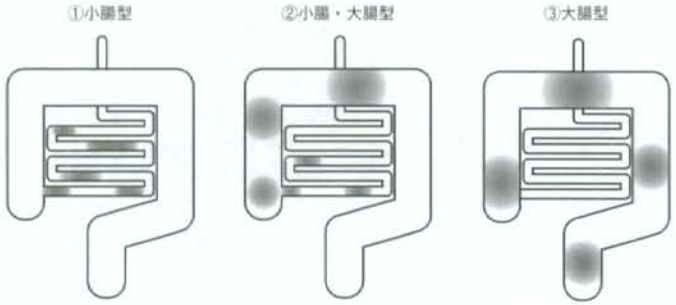
3. クローン病の治療に際して

クローン病では、腸管の病変（潰瘍、狭窄、瘻孔など）の種類や、病変部位、さらには重症度（炎症や症状の強さ）によって、いろいろな薬の種類やその投与法（内科的治療）、さらには外科的治療が選択されます。

1）あなたの病変の部位は

クローン病の病変（潰瘍、狭窄、瘻孔など）は、消化管のどの部位にも生じます。病変の部位により、小腸だけに病変がみられる小腸型、小腸だけでなく大腸にも病変がみられる小腸・大腸型、大腸だけに病変が限られる大腸型に分けられます。

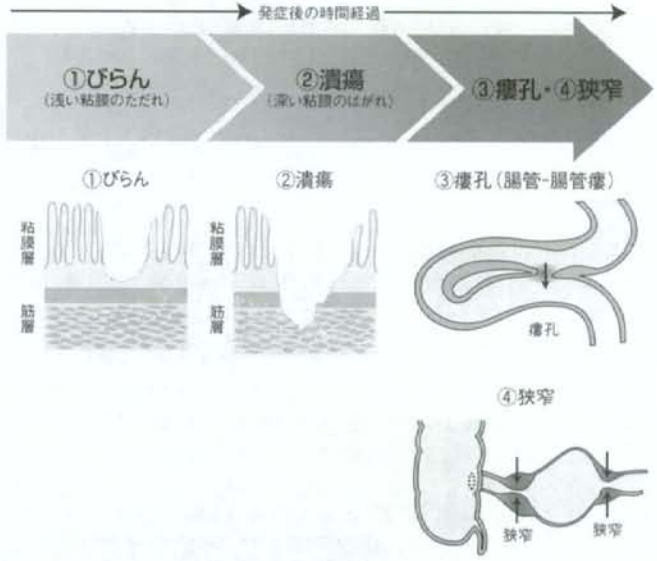
■ 病変部位による分類



2）あなたの病変の状態は

クローン病の炎症は粘膜表面にとどまらず深くまでおよぶことから腸管に孔が開き、腸管と腸管あるいは腸管と皮膚が孔でつながる瘻孔と呼ばれる病変が生じることがあります。また炎症を繰り返すことで腸管が狭くなる狭窄と呼ばれる病変や、さらには狭くなった部位を食べたものなどが通過できなくなる閉塞なども起こることがあります。クローン病は肛門部に病変を生じることも多く、肛門周囲に膿がたまったり（肛門周囲膿瘍）、直腸と肛門周囲に孔がつながる痔瘻などが現れることもあります。

■ 病変の特徴



4. クローン病の内科的治療

1)知っておくべき治療の位置づけ

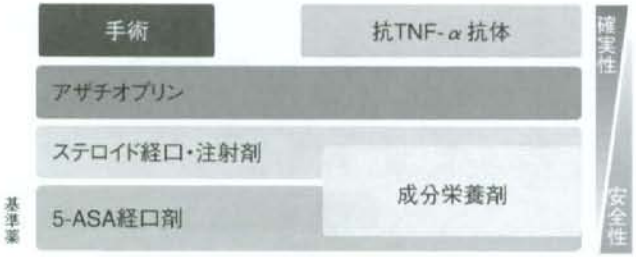
クローン病治療の目的は、腸管の炎症を抑えて症状を鎮め、寛解に導くとともに栄養状態の改善を図り、寛解状態を長期に継続することです。

