BaP激活AhR调控FGF-21影响HepG2细胞脂质代谢的机制

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摘要: 探究不同浓度的苯并芘 (benzo[*a*]pyrene, BaP) 对HepG2细胞中成纤维细胞生长因子21 (fibroblast growth factor 21, FGF-21) 和脂质代谢及相关基因表达的影响, 以及这些作用是否由芳香烃受体 (aryl hydrocarbon receptor, AhR) 所介导。采用15、30和60 μmol·L^{-1} BaP作用HepG2细胞24 h, 检测HepG2细胞中总胆固醇 (total cholesterol, TC)、甘油三酯 (triglyceride, TG) 和低密度脂蛋白胆固醇 (low-density lipoprotein cholesterol, LDL-C) 的含量, qPCR和Western blot检测FGF-21、*β*-氧化相关基因 [过氧化物酶体增殖物激活受体*α* (peroxisome proliferator-activated receptor *α*, PPAR*α*)、肉碱棕榈酰转移酶1*α* (carnitine palmitoyltransferase 1*α*, CPT-1*α*)、过氧化物酶体增殖物激活受体*γ*共激活因子1*α* (peroxisome proliferator-activated receptor gamma coactivator 1*α*, PGC-1*α*)] 和脂质合成相关基因 [过氧化物酶体增殖物激活受体*γ* (peroxisome proliferator-activated receptor *γ*, PPAR*γ*)、CCAAT/增强子结合蛋白*α* (CCAAT/enhancer-binding protein *α*, C/EBP*α*)、硬脂酰辅酶A去饱和酶1 (stearoyl-CoA desaturase 1, SCD1)] 的mRNA和蛋白表达。通过慢病毒转染构建沉默AhR的稳转细胞株, 按分组加入30 μmol·L^{-1} BaP进行给药处理, 油红O染色法检测脂质含量, 检测FGF-21、*β*-氧化相关基因和脂质合成相关基因的mRNA和蛋白表达。与control组相比, 15、30和60 μmol·L^{-1} BaP都可使HepG2细胞中TC、TG和LDL-C的含量升高; 60 μmol·L^{-1} BaP组HepG2细胞中FGF-21和*β*-氧化相关基因mRNA和蛋白表达增加程度不及30 μmol·L^{-1} BaP组明显, 但脂质合成相关基因的mRNA和蛋白的表达升高。沉默AhR并给予30 μmol·L^{-1} BaP后HepG2细胞中脂质含量、TC、TG和LDL-C的含量降低, FGF-21、*β*-氧化相关基因和脂质合成相关基因的mRNA和蛋白表达也均降低。不同剂量BaP都可通过AhR造成HepG2细胞脂质含量和FGF-21表达增加, 其中低剂量的BaP促进脂质合成作用相对较弱, 但可显著提高脂肪酸*β*-氧化水平; 而高剂量BaP对FGF-21的诱导作用弱于低剂量组, 可提高脂质合成水平, 而促进脂肪酸*β*-氧化作用相对较弱。综上, BaP对FGF-21、脂质代谢及相关基因的作用都是由AhR所介导的。

关键词: 苯并芘; 芳香烃受体; 成纤维细胞生长因子21; 慢病毒转染; 脂质代谢

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Mechanism by which benzo[*a*]pyrene activates AhR to regulate FGF-21 and affect lipid metabolism in HepG2 cells

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Abstract: This study investigated the effects of different doses of benzo[*a*]pyrene (BaP) on the expression of

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fibroblast growth factor 21 (FGF-21), lipid metabolism, and related genes in HepG2 cells, as well as whether these effects are mediated by the aryl hydrocarbon receptor (AhR). HepG2 cells were treated with 15, 30, and 60 μmol·L^{-1} BaP for 24 h. The levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in HepG2 cells were measured. The mRNA and protein expression levels of FGF-21, *β*-oxidation-related genes [peroxisome proliferator-activated receptor *α* (PPAR*α*), carnitine palmitoyltransferase 1*α* (CPT-1*α*), and peroxisome proliferator-activated receptor gamma coactivator 1*α* (PGC-1*α*)], and lipid synthesis-related genes [peroxisome proliferator-activated receptor *γ* (PPAR*γ*), CCAAT/enhancer-binding protein *α* (C/EBP*α*), and stearoyl-CoA desaturase 1 (SCD1)] were detected by qPCR and Western blot. A stable cell line with AhR silencing was established *via* lentiviral transduction, and the cells were treated with 30 μmol·L^{-1} BaP. Lipid content was assessed by oil red O staining, and the mRNA and protein expression levels of FGF-21, *β*-oxidation-related genes, and lipid synthesis-related genes were measured. Compared with the control group, all three doses of BaP (15, 30, and 60 μmol·L^{-1}) increased the levels of TC, TG, and LDL-C in HepG2 cells. Compared with the 30 μmol·L^{-1} BaP group, the 60 μmol·L^{-1} BaP group showed higher levels of TC, TG, and LDL-C, lower mRNA and protein expression of FGF-21 and *β*-oxidation-related genes, and higher mRNA and protein expression of lipid synthesis-related genes. Following AhR silencing and treatment with 30 μmol·L^{-1} BaP, lipid content, as well as the levels of TC, TG, and LDL-C, decreased in HepG2 cells, and the mRNA and protein expression levels of FGF-21, *β*-oxidation-related genes, and lipid synthesis-related genes were all reduced. Different doses of BaP increased lipid content in HepG2 cells. However, low-dose BaP activated AhR, thereby relatively increasing FGF-21 expression, reducing lipid synthesis, and enhancing fatty acid *β*-oxidation. In contrast, high-dose BaP activated AhR but relatively decreased FGF-21 expression, increased lipid synthesis, and reduced fatty acid *β*-oxidation. The effects of BaP on FGF-21, lipid metabolism, and related genes are all mediated by AhR.

