

非瑟素对HBV复制诱导的细胞氧化损伤的保护作用及其机制研究

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摘要 该文旨在研究sirtuin家族激活剂非瑟素(fisetin)对乙型肝炎病毒(hepatitis B virus, HBV)复制导致的氧化损伤过程中的保护作用, 并初步分析其分子机制。在Huh-7细胞中转染HBV表达质粒pCH9/3091并同时用N-乙酰半胱氨酸(n-acetyl-cysteine, NAC)处理细胞, MitoSOX™ Red试剂检测细胞线粒体活性氧类(reactive oxygen species, ROS)水平, DCFH-DA探针法检测细胞内ROS水平, 丙二醛(malondialdehyde, MDA)试剂盒检测细胞MDA水平, Western blot检测细胞超氧化物歧化酶1(superoxide dismutase 1, SOD1)和SOD2的蛋白质水平。Huh-7细胞转染pCH9/3091的同时用非瑟素处理细胞, 并检测细胞ROS、MDA、SOD1和SOD2蛋白质水平; Huh-7细胞转染pCH9/3091并用非瑟素处理细胞或同时沉默SOD2, 用细胞免疫荧光及Western blot检测 γ -H₂AX(Phosphorylated Histone H2AX)的形成; MTS[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]实验分析氧化应激条件下非瑟素对HBV复制细胞活率的影响; 进一步应用Western blot检测丝裂原激活的蛋白激酶(mitogen-activated protein kinase, MAPK)家族磷酸化水平及总蛋白水平的变化。结果显示, HBV复制明显增加细胞线粒体和细胞内ROS水平和细胞MDA水平, 并且也明显降低SOD1和SOD2水平。NAC处理HBV复制细胞后, 线粒体内和细胞内的ROS和细胞MDA水平明显减少。非瑟素处理HBV复制细胞后, HBV复制对细胞内ROS和MDA水平促进作用明显减弱; 此外, HBV复制对细胞内SOD1和SOD2水平的抑制作用也明显减弱。非瑟素处理HBV复制细胞后, HBV复制对 γ -H₂AX形成的促进作用明显减弱, 而SOD2沉默则减弱了非瑟素对HBV复制细胞中 γ -H₂AX形成的抑制作用。在氧化应激条件下, 非瑟素明显减弱HBV复制对细胞活力的影响, SOD2沉默则减弱了非瑟素对HBV复制细胞活率的影响。非瑟素拮抗HBV表达对JNK(c-Jun N-terminal kinase)和p38(p38 kinase)磷酸化的促进作用, 而SOD2沉默减弱了非瑟素对HBV复制细胞中JNK和p38磷酸化的抑制作用。该研究结果表明, 非瑟素可能通过促进SOD2的表达拮抗HBV复制导致的氧化应激反应, 并可能通过抑制JNK及p38的激活减少细胞氧化损伤, 从而发挥保护细胞的作用。

关键词 sirtuin家族; 非瑟素; 乙型肝炎病毒; 氧化应激

The Protective Role of Fisetin in Oxidative Damage Induced by HBV Replication and Its Mechanism

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Abstract This study was aimed to investigate the protective role of sirtuins activator fisetin in oxidative damage induced by hepatitis B virus (HBV) replication and its mechanism. Huh-7 cells were transfected with HBV expressing plasmid pCH9/3091 or treated with n-acetyl-cysteine (NAC). The mitochondria and cytosolic reactive oxygen species (ROS) levels were measured by MitoSOX™ Red reagent and DCFH-DA probe assay, respectively. The malondialdehyde (MDA) level was detected by MDA assay kit. The superoxide dismutase 1 (SOD1) and SOD2 protein levels were measured by Western blot. Furthermore, the Huh-7 cells transfected with pCH9/3091 were treated with fisetin, then the levels of ROS, MDA, SOD1 and SOD2 were determined. In addition, the Huh-7 cells transfected with pCH9/3091 were treated with fisetin or simultaneously silencing the SOD2 expression. The phosphorylated Histone H2AX (γ -H₂AX) formation was analyzed by cyto-immunofluorescence and Western blot. Under the condition of oxidative stress, the effect of fisetin and SOD2 silencing to the cell viability of HBV expressing cells was measured by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay. Moreover, the levels of total mitogen-activated protein kinases (MAPKs) and phosphorylated MAPKs were detected by Western blot. The results showed that HBV replication significantly increased the ROS and MDA levels, and significantly decreased the SOD1 and SOD2 protein levels in Huh-7 cells. NAC decreased the ROS and MDA levels in HBV expressing cells. Fisetin attenuated the increase of ROS and MDA levels, and the decrease of SOD1 and SOD2 protein levels. Moreover, fisetin inhibited the enhancement of γ -H₂AX formation in HBV replication cells. SOD2 silencing attenuated the inhibitory role of fisetin in γ -H₂AX formation in HBV replication cells. Furthermore, under the oxidative stress condition, fisetin significantly attenuated the effect of HBV replication on cell viability. SOD2 silencing decreased the effect of fisetin on the viability of HBV replication cells. In addition, fisetin significantly antagonized the promotion effect of HBV expression to the phosphorylation of c-Jun N-terminal kinase (JNK) and p38 kinase (p38). SOD2 silencing attenuated the inhibitory role of fisetin in the phosphorylation of JNK and p38. These results indicated that fisetin could attenuated the oxidative stress response induced by HBV replication possibly through enhancing the expression of SOD2. Moreover, fisetin could decrease the oxidative damage possibly by inhibiting the activation of JNK and p38, finally play a protection role in HBV replication cell.