このような寛解導入ならびに寛解維持には、基本的に栄養療法と薬物療法を中心とした内科的治療が行われ、内科的治療で効果が得られない症状や合併症に対しては外科的治療が行われます。

栄養療法としては、活動期では主に成分栄養剤を用いた経腸栄養法や静脈から栄養剤を投与する完全中心静脈栄養法が行われ、寛解維持療法としては在宅経腸栄養法が行われます。

薬物療法としては、基準薬として5-アミノサリチル酸(5-ASA)製剤が寛解導入ならびに寛解維持療法として使用されます。炎症が強い場合はステロイドの経口剤が用いられ、ステロイドが減量・中止できない場合には免疫調節剤が使用されます。また5-ASA製剤やステロイドで改善がみられない場合や肛門に病変がある患者さんには抗菌剤が用いられることもあります。このような治療薬で効果がみられない場合はより強力な薬として抗TNF-α抗体製剤が選択されます。

■ クローン病における各種治療薬・治療法の位置づけ



2)服薬遵守を知っていますか

クローン病は、再燃・再発を予防するために長期にわたって薬の服用が必要です。

- 重要なことは、症状がない寛解期でも、医師の指示通りにきちんと服薬を遵守することが再燃・再発を予防し、長期にわたって寛解を維持することができることです。
- 正確に服薬の状況を医師に話すことは、個々に適応する治療法の選択につながります。
- もし、飲み忘れが多かったり、錠数が多いと感じていたり、経腸栄養剤の服用が継続しづらい場合は、医師と相談して、服薬を継続できるような工夫をしてみることが大切です。

参考

腹痛や下痢などの症状がある活動期には、きちんと医師の指示どおりに薬を服用できますが、症状がない寛解期に長期間にわたって薬を服用し続けること(寛解維持療法)は難しくなるようです。

潰瘍性大腸炎の成績ですが、2年間にわたって5-アミノサリチル酸製剤による寛解維持療法を受けている患者さんの服薬状況を調査した結果、指示されたとおりにきちんと服薬を守っていた患者さんでは約9割が寛解を維持できていました。一方、服薬を守っていなかった患者さんは、寛解維持率が約40%と低く、6割の患者さんで再燃したことが報告されています。また服薬を守れない理由として、飲み忘れ(50%)、錠数が多いこと(30%)、薬の必要性を感じないこと(20%)が挙げられています。

3) 知っておきたい治療薬・治療法

多くの患者さんは、いろいろな治療薬や治療法を用いて寛解導入や寛解を維持することが可能です。しかし、薬や治療法は効果を発揮する反面、副作用を生じる可能性もあります。したがって、それぞれの薬や治療法の特徴などを正しく理解することが大切です。

●栄養療法

栄養療法は、栄養状態の改善や腸管の安静のみならず、腸管の炎症も抑えます。症状が非常に強い場合や高度な狭窄がある場合、さらには広範囲に小腸に病変が存在する場合などには、中心静脈から栄養分を投与する完全中心静脈栄養法が行われ、病勢が沈静化すれば成分栄養剤を用いた経腸栄養法が行われます。

(経腸栄養法)

活動期の経腸栄養法には基本的には成分栄養剤(エレンタール®)が用いられますが、消化態栄養剤(ツインライン®)が用いられることもあります。鼻から十二指腸までチューブをとおし、注入ポンプなどを用いて栄養剤を注入します。寛解期には成分栄養剤、消化態栄養剤あるいは半消化態栄養剤(エンシュア・リキッド®、ラコール®など)により1日の必要カロリーの約半分を摂取する在宅経腸栄養法が用いられます。