Key words: benzo[*a*]pyrene; aryl hydrocarbon receptor; fibroblast growth factor 21; lentiviral transfection; lipid metabolism

脂类在机体能量储存中发挥关键作用, 还可参与细胞信号的传递过程, 脂质稳态的维持与脂肪、肝脏等核心代谢组织的功能运转密切相关[1,2]。许多研究证明, 脂质代谢紊乱会导致过度的脂质积累, 促进代谢功能障碍相关脂肪性肝病 (metabolic dysfunction-associated steatotic liver disease, MASLD) 的发生发展[3,4]。MASLD是一个不断发展的肝病, 严重时可致肝功能衰竭和肝细胞癌[5,6], 常常会伴随心血管疾病[7]、糖尿病[8]等代谢性疾病。

吸烟会诱发机体脂质代谢紊乱, 影响脂肪肝的发生发展以及晚期肝病患者的预后[9-11], 并且香烟烟雾中的多环芳烃 (polycyclic aromatic hydrocarbons, PAHs) 是公认的具有肝毒性且致畸致癌的有机环境污染物[12-14]。苯并芘 (benzo[*a*]pyrene, BaP) 是PAHs中最常见的化合物, 常常存在于炭烤食物、汽车尾气、香烟烟雾等人类生存的环境介质中, 具有高度亲脂性, 被认为是肿瘤、炎症和代谢性疾病的诱因。BaP可导致肝脂肪变性, 肝脂含量显著增加, 有研究表明BaP与芳香烃受体 (aryl hydrocarbon receptor, AhR) 结合可诱发小鼠自发性肝细胞脂肪变性[15,16]。

AhR为一种核转录因子, 通过内源或外源性配体激活后, 入核形成特定的核蛋白发挥作用。PAHs包括BaP、2, 3, 7, 8-四氯二苯-*p*-二噁英 (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin, TCDD) 等都是经典的AhR外源性配体, BaP的体内代谢过程经由AhR介导[17]。在未激活的状态下, AhR和分子伴侣HSP90、p23、XAP2在细胞质形成无活性的复合物, 当BaP与AhR结合后, AhR脱离复合物与芳香烃受体核转位蛋白 (aryl hydrocarbon receptor nuclear translocator, ARNT) 形成异二聚体, 转移到细胞核内, 与目标基因启动子区域的异源物质反应元件 (xenobiotic- or dioxin-responsive elements, XREs/DREs) 结合, 引发多种生理及毒理作用[18]。并且AhR在肝脏脂质代谢方面具有重要的作用, 有研究表明[19], 激活色氨酸代谢产物介导的AhR, 可抑制核因子*κ*B (NF-*κ*B) 通路, 下调脂肪酸合成酶 (fatty acid synthetase, FAS)-固醇调节元件结合蛋白1c (sterol regulatory element-binding protein-1c, SREBP-1c) 通路, 从而调节炎症和脂质代谢。且肠道微生物群与AhR之间的相互作用也可调节宿主的脂质代谢[20]。

成纤维细胞生长因子21 (fibroblast growth factor FGF-21) 是一种参与脂质代谢的新型调节因子, 可参与并调控脂质代谢[21]。有研究表明AhR与FGF-21的转录调控有关。Girer等[22]研究表明FGF-21启动子中有3段XRE结构域, 分别为XRE1、XRE2和XRE3。项目组前期的研究发现, 低剂量的BaP可调控AhR与FGF-21启动子区域中XRE1结合, 而高剂量的BaP可

调控AhR与FGF-21启动子区域中XRE3结合[23]。但有关BaP调控FGF-21及对下游脂质代谢相关基因表达的影响是否由AhR介导尚无明确的定论。

本研究旨在明确BaP对HepG2细胞脂质代谢的影响及其分子机制, 重点阐明AhR信号通路与FGF-21在其中的调控作用。实验通过慢病毒转染构建沉默AhR的HepG2稳转细胞株, 进一步验证BaP对FGF-21和脂质代谢及相关基因的表达是由AhR介导的分子机制, 以期补充环境污染物致肝脂代谢异常的理论依据, 为MASLD的治疗提供新的药物治疗靶点和策略, 预防MASLD等代谢性疾病发生和发展。

材料与方法

实验细胞 人肝癌细胞株 (HepG2) 购自北京协和医学院。

试剂 苯并芘粉末 (美国Sigma公司, 批号: B1760); 改良油红O染色试剂、1 mol·L^{-1} Tris-HCl (pH6.8)、1 mol·L^{-1} Tris-HC1 (pH8.8) (上海碧云天生物技术有限公司, 批号分别为C0158S、ST768、ST788); 甘氨酸、十二烷基磺酸钠、Tris-base (北京索莱宝科技有限公司, 批号分别为15121223、13120529、15032698); BCA蛋白定量分析试剂盒 (美国Thermo Fisher Scientific, 批号: 23227); 总胆固醇 (total cholesterol, TC)、甘油三酯 (triglyceride, TG) 和低密度脂蛋白胆固醇 (low-density lipoprotein cholesterol, LDL-C) 试剂盒 (南京建成生物工程研究所, 批号分别为A110-1-1、A111-1-1、A113-1-1); 抗体 [硬脂酰辅酶A去饱和酶1 (stearoyl-CoA desaturase 1, SCD1)、过氧化物酶体增殖物激活受体γ (peroxisome proliferator-activated receptor γ, PPARγ)、CCAAT/增强子结合蛋白α (CCAAT/enhancer-binding protein α, C/EBPα)、过氧化物酶体增殖物激活受体α (peroxisome proliferator-activated receptor α, PPARα)、肉碱棕榈酰转移酶1α (carnitine palmitoyltransferase 1α, CPT-1α)、过氧化物酶体增殖物激活受体γ共激活因子1α (peroxisome proliferator-activated receptor gamma coactivator 1α, PGC-1α)、β-actin] 购自英国Abcam公司, 批号分别为ab232368、ab178860、ab138491、ab76026、ab80588、ab68477、ab8226。

仪器 BIO-RAD基础电泳仪 (伯乐生命医学产品有限公司, 型号: 1645050); Odyssey红外激光成像系统 (美国LI-COP公司, 型号: CLX-0493); 多模式微孔板检测系统 (美国Perkin Elmer公司, Enspire); PCR仪 (型号: Arktik)、五通道实时定量荧光PCR仪 (型号: Pikoreal 96) 购自美国Thermo Fisher公司。

细胞分组与处理 取生长至80%密度的HepG2细胞铺于6孔板内, ① 设置0、15、30、60 μmol·L^{-1}浓度BaP组, 按分组加入相应浓度的BaP。② 设置control组、BaP组、shControl+BaP组、shAhR+BaP组, 按分组加入30 μmol·L^{-1} BaP。用高糖DMEM培养并于37 ℃、5% CO_2培养箱中放置24 h后, 进行后续实验, 重复实验3次。

细胞TC、TG、LDL-C含量检测 按试剂盒操作说明进行检测, 设置相应孔数和样本, 于酶标仪测定吸光度, 计算TC、TG、LDL-C含量。

油红O染色 取状态良好的细胞加入1 mL多聚甲醛将细胞固定30 min。避光加入400 μL油红O染色液染色20 min, PBS清洗3次, 最后于光学显微镜下观察拍照, 采用HALO软件进行定量分析, 测定脂滴阳性面积百分比。

