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PM2.5 通过抑制 FGF21/AMPK α 2 通路促发非酒精性脂肪肝*

周 蓓 毛维维 巩伟伟 韩冬梅 舒 丹

(上海市第一康复医院(同济大学医学院) 上海 200090)

摘要 目的:探讨 PM2.5 对非酒精性脂肪肝(NAFLD)的影响及与 FGF21/AMPK α 2 通路的关系。**方法:**根据气管滴注的 PM2.5 浓度随机将 40 只 6 周龄雄性小鼠分为 4 组:对照组(生理盐水, 10 只)和低毒组(6.25 μ g/mL)、中毒组(12.5 μ g/mL)、高毒组(25.0 μ g/mL), 10 只/组,持续染毒 19 天。10 周龄起给予高脂饲料喂养 6 周,构建 NAFLD 小鼠模型。分别于染毒前、染毒后、高脂喂养 6 周测定循环 TG、TC 及 FGF21 水平。处死小鼠,取肝细胞培养后随机分两组,分别转染 Ad-FGF21 与对照病毒 Ad-GFP 16 h,后加入 PM2.5 混悬液进行染毒共培养 36 h,测定两组细胞存活率,肝细胞内 TC 及 TG、FGF21、AMPK α 2 含量。**结果:**染毒后及高脂喂养 6 周后,各组血清 TG、TC 较染毒前明显升高,血清 FGF21 水平降低,且随着染毒浓度的增加,血清 TG、TC 亦相应升高,FGF21 随之降低 ($P<0.05$)。与染毒后比较,高脂喂养 6 周后各组血清 TG、TC 均显著升高,血清 FGF21 显著降低 ($P<0.05$)。与 Ad-GFP 组比较,Ad-FGF21 组 PM2.5 染毒后肝细胞存活率显著升高,TG、TC 水平明显降低,而肝细胞 FGF21、AMPK α 2 表达含量则显著升高($P<0.05$)。**结论:**幼年期 PM2.5 暴露可加速成年后 NAFLD 发生,且与暴露浓度密切相关,PM2.5 可能通过抑制肝脏 FGF21/AMPK α 2 信号通路促进肝细胞脂质沉积和 NAFLD 的发生发展。

关键词:PM2.5; FGF21/AMPK α 2 通路; 非酒精性脂肪肝

中图分类号:R-33; R575.5 **文献标识码:**A **文章编号:**1673-6273(2020)11-2032-04

PM 2.5 Promotes the Incidence of Nonalcoholic Fatty Liver Disease Via Inhibiting FGF21/AMPK α 2 Pathway*

ZHOU Qian, MAO Wei-wei, GONG Wei-wei, HAN Dong-mei, SHU Dan

(Shanghai First Rehabilitation Hospital (Tongji University Medical College), Shanghai, 200090, China)

ABSTRACT Objective: To explore the influence of PM 2.5 on the nonalcoholic fatty liver disease(NAFLD) and its correlation with FGF21/AMPK α 2 pathway. **Methods:** 40 6-week-old male mice were randomly divided into 4 groups according to the concentration of PM2.5 in trachea drip: control group (10 mice with normal saline), low toxicity group (6.25 μ g/mL), medium toxic group (12.5 μ g/mL), high toxicity group (25.0 μ g/mL), 10 mice/group, continued exposure for 19 days. A NAFLD mouse model was established by feeding high-fat diet for 6 weeks from 10 weeks of age. Levels of circulating TG, TC and FGF21 were measured before, after contamination and after high fat feeding for 6 weeks. Mice were sacrificed, and liver cells were cultured and randomly divided into two groups, respectively transfected with Ad-FGF21 and control virus Ad-GFP for 16h. After that, PM2.5 suspension was added for a total culture of 36 h, and the survival rate of the two groups was measured, and the contents of TC, TG, FGF21 and AMPK alpha 2 in liver cells were measured. **Results:** After the contamination and 6 weeks of high-fat feeding, serum TG and TC in each group were significantly increased compared with those before the contamination, and serum FGF21 levels were decreased. With the increase of the concentration of the contamination, serum TG and TC were also increased, and serum FGF21 was decreased ($P<0.05$). After 6 weeks of high-fat feeding, serum TG and TC in each group were significantly increased and serum FGF21 significantly decreased ($P<0.05$). Compared with the Ad-GFP group, the survival rate of hepatocytes in the Ad-FGF21 group was significantly increased after PM2.5 exposure, TG and TC levels were significantly reduced, and the FGF21 and AMPK α 2 hepatic expression levels were significantly increased ($P<0.05$). **Conclusions:** Childhood PM2.5 exposure can accelerate the development of NAFLD in adulthood and is closely related to the exposure concentration. PM2.5 may promote hepatic lipid deposition and the development of NAFLD by inhibiting the liver FGF21/AMPK α 2 signaling pathway.