Keywords sirtuins; fisetin; HBV; oxidative stress

乙型肝炎病毒(hepatitis B virus, HBV)是世界范围内广泛流行的一种嗜肝DNA病毒,感染了3亿5千万人,严重危害人类的健康^[1]。HBV感染可通过多种途径影响宿主细胞的生理进程及宿主免疫应答,最终导致肝细胞损伤。一方面,HBV基因组可以整合入宿主基因,并且调控宿主基因的转录,进而调控宿主细胞信号通路^[2],影响细胞凋亡^[3]、细胞周期^[4]、细胞增殖^[5]等。另一方面,HBV感染引起T细胞免疫应答,诱发肝细胞坏死,导致肝炎的发生;而HBV如果能够逃脱宿主的免疫应答,长久存活于肝细胞内,则会导致持续的肝细胞损害,引发肝脏疾病^[6]。此外,也有文献报道,HBV感染可引起细胞氧化应激反应,导致肝细胞氧化损伤,最终促进了肝脏疾病发生^[7]。因此,鉴定新的分子、保护在HBV感染过程中受氧化损伤的肝细胞具有重要的意义。

沉默信息调节因子家族(sirtuins)是近来发现的烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NAD⁺)依赖性的第III类组蛋白去乙酰化酶,人类sirtuin家族共有7个成员即SIRT1~SIRT7,它们在定位在细胞的不同部位,发挥着多种功能。研究表明,sirtuins参与了多种生物学功能的调节,如细胞存活、老化、增殖、凋亡、DNA修复、细胞代谢等^[8]。近年来,sirtuin家族与细胞氧化应激反应的关系也逐渐引起了人们的关注。SIRT1可去乙酰化参与细胞应激反应的关键蛋白质,如FoxO(forkhead box O)、PGC-1 α (peroxisome proliferator-activated receptor- γ coactivator-1 α)、NF- κ B(nuclear factor-kappa B)等,从而降低细胞的氧化应激反应^[9]。SIRT2能去乙酰化并活化葡萄糖-6-磷酸脱氢酶,激活磷酸戊糖途径,从而产生NADPH,拮抗氧化损

伤^[10]。SIRT3能去乙酰化OGG1(8-oxoguanine DNA glycosylase), 保护氧化损伤的线粒体DNA, 抑制氧化应激导致的细胞死亡^[11]。也有多篇文献报道了SIRT4~SIRT7与氧化应激存在密切的关系^[12-15]。非瑟素(fisetin)是sirtuin家族的活化物^[16], 目前尚没有研究证实非瑟素与HBV复制诱导的氧化应激的关系。因此, 本研究将重点关注非瑟素对HBV复制诱导的氧化应激及氧化损伤的调节作用并初步分析其分子机制。

1 材料与方 法

1.1 材 料

Huh-7细胞购于ATCC(American Type Culture Collection)。HBV复制质粒pCH9/3091(pCH9/3091含有HBV 1.1×全基因组DNA, 由CMV(cytomegalovirus)启动子驱动HBV前基因组RNA(pgRNA)转录, 该质粒在转入肝源性细胞后可支持HBV复制, 由第三军医大学林兰教授赠送。shCont和shSOD2质粒购于吉凯基因公司。ERK(extracellular signal-regulated kinase)抗体、ERK5抗体、JNK(c-Jun N-terminal kinase)抗体、p38(p38 kinase)抗体、SOD1(superoxide dismutase 1)抗体、SOD2抗体、 γ -H₂AX(phosphorylated Histone H2AX)抗体购于Cell Signaling Technology公司。 β -actin抗体购于Santa Cruz Biotechnology公司。胎牛血清购于Gibco公司。DMEM液体培养基购于Corning公司。NAC和活性氧检测试剂盒购于碧云天生物技术有限公司。sirtuin激活剂非瑟素购于Selleck公司。MDA试剂盒购于南京建成生物工程研究所。MTS试剂和MitoSOX™ Red试剂购于Promoga公司。质粒转染试剂购于Roche公司。