※経鼻チューブを用いなくても経口摂取が可能な場合は、経口的に服用することが可能です。

【成分栄養剤】

商品名 エレンタール®

特 徴 たんぱく源としてアミノ酸を用い、脂肪をほとんど含有せず、極めて低残渣で吸収されやすい栄養剤です。クローン病の寛解導入・寛解維持療法に用いられます。
※アミノ酸臭のために経口的に服用しづらい場合は、フレーバーなどが用いられます。

投与量 活動期には1日の必要カロリー(2,000kcal)のすべてを栄養剤によって摂りますが、寛解導入後は徐々に栄養剤の量を減らし、その分を低脂肪・低残渣食でおぎないます。寛解維持療法としては、必要カロリーの半分程度を栄養剤で摂取します。

副作用 主な副作用:下痢、腹部膨満感、吐き気、嘔吐、腹痛、発疹など。
＊基本的には通常の薬と異なり、栄養剤ですので問題となる副作用は少ないことが特徴です。
＊これらの症状を含め気になる症状などが発現した場合は医師や薬剤師に相談してください。

■エレンタール®

■エレンタール®(ボトル)

(他の栄養剤の特徴)
成分栄養剤との違いは、たんぱく源と脂肪の含量が異なります。分解されていないたんぱく質は吸収するために消化が必要ですし、腸管への刺激となる可能性もあります。また、脂肪が多いと腸管の運動を活発にさせる要因となります。
成分栄養剤に比べて、経口で飲みやすくなっていますが、症状などにあわせて、医師と相談して選択する必要があります。

【消化態栄養剤】

商品名 ツインライン®

特 徴 たんぱく源はアミノ酸と消化吸収されやすいように卵を分解したたんぱく質から構成されています。脂肪は成分栄養剤よりも多く含まれています。

【半消化態栄養剤】

商品名 エンシュア・リキッド®、ラコール®など

特 徴 たんぱく源として分解されていないたんぱく質と分解されたたんぱく質を含んでおり、脂肪は成分栄養剤より多く含まれています。

● 薬物療法

①5-アミノサリチル酸(5-ASA)経口製剤

5-ASAを有効成分とする薬で、腸管の炎症を抑えます。多くの患者さんは活動期の症状改善と寛解維持を目的に服用しています。

代表的な薬にメサラジン経口剤とサラゾスルファピリジン経口剤があります。

【メサラジン経口剤】

商品名 ペンタサ®錠250、ペンタサ®錠500など

特　徴 クローン病の基準薬として、活動期の症状を抑えるためと、再燃・再発を予防するための寛解維持療法に広く用いられる薬です。手術後の再発予防にも用いられます。
この薬はサラゾスルファピリジンを改良し、副作用となる成分を取り除き、有効成分(5-ASA)だけを含有する薬です。その安全性の高さから、成人だけでなく小児にも適応があります。
この薬は小腸から大腸にわたって薬を放出するために病変の部位にかかわらず効果が期待できます。

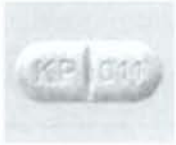
投与量 基本的な投与量は1日1,500mg～3,000mgです。
※高い治療効果を得るために、1日3,000mgが投与されます。
※服薬遵守を改善するために、500mgの錠剤が開発されています。

副作用 主な副作用：発疹、吐き気、下痢、腹痛、血便、発熱など
稀な副作用：間質性肺炎(発熱・呼吸困難・から咳を伴う)、心筋炎(胸部痛・発熱・呼吸困難を伴う)、間質性腎炎(発熱・尿量減少を伴う)、血球減少(貧血・出血傾向を伴う)、膵炎(激しい上腹部や腰背部の痛み・吐き気を伴う)など

＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

■ペンタサ®錠250

■ペンタサ®錠500



【サラゾスルファピリジン経口剤】

商品名 サラゾピリン®錠など

特　徴 大腸に病変がある場合の活動期の症状を抑えるためと、再燃を予防するための寛解維持療法に用いられている薬です。
※サラゾスルファピリジンは大腸内の腸内細菌によって有効成分の5-アミノサリチル酸と副作用の主な原因となるスルファピリジンに分解されます。したがってこの薬は大腸の病変にのみ効果が期待できます。