沉默AhR稳转细胞株构建 慢病毒载体购自生工生物工程 (上海) 股份有限公司 (货号: WZ139)。接种HepG2细胞至6孔板, 感染细胞时每孔细胞量为2×10^5个, 感染复数 (multiplicity of infection, MOI) = 100。向各个实验组添加polybrene, 使其终浓度为5 μg·mL^{-1}, 并将所需的病毒液加入各孔, 轻摇混匀放置于37 ℃、5% CO_2培养箱中培养。转染6 h后观察细胞形态, 如有毒性反应则对其进行换液; 如无异常现象, 则可延长时间最多至24 h换液处理。48 h后通过荧光显微镜, 观察细胞荧光。在细胞培养48 h后, 弃除细胞上清液, 更换为含2 μg·mL^{-1}嘌呤霉素的筛选培养基, 于CO_2孵箱中继续培养。药筛周期结束后, 撤去含药培养基更换新鲜培养基, 培养48 h后恢复细胞状态。

qPCR 取状态良好的细胞按试剂盒操作提取总RNA, 采用逆转录试剂盒转cDNA, 将10 μL total RNA、2.5 μL primer mix、25 μL nuclease-free water、10 μL 5× RT buffer, 2.5 μL RT enzyme mix在冰上混匀配制成50 μL反应液, PCR反转录仪 (37 ℃ 15 min, 98 ℃ 5 min) 完成反转录, 于−20 ℃保存待用。将1 μL cDNA、0.4 μL上、下游引物、5 μL SYBR Green PCR Master Mix、3.2 μL RNase free water混合均匀配成10 μL反应体系, 设定qPCR仪程序为: 95 ℃预变性30 s, 随后进行40个扩增循环 (95 ℃, 5 s; 60 ℃, 10 s; 72 ℃, 15 s); 根据溶解曲线, 采集数据C_T值, 目的基因相对表达量分析方法基于$2^{-\Delta\Delta CT}$原理进行, 以β-actin作为内参对照, 每个样本平行重复5次, 取平均值进行分析。引物序列见表1。

Western blot 取生长状态良好的细胞提取总蛋白, SDS-PAGE凝胶电泳对蛋白进行分离, 根据凝胶大小剪取相应大小的聚偏二氟乙烯膜 (polyvinylidene fluoride, PVDF), 转膜夹放置顺序为: 阳极电板、滤纸、

Table 1　Primer sequences. SCD1: Stearoyl-CoA desaturase 1; PPAR*γ*: Peroxisome proliferator-activated receptor *γ*; C/EBP*α*: CCAAT/enhancer-binding protein *α*; PPAR*α*: Peroxisome proliferator-activated receptor *α*; CPT-1*α*: Carnitine palmitoyltransferase 1*α*; PGC-1*α*: Peroxisome proliferator-activated receptor gamma coactivator 1*α*; AhR: Aryl hydrocarbon receptor

Gene	Species	Primer sequence (5'-3')
CPT-1α	Human	Forward primer 5'-TCCACCGTTTGACTTGTGACCC-3'
		Reverse primer 5'-CCCTTTATCCATCCATTAGGAGCCGAT-3'
PPARα	Human	Forward primer 5'-ACAGATGACGACCAGGACAC-3'
		Reverse primer 5'-AAACGGGATTGCATTGTGTGA-3'
PGC-1α	Human	Forward primer 5'-AGGCGGTGCTCGCTTGTG-3'
		Reverse primer 5'-GGGATAGATAAAACAGGATGCGA-3'
SCD1	Human	Forward primer 5'-CAGTTCCTACACGACCACCACTA-3'
		Reverse primer 5'-GGACGGATGTCTTCTTCCAGAT-3'
PPARγ	Human	Forward primer 5'-GATCCTCCTGTTGACCCAGA-3'
		Reverse primer 5'-TCAAAGGAATGGGAGTGGTC-3'
C/EBPα	Human	Forward primer 5'-CCAGAGGATGGTTTCGGGTC-3'
		Reverse primer 5'-GCGGAAAGTCTCTCGGTCTC-3'
AhR	Human	Forward primer 5'-AGCTACTCCACTTCAGCCAC-3'
		Reverse primer 5'-TTCGGATATGGGACTCGGCA-3'
β-actin	Human	Forward primer 5'-CTACCTCATGAAGATCCTGACC-3'
		Reverse primer 5'-CACAGCTTCTCTTTGATGTCAC-3'

PVDF膜、含目的蛋白的凝胶、滤纸、阴极电板, 设置电流400 mA, 1 h。转膜结束后将PVDF膜于封闭液中封闭30 min, 4 ℃一抗孵育过夜 (*β*-actin抗体浓缩液按照1∶10 000用抗体稀释液进行稀释, 其余一抗浓缩液按照1∶1 000用抗体稀释液进行稀释), 第二日取出条带用TBST洗膜3次, 避光放入二抗中 (二抗浓缩液按照1∶1 000用抗体稀释液进行稀释), 室温孵育1 h, TBST洗膜3次, TBS洗膜1次, 使用红外激光扫描成像系统进行扫描, 使用ImageJ进行数据可视化分析。

统计学处理　数据采用GraphPad Prism 7.0软件分析, 两组间比较采用独立样本*t*检验, 多组数据进行单因素方差分析 (one-way ANOVA) 评估各实验组间的差异性, 数据采用均数 ± 标准差 ($\bar{x} \pm s$) 表示, $P < 0.05$认为差异具有统计学意义。

结果

1　不同浓度BaP升高HepG2细胞中TC、TG和LDL-C含量

检测HepG2细胞TG、TC和LDL-C的含量, 与control相比, 给予15、30和60 μmol·L^{-1} BaP后HepG2细胞中TG (图1A)、TC (图1B) 的含量显著上升, LDL-C含量在15和60 μmol·L^{-1} BaP组显著上升, 而30 μmol·L^{-1} BaP组虽无统计学差异, 但仍表现出上升趋势 (图1C), 说明不同浓度BaP都可造成HepG2细胞脂质含量增加, 与30 μmol·L^{-1} BaP相比, 60 μmol·L^{-1} BaP组HepG2细胞中TG、TC和LDL-C含量升高, 15 μmol·L^{-1}和30 μmol·L^{-1} BaP相比, TG、TC和LDL-C含量无显著性。