1.2 细胞培养和转染

Huh-7细胞培养于含有10%胎牛血清、1%青/链霉素的DMEM培养基中, 在37 °C、5% CO₂孵箱中常规培养。质粒转染按说明书操作, 具体如下: 用无抗生素培养基给细胞换液, 2 μ g质粒混匀于200 μ L opti-MEM培养基中, 混匀后加入6 μ L转染试剂(质粒与转染试剂的比例为1:3), 充分混匀后室温静置15 min均匀滴加质粒于6孔板后放置于孵箱中。

1.3 HBV复制中间体提取和Real-time PCR

Huh-7细胞转染后5 d, PBS洗涤, 用0.5 mL细胞裂解液[10 mmol/L Tris-HCl(pH8.0), 1 mmol/L EDTA, 1% NP-40, 2%蔗糖]于37 °C裂解细胞15 min, 离心后取

上清。在上清中加入40 U/mL DNase I和10 mmol/L MgCl₂, 37 °C孵育4 h。加入200 μ L 35% PEG-8000(含1.5 mol/L NaCl)冰浴1 h。12 000 r/min、4 °C离心5 min, 弃上清。加入0.5 mL蛋白酶K消化液[0.5% SDS, 150 mmol/L NaCl, 25 mmol/L Tris-HCl (pH 8.0), 10 mmol/L EDTA]和0.5 mg/mL蛋白酶K, 45 °C过夜。然后用酚氯仿抽提, 异丙醇沉淀, 70%乙醇洗涤, TE缓冲液溶解HBV复制中间体。按照SYBR Green说明书配制反应体系, 进行HBV复制中间体绝对定量检测。HBV特异性上游引物: 5'-CCT AGT AGT CAG TTA TGT CAA C-3'; 下游引物: 5'-TCT ATA AGC TGG AGG AGT GCG A-3'。

1.4 Western blot

用含蛋白酶抑制剂的RIPA裂解液裂解细胞, 用BCA蛋白质定量试剂盒测定蛋白质浓度。取等量(30 μ g)细胞总蛋白于10%的SDS-PAGE中分离蛋白质。电泳后, 蛋白质在湿式转移电泳槽中被转移至硝酸纤维素膜。5%脱脂牛奶室温封闭膜1 h, 然后在4 °C摇床过夜孵育相应的一抗(用封闭液按1:2 000稀释)。用TBS-T洗膜3次后, 用辣根过氧化物酶标记的羊抗兔或羊抗鼠二抗(以封闭液按1:3 000稀释)室温摇床孵育2 h。用TBS-T洗膜3次后, 用ECL试剂显影。以GAPDH为内参。

1.5 线粒体ROS检测

Huh-7细胞接种于6孔板中, 转染HBV质粒后3 d, 用预热的PBS洗涤细胞, 然后加入1 mL含有5 μ L MitoSOX™ Red的PBS覆盖细胞, 37 °C避光孵育10 min。然后用预热的PBS洗涤细胞3次后, 在荧光显微镜下观察并拍照。

1.6 细胞内ROS水平检测

Huh-7细胞接种于6孔板中, 转染HBV质粒后3 d, 去除细胞培养液, 加入1 mL 10 μ mol/L DCFH-DA溶液, 37 °C细胞培养箱内孵育20 min, 无血清细胞培养液洗涤细胞3次, 在荧光显微镜下观察并拍照。

1.7 MDA检测

MDA检测按说明书操作, 具体如下: 将等量的标准品和样品分别加入试管中, 再加入试剂一、二、三, 混匀, 95 °C沸水浴40 min, 3 500 r/min离心10 min, 取上清, 532 nm波长处测D值。

1.8 细胞免疫荧光

接种Huh-7细胞至含盖玻片的6孔板中。转染72 h后, 用4%多聚甲醛室温固定15 min, 然后用0.1%

Triton X-100室温孵育10 min, 再用1% BSA室温封闭45 min。4 °C过夜孵育相应的一抗(按1:100稀释), 用PBS洗涤3次, 室温孵育二抗2 h。最后, 细胞核用DAPI在室温染色10 min。在荧光显微镜下观察并拍照。

1.9 MTS检测细胞活率

接种细胞于6孔板中, 转染后24 h, 接种细胞于96孔板中, 培养24 h后经不同处理后, 继续培养72 h后加入MTS溶液, 37 °C避光孵育1 h, 将96孔板置于酶标仪中, 490 nm波长处读取D值。

