Biochemical and Biophysical Research Communications 422 (2012) 697–702

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Reduction of DNA hydroxymethylation in the mouse kidney insulted by ischemia reperfusion

Ning Huang [a], Li Tan [b], Zhanggang Xue [a], Jing Cang [a,*], Hao Wang [a,*]

[a] Department of Anesthesiology, Zhongshan Hospital, Shanghai Medical College of Fudan University, 180 Fenglin Road, Shanghai 200032, PR China
[b] Laboratory of Epigenetics, Institutes of Biomedical Sciences, Shanghai Medical College of Fudan University, 138 Yixueyuan Road, Shanghai 200032, PR China

ARTICLE INFO

Article history:
Received 30 April 2012
Available online 22 May 2012

Keywords:
Mouse kidney
Ischemia reperfusion
DNA hydroxymethylation
Tet2

ABSTRACT

Ischemia reperfusion (IR) is a frequent pathological injury to the perioperative patients. The molecular mechanism underlying IR injury is still not well characterized. In this study, we investigated the effect of IR injury on DNA hydroxymethylation in mouse kidney. Dot blot and immunochemistry analysis showed that the global level of 5-hydroxymethylcytosine (5hmC) was reduced in mouse kidney insulted by IR; however, the 5-methylcytosine (5mC) level had no significant change. hMeDIP-qPCR validated that IR injury also decreased the 5hmC enrichment at promoter regions of *Cxcl10* and *Ifngr2* genes. RT-qPCR analysis revealed that the mRNA levels of *Cxcl10* and *Ifngr2* increased in IR-injured kidney. In addition, mRNA expression of both *Tet1* and *Tet2* but not *Tet3* was dramatically downregulated in IR-injured kidney. Taken together, our data provided the first evidence that IR injury influences DNA hydroxymethylation and Tet gene expression in mouse kidney, which may contribute to the regulation of gene transcription during renal IR injury.

1. Introduction

Ischemia reperfusion (IR) is a frequent pathological injury to perioperative patients, especially to those undergoing organ transplantation [1]. In last decades, many cellular and molecular mechanisms have been raised to explain IR injury, such as hypoxia, oxidative stress, calcium overloading and sterile inflammation [1,2]. In addition to the damage observed on cellular and organ levels, significant changes in gene expression have also been detected during IR injury [3–5]. It draws attention to the question how IR injury causes transcriptional reprogramming. Recent great advances in epigenetics provide a link between pathological processes including IR injury and the regulation of gene expression [6].

As an important epigenetic modification, DNA methylation (5-methylcystosine, 5mC) regulates genomic functions, such as gene transcription, X-chromosome inactivation, imprinting, genetic mutation and chromosome stability [7]. Cell-specific DNA methylation patterns are precisely established during mammalian embryonic development and abnormalities in DNA methylation have been widely reported in lots of human diseases including cancers [8,9]. Although research studied alterations of DNA methylation and related enzymes (DNA methyltransferase, DNMTs) during IR injury is very limited, it still shows strong correlation between them. The area of brain IR injury in $Dnmt^{S/+}$ mice was smaller than that in wild-type mice after 30 min middle cerebral artery occlusion (MCAO) and reperfusion, and 5-aza-2′-deoxycytidine (DNMTs inhibitor) can protect wild-type mice from IR injury [10]. Expression of estrogen receptor alpha (ERα) which conferred to brain protection after MACO in female rats was enhanced due to the decrease of DNA methylation in its promoter regions [11]. DNA methylation was reduced significantly in putative regulatory sites within the C3 promoter of the kidney undergone 4 h ischemia for transplantation and dysregulated gene expression of C3 [12]. In summary, DNA methylation has participated in IR injury indeed.

Recently, Rao and colleagues reported that TET family proteins can catalyze the oxidation of 5mC into 5-hydroxymethylcytosine (5hmC) [13]. TET proteins can also oxidize either 5mC or 5hmC into 5-formylcytosine (5fC) and/or 5-carboxylcytosine (5caC) [14,15]. 5hmC is the most abundant form among the three 5mC oxidative derivatives (5hmC, 5fC, 5caC) and can be detected in almost all organs and tissues. However, unlike 5mC, 5hmC level varies among different organs and tissues [16]. 5hmC may function as a new epigenetic modification above 5mC or as an intermediate state during DNA demethylation [17,18]. Although the exact role of 5hmC in gene regulation is not well defined, TET-catalyzed DNA hydroxymethylation may be a key regulatory mechanism of DNA methylation and demethylation.

