综述

肝脏衰老中凋亡的调控机制

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摘要 肝脏是机体重要的代谢器官, 在机体全身衰老中尤为重要。脂肪肝、肝硬化和肝癌等老年常见病都与肝脏衰老密切相关。细胞凋亡作为一种细胞自我清除的保护机制, 在生物机体衰老过程中不可或缺。越来越多的研究证据表明, 凋亡在肝脏衰老中起着重要作用。适度的凋亡对于肝脏衰老是必要的; 过度凋亡会造成功能细胞的大量丧失、疾病恶化, 甚至最后导致肝功能衰竭; 凋亡不足则会使损伤的细胞积蓄, 导致细胞坏死或癌变。因此, 维持细胞凋亡在衰老肝脏中的适度平衡可延缓或减轻肝脏衰老对机体的影响。该文针对肝脏衰老过程中凋亡的调控机制包括氧化应激、基因不稳定性、脂肪毒性、内质网应激、营养感应失调等的研究进展进行了分析总结。

关键词 凋亡; 衰老; 肝脏; 氧化应激; 基因不稳定性

Mechanisms Underlying Apoptosis Process in Liver Aging

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Abstract Liver is the metabolic organ of the human body, which renders it particularly significant in the body aging. Fatty liver, liver cirrhosis, liver cancer as well as other senile liver diseases are closely related to liver aging. More and more research evidences suggest that apoptosis, as a self-eliminating mechanism, play a vital role in liver aging. Maintaining apoptosis to a certain extent is essential for liver aging: excessive apoptosis of liver cell leads to liverdisfunction, liver diseases aggravation and ultimately liver failure; whereas apoptosis incapacity of liver cell may cause the accumulation of damaged cells and lead to its necrosis or hepatocarcinoma. Therefore, only to maintain a delicate balance of liver cell apoptosis during liver aging, the maximum senescence delay or the minimum impact of aging on the body can be achieved. Here we select several current research focuses, namely, oxidative stress, genomic instability, lipotoxicity, endoplasmic reticulum stress and nutrient sensing dysregulation to elaborate the mechanisms underline apoptosis in liver aging.

Keywords apoptosis; aging; liver; oxidative stress; genomic instability

衰老作为一种自然规律, 表现为细胞增殖能力减弱、生理功能减退与免疫功能降低等特征。肝脏的衰老亦是如此, 随着增龄, 其表现出血液灌流下降、代谢能力下降、肝纤维化和肝癌发生率增加等一系列病理生理变化。凋亡(apoptosis)在其中扮演着重要角色, 可清除肝内衰老或坏死的细胞, 使细胞

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主动降解DNA和蛋白质, 在胞膜外显露出磷脂酰丝氨酸, 并最终诱导相邻巨噬细胞包围吞噬细胞自身。目前已有不少相关肝脏衰老过程中凋亡的研究报道, 本文将近年来肝脏衰老过程中凋亡的调控机制进展作一综述。

1 凋亡与衰老

老化是指全身细胞和器官逐渐退化的长期过程。衰老(aging)是指细胞的衰老, 通常指老化的结局, 是细胞临近死亡的短暂阶段。

伴随细胞老化的渐进过程, 细胞本身受到多方面的攻击与损伤。为了避免癌变, 细胞面临着“衰老”或“凋亡”的命运抉择。虽然二者共同的结局都是死亡, 但与细胞凋亡不同, 细胞衰老能够通过衰老相关分泌表型(senescence-associated secretory phenotype, SASP)分泌各种细胞因子、趋化因子与基质重塑酶来产生炎症, 从而影响周围微环境, 甚至促进肿瘤生成。然而, 细胞凋亡能在维持微环境稳态的前提下降解细胞自身, 被认为是一种有益的细胞保护反应[1]。此外, 凋亡亦是抑制生物体衰老的一种可能, 如抗凋亡蛋白Bcl-2(B cell lymphoma-2)和Bcl-xL(B-cell lymphoma-extra large)的抑制剂ABT263通过促进凋亡清除衰老细胞, 恢复衰老小鼠造血干细胞的活力[2], FOXO4-DRI(D-retro inverso-isoform of Forkhead box O4)通过选择性地促进高分泌SASP衰老细胞凋亡也恢复了衰老小鼠一些器官的活力[3]。凋亡也被视为机体的一种抗癌机制, 细胞内凋亡信号的衰退是癌症的表现之一[4]。可见, 凋亡是生物体对抗衰老的一种自我保护机制。