Key words: PM2.5; FGF21/AMPK α 2 pathway; Non-alcoholic fatty liver disease

Chinese Library Classification(CLC): R-33; R575.5 **Document code:** A

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前言

非酒精性脂肪肝 (non-alcoholic fatty liver disease, NAFLD) 是过量饮酒患者出现以弥漫性肝细胞大泡性脂肪变性为特

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作者简介: 周蓓 (1980-), 女, 硕士, 主治医师, 研究方向: 非酒精性脂肪肝; 电话: 021-65432021, E-mail: zhou_9797@126.com

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征的代谢应激性肝损伤,部分患者最终可进展为肝硬化或肝癌^[1-3]。NAFLD的致病机制尚不十分清楚,但多数观点认为其与胰岛素抵抗(insulin resistance, IR)和遗传易感密切相关,已成为慢性肝病、肝功能指标异常的首要原因^[4,5]。

大量研究证实长期暴露于PM2.5(空气动力学直径<2.5 μm的颗粒物)空气污染环境中可导致肺癌、慢性阻塞性肺病等疾病的发病率和死亡率显著增加,严重危害人群健康^[6-8]。近年来,有研究显示细颗粒物暴露可加剧机体的氧化应激和IR,两者均为NAFLD发生的关键病理生理过程,但PM2.5与NAFLD的关系,尤其是PM2.5对NAFLD影响的作用机制尚缺乏相关研究^[9,10]。

本研究采用小鼠染毒模型,探究幼年PM2.5暴露对成年NAFLD发生发展的影响,并在细胞水平明确成纤维细胞生长因子21(FGF21)/腺苷酸活化蛋白激酶(AMP-activated protein kinaseα2, AMPKα2)通路在其中的作用,以期为NAFLD的预防及治疗提供理论依据。

1 材料与方法

1.1 实验动物

6周龄C57BL/6健康雄性小鼠40只,体质量100-150 g,平均(237.5±21.7)g,购自上海斯莱克实验动物中心。普通饮食习服喂养,温度23±2℃,湿度60±5%,12 h明暗交替。

1.2 建立PM2.5染毒及NAFLD小鼠模型

适应性喂养1周后,根据气管滴注的PM2.5浓度随机分为4组:对照组(生理盐水,10只)和低毒组(6.25 μg/mL)、中毒组(12.5 μg/mL)、高毒组(25.0 μg/mL),10只/组。PM2.5由同济大学公共卫生学院提供,样品来自上海市交通区,隔天1次,持续染毒19天。10周龄起给予高脂饲料喂养6周,自由饮水,饲料购自美国Research Diets公司。高脂喂养6周后形成中重度大泡性脂肪肝变,且丙氨酸氨基转移酶明显升高,造模成功。所有实验操作过程均严格遵循我院动物实验管理和规定。

1.3 检测指标

1.3.1 血脂及血清 FGF21 水平测定 采用全自动生化分析仪,分别于染毒前、染毒后、高脂喂养6周测定循环三酰甘油(triacylglycerol, TG)、总胆固醇(total cholesterol, TC)。采用ELISA试剂盒(Abcam公司)检测 FGF21 水平。