Since DNA methylation plays key roles in lots of pathological conditions and diseases, we proposed that DNA hydroxymethylation may be also involved in most pathological process, including IR injury. In this study, we therefore investigated the effect of IR injury on DNA hydroxymethylation and the expression of

* Corresponding authors.
E-mail addresses: cangjing1998@yahoo.com.cn (J. Cang), wang.hao2@zs-hospital.sh.cn (H. Wang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.061

corresponding diaoxygenases in mouse kidney. We determined that global 5hmC levels and *Tet2* expression were reduced in mouse kidney insulted by IR.

2. Materials and methods

2.1. Mouse model for kidney IR injury

6–8 weeks C57BL/6 male mice weighing 16–20 g each ($n = 5$) were used in our experiments. The studies were approved by the Institutional Animal Care and Use Committee of Zhongshan Hospital. Mice were raised under normal 12-h cyclic illumination with free access to water and standard mouse chow for 1 week before intervention. Pentobarbital sodium-anesthetized mice via peritoneal injection (70 mg/kg body weight) were placed in lateral position under a warming light to maintain body temperature between 36 and 38 °C. Additional pentobarbital sodium was given when needed based on the response to tail pinch. Kidneys were exposed via bilateral flank incisions. The left kidneys were subjected to 40 min of renal ischemia (marked as IR) and the right kidneys were just exposed as control (marked as Control). Ischemia was induced by clamping renal pedicles with nontraumatic microaneurysm clamps. The kidneys were covered with humidified pledget during ischemia. IR was subjected to 40 min ischemia and reperfusion was confirmed visually. All mice were sacrificed 24 h after reperfusion. Kidneys were either snap-frozen in liquid nitrogen for RNA and DNA extraction or fixed with 4% paraformaldehyde for histological studies.

2.2. Histopathology and Immunohistochemistry (IHC) analysis

To validate the histopathological changes of renal ischemia reperfusion injury, kidneys were fixed by 4% paraformaldehyde for 24 h before being dehydrated by 70% alcohol. The paraffin-embedded specimens were cut into 5-μm sections and stained with hematoxylin-eosin (HE). Histological changes of IR injury were evaluated by observation of tubular injury in 5 random individual high-power fields (maginification × 400) per sample.

For IHC analysis, sections were dewaxed and rehydrated at first. Sections were then heated in a pressure cooker for 25 min in target retrieval solution (Dako). For 5mC or 5hmC detection, sections were incubated respectively with monoclonal mouse anti-5mC antibodies (Eurogentec, 1:500 dilution) or polyclonoal rabbit anti-5hmC antibodies (Active motif, 1:1000 dilution) for 1 h at room temperature. A horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, USA) was then applied and incubated at 37 °C for 1 h. Sections were developed with DAB kit and stopped with water. Five randomly selected fields from each sample were microscopically examined.

2.3. DNA extraction and anti-5hmC and anti-5mC dot blotting

Genomic DNA was extracted from snap-frozen tissues using DNeasy Blood & Tissue Kit (Qiagen) by following the manufacturer's instructions. DNA concentrations were determined throughout by fluorometry using the HS dsDNA kit and Qubit Fluorometer (Invitrogen). The procedure for the dot-blot assay was modified from a procedure described previously [19]. Briefly, DNA was spotted on a nylon membrane (Hybond-N+, GE) and placed in an ultraviolet crosslink equipment (Hoefer) for 5 min. Subsequently, the membrane was blocked with 5% milk in TBS-Tween 20 for 1 h and incubated with the primary antibody (anti-5mC or anti-5hmC) at 4 °C overnight. After incubation with a horse radish peroxidase-conjugated secondary antibody (anti-mouse IgG for 5mC and anti-rabbit IgG for 5hmC) for 1 h at room temperature, the membrane was washed with TBS-Tween 20 for three times and then detected by an enhanced chemiluminescence Western blotting analysis system (Kodak). In addition, the dot-blot membrane was hybridized with 0.02% methylene blue in 0.3 M sodium acetate (pH 5.2) to stain DNA. The dot blot intensity was quantified by Image-J software (NIH).

2.4. Reverse transcription and quantitative real-time PCR (RT-qPCR)

Total RNA were extracted from snap-frozen tissues using RNeasy Plus Mini kit (Qiagen) according to the manufacturer's instruction. Reverse transcription of cDNA was performed using PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara) and quantitative real-time PCR was performed using iQ5 (Bio-Rad) with SYBR Green (Takara). *Gapdh* was used as an endogenous control. Primer sequences are listed in Supplementary Table S1.