2　不同浓度的BaP上调HepG2细胞中FGF-21表达

使用不同浓度BaP处理后, 与control相比, HepG2细胞中FGF-21的蛋白表达均增加 (图2)。其中60 μmol·L^{-1} BaP组诱导FGF-21蛋白上调的作用弱于30 μmol·L^{-1} BaP组, 表明低剂量和高剂量的BaP均影响FGF-21的表达, 但程度不同。

3　不同浓度的BaP上调HepG2细胞中脂质代谢相关基因的表达

给予不同浓度的BaP后, 与control相比, HepG2细胞中*β*-氧化相关基因的表达呈现不同程度的升高, 但

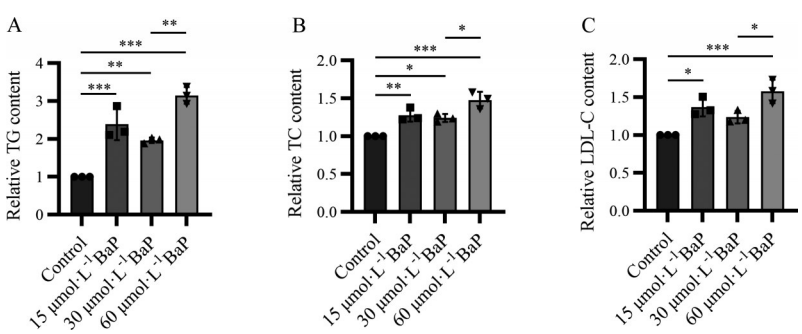


Figure 1　Increasing concentrations of BaP elevate the levels of TG (A), TC (B), and LDL-C (C) in HepG2 cells. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. BaP: Benzo[*a*]pyrene; TG: Triglyceride; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol

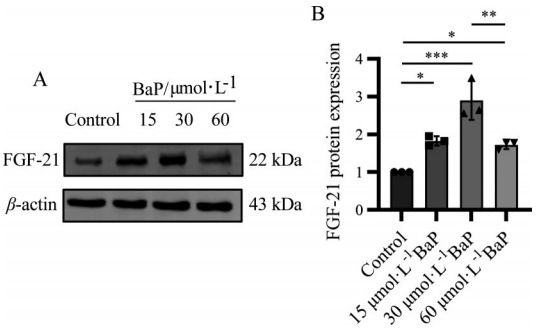


Figure 2 Increasing concentrations of BaP upregulate the expression of FGF-21 in HepG2 cells. A: FGF-21 protein band pattern; B: FGF-21 protein expression statistics. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. FGF-21: Fibroblast growth factor 21

60 μmol·L^{-1} BaP处理的HepG2细胞中β-氧化相关基因的mRNA和蛋白的表达增加程度不及30 μmol·L^{-1}组明显 (图3A、B、E), 结果表明, 不同浓度BaP均可升高HepG2细胞的β-氧化水平, 但60 μmol·L^{-1} BaP的升高作用相对较弱。与control相比, HepG2细胞中脂质合成相关基因的表达也呈现不同程度的升高, 与30 μmol·L^{-1} BaP组相比, 60 μmol·L^{-1} BaP显著升高了脂质合成相关基因的mRNA和蛋白表达 (图3C、D、F), 结果表明, 60 μmol·L^{-1} BaP升高HepG2细胞脂质合成水平的作用强于15和30 μmol·L^{-1} BaP。

4 沉默AhR可改善BaP诱导的HepG2细胞脂质蓄积

使用慢病毒转染HepG2细胞, 并采用qPCR和Western blot验证实验结果, 与shControl相比, shAhR组AhR的mRNA和蛋白表达均减少 (图4A～C), 干扰效率约78%, 表明沉默AhR稳转细胞株构建成功, 因此, 可用此细胞进行后续实验。油红O染色结果显示 (图4D、E), 给予HepG2细胞30 μmol·L^{-1} BaP后, 脂质含量升高, 沉默AhR后, shAhR+BaP组中HepG2细胞脂质含量降低, 表明BaP通过AhR升高了HepG2脂质的含量。试剂盒检测HepG2细胞中TC、TG和LDL-C的含量 (图4F～H), 与shControl+BaP组相比, shAhR+

