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文献综述 REVIEW

NOX介导的MAPK、PI3K/Akt信号通路与肝纤维化的研究进展

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NOX-mediated MAPK and PI3K/Akt signaling pathways and liver fibrosis

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Abstract

Hepatic satellite cells (HSCs) are the main cell type involved in the development of liver fibrosis and have been recognized as the important cellular source of extracellular matrix (ECM). NADPH oxidase (NOX) catalyzes the generation of reactive oxygen species (ROS), regulates signal transduction in HSCs, and thereby plays a key role in the pathogenesis of hepatic fibrosis. ROS generated by NOX promotes proliferation and inhibits apoptosis of HSCs by activation of mitogen-activated protein kinase and phosphatidylinositol-3 kinase/Akt signaling pathways, thus contributing to the development of liver fibrosis. Inhibition of NOX activation to generate ROS and NOX-mediated signal transduction induces HSC apoptosis. Therefore, drugs that target specific NOX can be expected to be useful in arresting the progression of liver fibrosis.

Key Words: NADPH oxidase; Mitogen-activated protein kinase; Phosphatidylinositol-3 kinase/Akt; Liver fibrosis

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摘要

肝星状细胞(hepatic stellate cells, HSC)是肝纤维化的主要细胞, 是细胞外基质(extracellular matrix, ECM)的重要来源. 烟酰胺腺嘌呤二核苷酸磷酸氧化酶(NADPH oxidase, NOX)产生活性氧(reactive oxygen species, ROS), 调控HSCs内信号转导, 在肝纤维化发病中起关键作用. NOX产生的ROS可介导丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)和磷脂酰肌醇-3激酶/蛋白激酶(phosphatidylinositol-3 kinase/Akt, PI3K/Akt)信号通路激活, 促进HSC增殖、抑制其凋亡, 导致肝纤维化形成. 抑制NOX产生ROS, 阻断相应的信号通路可诱导HSC凋亡. 因此, 探索出以NOX为作用靶点的抗纤维化药物意义重大.

关键字: NADPH氧化酶; 丝裂原活化蛋白激酶; 磷脂酰肌醇-3激酶/蛋白激酶; 肝纤维化

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0 引言

肝星状细胞(hepatic stellate cells, HSC)内的活性氧(reactive oxygen species, ROS)通过氧化敏感的蛋白激酶和转录因子调控各种介质的活化和表达, 诱导肝纤维化[1,2]. 烟酰胺腺嘌呤二核苷酸磷酸氧化酶(NADPH oxidase, NOX)是细胞内ROS产生的主要酶来源, 在肝纤维化中起重要作用, 也许因此成为治疗肝纤维化的重要新型靶点[3]. NOX能针对刺激因素如血小板源性生长因子(platelet-derived growth factor, PDGF)、瘦素(leptin, LP)等通过活化多种信号通路调控HSC的增殖、激活, 促进肝纤维化形成[4]. 因此, 本文将对HSC内NOX产生ROS介导的丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)、磷脂酰肌醇-3激酶/蛋白激酶(phospha-

■背景资料
肝纤维化是各种病因所致慢性肝损伤的创伤愈合反应, 持续的炎症与纤维形成最终导致肝硬化. 目前, 抗病毒治疗、抗血吸虫、戒酒等病因治疗可延缓或阻止肝纤维化的发展. 肝星状细胞(HSC)的激活、转化被认为是肝纤维化发病的中心事件. 氧化应激是肝纤维化的主要病因, 阻断活性氧(ROS)产生可能发挥抗纤维化效应, 而NADPH氧化酶(NOX)为肝脏中ROS的重要来源, 在肝纤维化中起重要作用. 近年来针对促纤维化因子及其介导的信号通路对肝纤维化的影响有了进一步研究, 阻断这些信号通路有望为肝纤维化的治疗带来新突破.

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■研发前沿
近年来HSC内的信号转导在肝纤维化中的作用得到广泛关注，进一步认识了NOX及其介导的信号通路与肝纤维化的关系，如激活NOX及其介导的信号通路可促进HSC增殖、转化，引起肝纤维化.

tidylinositol-3 kinase/Akt, PI3K/Akt)信号通路与肝纤维化的关系作一综述.

1 NADPH氧化酶的组成、分布及功能

哺乳动物类细胞中ROS产生的简单例子是可催化呼吸爆炸的巨噬细胞型NOX, 他表达于所有典型的巨噬细胞, 也表达于B淋巴细胞和T淋巴细胞[5]. NOX是由位于细胞膜的催化亚基gp91phox(又为NOX2)和调节亚基p22phox及其他位于细胞质的调节亚基p47phox、p40phox、p67phox和小GTP连接酶Rac(即Rac1)[6]组成的多蛋白复合体. 一旦被刺激, 胞质蛋白移至胞膜, 与膜蛋白相互作用后, 激活NOX[7]. 活化的NOX产生大量胞外超氧化物(如ROS), 在抵制微生物感染的宿主防御中起关键作用[8].