2.5. Hydroxy-Methylated DNA immunoprecipitation (hMeDIP)

hMeDIP assays were performed as described [20] using 4 μg of denatured sonicated DNA in 300 μl of binding buffer and 4 μg of 5hmC antibody. The samples were incubated for four hours at 4 °C before addition of 20 μl of equal volume mixed ProteinA/G beads (Millipore). After 2 h of incubation, the samples were washed 3 times and bound DNA was eluted by incubation for 2 h at 55 °C in 500 μl of 50 mM Tris–HCl, 10 mM EDTA, 0.5% SDS and 20 μg proteinase K. The DNA was purified using a QIAquick PCR purification kit (Qiagen). Independent hMeDIP assay of each animal was done at the same time. Locus-specific hMeDIP–qPCR was performed using immunoprecipitated DNA and normalized by input. The primers were designed to locate at the region covered ±2 kb around TSS of genes (sequences are listed in Supplementary Table S2).

2.6. Statistical analysis

All data were represented as Mean ± SD. SPSS 16.0 statistical package were used for statistical analysis (Paired t-test). All statistical tests were considered significant at an $\alpha = 0.05$ ($P < 0.05$).

3. Results

3.1. Establishment of mouse model for renal IR injury

Mouse model for renal IR injury was evaluated by hispathological changes and sensitive gene markers of tubular injury. A global injury was assessed by HE staining which showed the increased number of necrotic tubular cells and tubular casts, the apparent loss of brush border of tubular cells and emergence of dilated tubules [21]. IR kidneys displayed serious tubular injury and large amounts of infiltrated blood cells both in the cortex and the medulla after 24 h reperfusion (Fig. 1A). In addition, the mRNA levels of *Ngal* and *Kim1* (two marker genes of renal IR injury confirmed by recent research [22,23]) were dramatically upregulated in IR-insulted kidneys (Fig. 1B). These data confirmed that the mouse model for renal IR injury was successfully established.

3.2. IR injury reduces the global 5hmC level in mouse kidney

Next, we examined whether IR injury influences DNA methylation and hydroxymethylation of mouse kidney. Both dot blotting and IHC analysis revealed that the global 5hmC level in mouse kidney was reduced upon IR injury ($P < 0.05$), whereas the global 5mC level had no significant change (Fig. 2A–E). Thus, our data

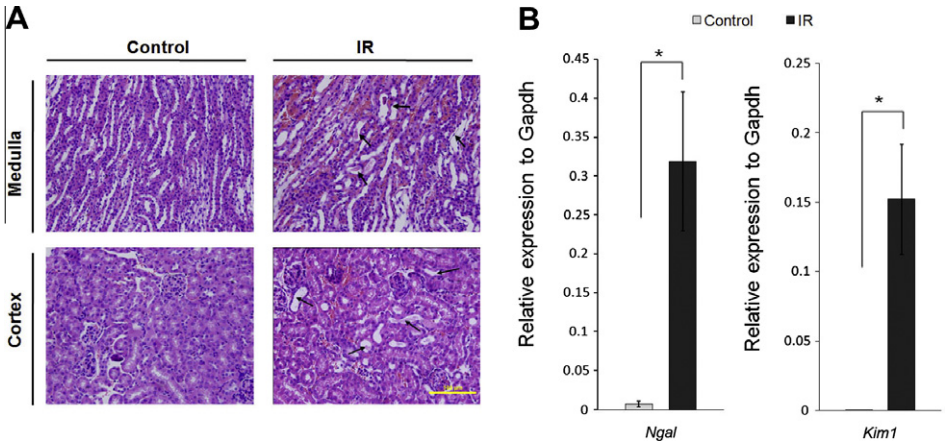

Fig. 1. IR injury in mouse kidney. (A) Histopathology analysis in different kidney sections (HE staining). Contrast with infiltration of blood cells and serious tubular injury, such as intratubular obstruction, severe tubular dilation and necrosis in the IR-treated kidney (marked with arrows), renal structure of the control exhibited normally. Scale bar is 100 μm. (B) RT-qPCR analysis of *Ngal* (left) and *Kim1* (right) in control and IR-treated mouse kidneys ($n = 5$). Data are represented as mean ± SD. $^{*}P < 0.05$.