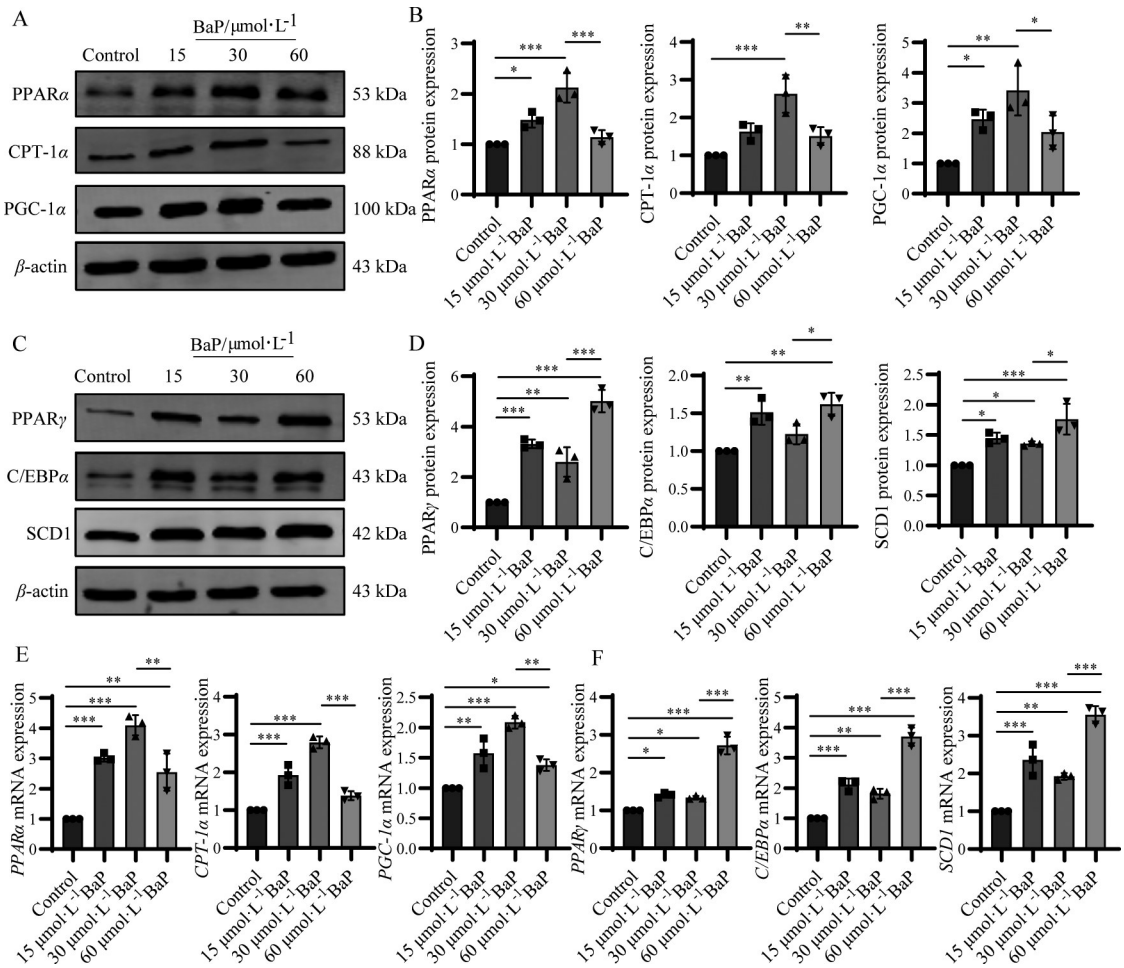


Figure 3 Increasing concentrations of BaP upregulate the expression of genes related to lipid metabolism in HepG2 cells. A: Protein band pattern of β-oxidation-related genes; B: Statistical graph of β-oxidation-related gene protein expression; C: Protein band pattern of lipid synthesis-related genes; D: Statistical graph of lipid synthesis-related gene protein expression; E: Statistical graph of β-oxidation-related gene mRNA expression; F: Statistical graph of lipid synthesis-related gene mRNA expression. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

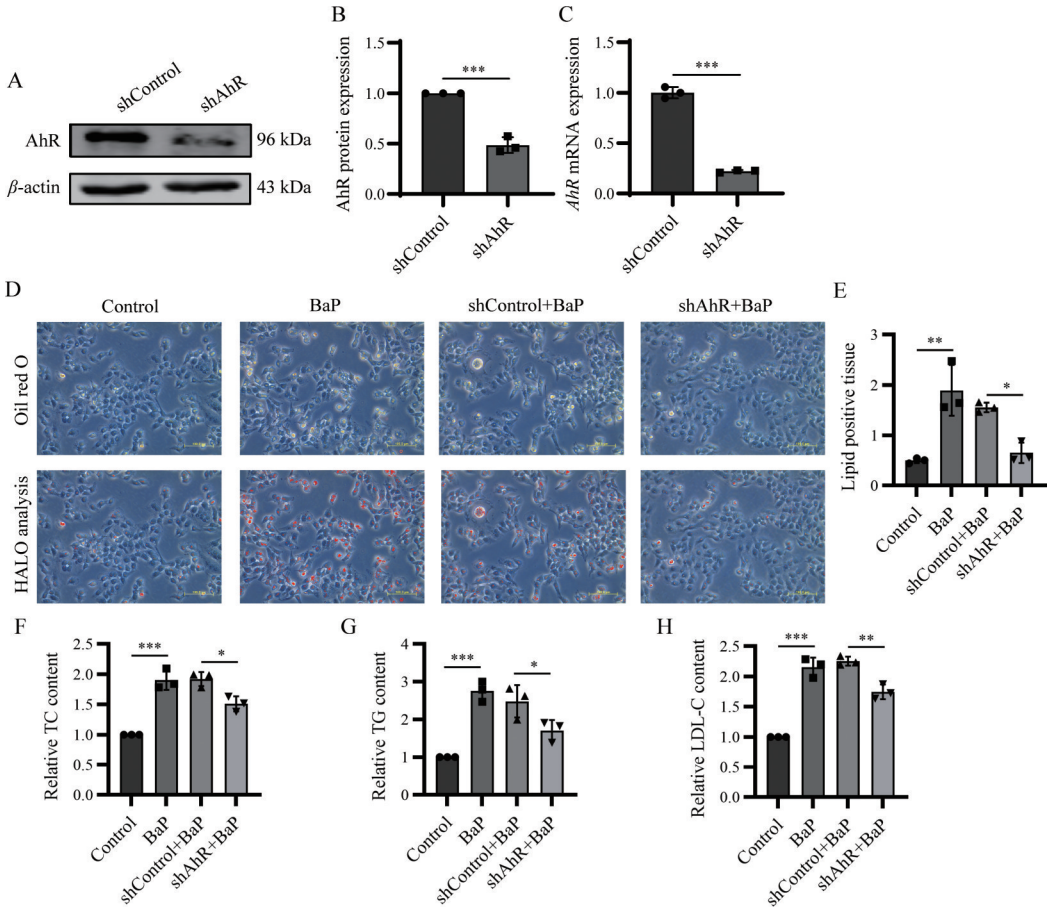


Figure 4　Silencing of AhR ameliorates BaP-induced lipid accumulation in HepG2 cells (40×). A: Band pattern of AhR silencing; B: Statistical analysis of AhR silencing interference effect by Western blot; C: Statistical analysis of AhR silencing interference effect by qPCR; D: Oil red O staining and HALO software analysis results; E: Statistical analysis of HALO software results; F: TC content; G: TG content; H: LDL-C content. $n = 3, \bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

BaP组中TC、TG和LDL-C的含量降低, 表明BaP导致HepG2细胞的脂质堆积是由AhR介导的。

5　BaP通过AhR影响HepG2细胞中FGF-21的表达

与control组相比, BaP组HepG2细胞中FGF-21的表达增加 (图5A、B)。与shControl+BaP相比, shAhR+BaP组FGF-21的蛋白表达减少, 结果说明, BaP促进FGF-21的表达是由AhR介导的。

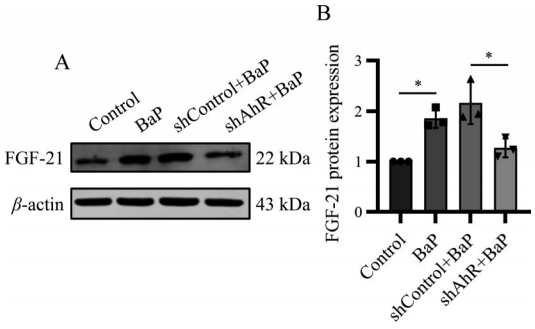


Figure 5　BaP affects the expression of FGF-21 in HepG2 cells through AhR. A: Protein blot; B: Protein expression statistics. $n = 3, \bar{x} \pm s$. $^{*}P < 0.05$