伴随着生物的衰老, 凋亡水平究竟是上调还是下降以及这种改变的意义何在？这些问题一直未有定论。Salminen等[5]认为, 老化细胞通过缺陷的p53通路, 增强NF-κB(nuclear factor-κB)/IAP(inhibitor of apoptosis)/JNK(c-Jun N-terminal kinases)轴的活性, 改变分子伴侣、miRNA、表观遗传调控等途径, 增强对凋亡的抵抗, 并且这种抵抗加速了老化的进程。Childs等[1]认为, 衰老细胞是否发生凋亡取决于细胞类型, 如衰老的内皮细胞更倾向凋亡, 而衰老的成纤维细胞与角质细胞更倾向逃避凋亡。肝脏内的肝细胞、胆管上皮细胞、星形细胞以及其他免疫细胞的衰老已被证明与老年性肝病的发病密切相关[6]。在衰老肝脏中, 过度的凋亡会导致在病毒性肝炎时功能细胞的丧失以及在肝纤维化时炎症的加剧, 甚至最后导致肝衰竭; 凋亡不足又会导致损伤细胞积聚, 诱发肝癌的发生[7]。因此, 我们认为, 凋亡同免疫反应类似, 过强或过弱对机体都是有害的。即使凋亡在生物衰老中具有保护作用, 但对肝脏而言, 维系促进与抑制凋亡途径的平衡才是延缓或抑制衰老的最佳途径。

2 肝衰老与氧化应激诱导的凋亡

“氧化应激”理论是指机体内合成活性氧类(reactive oxygen species, ROS)与清除ROS的状态失衡, 其核心是ROS。近二十年来, 该理论从单纯认为ROS具有损害细胞结构促进衰老的观点发展到认为ROS具有两面性的毒物兴奋效应(hormesis)的观点。ROS主要由线粒体的电子传递链(electron transport chain, ETC)产生, 与线粒体的功能障碍密切相关。传统的观点认为, 伴随衰老的线粒体功能障碍会干扰正常的氧化磷酸化途径, 使氧分子得不到充分的还原而产生大量有害的ROS, 损害细胞内的DNA、蛋白质、脂质。现在的观点认为, 低浓度的ROS参与氧化还原信号转导(redox signaling), 调控着细胞增殖以及其他正常的生理功能; 而高浓度的ROS则损害了氧化与抗氧化机制的平衡, 造成细胞结构与功能的损害, 甚至死亡[8]。在恒河猴与鼠类肝脏的生理性衰老过程中均发现了大量氧化应激产物[9-10]。鉴于肝脏作为生物体内氧化代谢的中枢, ROS在其中的积聚损伤, 相比其他大部分组织器官更为严重。肝衰老过程中有关氧化应激致细胞凋亡的机制涉及caspase活性升高、抗氧化功能减退、线粒体功能障碍、Nrf2(nuclear factor E2-related factor 2)衰退诱导的AIF(apoptosis inducing factor)途径(图1)。

Caspase是经典凋亡途径的主要起始者与执行者, 以未激活酶原单体的形式存在于胞质, 可被ROS信号通过蛋白质水解特异位点而激活[11]。Zhang等[12]发现, 衰老Fisher 344大鼠肝脏细胞对氧化应激比年轻大鼠更为敏感, 他们检测到caspase-3、caspase-6、caspase-7、caspase-2、caspase-9的活性增高, *Fas*(TNF receptor superfamily)mRNA有升高趋势, 但并未检测到激活的caspase-8。他们的结果提示, 外源性凋亡可能在生理性的衰老凋亡中并不起主要作用。细胞内各种内源性抗氧化系统的功能减退与线粒体的功能障碍也是造成衰老氧化应激的原因