1.3.2 肝细胞脂质沉积检测 高脂喂养6周后处死小鼠,取肝细胞培养于DMEM培养基。随机分两组,分别转染过表达 FGF21 重组腺病毒载体(Ad-FGF21)与对照病毒 Ad-GFP 16 h,后均于培养基加入PM2.5混悬液进行染毒共培养,36 h后测定细胞存活率(%),测定肝细胞内TC及TG。

1.3.3 Western blot 法检测肝细胞 FGF21、AMPK α2 含量 使用RIPA裂解液进行细胞裂解抽提总蛋白,采用BCA法进行蛋白定量,并加入相应体积的6×SDS-PAGE Loading buffer,取30 μg蛋白上样,在8%的SDS-PAGE中进行电泳2 h,然后将蛋白转膜至甲醇预处理的PVDF膜1 h,以5%脱脂奶粉在室温下封闭1 h,加入1:1000稀释的一抗(FGF21、AMPKα2及β-actin抗体均购自Abcam公司),4℃孵育过夜,次日PBST漂洗3次后,加入1:5000稀释的HRP标记的二抗(Abcam公司)室温孵育2 h,用ECL化学发光液并进行暗室曝光。

1.4 统计学方法

运用SPSS17.0版统计软件包进行数据处理,计量资料以($\bar{x} \pm s$)表示,组间比较采用t检验或单因素方差分析,以P<0.05视为差异有统计学意义。

2 结果

2.1 各组不同时间点 TG、TC 及 FGF21 水平比较

染毒前,各组TG、TC、FGF21水平比较差异均无统计学意义(P>0.05);染毒后及高脂喂养6周后,各组TG、TC水平较染毒前明显升高,FGF21水平显著降低,且随着染毒浓度的增加,TG、TC亦相应升高,FGF21随之降低(P<0.05)。与染毒后比较,各组高脂喂养6周后TG、TC均显著升高,FGF21显著降低,差异均有统计学意义(P<0.05)。见表1。

表1 各组不同时间点 TG、TC 及 FGF21 水平的比较

Table 1 Comparison of TG, TC and FGF21 levels at different time points among different groups

Time	Groups	N	TG(mmol/L)	TC(mmol/L)	FGF21(pg/mL)
Before poisoning	Control group	10	0.35±0.09	1.21±0.18	136.35±10.35
	Low toxicity group	10	0.39±0.11	1.23±0.11	134.42±9.44
	Middle toxicity group	10	0.37±0.13	1.19±0.15	138.63±10.40
	High toxicity group	10	0.36±0.14	1.20±0.13	140.89±12.51
After poisoning	Control group	10	0.37±0.12	1.24±0.20	137.19±9.33
	Low toxicity group	10	0.51±0.14 [°]	1.35±0.19 [°]	121.29±12.31 [°]
	Middle toxicity group	10	0.66±0.17 ^{°°}	1.52±0.17 ^{°°}	112.36±15.34 ^{°°}
	High toxicity group	10	0.87±0.21 ^{°°°}	1.70±0.24 ^{°°°}	101.44±13.36 ^{°°°}
High fat feeding for 6 weeks	Control group	10	0.52±0.19 [°]	1.50±0.31 [°]	124.17±0.37 [°]
	Low toxicity group	10	0.87±0.20 ^{°°}	1.89±0.35 ^{°°}	112.15±12.29 ^{°°}
	Middle toxicity group	10	1.03±0.23 ^{°°°}	2.21±0.33 ^{°°°}	99.20±14.34 ^{°°°}
	High toxicity group	10	1.39±0.29 ^{°°°°}	2.65±0.41 ^{°°°°}	87.19±10.32 ^{°°°°}

Note: Compared with before poisoning, [°] P<0.05; Compared with the control group, [°] P<0.05; Compared with low toxicity group, [°] P<0.05; Compared with middle toxicity group, [°] P<0.05.

