BBA - Gene Regulatory Mechanisms 1861 (2018) 117–124

Contents lists available at ScienceDirect

BBA - Gene Regulatory Mechanisms

journal homepage: www.elsevier.com/locate/bbagrm

Epigenetic alterations contribute to promoter activity of imprinting gene *IGF2*

Qi-Fan Zheng[a,b,1], Bin Xu[a,b,*,1], Hui-Min Wang[a], Li-Hong Ding[a], Jin-Yang Liu[a], Ling-Yu Zhu[a], Huan Qiu[a], Li Zhang[a,b], Guang-Yi Ni[a,b], Jing Ye[a], Shu-Bin Gao[a,b], Guang-Hui Jin[a,b,*]

[a] Department of Basic Medical Sciences, Medical College, Xiamen University, Chengzhi building 110, Xiang'an South Road, Xiamen 361102, PR China
[b] Fujian Provincial Key Laboratory of chronic liver disease and hepatocellular carcinoma, Xiamen University, Chengzhi building 110, Xiang'an South Road, Xiamen 361102, PR China

ARTICLE INFO

Keywords:
MEN1
MLL
H3K4 methylation
DNA methylation
Imprinting

ABSTRACT

The expression of insulin-like growth factor 2 (*IGF2*), a classical imprinting gene, didn't completely correlate with its imprinting profiles in hepatocellular carcinoma (HCC). The mechanistic importance of promoter activity in regulation of *IGF2* has not been fully clarified. Here we show that histone 3 lysine 4 trimethylation (H3K4me3) modified by menin-MLL complex of *IGF2* promoter contributes to promoter activity of *IGF2*. The strong binding of menin and abundant H3K4me3 at the DNA demethylated P3/4 promoters were observed in Hep3B cells with the robust expression of *IGF2*. In *IGF2*-low-expressing HepG2 cells, menin didn't bind to DNA hypermethylated P3/4 regions; however, menin overexpression inhibited DNA methylation and promoted H3K4me3 at the P3/4 as well as *IGF2* expression in HepG2. In addition, the H3K4me3 at P3/4 locus was activated in primary HCC specimens with high *IGF2* expression. Furthermore, inhibition of the menin/MLL interaction via MI-2/3 reduced *IGF2* expression, inhibited the IGF1R-AKT pathway, and significantly repressed HCC with robust expression of *IGF2*. Taken together, we conclude that H3K4me3 of P3/4 locus mediated by the menin-MLL complex is a novel epigenetic mechanism for releasing *IGF2*.

1. Introduction

Genomic imprinting is a classical epigenetic mechanism for controlling allelic gene expression. Insulin-like growth factor 2 (*IGF2*) was the first identified imprinted gene [1], and itis reciprocally imprinted with the adjacent gene *H19*, a long non-coding RNA, sharing a set of enhancers downstream of *H19* [1]. DNA methylation and the chromatin looping conformation are well studied within the context of the imprinted *IGF2* gene. In the well-established imprinting models, the insulator CCCTC-binding factor (CTCF) binds to unmethylated imprinting control regions (ICRs), blocking the interaction of enhancers with the *IGF2* promoter and resulting in monoallelic silencing of *IGF2* expression in maternal allele [2,3]. By contrast, the paternal ICR is methylated, which blocks the binding of CTCF, and a common enhancer promotes *IGF2* expression but not that of *H19* [2,3].

Aberrant imprinting of *IGF2* is an important molecular hallmark of many human cancers, including hepatocellular carcinoma (HCC) [4]. However, although 89% of cases exhibited altered methylation of ICR loci [5], the expression of *IGF2* did not completely correlate with its imprinting profiles in HCC, and high expression of *IGF2* was found in some HCC samples with an imprinted *IGF2* gene [6], suggesting the interesting hypothesis that additional mechanisms are involved in regulating *IGF2* transcription. *IGF2* consists of nine exons and is transcribed from four different promoters (P1–P4) [7]. The overexpression of *IGF2* is associated with the upregulation of fetal transcripts driven by the P3 and P4 promoters in HCC. Tang et al. found that demethylation of a P4 promoter locus contributes to P4 transcriptional activation at the early stage of hepatocarcinogenesis, and the abnormal hypomethylation of P4 promoter loci in patients with HCC was associated with a poor prognosis [8]. However, the mechanistic importance of promoter activity to *IGF2* upregulation during hepatocarcinogenesis has not been fully clarified.