之一[13]。这二者联系密切, 抗氧化能力减退可导致ROS积累、线粒体膜电位(Δψm)与通透性的下降, 促进促凋亡因子的释放。ETC障碍则导致更多ROS的产生, 损伤细胞结构与功能, 或直接激活凋亡信号调节激酶1(apoptosis signal-regulating kinase 1, ASK1), 再由此激活JNK通路诱导凋亡。甚至有研究表明, 在与ROS无关的前提下, 仅有抗氧化能力减退也可以导致凋亡, 如谷胱甘肽外流(GSH efflux)导致的凋亡[14]。无论是硫氧还蛋白2(thioredoxin 2)[15]还是锰超氧化物歧化酶(manganese superoxide dismutase, *MnSOD*)[16]基因缺失的小鼠, 其肝脏细胞内氧化应激产物与凋亡的水平都显著提高。硫氧还蛋白2缺失的小鼠表现出线粒体功能障碍与氧化应激增加, 二者共同促进肝脏细胞凋亡; 而*MnSOD*缺失小鼠因线粒体损伤, 其线粒体通透转运通道(mitochondrial permeability transition pore, mtPTP)更易形成, 导致线粒体内如细胞色素c之类的促凋亡因子释放。反之, 在右旋半乳糖(D-galactose)小鼠衰老模型中, 给予水飞蓟油(silybum marianum oil)抗氧化物的小鼠[17], 其肝脏细胞抗氧化能力增强、caspase-3减少、抗凋亡蛋白Bcl-2活性增加, 凋亡减少; 给予白藜芦醇葡萄糖甙(polydatin)抗氧化物的小鼠[18], 其肝脏细胞氧化应激与炎症反应减轻、Bcl-2/Bax(Bcl-2-associated X)的比例上调、caspase-3表达增强, 凋亡被抑制。以上两者均表明, 肝脏内的抗氧化能力下降与凋亡有关。转录因子Nrf2活性减少被认为与肝脏衰老有关, Nrf2调控着多种抗氧化酶的转录与翻译, 是细胞对氧化应激的一种适应性保护机制[19]。Ariza等[20]在*Nrf2*缺失的鼠胚胎成纤维细胞中发现, 线粒体功能障碍导致膜通透性升高。在*Nrf2*缺失的小鼠肝组织中发现更多凋亡与分解的DNA片段, 凋亡诱导因子AIF在不引起caspase激活的情况下进入核内, 直接降解DNA, 导致凋亡。

3 肝衰老与基因不稳定性诱导的凋亡

基因不稳定性一直是衰老原因的解释机制之一。肝脏的高度代谢活动决定了其将受到多种内外源性的攻击, 如水解、氧化、烷化、离子化、紫外辐射等[21], 加上伴随衰老的各种DNA修复机制的功能减弱[22], 受损的DNA积累在所难免。虽然存在各种DNA损伤形式, 但细胞内存在对受损DNA的监测机制, 如ATM(axia telangiectasia mutated)、ATR(axia telangiectasia and Rad3 related)与DNA-PK(DNA-depen-dent protein kinase)。这些激酶可以磷酸化一系列蛋白质以产生DNA损伤反应(DNA damage response, DDR), 通过p53或BRCA1/2(breast cancer 1/2)通路决定细胞是进行自我修复还是凋亡[23]。其中, 位于端粒的DNA因被端粒蛋白复合体(shelterin)保护而较为特殊, 无法被细胞监测到DNA的损伤, 所以这种持续性损伤更易诱导细胞的衰老