2.2 肝细胞过表达 FGF21

我们取小鼠肝细胞培养于 DMEM 培养基, 分别转染过表达 FGF21 重组腺病毒载体(Ad-FGF21)与对照病毒 Ad-GFP 16 h。如图 1 所示, 过表达细胞 FGF21 水平显著高于对照细胞表明我们成功构建了 FGF21 过表达模型。

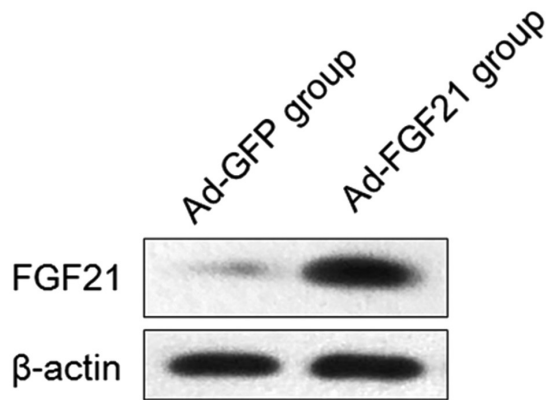


Fig.1 Overexpression of FGF21 in mice hepatocyte

2.3 过表达 FGF21 后 PM2.5 对肝细胞存活率的影响

与 Ad-GFP 组比较, Ad-FGF21 组 PM2.5 染毒后肝细胞存活率显著升高, 差异有统计学意义($P < 0.05$), 见图 2。

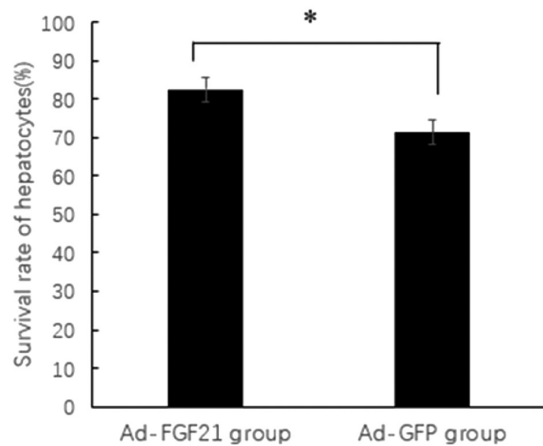


Fig.2 Overexpression of FGF21 increased the survival rate of hepatocytes after PM2.5 poisoning

Note: Compared with Ad-GFP group, $*P < 0.05$.

2.4 过表达 FGF21 后 PM2.5 对肝细胞脂质沉积的影响

与 Ad-GFP 组比较, Ad-FGF21 组 PM2.5 染毒后 FGF21、AMPK $\alpha 2$ 含量则显著升高(图 3), TG、TC 水平明显降低(图 4), 差异均有统计学意义($P < 0.05$)。

3 讨论

NAFLD 是导致肝细胞癌的重要因素之一, 在我国的发病率超过 10%, 且呈逐年增长趋势, 但仍缺乏特殊有效的预防和治疗方案^[11-13]。"二次打击"学说是 NAFLD 发病及病理机制的经典学说, 认为肝细胞内脂质沉积是 NAFLD 发生的第一次打击, 该阶段尚未出现炎症改变或症状较轻。在此基础上在各种肝毒性物质的刺激下, 肝细胞与库普弗细胞被活化, 炎症细胞因子释放引起氧化应激, 诱发局部肝细胞的炎症反应、脂质过氧化及细胞损伤, 形成第二次打击, 最终导致 NAFLD 的发生^[14-16]。