1.10 统计学处理

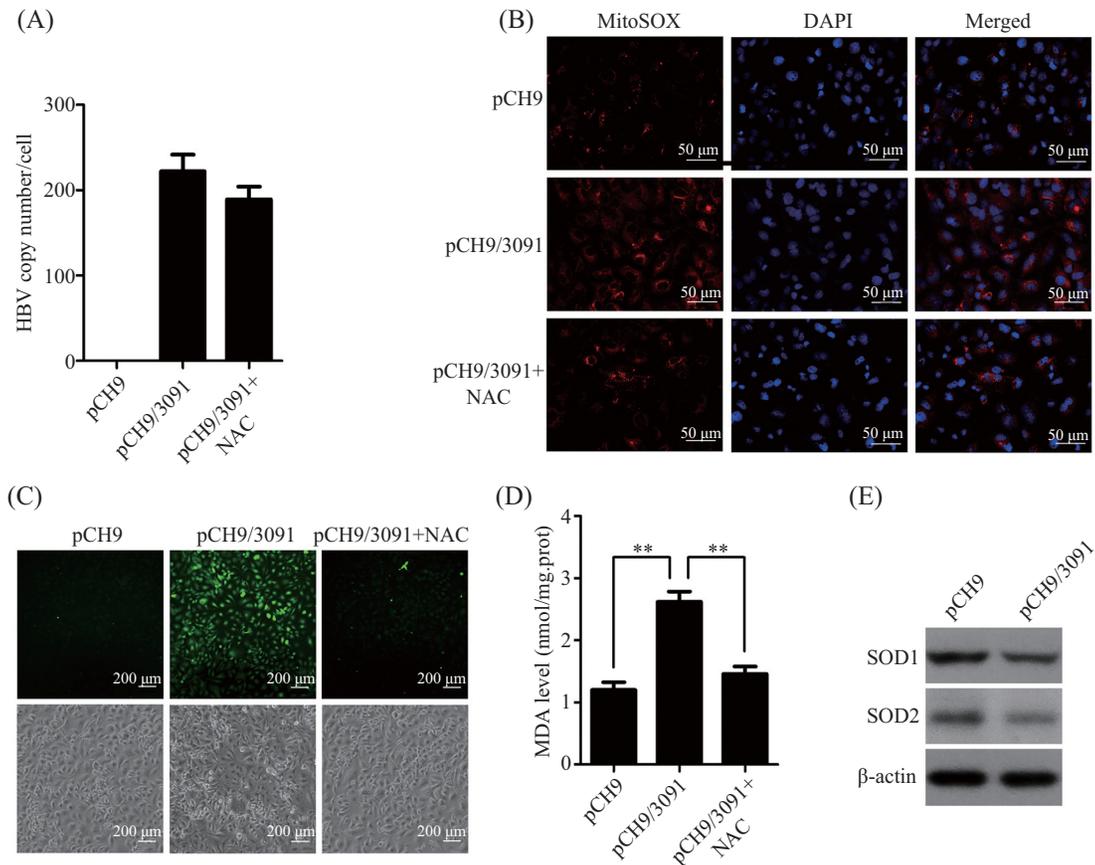
采用SPSS 20.0统计软件进行统计学分析。数据均采用均数±标准差(mean±S.D.)表示, 多样本均数间的显著性检验用方差分析, 两样本间均数比较

采用配对t检验, $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 HBV复制对细胞氧化应激反应指标的影响

为了验证HBV复制对细胞氧化应激反应的影响, 我们在Huh-7细胞中瞬时转染了对照质粒pCH9及HBV表达质粒pCH9/3091, 并同时用2 mmol/L NAC处理转染pCH9/3091的细胞, Real-time PCR检测结果显示, 转染pCH9/3091后, Huh-7细胞中存在较高复制水平的HBV(图1A)。利用MitoSOX™ Red试剂检测结果显示, HBV复制细胞线粒体中的ROS水平明显高于对照细胞, NAC处理后, HBV复制细胞线粒体ROS水平明显降低(图1B)。DCFH-DA探针法检测结果显示, HBV复制细胞内的ROS水平



A~D: Huh-7细胞转染pCH9/3091或用2 mmol/L NAC处理。A: Real-time PCR检测细胞中HBV复制水平; B: MitoSOX™ Red试剂分析细胞线粒体中的ROS水平; C: DCFH-DA探针法分析细胞内的ROS水平; D: MDA检测试剂检测细胞中的MDA水平, $**P < 0.01$ 。E: Huh-7细胞转染pCH9和pCH9/3091, Western blot检测SOD1和SOD2蛋白质水平。

A~D: Huh-7 cells were transfected by pCH9/3091 or treated with 2 mmol/L NAC. A: the HBV replication level in the cells was detected by Real-time PCR; B: ROS level in the cellular mitochondria was examined by MitoSOX™ Red fluorescence; C: the cytosolic ROS was analyzed by DCFH-DA probe assay; D: MDA level was examined by MDA assay kit, $**P < 0.01$. E: the protein levels of SOD1 and SOD2 in Huh-7 cells transfected by pCH9 or pCH9/3091 were measured by Western blot.