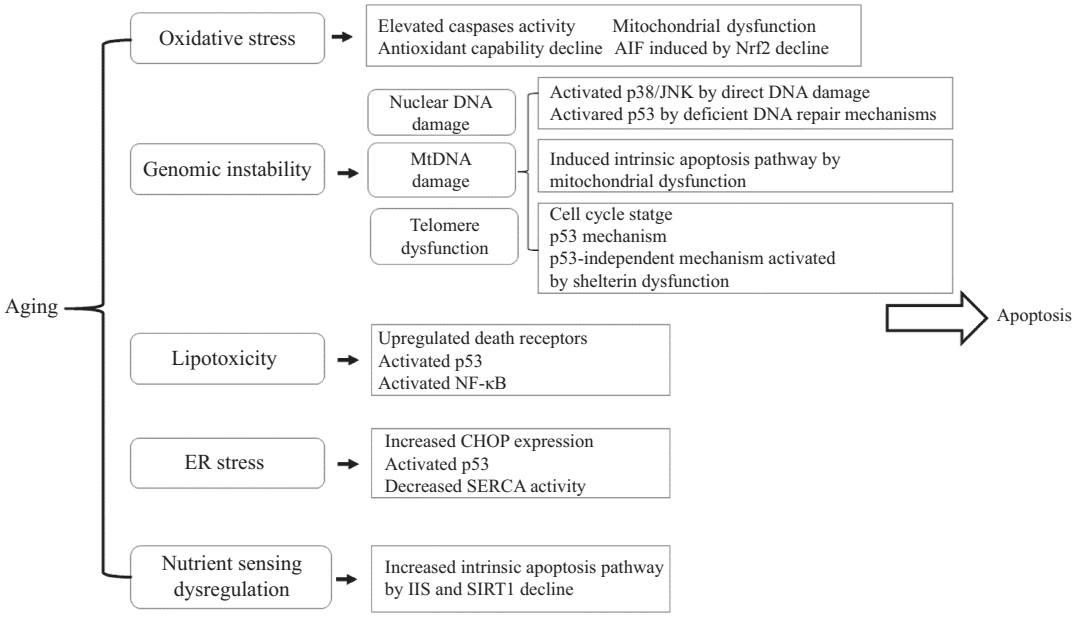

图1 肝脏衰老中凋亡的调控机制

Fig.1 Mechanisms underlying apoptosis process in liver aging

与凋亡[24]。在此, 我们将从核DNA损伤、mtDNA损伤和端粒功能障碍三个方面来阐述基因不稳定性对衰老肝脏中凋亡的影响。

3.1 核DNA损伤

肝癌在老年人群中多发, 肝脏的代谢产物或外来有害因素直接损伤DNA从而使肝细胞更易癌变, 通过凋亡可避免这一过程。衰老过程中, 核DNA损伤导致肝脏凋亡的调节机制涉及DNA直接损伤激活的p38/JNK通路与DNA修复缺陷激活的p53通路。Suh等[25]向雄性大鼠腹膜内注射甲磺酸甲酯(methyl methanesulfonate, MMS), MMS无需代谢即可直接烷基化细胞中的DNA。在等量核DNA损伤的情况下, MMS是大鼠脑癌的强诱发剂, 却对肝癌的诱发效果甚微, 但通过激活p38与JNK通路诱发肝脏细胞大量凋亡, 这说明不同细胞对相同刺激产生的凋亡倾向是不同的。在此过程中, MMS激活的通路磷酸化SEK1(SAPK or extracellular signal-regulated protein kinase kinase 1)/MKK4(MAPK kinase 4)的Thr223, 后者随后又激活了p38与JNK通路, 二者通过下游的ATF-2(activating transcription factor-2)和c-Jun等信号诱导凋亡。此外, 伴随着衰老, DNA修复机制功能逐渐减退[26]。当检测到DNA损伤时, 磷脂酰肌醇3激酶样激酶ATM可磷酸化Chk2(checkpoint kinase 2), ATR可磷酸化Chk1(checkpoint kinase 1), Chk1与Chk2再共同磷酸化转录因子p53的Ser20以激活p53; 根据DNA损伤水平的高低, p53决定转录如*DDB2*(DNA damage-binding protein 2)、*XPC*(xeroderma pigmentosum, complementation group C)、*Fen1*(flap structure-specific endonuclease 1)等抑凋亡基因修复细胞, 还是由*Fas-R*(Fas receptor)、*Bax*、*Puma*(p53 upregulated modulator of apoptosis)的促凋亡基因诱发凋亡[23]。Xpd(TTD)雌性小鼠因核苷酸切除修复缺陷表现为早衰, 但肝癌的发生几率较小, Park等[27]发现, caspase-3与p53蛋白质在该类衰老小鼠的肝脏中大量表达, p53引起了凋亡的增加。