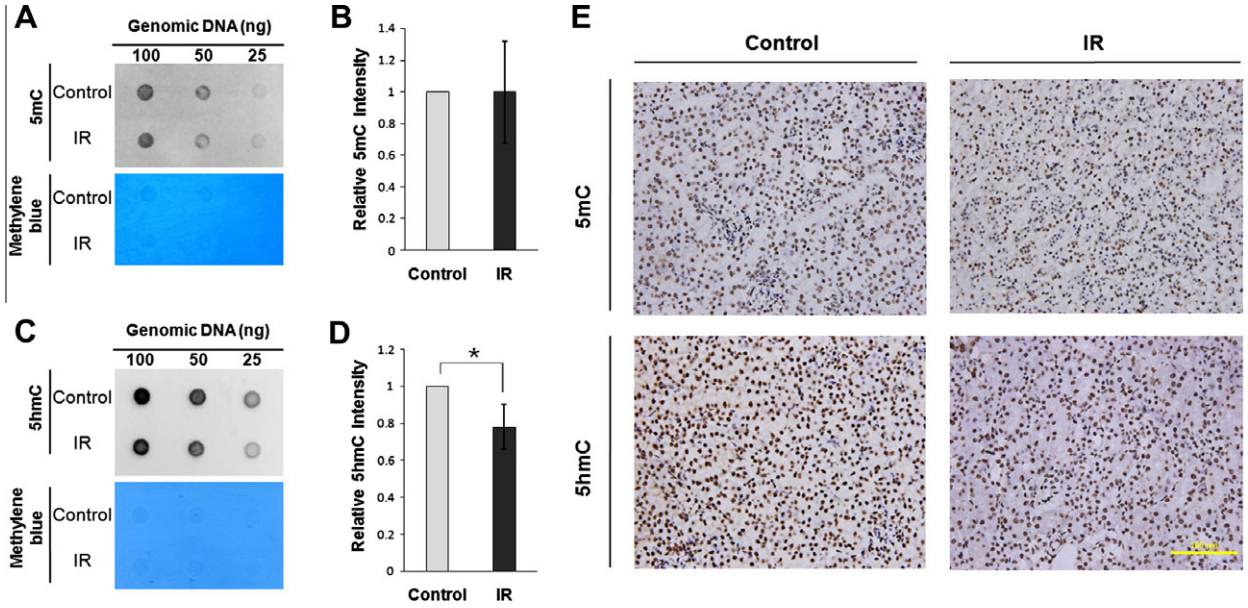

Fig. 2. IR injury reduces the global 5hmC level in mouse kidney. (A and C) Upper panel shows the representative anti-5mC and anti-5hmC dot blot for the DNA extracted from control and IR kidneys, respectively. Bottom panel shows the methylene blue staining to validate the equal loading amount of DNA. (B and D) Diagram showing the relative intensity of 5mC and 5hmC signal in dot blot separately. Data are represented as mean ± SD ($n = 5$). $^{*}P < 0.05$. (E) immunohistochemistry analysis using 5mC Ab and 5hmC Ab shows unchanged global 5mC level but decreased global 5hmC level in the IR kidney. Scale bar is 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicated that the global level of 5hmC but not 5mC in mouse kidney is sensitive to IR injury.

3.3. IR injury decreases the 5hmC enrichment at two gene loci in mouse kidney

To determine whether IR injury alters the 5hmC levels at specific gene loci in mouse kidney, we selected two genomic loci at proximal promoter regions of the gene *Cxcl10* and *Ifngr2* based on our hMeDIP-seq data of mouse kidney (Huang et al. unpublished data). As shown in Fig. 3A, 5hmC enrichment at both gene loci decreased in IR-insulted mouse kidney. In addition, RT-qPCR analysis revealed that the mRNA levels of the two genes were upregulated upon IR injury (Fig. 3B), suggesting that the change in 5hmC enrichment at promoter regions may associate with gene transcriptional activity.

3.4. Tet2 expression is down-regulated in IR-insulted mouse kidney

As 5hmC generation is catalyzed by Tet family proteins, we examined whether IR injury has impact on the expression of Tet family genes in mouse kidney. Interestingly, mRNA expression of *Tet1* and *Tet2* but not *Tet3* was significantly downregulated in IR-injured kidney (Fig. 4). Since *Tet1* was expressed at low level in normal mouse kidney (Fig. 4), the decrease in *Tet2* expression may contribute to the downregulation of 5hmC in mouse kidney upon IR injury.

4. Discussion

In this study we revealed that IR injury influenced DNA hydroxymethylation in mouse kidney. As a common pathological injury during perioperative period, IR challenged the homeostasis and