除了细胞膜亚基NOX2, 还有6种NOX同系物, 为非巨噬细胞型NOX(NOX1、NOX3、NOX4、NOX5、DUOX1和DUOX2). NOX1主要表达于消化道, 在调控NOX2中起重要作用. NOX4, 主要表达于肾脏, 能依赖其他可诱导的NOX亚基活化, 产生一定的ROS. 另外, NOX3表达于内耳, NOX5表达于淋巴组织. NOX2的其他2个同系物近来被证实, 为特异成熟亚基DUOX1和DUOX2, 主要表达于甲状腺, 是以额外的过氧化物存在为特征[9,10]. NOXO1为P47phox的同系物, 不过他缺乏自身抑制结构域. NOXA1为P67phox的同系物, 有类似的组织结构域. 所有NOX家族均为跨膜蛋白, 可将电子传递给氧分子产生超氧化物[11-15].

总之, NOX为针对各种刺激因素产生ROS的多蛋白复合物, 在活化信号通路方面起关键作用, 与肝纤维化密切相关[4]. 先前已报道在缺乏p47phox的小鼠实验中发现其能减轻胆管结扎(bile duct ligation, BDL)诱导的肝损伤和纤维化[16]. 在过度表达Rac1的转基因小鼠中发现其可促进HSC激活, 加重四氯化碳(CCL_4)诱导的肝损伤和纤维化[17]. 缺乏NOX2将减轻CCl_4诱导的小鼠肝纤维化[18]. 再者, 近来的研究也显示NOX2在HSC激活和凋亡肝细胞的吞噬中起关键作用[19]. NOX1可诱导HSC活化, 且在敲除NOX1基因的小鼠实验中显示NOX1可通过产生ROS, 激活Akt/人叉头框蛋白4(forkhead box4, FOXO4)/p47kipl信号通路, 进而促进HSC增殖, 加快BDL诱导的肝损伤后肝纤维化形成[20]. 最近发现NOX4在肝纤维化中的重要作用, 用抑制剂GKT137831(为新型NOX4/NOX1抑制剂)或敲除NOX4基因可减轻BDL诱导的小鼠肝纤维化[21]. 另外, 在长期门脉高压致肝硬化大鼠中发现P22phox表达上调[22]. 由此可见, NOX在肝纤维化中起关键作用, 可能为其成为抗纤维化综合治疗的新型靶点提供重要依据.

2 NADPH氧化酶介导活性氧产生

氧化还原酶可催化电子从还原剂传递给氧化剂. 在生物体内, 这些化学反应总是伴随着超氧化物形成, 分子氧获得电子主要产生ROS[3]. 多种氧化还原酶已被证实为ROS的潜在来源, 包括环氧酶[23]、氮氧合酶[24]、脂氧合酶[25]、细胞色素P450[26]、黄嘌呤氧化酶[27]、线粒体NADPH[28](辅酶Q)及NOX[29]. 细胞内ROS主要由NOX产生, 因为该酶专职产生ROS, 而其他酶仅通过特殊的催化方式且作为副产物产生ROS[30]. NOX通过产生ROS参与宿主防御和炎症, 细胞信号转导, 基因表达, 细胞生长发育、死亡和衰老, 生物合成和蛋白反应(如甲状腺激素合成), 血管生成等[15]. 在病理情况下, 大部分NOX相关的疾病均因ROS增加所致, 仅仅少数由ROS减少引起, 且大部分为慢性病[31], 如糖尿病肾病[32-34]、肺动脉高压[35,36]、心肌梗死[37,38]、缺血性中风[39,40]、阿尔茨默症[41,42]及帕金森病[43]等.

3 NADPH氧化酶产生活性氧介导的MAPK、PI3K/Akt信号通路与肝纤维化

3.1 MAPK的组成与活化 MAPK是信号转导中一个重要的丝氨酸/苏氨酸蛋白激酶通路. MAPK一般分为4个亚族: 细胞外信号调节激酶1/2(也为ERK1/2, P44/42), P38MAPKs, 应激活化蛋白激酶/C-Jun氨基端激酶(SAPK/JNK), 大丝裂原活化蛋白酶BMK1/ERK5[44]. MAPK通路由MAPKKK、MAPKK和MAPK 3个蛋白激酶组成. 这些蛋白激酶被依次活化, MAPKKK磷酸化MAPKK上的丝氨酸位点, 活化的MAPKK磷酸化MAPK的苏氨酸/酪氨酸位点[45]. 多种MAPKKK-MAPKK-MAPK通路已被广泛认识. Raf-MEK1/2-ERK1/2、MLK3-MKK3/6-P38、ASK1-MKK4/7-JNK及BMK1/ERK5通路均能被氧化应激、生长因子激活[46,47]. MAPK均在蛋白激酶Ⅷ亚区苏氨酸(threonine, Thr)和酪氨酸(tyrosine, Tyr)位点被双重磷酸化, 并能磷酸化胞核、胞质蛋白并改变其功能, 也能被特异性磷酸酶去磷酸化而失活. 因此, 磷酸化和去磷酸化之间的平衡可通过这些通路调控信号转导.