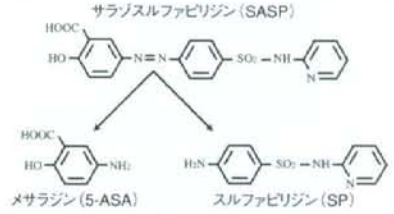
投与量 基本的な投与量は1日2,000mg～3,000mgですが、再燃時には4,000mgを投与する場合もあります。

副作用 主な副作用：腰背部痛、腫れ、むくみ、血尿、発疹、かゆみ、光線過敏症、関節痛、紅斑、顔面潮紅、蕁麻疹など
稀な副作用：貧血症状(立ちくらみ・頭痛を伴う)、再生不良性貧血(発熱・出血傾向を伴う)、皮膚粘膜眼症候群(高熱・皮膚が赤くなる・口内炎を伴う)、間質性肺炎(発熱・咳・痰・呼吸困難を伴う)、腎不全(尿量減少・手足や顔のむくみ・倦怠感を伴う)など

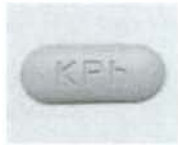
＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

＊下記の事項についても、医師や薬剤師に相談してください。
・男性では可逆性の男性不妊が報告されています。配偶者の受胎を希望される場合
・尿や汗の肌着への着色や、稀にソフトコンタクトレンズへ着色する場合

■サラゾスルファピリジンとメサラジンの構造式



■サラゾピリン®錠



②ステロイド経口剤

ステロイド経口剤は、活動期の炎症を抑えて症状を改善するために用いられます。

ステロイド経口剤は全身的な作用により、炎症反応や免疫反応を強力に抑制するため高い効果が得られます。しかし、長期に大量に使用すると副作用が問題となることから、効果が得られれば徐々に減量して投与を中止します。また寛解を維持する効果は認められていないため、寛解維持療法には使用されません。

※この薬を自分の判断で急に投与を中止すると症状の悪化などを引き起こす場合があります。必ず医師の指示に従い服用してください。

【プレドニゾロン経口剤】

商品名 プレドニン®錠など

特　徴 ステロイドとしてプレドニゾロンを有効成分とする薬です。

投与量 1日40mg～60mgが経口投与で用いられます。

副作用 主な副作用：月経異常、下痢、吐き気、食欲不振、食欲亢進、幸福感、不眠、頭痛、めまい、満月様顔貌、いかり肩、むくみ、血圧上昇、にきび、多毛、脱毛、皮下出血、視力低下、皮膚のすじ状の変化、かゆみ、発疹など

稀な副作用：続発性副腎不全（身体のだるさ・吐き気・血圧低下を伴う）、糖尿病（喉の渇き・尿量増加を伴う）、精神変調（精神状態の不安定・不眠・けいれんを伴う）、骨粗鬆症（背中や腰の痛み・足や腕のつけ根の痛みを伴う）、緑内障（視力低下・眼のかすみを伴う）、血栓症（手足のしびれ・足のむくみ・痛み・胸の痛みを伴う）など

＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

■プレドニン®錠5mg

③抗菌剤

5-アミノサリチル酸製剤やステロイド剤で効果が見られない場合や肛門周囲に膿がたまる（肛門周囲膿瘍）などの肛門部病変がある場合には抗菌剤（メトロニダゾールやシプロフロキサシンなど）が用いられることがあります。