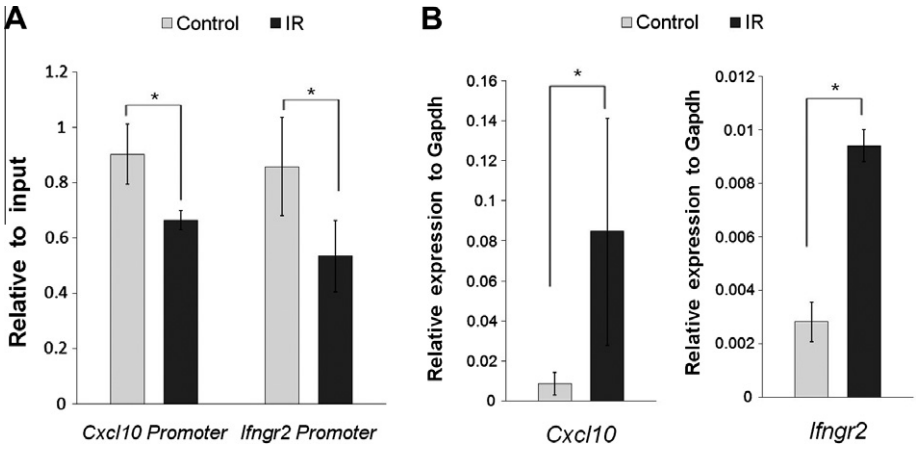

Fig. 3. IR injury decreases the 5hmC enrichment at gene loci in mouse kidney. (A) hMeDIP-qPCR analysis of the 5hmC enrichment at *Cxcl10* and *Ifngr2* promoter regions in genomic DNA of Control and IR mouse kidney ($n = 5$). $^*P < 0.05$. (B) RT-qPCR analysis of the mRNA levels of *CxCl10* and *Ifnngr2* in Control and IR mouse kidney ($n = 5$). $^*P < 0.05$.

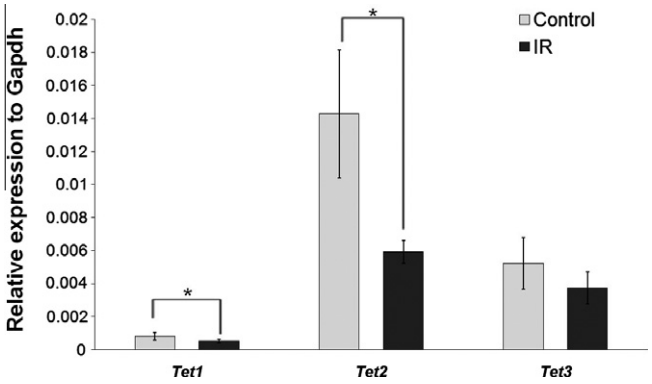

Fig. 4. IR injury represses *Tet1* and *Tet2* expression in mouse kidney. RT-qPCR analysis of the mRNA levels of Tet family genes in Control and IR mouse kidneys ($n = 5$). $^*P < 0.05$.

plasticity of vital organs like kidney, heart, lung, liver, and brain [1]. Although the molecular mechanisms underlying IR injury have been widely investigated in many aspects, little is known about its relationship with epigenetic regulatory system. When the kidney is undergoing ischemia, endothelial cell-cell junctions are disrupted firstly, which can cause increased microcirculatory permeability and interstitial edema [24]. At the same time, endothelium itself is also injured by hypoxia and accumulated peroxide. Second, infiltrated leukocytes adhere to the injured endothelium and trigger the sterile inflammation which augments the dysfunction of microcirculatory [25]. The tubular cells are not only the victim of inflammation but also actively involved in proinflammation by expressing chemotactic cytokines, Toll-like receptors, complement and complement receptors [26]. On the other hand, hypoxia and inflammation activate cell signaling pathway associated with tubular cell death, which construct the typical histopathological changes of renal IR injury [27]. Owing to the numerous transcriptional changes in the process, a few literatures concentrated mainly on the DNA methylation and histone modification of selected proinflammatory genes [12,28,29]. These results, accompanied by our data, indicated that epigenetic regulatory system is actually implicated in the renal IR injury.

As the primary finding of this work, we observed ~20% reduction in the global 5hmC levels in IR-insulted mice kidneys. It is known that fluctuation in 5mC (the 5hmC precursor) amount is a key element to determine 5hmC level. Knockouts of *Uhrf1* or *Dnmt* genes that are required for DNA methylation (maintenance or *de novo* DNA methylation) can impair 5hmC levels in mouse ES cells [30]. However, though we observed significant reduction in 5hmC in IR-insulted kidneys, we did not detect any significant change of the global 5mC. Therefore, IR-induced 5hmC decrease should be caused by other factors. Until now, there are merely several reports concerned about locus-specific DNA methylation in IR injury. Most of them noticed diminished 5mC at specific regions upon IR injury [11,12,31]. Only Endres et.al reported the global change of DNA methylation and showed increased DNA methylation in the wounded brain area [10]. These controversial results of 5mC, including ours, may be partially aroused by different degree and duration of the injury. The dissimilarity of tissue types and analysis methods may also engage.