3.2 mtDNA损伤

mtDNA损伤是导致衰老的原因之一。处于氧化应激的中心、缺乏组蛋白的保护以及有限的修复机制都使mtDNA相比核DNA更易受到损伤[28]。低程度的mtDNA损伤能诱导细胞衰老, 而高程度的mtDNA损伤能诱发凋亡[29]。许多研究已证实, 在人与小鼠的多种细胞中, mtDNA损伤通过内源性(线粒体)途径产生凋亡[30-32]。伴随着肝脏衰老中的mtDNA损伤是多面的: 在衰老大鼠的肝脏细胞中发现mtDNA含量减少[33], 在衰老恒河猴肝脏细胞中发现有更多的mtDNA损伤与线粒体功能障碍[10], 在衰老小鼠中发现mtDNA损伤可以加速肝脏衰老[34], 在衰老大鼠的肝脏细胞中发现mtDNA的含量与功能的稳定有益长寿[35]。通过氧化应激引起mtDNA损伤的方式已在前面叙述, 这里需要强调的是, 造成与衰老相伴发生的mtDNA损伤的因素不止氧化应激一种[36]。Kujoth等[37]利用mtDNA校正功能缺陷的线粒体DNA聚合酶G(mitochondrial DNA polymerase G)缺失小鼠研究发现, mtDNA损伤加速了肝脏衰老, 衰老增加了活化的caspase-3水平与凋亡程度, 伴之以肝功能下降, 但其中并未检测到氧化应激导致的mtDNA损伤。他们认为, mtDNA损伤不一定会导致氧化应激加重, “毒性循环”理论(氧化应激导致mtDNA损伤, mtDNA损伤进一步导致氧化应激, 而致细胞凋亡)是存在问题的, 但mtDNA损伤仍会造成线粒体功能障碍, 导致线粒体膜通透性下降, 释放细胞色素c等促凋亡因子激活caspase-3, 造成线粒体(内源性)途径的凋亡。

3.3 端粒功能障碍

端粒一直是衰老的一大研究热点, 无论是有增殖功能的细胞因DNA不断复制导致的端粒缩短, 还是因伴随衰老的端粒酶的活性减弱, 端粒都与细胞的衰老密切相关。在无肝病史的人体肝脏生理性衰老过程中, 端粒长度仅限于在Kupffer细胞和星形细胞中下降, 而在肝细胞等其他细胞中维持稳定[38]。端粒的功能障碍主要体现在病理性的衰老肝脏中, 如人体移植肝脏的细胞表现出加速的端粒缩短与衰老[39], 端粒的功能障碍促进了慢性肝病与肝癌的发生[40]。特别是在端粒失去保护的情况下, 端粒的功能障碍造成DDR, 激活了细胞的凋亡。在肝脏衰老过程中由端粒功能障碍导致凋亡的机制涉及端粒蛋白复合体功能障碍激活的非p53途径、端粒酶缺失激活的p53途径, 而且与肝脏细胞所处的分裂状态有关。

端粒重复序列结合因子2(telomeric repeat-binding factor 2, TRF2)是端粒蛋白复合体的成分之一, 抑制TRF2活性可以导致端粒的功能障碍。Lechel等[41]利用显性阴性蛋白抑制12~14周雌性小鼠肝脏细胞内正常的TRF2活性, 发现轻度的端粒功