图1 HBV复制对细胞氧化应激反应的影响

Fig.1 The effect of HBV replication on cellular oxidative stress response

明显高于对照细胞, NAC处理后, HBV复制细胞内的ROS水平明显降低(图1C)。MDA是一种脂质氧化的天然产物, 细胞氧化应激时会发生脂质氧化, 因此MDA水平可反映细胞氧化应激水平。MDA检测试剂盒分析结果显示, HBV复制能明显增高细胞内的MDA水平, 而NAC能明显抑制HBV复制对MDA水平的促进作用(图1D), 差异具有统计学意义($P<0.01$)。此外, Western blot检测也发现, HBV复制细胞中SOD1和SOD2蛋白质水平明显降低(图1E), 这些结果揭示, HBV能明显促进细胞的氧化应激反应。

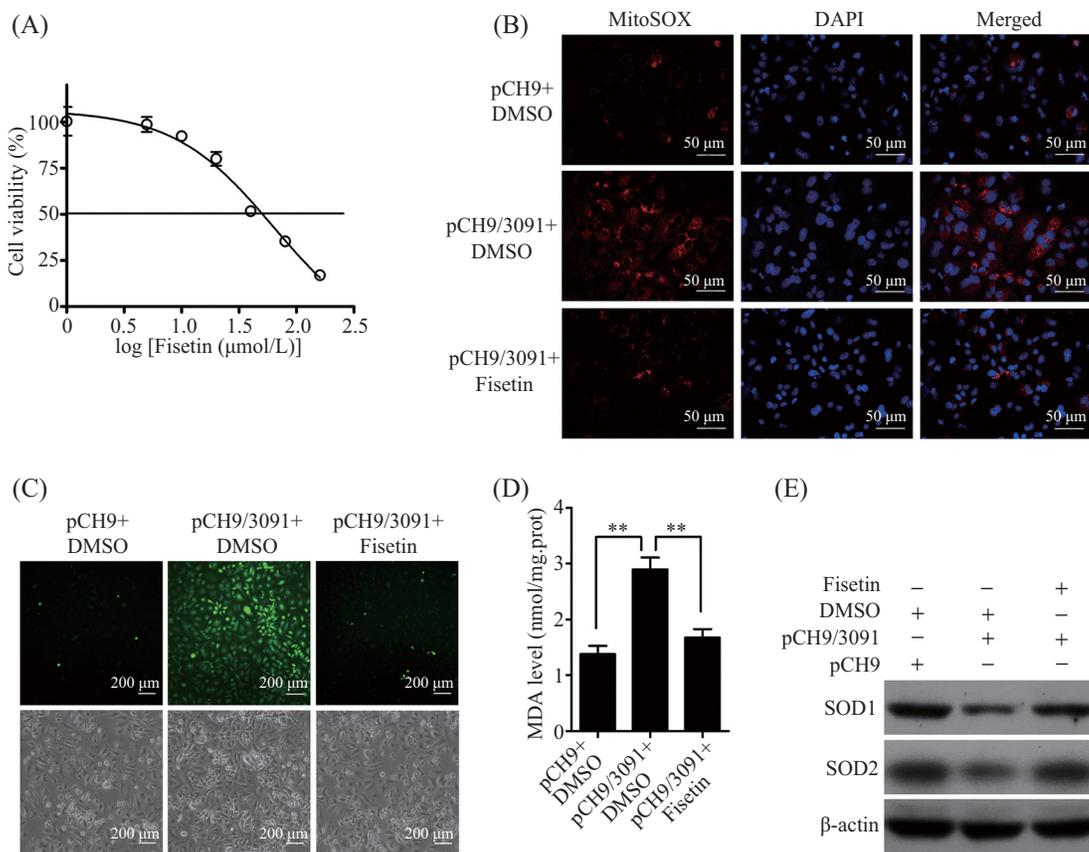
2.2 Sirtuin家族激活剂非瑟素对HBV复制诱导的氧化应激反应的影响

Sirtuin家族多个成员对氧化应激反应具有重要的调控作用, 为了研究 sirtuin 家族激活剂非瑟素在

HBV复制所诱导的氧化应激反应中的作用, 我们首先用MTS实验分析了非瑟素的细胞毒性。结果显示, 非瑟素的 IC_{50} 约是 $65 \mu\text{mol/L}$ (图2A)。其次, 在Huh-7细胞中瞬时转染pCH9/3091的同时用 $10 \mu\text{mol/L}$ 非瑟素处理细胞。通过一系列实验检测发现, 非瑟素处理后, HBV复制细胞线粒体和细胞内的ROS水平明显降低(图2B和图2C), MDA的水平也明显下降($P<0.01$)(图2D), SOD1和SOD2蛋白质水平明显回升(图2E)。这些结果证实, 非瑟素可能通过上调SOD1和SOD2水平而减弱了HBV复制所诱导的细胞氧化应激反应。