3.2 PI3K/Akt组成与活化 PI3K/Akt是信号转导中另一个重要的丝氨酸/苏氨酸蛋白激酶通路. PI3K是由85 kDa的调节亚基(P85)和110 kDa的催化亚基(P110)组成的异二聚体. 经生长因子如PDGF激活后, PI3K能催化磷脂酰肌醇的3-OH端磷酸化, 产生磷脂酰肌醇三磷酸盐(phosphatidylinositol trisphosphate, PIP3)[48]. PIP3聚集磷脂酰肌醇依赖激酶1(phosphoinositide-dependent protein kinase 1, PDK1), 在细胞膜磷酸化, 进而激活Ser/Thr位点激活Akt, 调控下游效应蛋白, 促进细胞增殖与存活[49,50]. Akt, 即蛋白激酶B(protein kinase B, PKB), 包括3种形式即Akt1/PKBα、Akt2/PKBβ和Akt3/PKBγ. 这3种形式有类似的磷酸化位点, 一个是氨基端的Thr308, 另一个是羧基端的Ser473. 这两个磷酸化位点对于Akt的完全激活是必不可少的. Akt的磷酸化因素包括糖原合成酶3(GSK-3), 叉头转录因子、坏蛋白及哺乳动物雷帕霉素靶点(the mammalian target of rapamycin, mTOR). Akt磷酸化GSK-3b、叉头及坏蛋白将这些蛋白失活, 而磷酸化mTOR可将其激活[44]. 肿瘤抑制基因(phosphatase and tensin homolog, PTEN)(从10号染色体上敲除的同源性磷酸酶张力蛋白), 是一种双重特异性蛋白和脂质磷酸酶, 通过去磷酸化减少PIP3, 从而负调控PI3K/Akt通路[50,51].

3.3 MAPK、PI3K/Akt与肝纤维化 NOX产生的ROS介导的MAPK和PI3K/Akt信号转导通路与肝纤维化密切相关. HSC内的ROS通过氧化敏感的蛋白激酶和转录因子调控各种介质的活化和表达. 各种促纤维化因素活化的NOX产生ROS, 在细胞信号通路中起关键作用[2]. Bataller等[16]首先报道血管紧张素Ⅱ(angiotensin, AngⅡ)与HSC上的受体结合后, 激活P47phox, 诱导ROS产生, 活化Akt和MAPK并增加活性蛋白1(activated protein1, AP-1)的DNA结合活性, 促进Ⅰ型胶原、转化生长因子(transforming growth factor, TGF)β1表达, 进而导致肝纤维化. 这与体内实验结果是一致的. Adachi等[52]随后发现PDGF可激活HSC内NOX产生ROS, 介导P38MAPK磷酸化, 进而促进HSC增殖, 导致肝纤维化. 在体内二甲基亚硝胺(dimethylnitrosamine, DMN)诱导的肝纤维化模型也证实了NOX在HSC激活和增殖中的作用. Proell等[53]研究显示TGF-β诱导NOX产生ROS, 促使活化型HSC分化为富含超氧化物歧化酶肌成纤维细胞(myofibroblast, MF), 提供谷胱甘肽以清除细胞内自由基, 加强细胞防御机制以保护细胞免于肝损伤. 之后De Minicis等[54]发现LP通过JAK激活NOX产生ROS, 活化Akt、ERK1/2等下游信号通路, 导致HSC增殖、纤维化形成. NOX抑制剂DPI、JAK抑制剂AG4T90或敲除P47phox亚基等NOX的药物和基因抑制可减弱这些效应, 并下调纤维化标志物如Ⅰ型胶原、炎性介质如单核细胞趋化蛋白-1(monocyte chemotactic protein-1, MCP-1)表达, 进而抑制HSC增殖. 最近Aleffi等[55]发现LP及PDGF也可通过激活NOX产生ROS, 介导mTOR通路, 增加血管内皮生长因子(vascular endothelial growth factor, VEGF)及低氧诱导因子-1α(hypoxia-inducible factor 1 alpha, HIF-1α)表达. 孕酮[56]、趋化因子受体[57]、凋亡小体[58]、高半胱氨酸[59]、胆汁酸[60]及高级聚糖化终产物[61]等均可激活HSC内的NOX产生ROS, 活化MAPK、PI3K/Akt及AP-1等信号通路, 引起HSC激活、增殖及纤维化形成. 因此, NOX在肝纤维化中起关键作用, 可能成为抗纤维化治疗的潜在药理学靶点.