近年来, 高敏感性的幼年期暴露于相关不利因素对成年后

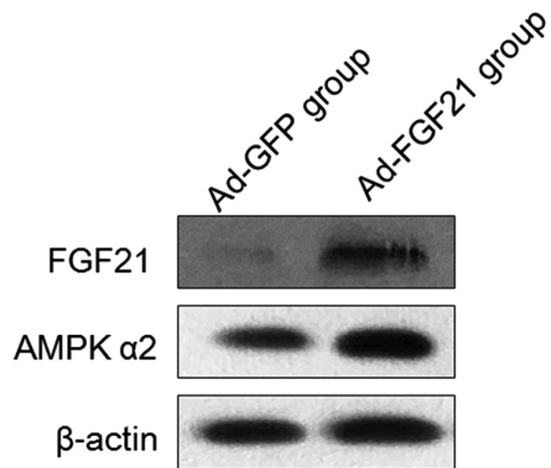


Fig.3 Influence of FGF21, AMPK $\alpha 2$ levels in hepatocytes after overexpression of FGF21

Note: Compared with Ad-GFP group, $**P < 0.01$.

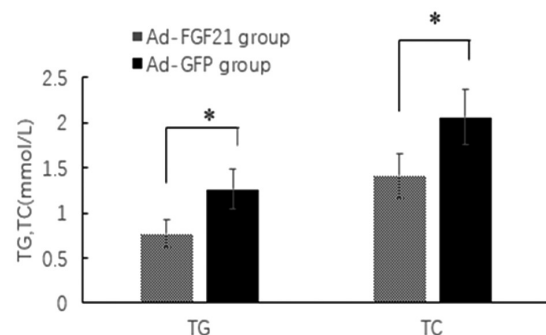


Fig.4 Influence of PM2.5 on the lipid deposition in hepatocytes after overexpression of FGF21

Note: Compared with Ad-GFP group, $*P < 0.05$.

慢性疾病发生发展的影响及作用机制愈引起关注, 临床学者也积极呼吁政府和公众应加强预防 PM2.5 等大气细颗粒物的迟发性影响^[17-19]。动物实验结果显示, PM2.5 吸入可使小鼠肝脏组织出现脂肪变^[20,21], 但 PM2.5 导致 NAFLD 加速与加重的作用机制仍不十分明确, 且其对机体的毒理学机制研究尚处于探索阶段。因此, 利用动物模型明确大气细颗粒物暴露对其成年后 NAFLD 发生发展的通路机制有重要的科学及社会意义。本实验发现, 经过 19 天染毒后, 各组 TG、TC 较染毒前明显升高, FGF21、AMPK 水平降低, 且随着染毒浓度的增加, TG、TC 亦相应升高, FGF21、AMPK 随之降低($P < 0.05$), 与李明等^[22]研究结果相符, 说明短期 PM2.5 暴露即可出现肝细胞脂质堆积, 诱发 NAFLD 发病机制的第一次打击。高脂喂养 6 周后, 各组的血脂紊乱进一步加剧, 循环 FGF21、AMPK 降低明显, 出现 NAFLD。由此可见, 幼年期 PM2.5 暴露可加剧其成年后肝脏的脂质沉积, 导致 NAFLD 的发生, 且严重程度与幼年期染毒的浓度密切相关^[23,24]。我们推测 FGF21 水平下降可通过影响下游的 AMPK 能量通路, 诱发脂质沉积。

有研究表明, FGF21 可通过改变脂代谢相关基因的表达, 对高脂饮食诱导的 NAFLD 具有保护作用^[25-27]。另有研究报道称, FGF21 下游 AMPK 信号通路对机体能量平衡及脂肪代谢起着关键性作用, 活化的 AMPK 可通过磷酸化抑制脂肪生成, 其中 AMPK $\alpha 2$ 主要在肝脏表达, 若 AMPK $\alpha 2$ 水平下降、作用减弱, 导致肝脏脂质沉积加剧^[28,29]。本研究发现, Ad-FGF21 组