Our RT-qPCR analysis showed that both *Tet2* and *Tet3* are expressed at intermediate levels in mouse kidney while *Tet1* is expressed at low level, showing accordance with previous research [32]. Therefore, we proposed that the 5hmC in mouse kidney should be generated predominantly by Tet2 and Tet3. Specifically, down-regulation of *Tet2* but not *Tet3* was observed in mouse kidney at 24 h after IR. It seems that the reduced *Tet2* expression may contribute to the decrease of 5hmC. However, we haven't established direct causal relationship between these two events in this work. Since Tet2 knockout mice grow normally except developing leukemia spontaneously at adult stage [33], it will be informative to investigate the exact Tet2 function during IR injury using *Tet2* knockout (or kidney-targeted *Tet2* knockout) mice.

Although IR injury-induced Tet2 repression may contribute to the decrease of 5hmC in mouse kidney, we cannot exclude other factors attributing to the decrease of 5hmC. Alpha-ketoglutarate (alpha-KG) and Fe (II) are the key co-factors required for the oxidative reaction which catalyzed by Tet family proteins [13]. It is possible that IR may alter the intracellular levels of alpha-KG or Fe (II). Recently, several labs have reported that 2-hydroxyglutarate (2-HG), a kind of oncogenic metabolites produced by the mutant IDH proteins, inhibits 5hmC generation by competition with alpha-KG [19]. In addition, we also noticed that *Ngal*, a kind of Fe (II) transporter is downregulated in IR kidneys which may alter the intracellular Fe (II) levels [23], then impair the Tet proteins-catalyzed 5mC oxidation. In addition, IR may cause oxidative stress and ROS accumulation, scavenge the intracellular O_2 and impair the Tet proteins-catalyzed 5mC oxidation. He et al. reported that ATP skews 5mC oxidation toward 5caC *in vitro* [15]. However, the 5fC and 5caC levels are too low to be detected in normal mouse kidney by conventional methods (such as HPLC or TLC) [14]. IR injury might skew 5mC oxidation toward 5fC and 5caC, or accelerate the further oxidation of 5hmC into 5fC and 5caC. With the

development of detection method for 5fC and 5caC, the mystery of IR-induced 5hmC decrease will be addressed in future.

Although recent studies have revealed the genome-wide distribution of 5hmC in mouse ES cells and brain tissues, the correlation between 5hmC enrichment and gene expression remains controversial [34–37]. We tested two genes (*Cxcl10* and *Ifngr2*) displaying 5hmC enrichment at their promoter regions for validation (based on our unpublished hMeDIP-seq data). As expected, we found that the 5hmC enrichment at the two gene mentioned above was significantly reduced in IR-insulted kidneys. Interestingly, both these two genes were up-regulated in IR kidneys. DNA methylation at the promoter regions can silence the gene transcription via recruiting 5mC binding proteins and/or histone deacetylases (HDAC) co-repressor complex and interfere the binding of transcriptional factors to DNA regulatory elements. It has been reported that 5hmC display different biochemical features compared with its precursor 5mC [35]. The alteration of DNA hydroxymethylation may partly contribute to the IR-induced changes in gene expression, or just as an epigenetic landmark which may have long-term effect on genomic functions. In the future, it will be of great interest to decipher the genome-wide change of 5hmC and 5mC in mice kidneys upon IR injury and their relationship with gene expression.

In addition, ischemia was often observed in malignant tumors where angiogenesis and vasculatures were not sufficient to support their growth [38]. Interestingly, both low 5hmC level and reduced Tet gene expression have been observed in many kinds of tumors [39,40]. IR injury and tumor related hypoxia may share some similar features or regulation mechanism in the down-regulation of 5hmC and Tet gene expression.

In summary, to our knowledge, this study established the first connection between DNA hydroxymethylation and IR injury. Although such epigenetic connection may expand our understanding in the molecular mechanisms of IR injury, the contribution of dynamic 5hmC change to kidney IR injury still needs further investigation.

Acknowledgments

We thank Dr. Chang Tong for critical reading of the manuscript. This work was supported by Grant from Shanghai Medical College for Young Teachers, Grant from Zhongshan Hospital for Young Doctors, and AstraZeneca Asian Anesthesiology Innovation Project to Wang H., Natural Science Fund of Shanghai (08411960300) to Cang J., Natural Science Fund of China (30872429) and Shanghai Science and Technology Development Fund (09JC1403500) to Xue Z.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.061.