2.3 非瑟素对HBV复制诱导的DNA损伤的影响

氧化应激是DNA损伤的重要原因, 而线粒体是细胞ROS产生的重要部位。为了研究非瑟素对HBV复制介导的氧化应激反应导致的DNA损伤的



A: MTS实验分析不同浓度非瑟素的细胞毒性; B~E: Huh-7细胞转染pCH9/3091或同时用 $10 \mu\text{mol/L}$ 非瑟素处理。B: MitoSOXTM Red试剂分析细胞线粒体中的ROS水平; C: DCFH-DA探针法分析细胞内的ROS水平; D: MDA检测试剂盒检测细胞中的MDA水平, ** $P<0.01$ 。E: Western blot检测细胞内SOD1和SOD2蛋白质水平。

A: the cytotoxicity of different concentration of fisetin was analyzed by MTS assay; B-E: Huh-7 cells were transfected by pCH9/3091 or treated with $10 \mu\text{mol/L}$ fisetin. B: ROS level in the cellular mitochondria was examined by MitoSOXTM Red fluorescence; C: the cytosolic ROS was analyzed by DCFH-DA probe assay; D: MDA level was examined by MDA assay kit, ** $P<0.01$. E: SOD1 and SOD2 protein levels were detected by Western blot.

图2 非瑟素对HBV复制诱导的氧化应激反应的影响

Fig.2 The effect of fisetin on the oxidative stress response induced by HBV replication

影响及定位在线粒体的SOD2在其中的重要作用,我们在Huh-7细胞中瞬时转染pCH9/3091的同时用10 $\mu\text{mol/L}$ 非瑟素处理细胞或同时沉默SOD2的表达。细胞免疫荧光检测发现,HBV复制细胞中DNA损伤标志物 $\gamma\text{-H}_2\text{AX}$ foci的形成明显多于对照细胞,非瑟素处理后的HBV复制细胞中 $\gamma\text{-H}_2\text{AX}$ foci的形成明显减少,而当SOD2沉默时, $\gamma\text{-H}_2\text{AX}$ foci的形成又明显增加($P<0.01$)(图3A和图3B)。这一结果证明,非瑟素通过增加SOD2蛋白质水平而减弱了HBV复制细胞的DNA损伤。

2.4 在氧化应激条件下非瑟素对HBV复制细胞的保护作用

为了进一步分析在氧化应激条件下,非瑟素对HBV复制细胞的保护作用,我们用含不同浓度的 H_2O_2 处理Huh-7细胞,使细胞处于氧化应激状态(同时转染pCH9/3091和用10 $\mu\text{mol/L}$ 非瑟素处理细胞或同时沉默SOD2的表达)。MTS实验分析发现,在氧化应激条件下HBV复制细胞的活力明显下降($P<0.05$),非瑟素作用时,HBV复制细胞的活率明显回升($P<0.05$),而同时SOD2沉默时,HBV复制细胞的活力又显著下降($P<0.05$)(图4)。这一发现表明,在HBV复制细胞氧化损伤时,非瑟素通过增加SOD2