PM2.5 染毒后肝细胞存活率较 Ad-GFP 组显著升高,且 TG、TC 水平明显降低,FGF21、AMPK $\alpha 2$ 含量则显著升高($P < 0.05$),再次说明 PM2.5 与 FGF21/AMPK $\alpha 2$ 通路存在相关性,同时也从分子层面证实过表达 FGF21 可通过影响下游的 AMPK 能量通路改善 PM2.5 所致肝脏脂质沉积,可能与激活 FGF21/AMPK- $\alpha 2$ 通路对脂代谢发挥正向调控作用有关。谭茂卿等^[30]研究构建敲除 AMPK 基因的小鼠模型,并给予高脂喂养,第 5 周后小鼠出现明显脂肪肝病变,而对照组小鼠在第 12 周才出现脂肪肝病变,说明抑制 FGF21/AMPK $\alpha 2$ 通路可加速肝脏脂质沉积。

综上所述,幼年期 PM2.5 暴露可加速成年后 NAFLD 发生,且与暴露浓度密切相关,PM2.5 可能通过抑制肝脏 FGF21/AMPK $\alpha 2$ 信号通路促进肝细胞脂质沉积和 NAFLD 的发生发展,通过激活 FGF21/AMPK $\alpha 2$ 信号通路的活性有望成为治疗 NAFLD 的重要分子途径。

参考文献(References)