References

[1] H.K. Eltzschig, T. Eckle, Ischemia and reperfusion–from mechanism to translation, Nat. Med. 17 (2011) 1391–1401.
[2] P.A. Grace, Ischaemia-reperfusion injury, Br. J. Surg. 81 (1994) 637–647.
[3] R. Schmidt-Kastner, B. Zhang, L. Belayev, L. Khoutorova, R. Amin, R. Busto, M.D. Ginsberg, DNA microarray analysis of cortical gene expression during early recirculation after focal brain ischemia in rat, Brain Res. Mol. Brain Res. 108 (2002) 81–93.
[4] J.B. Kim, C.S. Piao, K.W. Lee, P.L. Han, J.I. Ahn, Y.S. Lee, J.K. Lee, Delayed genomic responses to transient middle cerebral artery occlusion in the rat, J. Neurochem. 89 (2004) 1271–1282.
[5] Y.D. Kim, N.W. Sohn, C. Kang, Y. Soh, DNA array reveals altered gene expression in response to focal cerebral ischemia, Brain Res. Bull. 58 (2002) 491–498.
[6] R. Bonasio, S. Tu, D. Reinberg, Molecular signals of epigenetic states, Science 330 (2010) 612–616.
[7] P.A. Jones, D. Takai, The role of DNA methylation in mammalian epigenetics, Science 293 (2001) 1068–1070.
[8] S. Feng, S.E. Jacobsen, W. Reik, Epigenetic reprogramming in plant and animal development, Science 330 (2010) 622–627.
[9] M. Ehrlich, DNA methylation in cancer: too much, but also too little, Oncogene 21 (2002) 5400–5413.
[10] M. Endres, A. Meisel, D. Biniszkiewicz, S. Namura, K. Prass, K. Ruscher, A. Lipski, R. Jaenisch, M.A. Moskowitz, U. Dirnagl, DNA methyltransferase contributes to delayed ischemic brain injury, J. Neurosci. 20 (2000) 3175–3181.
[11] J.M. Westberry, A.K. Prewitt, M.E. Wilson, Epigenetic regulation of the estrogen receptor alpha promoter in the cerebral cortex following ischemia in male and female rats, Neuroscience 152 (2008) 982–989.
[12] M.D. Parker, P.A. Chambers, J.P. Lodge, J.R. Pratt, Ischemia- reperfusion injury and its influence on the epigenetic modification of the donor kidney genome, Transplantation 86 (2008) 1818–1823.
[13] M. Tahiliani, K.P. Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L.M. Iyer, D.R. Liu, L. Aravind, A. Rao, Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, Science 324 (2009) 930–935.
[14] S. Ito, L. Shen, Q. Dai, S.C. Wu, L.B. Collins, J.A. Swenberg, C. He, Y. Zhang, Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine, Science 333 (2011) 1300–1303.
[15] Y.F. He, B.Z. Li, Z. Li, P. Liu, Y. Wang, Q. Tang, J. Ding, Y. Jia, Z. Chen, L. Li, Y. Sun, X. Li, Q. Dai, C.X. Song, K. Zhang, C. He, G.L. Xu, Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA, Science 333 (2011) 1303–1307.
[16] D. Globisch, M. Munzel, M. Muller, S. Michalakis, M. Wagner, S. Koch, T. Bruckl, M. Biel, T. Carell, Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates, PLoS One 5 (2010) e15367.
[17] S.C. Wu, Y. Zhang, Active DNA demethylation: many roads lead to Rome, Nat. Rev. Mol. Cell Biol. 11 (2010) 607–620.
[18] M. Munzel, D. Globisch, T. Carell, 5-Hydroxymethylcytosine, the sixth base of the genome, Angew. Chem. Int. Ed. Engl. 50 (2011) 6460–6468.
[19] W. Xu, H. Yang, Y. Liu, Y. Yang, P. Wang, S.H. Kim, S. Ito, C. Yang, M.T. Xiao, L.X. Liu, W.Q. Jiang, J. Liu, J.Y. Zhang, B. Wang, S. Frye, Y. Zhang, Y.H. Xu, Q.Y. Lei, K.L. Guan, S.M. Zhao, Y. Xiong, Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases, Cancer Cell 19 (2011) 17–30.
[20] M. Weber, J.J. Davies, D. Wittig, E.J. Oakeley, M. Haase, W.L. Lam, D. Schubeler, Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells, Nat. Genet. 37 (2005) 853–862.
[21] P. Hill, D. Shukla, M.G. Tran, J. Aragones, H.T. Cook, P. Carmeliet, P.H. Maxwell, Inhibition of hypoxia inducible factor hydroxylases protects against renal ischemia-reperfusion injury, J. Am. Soc. Nephrol. 19 (2008) 39–46.
[22] P. Devarajan, Biomarkers for the early detection of acute kidney injury, Curr. Opin. Pediatr. 23 (2011) 194–200.
[23] J. Mishra, Q. Ma, A. Prada, M. Mitsnefes, K. Zahedi, J. Yang, J. Barasch, P. Devarajan, Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury, J. Am. Soc. Nephrol. 14 (2003) 2534–2543.
[24] T.A. Sutton, Alteration of microvascular permeability in acute kidney injury, Microvasc. Res. 77 (2009) 4–7.
[25] H.R. Jang, H. Rabb, The innate immune response in ischemic acute kidney injury, Clin. Immunol. 130 (2009) 41–50.
[26] J.V. Bonventre, L. Yang, Cellular pathophysiology of ischemic acute kidney injury, J. Clin. Invest. 121 (2011) 4210–4221.
[27] B.J. Padanilam, Cell death induced by acute renal injury: a perspective on the contributions of apoptosis and necrosis, Am. J. Physiol. Renal. Physiol. 284 (2003) F608–627.
[28] R.A. Zager, A.C. Johnson, Renal ischemia-reperfusion injury upregulates histone-modifying enzyme systems and alters histone expression at proinflammatory/profibrotic genes, Am. J. Physiol. Renal Physiol. 296 (2009) F1032–1041.
[29] R.A. Zager, A.C. Johnson, K. Becker, Acute unilateral ischemic renal injury induces progressive renal inflammation, lipid accumulation, histone modification, and "end-stage" kidney disease, Am. J. Physiol. Renal Physiol. 301 (2011) F1334–1345.
[30] G. Ficz, M.R. Branco, S. Seisenberger, F. Santos, F. Krueger, T.A. Hore, C.J. Marques, S. Andrews, W. Reik, Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation, Nature 473 (2011) 398–402.
[31] H.A. Lee, S.H. Hong, J.W. Kim, I.S. Jang, Possible involvement of DNA methylation in NKCC1 gene expression during postnatal development and in response to ischemia, J. Neurochem. 114 (2010) 520–529.
[32] A. Szwagierczak, S. Bultmann, C.S. Schmidt, F. Spada, H. Leonhardt, Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA, Nucleic Acids Res. 38 (2010) e181.
[33] K. Moran-Crusio, L. Reavie, A. Shih, O. Abdel-Wahab, D. Ndiaye-Lobry, C. Lobry, M.E. Figueroa, A. Vasanthakumar, J. Patel, X. Zhao, F. Perna, S. Pandey, J. Madzo, C. Song, Q. Dai, C. He, S. Ibrahim, M. Beran, J. Zavadil, S.D. Nimer, A. Melnick, L.A. Godley, I. Aifantis, R.L. Levine, Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation, Cancer Cell. 20 (2011) 11–24.
[34] S.G. Jin, X. Wu, A.X. Li, G.P. Pfeifer, Genomic mapping of 5-hydroxy methylcytosine in the human brain, Nucleic Acids Res. 39 (2011) 5015–5024.
[35] C.X. Song, K.E. Szulwach, Y. Fu, Q. Dai, C. Yi, X. Li, Y. Li, C.H. Chen, W. Zhang, X. Jian, J. Wang, L. Zhang, T.J. Looney, B. Zhang, L.A. Godley, L.M. Hicks, B.T. Lahn,