能障碍导致肝脏细胞的衰老, 重度的端粒功能障碍导致肝脏细胞的凋亡, 而且该实验中的凋亡是p53非依赖性的。其他研究表明, 当端粒失去其外包盖的保护时, 除了p53依赖性途径外, 聚ADP核糖聚合酶-1[poly(ADP-ribose) polymerase-1, PARP-1]以及p53的同系物p73都参与凋亡[42]。最近的研究也显示, p73途径引起的凋亡在端粒功能障碍中起着重要作用[p73可以通过激活Bax、p53正向凋亡调控因子(p53 upregulated modulator of apoptosis, PUMA)与caspase-3来引起凋亡], 但缺乏肝脏方面的研究证据[43]。与Lechel等[41]的实验结果不同, Lazzerini等[44]条件性敲除小鼠肝脏细胞中的*TRF2*基因后并未发现p53的激活与细胞的凋亡。他们认为, 处于G_0期的肝细胞对于端粒功能障碍更不敏感, 所以凋亡可能也与细胞所处的分裂状态有关。端粒功能障碍亦能导致线粒体功能障碍, 激发线粒体途径的凋亡。例如, 调控线粒体功能代谢的主要调节因子(peroxisome proliferator-activated receptor gamma coactivator, PGC)的活性随着年龄的增大逐渐降低。Sahin等[45]在端粒功能障碍的端粒酶RNA组分缺失(telomerase RNA component, $Terc^{-/-}$)和端粒酶逆转录酶缺失(telomerase reverse transcriptase, $Tert^{-/-}$)小鼠中发现, p53通路激活, 激活的p53结合并抑制了PGCs的启动子, 引起肝细胞线粒体功能障碍, 导致凋亡。

4 肝衰老与脂肪毒性诱导的凋亡

脂肪毒性是指游离脂肪酸(free fatty acids, FFA)因体内代谢功能障碍而在非脂肪组织中大量积累导致的细胞功能障碍或死亡。流行病学研究显示, 非酒精性脂肪肝病(nonalcoholic fatty liver disease, NAFLD)与非酒精性脂肪肝炎(non-alcoholic steatohepatitis, NASH)在老年人群中高发[46-47]。随着年龄的增大, 衰老伴随的胰岛素抵抗导致肝脏的脂质代谢能力减低, FFA在肝细胞积蓄; 同时, 肝细胞的抗氧化能力下降亦导致游离脂肪酸的氧化应激; 二者共同导致了肝细胞的凋亡。凋亡被认为是NASH这类疾病炎症发生的主因之一, 衰老的肝细胞因衰老相关分泌表型(senescence-associated secretory phenotype, SASP)分泌促炎因子加强了炎症反应, 诱导细胞凋亡[48]。衰老过程中, 脂肪毒性导致肝脏凋亡的调节机制涉及上调死亡受体、激活p53、NF-κB信号途径。

外源性凋亡受体高表达是脂肪毒性介导的肝脏损伤的重要特点之一。Malhi等[49]发现, 过高的FFA导致体外培养的肝脏细胞(Huh-7细胞系、HepG2细胞与初代大鼠肝脏细胞)通过激活JNK通路, 上调DR5(death receptor 5)介导的TRAIL(tumour necrosis factor-related apoptosis-inducing ligand)外源性凋亡。Volkmann等[50]发现, 体外提取的衰老病人(如肝脂肪变性)的肝细胞上调TRAIL受体表达的同时上调了促凋亡蛋白Bcl-2的水平。Ribeiro等[51]发现, 在脂肪性肝炎病人肝细胞激活的NF-κB导致TNF-α(tumor necrosis factor-α)与Fas受体表达增多; 虽然内源性凋亡途径的抗凋亡蛋白Bcl-2有所升高, 但仍是不敌外源性的死亡受体的促凋亡作用, 提示外源性途径的凋亡可能在此过程中起主要作用。Farrell等[52]认为, 在蛋氨酸与胆碱缺乏(methionine and choline-deficien, MCD)的NASH模型小鼠肝脏中, p53通路可能介导了内源性的线粒体凋亡途径与外源性的TRAIL受体凋亡途径。p53可抑制抗凋亡蛋白Bcl-xL, 造成Bid(BH3 interacting-domain death agonist)裂解为tBid(truncated Bid), 从而启动线粒体凋亡途径; 还可激活p21, 抑制抗凋亡的D细胞周期蛋白激酶(cyclin D kinase)活性以暂停细胞周期; 亦可上调TRAIL等外源性死亡受体的表达。以上三者共同促进凋亡。Yan等[53]发现, 安石榴苷(punicalagin)可通过激活Keap1-Nrf2信号通路来减轻FFA导致的肝癌细胞凋亡。如前所述, Nrf2起着抗氧化应激的作用, 其在正常情况下会被Cul3-Keap1泛素E3连接酶泛素化, 再被蛋白酶体降解; 但在氧化应激时Keap1被修饰, 导致Nrf2稳定转录出对细胞有保护作用的基因, 减轻了凋亡。