6　BaP通过AhR影响HepG2细胞中脂质代谢相关基因的表达

与control相比, BaP组β-氧化相关基因PPARα、CPT-1α、PGC-1α (图6A、B、E) 和脂质合成相关基因PPARγ、C/EBPα、SCD1 (图6C、D、F) 的mRNA和蛋白的表达均有不同程度的升高, 沉默AhR后, 脂质代谢相关基因的mRNA和蛋白的表达减少, 结果显示, BaP影响HepG2细胞脂质代谢相关基因的表达是由AhR介导的。

讨论

近20年来, 肝脏脂质代谢相关疾病如肝炎、肝纤维化、MASLD患病率逐渐增加, MASLD影响着全球约四分之一的成年人, 造成严重的健康负担[24-26]。高脂饮食、不健康的生活习惯和工业污染等多方面因素作用, 肝脏脂质代谢紊乱所带来的相关疾病逐渐增加。香烟烟雾、工业废气、碳烤食品无不加重着肝脏的代谢负担, 而其中危害性最大的为PAHs[27,28]。BaP是深入

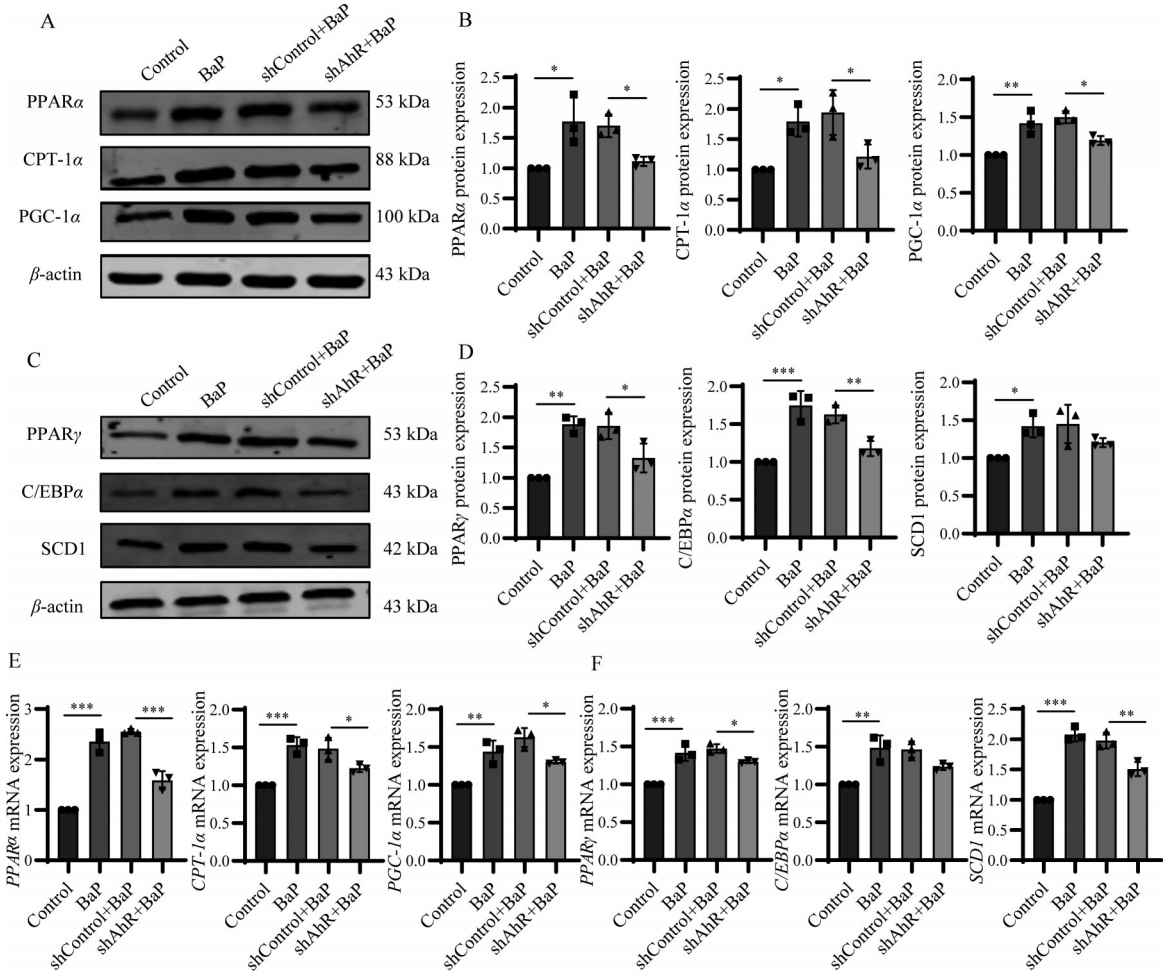


Figure 6 BaP affects the expression of genes related to lipid metabolism in HepG2 cells through AhR. A: Protein band pattern of *β*-oxidation-related genes; B: Statistical graph of *β*-oxidation-related gene protein expression; C: Protein band pattern of lipid synthesis-related genes; D: Statistical graph of lipid synthesis-related gene protein expression; E: Statistical graph of *β*-oxidation-related gene mRNA expression; F: Statistical graph of lipid synthesis-related gene mRNA expression. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

研究的PAHs之一, 是人类生活各个领域中广泛存在的有机污染物[29,30]。本课题组前期研究发现, 连续给予小鼠低剂量的BaP 12周后, 肝脏组织发生病变, 脂肪含量增加, 并发生了纤维化[31]。实验前期采用MTT试剂盒测定不同浓度BaP对HepG2细胞活力的影响, 当BaP浓度为0～64 μmol·L^{-1}时对细胞活性无抑制作用, 当BaP浓度高于64 μmol·L^{-1}时对细胞活性呈现抑制作用[23], 因此选用0、15、30和60 μmol·L^{-1} BaP进行后续的实验。在给予HepG2细胞15、30、60 μmol·L^{-1} BaP后, HepG2细胞发生了不同程度的脂质堆积。