- [1] Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review[J]. World Journal of Hepatology, 2017, 9(16): 715-732
- [2] Chi ZC, Gastroenterology DO, Hospital QM. Pathogenesis of non-alcoholic fatty liver disease [J]. World Chinese Journal of Digestology, 2017, 25(8): 670
- [3] Rocha ALL, Faria LC, Guimarães TCM, et al. Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis[J]. Journal of Endocrinological Investigation, 2017, 40(12): 1-10
- [4] Rinella ME. Nonalcoholic fatty liver disease: a systematic review[J]. JAMA, 2015, 313(22): 2263-2273
- [5] Diehl AM, Day C. Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis[J]. N Engl J Med, 2017, 377(21): 2063-2073
- [6] Tarantino G, Capone D, Finelli C. Exposure to ambient air particulate matter and non-alcoholic fatty liver disease[J]. World J Gastroenterol, 2013, 19(25): 3951-3956
- [7] Kim JW, Park S, Lim CW, et al. The role of air pollutants in initiating liver disease[J]. Toxicol Res, 2014, 30(2): 65-70
- [8] 曹璇. PM2.5 对心血管病人血清 TNF- α 、IL-2、IL-8 以及 ICAM-1 水平影响的研究[D]. 吉林大学, 2017
- [9] 谢菁菁, 邱俊侠, 王婴, 等. PM2.5 对 HepG2 细胞脂质堆积影响及机制研究[J]. 环境与健康杂志, 2015, 32(6): 509-512
- [10] Li M, Li YM. Fine particulate matter and nonalcoholic fatty liver disease[J]. Zhonghua Gan Zang Bing Za Zhi, 2016, 24(9): 713-715
- [11] Molleston JP, Schwimmer JB, Yates KP, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels [J]. The Journal of pediatrics, 2014, 164(4): 707-713
- [12] Tarantino G, Capone D, Finelli C. Exposure to ambient air particulate matter and non-alcoholic fatty liver disease [J]. World journal of gastroenterology, 2013, 19(25): 3951-3956
- [13] Khoonsari M, Azar MMH, Ghavam R, et al. Clinical Manifestations and Diagnosis of Nonalcoholic Fatty Liver Disease[J]. Iranian Journal of Pathology, 2017, 12(2): 99-105
- [14] Jia Q, Xia Y, Zhang Q, et al. Dietary patterns are associated with prevalence of fatty liver disease in adults[J]. Eur J Clin Nutr, 2015, 69(8): 914-921
- [15] Periasamy S, Chien SP, Chang PC, et al. Sesame oil mitigates nutritional steatohepatitis via attenuation of oxidative stress and inflammation: a tale of two-hit hypothesis [J]. J Nutr Biochem, 2014, 25(2): 232-240
- [16] Kelishadi R, Poursafa P. Obesity and Air Pollution: Global Risk Factors for Pediatric Non-alcoholic Fatty Liver Disease [J]. Hepatitis Monthly, 2011, 11(10): 794-802
- [17] Giovanni T, Domenico C, Carmine F. Exposure to ambient air particulate matter and non-alcoholic fatty liver disease[J]. World Journal of Gastroenterology, 2013, 19(25): 3951-3956
- [18] Hashemzadeh B, Idani E, Goudarzi G, et al. Effects of Pm2.5 and No2 on the 8-Isoprostane and Lung Function Indices of FVC and FEV1 in Students of Ahvaz City, Iran [J]. Saudi Journal of Biological Sciences, 2016: S1319562X16301656
- [19] Shekarzifard M, Faghih-Imani A, Hatzopoulou M. An examination of population exposure to traffic related air pollution: Comparing spatially and temporally resolved estimates against long-term average exposures at the home location [J]. Environmental research, 2016, 147(15): 435-444
- [20] Zheng Z, Xu X, Zhang X, et al. Exposure to ambient particulate matter induces a NASH-like phenotype and impairs hepatic glucose metabolism in an animal model[J]. J Hepatol, 2013, 58(1): 148-154
- [21] Vesterdal LK, Danielsen PH, Folkmann JK, et al. Accumulation of lipids and oxidatively damaged DNA in hepatocytes exposed to particles[J]. Toxicol Appl Pharmacol, 2014, 274(2): 350-360
- [22] 李明, 谢菁菁, 吴彤, 等. PM2.5 对肝细胞脂质代谢和氧化应激的影响[J]. 中国当代医药, 2016, 23(9): 9-11
- [23] Videla LA, Vargas R, Riquelme B, et al. Thyroid Hormone-Induced Expression of the Hepatic Scaffold Proteins Sestrin2, β -Klotho, and FRS2 α in Relation to FGF21-AMPK Signaling [J]. Exp Clin Endocrinol Diabetes, 2018, 126(03): 182-186
- [24] Yang H, Feng A, Lin S, et al. Fibroblast growth factor-21 prevents diabetic cardiomyopathy via AMPK-mediated antioxidation and lipid-lowering effects in the heart [J]. Cell Death & Disease, 2018, 9(2): 196-203
- [25] 杨家耀, 时昭红, 马威, 等. 附子理中汤通过激活 AMPK 通路及抑制 NF- κ Bp65 通路降低非酒精性脂肪肝大鼠肝脏损伤[J]. 中国中药杂志, 2018, 43(15): 3176-3179
- [26] Fu X, Zhao J X, Zhu M J, et al. AMP-Activated Protein Kinase $\alpha 1$ but Not $\alpha 2$ Catalytic Subunit Potentiates Myogenin Expression and Myogenesis [J]. Molecular & Cellular Biology, 2013, 33(22): 4517-4522
- [27] Yong Z, Ying W, Yu Q, et al. Ampelopsin Improves Insulin Resistance by Activating PPAR γ and Subsequently Up-Regulating FGF21-AMPK Signaling Pathway[J]. Plos One, 2016, 11(7): e0159191
- [28] Zhang HA, Yang XY, Xiao YF. AMPK $\alpha 1$ overexpression alleviates the hepatocyte model of nonalcoholic fatty liver disease via inactivating p38MAPK pathway [J]. Biochem Biophys Res Commun, 2016, 474(2): 364-370
- [29] Kamikubo R, Kai K, TsujiNaito K, et al. Beta-Caryophyllene attenuates palmitate-induced lipid accumulation through AMPK signaling by activating CB2 receptor in human HepG2 hepatocytes [J]. Mol Nutr Food Res, 2016, 60(10): 2228-2242
- [30] 谭茂卿, 吴凡伟, 杜震生, 等. 从 " 治未病 " 思路用中医扶阳大法治疗脂肪肝临床疗效分析[J]. 河南中医, 2013, (B04): 10-11