The site-specific histone modifications are a pivotal epigenetic mechanism for controlling gene transcription [9]. A recent report showed that histone 3 lysine 27 trimethylation (H3K27me3) modified by polycomb group (PcG) is necessary for the establishment of *IGF2* imprinting [10]. In Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS), bivalent H3K9me3 and H4K20me3 marks are

* Corresponding authors at: Medical College of Xiamen University, Chengzhi building 110, Xiang'an South Road, Xiamen, Fujian 361102, PR China.
E-mail addresses: binxu198@xmu.edu.cn (B. Xu), ghjin@xmu.edu.cn (G.-H. Jin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbagrm.2017.12.007
Received 22 April 2017; Received in revised form 20 December 2017; Accepted 20 December 2017
Available online 03 February 2018
1874-9399/ © 2018 Elsevier B.V. All rights reserved.

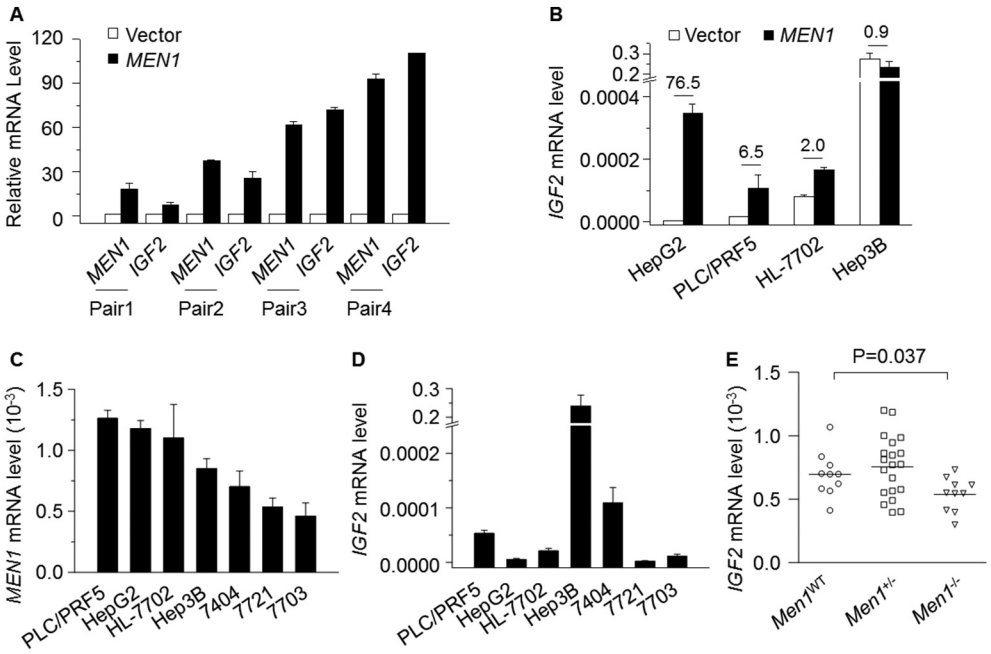

Fig. 1. The expression of *IGF2* was greatly induced by menin in HCC.
(A) Relative mRNA levels of *MEN1* and *IGF2* were determined by qRT-PCR in 4 independent pairs of vector-containing or *MEN1*-overexpressing HepG2 cells with the *pLNCX2* retrovirus system. (B) The HCC cells were stably transfected with the empty vector or *MEN1*-over-expressing plasmid via the *PLNCX2* retrovirus, and the mRNA levels of *IGF2* were determined. (C and D) The basal level of *MEN1* or *IGF2* mRNA expression were detected in HCC cell lines. Data were represented as the mean ± SD, n = 3. (E) The *IGF2* mRNA expression was detected by qRT-PCR in wild-type, *MEN1* homozygous or heterozygous deletion mice at embryonic day 11–12.5. Two-tailed independent sample *t*-test, $p = 0.037$.