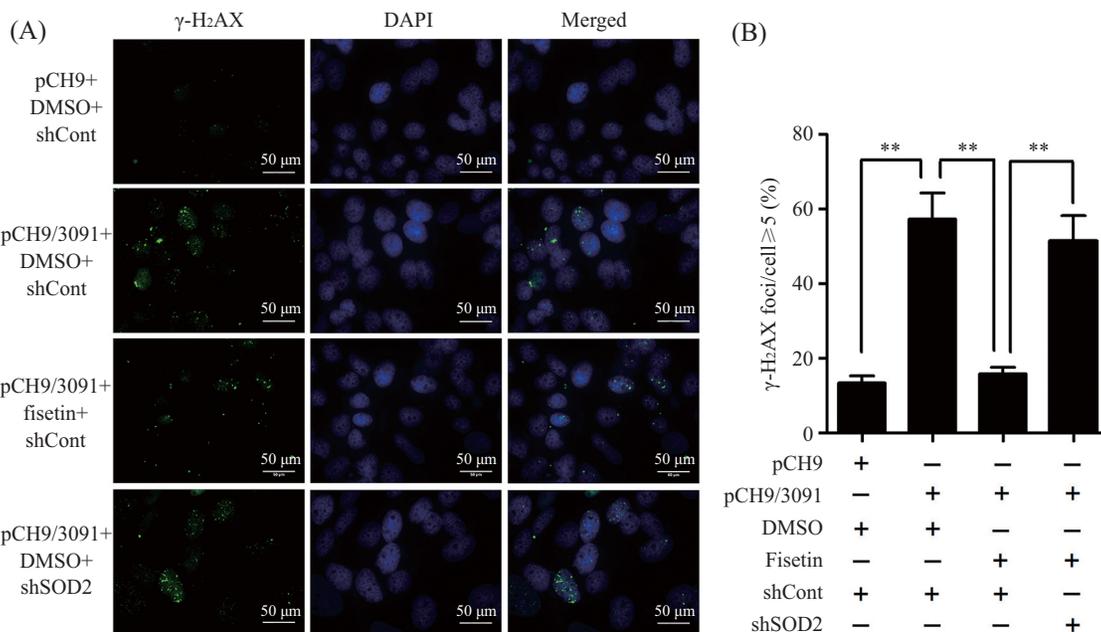
的表达发挥了保护细胞的作用。

2.5 非瑟素对促分裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)家族成员活化的影响

MAPK通路在氧化应激导致的氧化损伤反应中具有重要的调控作用,为了初步研究非瑟素保护HBV复制细胞的分子机制,我们检测了转染pCH9/3091和用10 $\mu\text{mol/L}$ 非瑟素处理或同时沉默SOD2的Huh-7细胞中MAPK家族成员(JNK、p38、ERK、ERK5)的活化形式磷酸化水平及总蛋白水平。Western blot检测结果显示,HBV复制细胞中JNK和p38的磷酸化水平显著增加,而JNK和p38的总蛋白水平和MAPK家族其他成员的磷酸化水平及总蛋白水平未见明显变化。非瑟素作用时HBV复制中JNK和p38的磷酸化水平明显降低,而当SOD2沉默时,JNK和p38的磷酸化水平又明显升高(图5)。这一结果表明, JNK/p38信号通路可能在非瑟素减弱HBV复制细胞氧化损伤过程中具有重要作用。

3 讨论

氧化应激被认为是疾病病理过程的重要原因,可导致多种致癌作用,包括引起基因组不稳定性

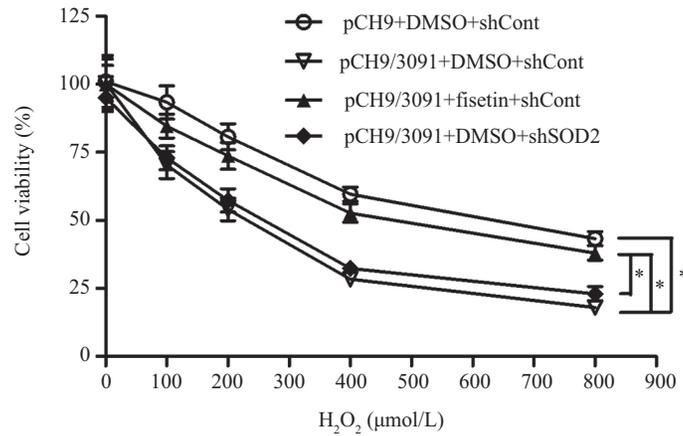


A: 细胞免疫荧光检测非瑟素和SOD2沉默对HBV复制促进 $\gamma\text{-H}_2\text{AX}$ 形成的影响; B: 非瑟素处理和SOD2沉默后HBV复制细胞中 $\gamma\text{-H}_2\text{AX}$ 形成阳性细胞($\gamma\text{-H}_2\text{AX}$ foci ≥ 5)百分比, $**P<0.01$ 。

A: the effect of fisetin and SOD2 silencing on the promotion of $\gamma\text{-H}_2\text{AX}$ induced by HBV replication detected by immunofluorescence; B: the percentage of $\gamma\text{-H}_2\text{AX}$ formation positive cells ($\gamma\text{-H}_2\text{AX}$ foci ≥ 5) in HBV expressing cells treated by fisetin and SOD2 silencing, $**P<0.01$.

图3 非瑟素和SOD2沉默对HBV复制促进 $\gamma\text{-H}_2\text{AX}$ 形成的影响

Fig.3 The effect of fisetin and SOD2 silencing on the promotion of $\gamma\text{-H}_2\text{AX}$ induced by HBV replication



* $P < 0.05$.