5 肝衰老与内质网应激诱导的凋亡

伴随肝脏衰老的进行, 未折叠蛋白反应(unfolded protein response, UPR)的保护效应开始弱于凋亡作用[54]。内质网内某些起着关键作用的分子伴侣和酶类功能下降, 如衰老小鼠肝脏中的蛋白质二硫键异构酶(protein disulfide isomerase, PDI)和免疫球蛋白重链结合蛋白质(immunoglobulin heavy chain binding protein, BiP)[55]以及其他损伤的积累, 导致内质网生产蛋白与折叠蛋白的不平衡, 形成内质网应激。激活内质网应激有助于形成UPR: 细胞

通过主动减少蛋白质生成、增加分子伴侣以加速蛋白的转运、降解未折叠与折叠错误蛋白等途径来自我适应这一刺激; 倘若刺激始终无法解决, 内质网应激则会主动地引起细胞凋亡来减小对周围组织的影响。在肝衰老过程中, 内质网应激导致凋亡的调节机制涉及上调CHOP(C/EBP homologous protein)表达, 激活p53和JNK通路以及下调肌浆网钙泵(sarco/endoplasmic reticulum Ca^{2+}-ATPase, SERCA)的活性。

能够促进凋亡的CHOP被发现在衰老大鼠肝脏中大量表达[56], 在慢性衰老小鼠肝脏中含量增加[57]。Enkhbold等[58]对衰老小鼠肝脏施以部分肝切除后, 相比年轻小鼠, 衰老小鼠肝脏细胞中CHOP的表达增多, 而且抗凋亡的衰老标记蛋白30(senescence marker protein 30, SMP30)的表达减少, CHOP增多与SMP30减少二者协同促进了凋亡。CHOP通过增强促凋亡基因与减弱抑凋亡基因的表达诱导凋亡, 如*DOCs*(down-stream of CHOP)、*Bcl-2*、*TRB3*(tribbles-related protein 3)[59]。SMP30的表达随衰老减少[60], 其下调增强了肝细胞对TNF-α与Fas外源性凋亡的敏感性[61]。在FFA导致的内质网应激背景下, Cazanave等[62]发现, 在体外培养的肝癌细胞(Huh-7细胞系)中CHOP可以协同激活蛋白-1(activator protein-1, AP-1)激活p53正向凋亡调控因子(PUMA)的表达, 进而通过线粒体途径诱导凋亡。SERCA的表达与活性下降在心脏与骨骼肌老年性疾病的发生中起着重要的作用[63]。SERCA在肥胖的鼠科动物肝脏中活性下降, 当内质网应激时, SERCA的活性减弱导致钙超载, 后者可激发线粒体的内源性凋亡途径, 导致肝脏细胞凋亡[64-65]。

6 肝衰老与营养感应失调诱导的凋亡

营养感应(nutrient sensing)是指细胞对不同营养物的感应及引发的相对应处理方式, 其失调在生物衰老中常见。与肝脏凋亡有关的营养感应失调有IIS(insulin and IGF-1 signaling)和SIRT1(sirtuin 1)信号, 在衰老过程中两者功能都减退。IIS与SIRT1都可通过增强细胞对营养物质的应激处理, 保护细胞的正常生理功能, 具有抑制凋亡的作用, 而且与能量限制(calorie restriction, CR)具有重要联系。CR是目前唯一被广泛认为能延缓生物衰老的机制, 最近更是确认了其对灵长类动物衰老的有益作用[66]。在肝衰老过程中, 营养感应失调诱导凋亡的调节机制涉及IIS衰退与SIRT1衰退造成的线粒体途径凋亡。