此外, 许多体内外实验发现P38MAPK、PI3K/AKT信号通路与HSC激活及肝纤维化相关. 早期Varela-Rey等[62]研究显示肿瘤坏死因子α(tumor necrosis factor α, TNF-α)减少大鼠HSC内P38MAPK磷酸化, 而TNFβ增加P38MAPK磷酸化, 进而调节Ⅰ型胶原表达. Cao等[63]发现DLPC可通过抑制氧化应激及过氧化氢依赖的P38MAPK活性, 从而阻止TGF-β1诱导的Ⅰ型胶原增加, 进而起抗纤维化效应. Lechuga等[64]研究发现P38MAPK信号通路参与TGF-β1介导的基质金属蛋白酶13(matrix metalloproteinase13, MMP-13)表达, 及上调Ⅰ型胶原表达. 近年来Ye等[65]体外研究证实全反式维甲酸(ATRA)通过阻断JNK及AP-1活性, 抑制HSC增殖及其胶原产生, 减少促纤维化基因如TIMP-1等mRNA表达, 并明显诱导MMP-3及MMP-13的mRNA表达, 起抗纤维化作用. Yang等[66]发现大麻素(Anandamide, AEA)通过β-甲基环糊精(一种胆固醇成分), 而非阻断大麻素受体CB1、CB2, 明显抑制PI3K/AKT磷酸化, 促进IL-2、IL-6释放, 进而诱导HSC坏死, 发挥抗纤维化效应. 随后Szuster-Ciesielska等[67]研究发现桦木醇和桦木酸可通过阻断ROS产生, 进而抑制JNK和p38MAPK信号通路, 使TGF-β等细胞因子表达下调, 减弱乙醇诱导的HSC激活及纤维化形成. 之后他们[68]还发现新型三嗪衍生物(IMT)也可抑制JNK、P38MAPK磷酸化, 进而拮抗乙醇诱导的大鼠

■相关报道
近年来已有很多研究显示各种促纤维化因子(如转化生长因子-β、瘦素等)通过激活NOX产生ROS, 介导MAPK、PI3K/Akt、JAK-STAT、NF-κB、AP-1等信号通路活化, 促进HSC增殖、转化, 导致肝纤维化形成.

■创新盘点
本文就HSC内NOX产生ROS及其介导的MAPK、PI3K/Akt信号通路作了一较新、系统而全面的阐述.

HSC活化, 提示其可能为防治肝纤维化的新型治疗靶点, 为其制备抗肝纤维化综合治疗方案提供重要依据.

4 结论

HSC的激活和增殖是肝纤维化的中心事件. HSC激活引起肝纤维化, 不管其病因, 最终将活化HSC. 活化型HSC为具有增殖性、纤维性及收缩性的肌成纤维细胞. HSC凋亡将导致纤维分解和肝纤维化恢复. 在生理情况下, HSC的增殖和凋亡保持平衡. 一旦这种平衡被慢性肝损伤破坏, 持续的HSC增殖及胶原分泌将促使肝纤维化形成. 因此, 抑制HSC增殖和诱导其凋亡是延缓或阻止纤维化的两种方法, 而MAPK、PI3K/Akt是促进HSC增殖、抑制其凋亡的两条重要信号通路. 先前用药物如维生素C[69]或饮食如异黄酮[70]、多酚[71]减少内源性ROS的方法大部分已消失, 可能因为内源性抗氧化剂(过氧化物歧化酶、过氧化氢酶、氧化酶等)较多且活性较强, 以至于外源性抗氧化剂不可能对稳定的ROS起主要作用, 除了在某些少量抗氧化剂的细胞和组织处. 因此, 期望阻断ROS产生的主要来源(如NOX)可对组织ROS水平起很大作用, 与传统的抗氧化治疗相比发挥更加好的治疗效应.

总之, 探索以NOX为特异性作用靶点, 阻断NOX产生ROS, 进而抑制MAPK、PI3K/Akt信号通路的药物对于阻断肝纤维化意义重大. 虽已发现早期抑制NOX的药物如氯化二亚苯基碘嗡, 但其为非特异性药物. 因此, 研制出低毒、高效的NOX抑制剂将成为目前生物学研究和临床诊疗的重要方向.

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■名词解释
NADPH氧化酶(NOX): 一种多蛋白复合物, 可针对各种促纤维化因子(如血管紧张素Ⅱ、转化生长因子-β、血小板源性生长因子(PDGF)及瘦素等)促进ROS形成.

■同行评价
本文密切联系常见病与多发病, 从信号传导与阻断机制探讨HSC与肝纤维化的发生及阻断, 对新药的研发有一定指导作用.

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