图4 H₂O₂处理下, MTS分析非瑟素和SOD2沉默对HBV复制细胞活力的影响
Fig.4 Under the H₂O₂ treatment, the effect of fisetin and SOD2 silencing on the viability of cells expressing HBV plasmid analyzed by MTS

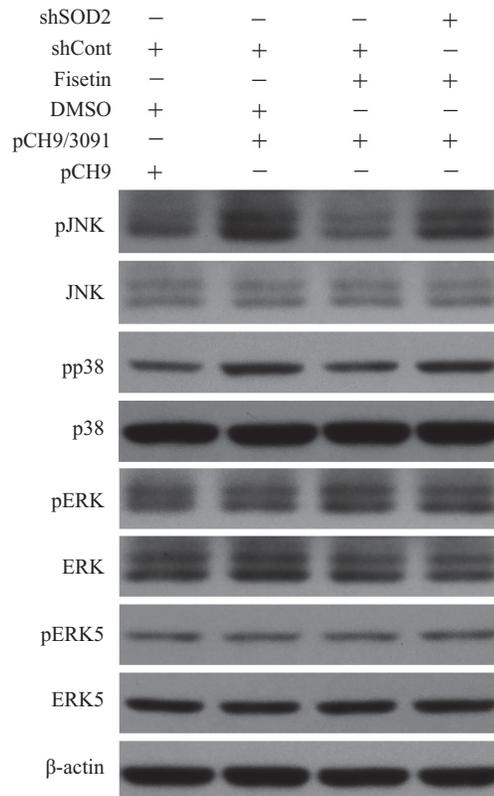


图5 Western blot检测经不同处理后的Huh-7细胞中MAPKs磷酸化水平

Fig.5 The levels of MAPKs phosphorylation in Huh-7 cells under different treatment measured by Western blot

增加、DNA突变、调控基因表达及表观遗传学修饰^[17]。多项研究表明,在HBV感染引起的急性或慢性肝炎中,氧化物增加或抗氧化物减少^[18-19]。本研究结果也证实,在HBV表达的细胞中氧化物ROS和MDA明显增加,而抗氧化物SOD1和SOD2的水平显著降低,抗氧化剂NAC能拮抗HBV复制诱导的氧化应激反应。我们进一步鉴定了一个新的细胞保护分

子sirtuin家族激活剂——非瑟素。我们证实,非瑟素能够明显减弱HBV复制引起的细胞ROS和MDA水平的增加,并且能明显增加HBV复制细胞SOD1和SOD2的蛋白质水平。线粒体是细胞内ROS的主要来源^[20],SOD2能够清除线粒体ROS^[21],因此,非瑟素增加SOD2的表达可能是其抑制HBV复制诱导的氧化应激反应的重要原因。ROS水平增加可进一步导

致细胞氧化损伤。例如, HBV编码蛋白HBx(hepatitis B virus x protein)可引起细胞ROS水平增加并导致线粒体DNA损伤, 这也被认为可能是肝癌发生的原因之一^[22]。本研究也发现, 在HBV表达细胞中 γ -H₂A_X的形成明显增加, 表明HBV引起的ROS水平增加导致了DNA氧化损伤。非瑟素能够减弱HBV复制导致的DNA氧化损伤, 而当SOD2沉默时, 非瑟素则不能减弱HBV复制导致的细胞氧化损伤。也有文献报道, ROS水平增加可引起细胞活力下降, 导致细胞死亡^[23]。我们的实验结果也证实, 在氧化应激条件下, HBV复制的细胞活力明显下降; 非瑟素作用时, HBV复制细胞的活力明显回升; 同时沉默SOD2时, HBV复制细胞的活力又明显下降。这就说明非瑟素可能通过SOD2对氧化损伤的HBV表达细胞发挥保护作用。

据文献报道, 氧化应激可激活MAPK家族成员, 进一步导致细胞氧化损伤^[24]。因此, 我们也检测了HBV复制诱导的氧化应激对MAPK家族成员激活的影响, 结果显示, HBV复制可激活JNK和p38, 而对其他成员的活化形式没有显著影响, 并且证实非瑟素抑制了HBV复制诱导的氧化应激反应导致的JNK和p38的激活, SOD2沉默则拮抗了非瑟素对JNK和p38激活的抑制作用。JNK/p38信号通路在氧化应激反应导致细胞损伤中发挥了重要作用。Thongnuanjan等^[25]报道, 非诺贝特通过抑制JNK/p38信号通路的活化减少了顺铂引起的ROS增加导致的细胞死亡。Ong等^[26]也报道, 2-甲氧基-1,4-萘醌通过增加ROS水平, 激活JNK/p38信号通路导致了细胞凋亡。此外, Li等^[27]也发现, 乌司他丁可通过抑制JNK及下游信号分子c-Jun(Jun proto-oncogene, AP-1 transcription factor subunit)的激活减弱了脂多糖引起的氧化损伤。这些研究报道及我们的实验结果表明, 非瑟素极有可能通过增加SOD2的表达, 减少了细胞氧化应激反应, 从而抑制JNK和p38的激活最终减少了细胞的氧化损伤。综上所述, 我们鉴定了一个新的细胞保护分子——非瑟素。非瑟素能够拮抗HBV复制诱导的氧化应激反应, 并可能通过JNK/p38信号通路减少细胞的氧化损伤, 这为完善HBV感染患者的治疗提供了新的策略。

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