FGF-21是FGF家族的内分泌成员, 是一种由肝脏分泌的激素, 对葡萄糖、脂质和能量代谢的稳态具有多效性作用[32]。有研究发现, 采用高脂高果糖高胆固醇饲料喂养14周可增强小鼠肝脏FGF-21分泌, 长期饮食导致脂质过度沉积则降低其水平, 通过运动可上调FGF-21分泌、维持线粒体稳态, 延缓MASLD进展[33]。FGF-21可通过多个靶点、多条通路影响机体糖脂代谢水平, 其中主要通过影响脂肪酸*β*-氧化和脂质合成相关因子来调控机体脂质代谢水平。脂肪酸*β*-氧化相关因子通常指PPARα、CPT-1α和PGC-1α, 它们是脂肪酸*β*-氧化的关键酶, 对脂肪酸*β*-氧化的速率起着决定性作用[34-36]。PPARγ、C/EBPα和SCD1是与脂质合成密切相关的调节因子, 它们在控制脂质合成和脂肪细胞分化中发挥重要作用[37,38]。本实验将不同浓度的BaP作用于HepG2细胞24 h后, 细胞中FGF-21和*β*-氧化相关因子PPARα、CPT-1α、PGC-1α都出现不同程度的上调, 其中低浓度BaP组上调较为显著。此外, 给予不同浓度BaP后脂质合成相关因子PPARγ、C/EBPα、SCD1出现不同程度的上调, 其中高浓度BaP上调较为显著。

BaP进入循环系统主要与AhR结合, 激活AhR及其相关信号通路。AhR对下游基因的影响主要是由入

核的AhR与相关基因启动子区域中XRE结合, 进而调控该基因的表达[39]。FGF-21启动子区域存在3段XRE片段[22,23], AhR-DNA复合物调控了FGF-21的表达, 进而影响下游脂质代谢相关因子的表达。Girer等[22]发现, AhR激动剂介导的FGF-21基因表达诱导作用具有瞬时性, FGF-21的显著高表达与AhR在近端XRE1上激动剂依赖性结合增强相关, 同时伴随AhR在远端XRE3上的结合消失。前期根据FGF-21启动子区域设计不同的截短型质粒, 并采用双荧光素酶报告基因实验探究了低、中、高浓度的BaP对FGF-21不同区域启动子活性的影响, 发现低浓度的BaP通过激活AhR促进包含XRE1序列的启动子区域活性, 达到促进FGF-21表达的目的, 高浓度的BaP通过激活AhR抑制包含XRE3的启动子序列区域的活性, 降低FGF-21的表达[23]。本实验通过沉默AhR观察FGF-21及相关因子的表达, 表明BaP对肝脏脂质代谢及相关基因和FGF-21的作用均由AhR介导, 通过3组剂量的对比分析, 可清晰观察到BaP经XRE1/XRE3元件双向调控FGF-21进而影响脂质代谢, 同时明确FGF-21表达水平与脂质紊乱程度之间的关联性。BaP持续暴露可导致AhR过度活化, 进而引起FGF-21表达紊乱, 打破脂质合成与分解的平衡, 诱发肝脏脂质代谢异常、胰岛素抵抗及血脂紊乱[23,40]。

在前期研究[23,31]的基础上, 本研究采用AhR基因沉默的方法进一步证实了BaP对HepG2细胞脂质代谢及相关基因和FGF-21的影响是由AhR所介导的, 本项目前期均采用AhR拮抗剂CH223191进行研究, 为了证明AhR在BaP引起FGF-21和脂质代谢及相关基因表达变化中的作用, 本课题采用沉默AhR基因的方法, 显著抑制AhR的作用, 证实了AhR在调控脂质代谢的关键作用, 也从另一个侧面证实了课题组前期采用AhR拮抗剂CH223191进行类似研究的正确性。存在不足的是, 本研究仅选用HepG2人肝癌细胞作为体外模型, 细胞模型较单一, 无法完全模拟体内复杂的肝脏微环境及多细胞间相互作用, 具有一定局限性。但该模型可直观反映BaP对肝细胞脂质代谢的直接效应, 为后续开展动物体内实验及临床转化研究提供了初步理论依据与实验基础。同时本研究也为MASLD等代谢性疾病的预防和治疗提供可参考的新型药物作用靶点, 并为MASLD的防治策略提供实验依据。

作者贡献: 王雪、杜雨露、张梦迪、胡玉霞、周树宏负责实验; 王雪、范蕾、皇甫卫忠、吕晓丽、李君负责数据整理; 王雪负责撰写文章; 常福厚、白图雅负责修改文章。

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References

[1] Seebacher F, Zeigerer A, Kory N, et al. Hepatic lipid droplet homeostasis and fatty liver disease [J]. Semin Cell Dev Biol, 2020, 108: 72-81.

[2] Du J, Li Y, Zhu X, et al. METTL1-mediated m^7G methylation of FoxO1 regulates lipid metabolism in metabolic dysfunction-associated fatty liver disease [J]. Metabolism, 2026, 174: 156420.

[3] Li Y, Yang P, Ye J, et al. Updated mechanisms of MASLD pathogenesis [J]. Lipids Health Dis, 2024, 23: 117.

[4] Zhang LJ, Li SY, Fan SL, et al. Hepatoprotective activity of mulberry extract in NAFLD mice for regulating lipid metabolism and inflammation identified *via* AMPK/PPAR-γ/NF-κB axis [J]. J Ethnopharmacol, 2025, 351: 120108.

[5] Park Y, Ko KS, Rhee BD. Non-alcoholic fatty liver disease (NAFLD) management in the community [J]. Int J Mol Sci, 2025, 26: 2758.

[6] Wang X, Zhang L, Dong B. Molecular mechanisms in MASLD/MASH-related HCC [J]. Hepatology, 2025, 82: 1303-1324.

[7] Deprince A, Haas JT, Staels B. Dysregulated lipid metabolism links NAFLD to cardiovascular disease [J]. Mol Metab, 2020, 42: 101092.

[8] De Cól JP, de Lima EP, Pompeu FM, et al. Underlying mechanisms behind the brain-gut-liver axis and metabolic-associated fatty liver disease (MAFLD): An update [J]. Int J Mol Sci, 2024, 25: 3694.

[9] Zhu Y, Gamare S, Polverino F, et al. CYP1B1 mediates cigarette smoke-induced lipid accumulation in alveolar type 2 cells [J]. FASEB J, 2025, 39: e71062.

[10] Yang Y, Yang C, Lei Z, et al. Cigarette smoking exposure breaks the homeostasis of cholesterol and bile acid metabolism and induces gut microbiota dysbiosis in mice with different diets [J]. Toxicology, 2021, 450: 152678.

[11] Henderson GC. Plasma free fatty acid concentration as a modifiable risk factor for metabolic disease [J]. Nutrients, 2021, 13: 2590.