associated with a hypermethylated ICR, whereas H3K4me2/H3K27me3 marks are associated with a hypomethylated ICR [11]. A maternally inherited allele exhibits more H3K4me3 and less H3K9me3 than the paternally inherited allele of ICRs in fetal germ cells [12]. These findings support a potential link between histone covalent modifications and *IGF2* imprinting regulation. Menin, the product of the *MEN1* gene, is a highly specific partner for the mixed-lineage leukemia (MLL) (humans) histone methyltransferase complex [13]. As an activator, the menin-MLL complex alters histone tail modifications and the transcription of target genes, such as *Hox* family genes, through H3K4me3 and/or H3K79me2, a process that is necessary for leukemogenesis [13]. We previously reported that the menin-MLL complex and H3K4me3 are activated in both human HCC specimens and DEN-induced HCC development in mice and that this activation is associated with a poor prognosis [14]. Supporting this notion, menin occupancy frequently coincides with H3K4me3 and epigenetically promotes the transcription of Yes-associated protein (Yap1) [14]. Interestingly, using *ChIP-on-chip* assays, we found that menin binds to promoter loci, which is accompanied by H3K4me3 and H3K79me2 at many imprinted genes, including *IGF2* [14]. In addition to this H3K4me3 modification, we also reported that the epigenetic action of menin is involved in H3K27me3 bivalent modifications [15]. *MEN1* knockout (*KO*) dramatically decreases H3K27me3 and DNA methylation at paired box 2 (*Pax2*) gene promoters and upregulates Pax2 [15]. These findings suggest comprehensive epigenetic actions of menin, further indicating a potential epigenetic role of menin in regulating *IGF2* transcription.

Here, we report an interesting epigenetic model in which H3K4me3 remodeling by menin/MLL at P3/4 regions exerts an important, yet previously unappreciated, function in the release of *IGF2* from silencing. Moreover, our results indicate that blocking *IGF2* transcription using small-molecule inhibitors of the menin-MLL interaction is a potential therapeutic strategy for HCC with an aberrant IGF2-IGF1R axis.

2. Material and methods

2.1. ChIP assays

ChIP assays were performed as previously described [14,16]. Briefly, 50 mg of tissue was cut with surgical scissors into small pieces and then processed with the indicated antibodies according to the protocol of the ChIP Assay Kit (Millipore). The primer pair sequences and antibodies for the ChIP assays are shown in Supplementary Table S1 and S2, respectively.

2.2. DNA methylation assays

DNA methylation was assayed by bisulfite modification performed according to a previous report [17], and pyrosequencing (PSQ) with the bisulfite-converted DNA was performed as described previously [18] using PyroMark Q96 ID Systems and PyroMarkCpG Software 1.0.11 (Sangon Biotech, Shanghai). Briefly, bisulfite modification of genomic DNA extracted from HCC cells and clinical HCC samples was performed using the CpGenome DNA modification kit (Chemicon International). The primers are listed in Supplementary Table S2.

2.3. Ethics statement

All the human tissue samples used were approved by Xiamen University Medical Ethics Committee and were performed according to the principles expressed in the Declaration of Helsinki and the international ethical guidelines for biomedical research involving human. Animal work was approved by Xiamen University Animal Ethics Committee and was performed according to the institutional and national guidelines.

2.4. Statistical analysis

All the statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA) version 17.0 software (SPSS, Inc.; Chicago, IL). Where appropriate, a two-tailed independent sample *t*-test was performed; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Results for parametric variables are expressed as the mean ± SD. In all cases, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Menin upregulates IGF2 expression

To initially interrogate the potential regulating action of menin on *IGF2* transcription, we performed real-time qPCR analysis in HCC cells stably transfected with the empty vector or a *MEN1* over-expressing plasmid. Using 4 independent pairs of *MEN1*-overexpressing HepG2

Download English Version:

https://daneshyari.com/en/article/8300402

Download Persian Version:

https://daneshyari.com/article/8300402

Daneshyari.com