P. Jin, C. He, Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine, Nat. Biotechnol. 29 (2011) 68–72.
[36] K. Williams, J. Christensen, M.T. Pedersen, J.V. Johansen, P.A. Cloos, J. Rappsilber, K. Helin, TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity, Nature 473 (2011) 343–348.
[37] Y. Xu, F. Wu, L. Tan, L. Kong, L. Xiong, J. Deng, A.J. Barbera, L. Zheng, H. Zhang, S. Huang, J. Min, T. Nicholson, T. Chen, G. Xu, Y. Shi, K. Zhang, Y.G. Shi, Genome-wide regulation of 5hmC, 5mC, and gene expression by Tet1 hydroxylase in mouse embryonic stem cells, Mol. Cell. 42 (2011) 451–464.
[38] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[39] S.G. Jin, Y. Jiang, R. Qiu, T.A. Rauch, Y. Wang, G. Schackert, D. Krex, Q. Lu, G.P. Pfeifer, 5-Hydroxymethylcytosine is strongly depleted in human cancers but its levels do not correlate with IDH1 mutations, Cancer Res. 71 (2011) 7360–7365.
[40] H. Yang, Y. Liu, F. Bai, J.Y. Zhang, S.H. Ma, J. Liu, Z.D. Xu, H.G. Zhu, Z.Q. Ling, D. Ye, K.L. Guan, Y. Xiong, Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation, Oncogene (2012).