【メトロニダゾール経口剤】

商品名 フラジール®内服錠250mg

特　徴 抗原虫薬ですが、クローン病にも使用されることがあります。

投与量 1日750mgが投与されます。

副作用 主な副作用：発疹、食欲不振、悪心、胃不快感、暗赤色尿など。

＊長期に服用すると末梢神経障害（手足のしびれ・痛み・感覚の麻痺を伴う）や中枢神経系障害（味覚異常、めまい・ふらつきを伴う）などが報告されています。

＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

【シプロフロキサシン経口剤】

商品名 シプロキサン®錠200mg

特　徴 感染症に使用される薬でクローン病にも使用されることがあります。

投与量 1日400mg～800mgが投与されます。

副作用 主な副作用：発疹、胃部不快感、下痢、嘔吐、食欲不振、光線過敏症、蕁麻疹など

＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

④免疫調節剤

免疫調節剤は、もともと臓器移植時の拒絶反応の抑制や白血病などの治療薬として開発されましたが、クローン病の治療にも有効なことが明らかにされたことから、近年国内でも使用されるようになりました。主に国内で使用される免疫調節剤として、アザチオプリンやメルカプトプリンの経口剤があります。

【アザチオプリン・メルカプトプリン経口剤】

商品名 イムラン®錠、アザニン®錠、ロイケリン®散

特　徴 ステロイドの減量・中止にともなって再燃する場合に、この薬が用いられます。症状を抑えながらステロイドの減量・中止が可能で、寛解を維持する効果も認められています。ただし、効果が現れるまでには2～3ヶ月を要します。
副作用として血球減少を生じる可能性が高いことから、この薬を服用する場合は、投与開始直後は頻回に、その後は定期的に血液検査を受ける必要があります。
※医師の指示にしたがって受診することが大切です。

投与量 イムラン®錠・アザニン®錠の投与量は1日50mg～100mgです。
ロイケリン®散の投与量は1日30mg～50mgです。

副作用 主な副作用：発疹、血管炎、腎機能障害、全身倦怠感、筋肉痛、関節痛、発熱、悪寒、めまいなど
稀な副作用：再生不良性貧血（貧血・出血症状・発熱を伴う）、ショック様症状（寒気・震え・立ちくらみを伴う）、肝機能障害（全身倦怠感・皮膚が黄色くなる・食欲不振を伴う）、間質性肺炎（発熱・から咳・呼吸困難を伴う）など

＊これらの症状を含め、気になる症状などが発現した場合は医師や薬剤師に相談してください。

■イムラン®錠

＊ロイケリン®散は、クローン病に対する保険適応はありません。

⑤抗TNF-α抗体製剤

クローン病ではTNF-αと呼ばれる炎症を引き起こす生体物質が過剰に作られています。このTNF-αの作用を中和するのが抗TNF-α抗体製剤です。

【インフリキシマブ注射剤】

商品名 レミケード®点滴静注用100

特　徴 この薬は、炎症が非常に強く、これまでの薬では効果が得られない場合や外瘻（腸管と皮膚が孔でつながる）がある場合に用いられます。またこの薬は免疫機能も強力に抑制するため、投与前に結核などの感染症がないかを確認して投与する必要があります。

投与量 活動期の炎症を抑えるためや外瘻病変がある場合に、体重1kgあたり5mgのレミケードを1回に2時間かけて静脈に注射します。投与間隔は0週、2週、6週の3回投与で、その後8週ごとの寛解維持療法が行われる場合もあります。

副作用 この薬は非常に高い治療効果を示す反面、結核や敗血症などを含む重篤な感染症や悪性腫瘍の発現も報告されており、使用にあたっては薬に対する十分な理解と注意が必要です。その他にも投与時反応と呼ばれる投与後2時間以内に呼吸困難・気管支痙攣・血圧上昇・血圧低下・血管浮腫・チアノーゼ・低酸素症・発熱・蕁麻疹などを伴うアナフィラキシー様症状、さらには投与後3日以上経過後に筋肉痛・発疹・発熱・多関節痛・そう痒・手・顔面浮腫・蕁麻疹・咽頭痛・頭痛などを伴う遅発性過敏症があらわれることもあります。

＊これらの症状を含め、気になる症状などが発現した場合は、直ちに医師や薬剤師に相談してください。

■レミケード®点滴静注用100