[12] Pouwels S, Sakran N, Graham Y, et al. Non-alcoholic fatty liver disease (NAFLD): A review of pathophysiology, clinical management and effects of weight loss [J]. BMC Endocr Disord, 2022, 22: 63.

[13] Zhang S, Wang Z, Zhang J, et al. Inhalable cigarette-burning particles: Size-resolved chemical composition and mixing state [J]. Environ Res, 2021, 202: 111790.

[14] Hu Z, Li Y, Yang Y, et al. Serum lipids mediate the relationship of multiple polyaromatic hydrocarbons on non-alcoholic fatty liver disease: A population-based study [J]. Sci Total Environ, 2021, 780: 146563.

[15] Li Y, Liang N, Tang T, et al. Low-dose benzo[*a*]pyrene exposure induces hepatic lipid deposition through LCMT1/PP2Ac-mediated autophagy inhibition [J]. Food Chem Toxicol, 2023,

179: 113986.

[16] Treviño LS, Katz TA. Endocrine disruptors and developmental origins of nonalcoholic fatty liver disease [J]. Endocrinology, 2018, 159: 20-31.

[17] Zhang F, Jiang Y, Chen P, et al. Benzo(*a*)pyrene induces cardiac hypertrophy *via* the aryl hydrocarbon receptor-mediated DNA damage [J]. Sci Total Environ, 2025, 1009: 181061.

[18] Nebert DW. Aryl hydrocarbon receptor (AHR): "Pioneer member" of the basic-helix/loop/helix per-Arnt-sim (bHLH/PAS) family of "sensors" of foreign and endogenous signals [J]. Prog Lipid Res, 2017, 67: 38-57.

[19] Zhao W, Guo M, Feng J, et al. *Myristica fragrans* extract regulates gut microbes and metabolites to attenuate hepatic inflammation and lipid metabolism disorders *via* the AhR-FAS and NF-*κ*B signaling pathways in mice with non-alcoholic fatty liver disease [J]. Nutrients, 2022, 14: 1699.

[20] Zheng W, Liu M, Lv X, et al. AhR governs lipid metabolism: The role of gut microbiota [J]. Front Microbiol, 2025, 16: 1442282.

[21] Lu W, Li X, Luo Y. FGF21 in obesity and cancer: New insights [J]. Cancer Lett, 2021, 499: 5-13.

[22] Girer NG, Carter D, Bhattarai N, et al. Inducible loss of the aryl hydrocarbon receptor activates perigonadal white fat respiration and brown fat thermogenesis *via* fibroblast growth factor 21 [J]. Int J Mol Sci, 2019, 20: 950.

[23] Zhang M, Lv X, Wang C, et al. Molecular mechanisms of dose-dependent regulation of hepatic lipid metabolism by BaP through modulation of AhR binding to XRE1 or XRE3 [J]. Front Pharmacol, 2025, 16: 1595566.

[24] Lazarus JV, Mark HE, Anstee QM, et al. Advancing the global public health agenda for NAFLD: A consensus statement [J]. Nat Rev Gastroenterol Hepatol, 2022, 19: 60-78.

[25] Zhou XD, Fan QY, Byrne CD, et al. Combination therapies for metabolic dysfunction-associated steatohepatitis: Challenges and opportunities [J]. Gut, 2026, 75: 815-825.

[26] Younossi ZM, Golabi P, Paik JM, et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): A systematic review [J]. Hepatology, 2023, 77: 1335-1347.

[27] Ziyaei K, Mokhtari M, Hashemi M, et al. Association between exposure to water sources contaminated with polycyclic aromatic hydrocarbons and cancer risk: A systematic review [J]. Sci Total Environ, 2024, 924: 171261.

[28] Li W, Xiao H, Wu H, et al. Analysis of environmental chemical mixtures and nonalcoholic fatty liver disease: NHANES 1999-2014 [J]. Environ Pollut, 2022, 311: 119915.

[29] Zhao C, Jin H, Lei Y, et al. The dual effects of benzo(*a*)pyrene/benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide on DNA methylation [J]. Sci Total Environ, 2024, 950: 175042.

[30] Zhu M, Hwang J, Xu C. Meta-analysis identifies key genes and pathways implicated in benzo[*a*]pyrene exposure response [J]. Chemosphere, 2024, 364: 143121.

[31] Lou W, Zhang MD, Chen Q, et al. Molecular mechanism of benzo[*a*]pyrene regulating lipid metabolism *via* aryl hydrocarbon receptor [J]. Lipids Health Dis, 2022, 21: 13.

[32] Giralt M, Gavaldà -Navarro A, Villarroya F. Fibroblast growth factor-21, energy balance and obesity [J]. Mol Cell Endocrinol, 2015, 418 Pt 1: 66-73.

[33] Yuan X, Sun W, Xu Y, et al. Altered mitochondrial unfolded protein response and FGF21 secretion in MASLD progression and the effect of exercise intervention [J]. Sci Rep, 2025, 15: 3686.

[34] Bougarne N, Weyers B, Desmet SJ, et al. Molecular actions of PPAR*α* in lipid metabolism and inflammation [J]. Endocr Rev, 2018, 39: 760-802.

[35] Schlaepfer IR, Joshi M. CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential [J]. Endocrinology, 2020, 161: bqz046.

[36] Zhou J, Waskowicz LR, Lim A, et al. A liver-specific thyromimetic, VK2809, decreases hepatosteatosis in glycogen storage disease type Ia [J]. Thyroid, 2019, 29: 1158-1167.

[37] Zhao J, Qian H, An Y, et al. PPAR*γ* and C/EBP*α* enable adipocyte differentiation upon inhibition of histone methyltransferase PRC2 in malignant tumors [J]. J Biol Chem, 2024, 300: 107765.

[38] Cruz-Color L, Hernández-Nazará ZH, Maldonado-González M, et al. Association of the PNPLA2, SCD1 and leptin expression with fat distribution in liver and adipose tissue from obese subjects [J]. Exp Clin Endocrinol Diabetes, 2020, 128: 715-722.

[39] Sato S, Shirakawa H, Tomita S, et al. Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver [J]. Toxicol Appl Pharmacol, 2008, 229: 10-19.

[40] Lu P, Yan J, Liu K, et al. Activation of aryl hydrocarbon receptor dissociates fatty liver from insulin resistance by inducing fibroblast growth factor 21 [J]. Hepatology, 2015, 61: 1908-1919.