生长激素(growth hormone, GH)由神经垂体分泌, 可激活体内细胞(主要是肝细胞)分泌胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)。IGF-1作用类同于胰岛素, 可增强细胞对葡萄糖的感知, 因此二者共同组成IIS通路, 但都随着衰老在生物体内含量逐渐下降[28]。Tresguerres等[67]发现, 给予GH能减少衰老大鼠肝脏细胞内的氧化应激, 通过减少线粒体内的NO参与的硝基氧化应激, 减轻了对线粒体的损害, 抑制细胞色素c的释放, 减少了凋亡。正常衰老的大鼠血清中IGF-1含量下降, Puche等[68]对衰老大鼠皮下注射IGF-1至体内正常浓度后, 发现IGF-1恢复了肝脏细胞的线粒体活性, 相比对照组衰老大鼠体内caspase-3与caspase-9的过表达, 注射了IGF-1的衰老大鼠肝脏中的凋亡反而减少。反之, Tirosh等[69]在长寿的转基因alpha MUPA小鼠中发现了低血清浓度的IGF-1与肝脏细胞内增强的线粒体途径凋亡。以上二者表明, 低浓度的IGF-1可促进衰老肝脏细胞凋亡, 正常浓度的IGF-1可抑制衰老肝脏细胞凋亡。IGF-1主要通过PI3K(phosphoinositide 3-kinase)-Akt(protein kinase B)通路调节Bcl-2家族蛋白的表达与功能, 维持了线粒体膜的稳定性与通透性, 避免了凋亡[70]。至于去乙酰化酶中的SIRT1(sirtuin 1), 其可通过维持基因的稳定以增强衰老细胞的代谢, 如线粒体生成、脂质代谢、增强对应激的适应[71], SIRT1被发现通过调节线粒体途径来抑制人软骨细胞的凋亡[72], CR激发的SIRT1的表达也被认为是CR延缓衰老的机制之一[71]。Andre等[73]发现, 减少SIRT1的活性在加重NASH病情的同时亦导致了大鼠肝脏细胞的凋亡。相反, Minor等[74]用SRT1720激活了肥胖小鼠体内的SIRT1之后, 发现SIRT1通路在肝脏内纠正了伴随衰老的不利基因的表达, 同时激活PGC-1α以增强线粒体的活性, 小鼠肝脏内炎症减少、凋亡受到抑制, 小鼠寿命延长。

7 总结与展望

肝脏是机体中代谢、免疫和合成的重要器官, 肝脏衰老在整个机体衰老过程中尤为重要。脂肪肝、肝硬化和肝癌等老年常见肝病都与肝脏衰老密切相关。经过近二十年的研究, 凋亡在衰老肝脏中究竟是上调还是下调一直未有定论。总的来说, 无论是病理性还是生理性衰老, 凋亡在肝脏衰老过程中总

体上是增加的, 而且内源性、外源性和其他途径都有涉及。虽然目前已有较多此方面的研究, 但几乎每个方面都还缺乏足够的证据以提供完整的答案, 如端粒功能障碍如何调控凋亡, 与衰老相关的内质网应激怎样导致凋亡。同时, 也仍存在许多其他的谜题, 如NASH是如何调控凋亡加重病情、凋亡在CR中的作用、硝基氧化应激在肝衰老凋亡中的作用[75]。即使是对凋亡本身, 我们也不能局限于以前的观点, 认为它可以避免炎症发生。新的研究告诉我们, 凋亡被认为是NASH这类疾病炎症发生的主因之一, 肝细胞可以通过凋亡导致炎症的加剧。鉴于老年性肝病的多发, 凋亡与肝癌、肝纤维化的密切联系, 阐明凋亡在衰老肝脏中的调控机制更具有现实的临床意义。

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