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脂肪变性诱发肝癌的机制——来自丙型肝炎病毒核心基因转基因小鼠的启示

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关键词

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饮食限制

丙型肝炎病毒核心蛋白

摘要

丙型肝炎病毒(HCV)感染是全球慢性肝炎、肝硬化和肝细胞癌(HCC)的主要病因。在HCV的结构蛋白中,HCV核心蛋白具有调控基因转录、脂质代谢、细胞增殖、细胞凋亡和自噬的能力,所有这些都与HCC的发展密切相关。携带HCV核心基因的转基因小鼠表现出与慢性丙型肝炎患者的临床特征相似的年龄依赖性胰岛素抵抗、肝脂肪变性和HCC。一些饮食习惯的调整,包括限制热量和富含饱和脂肪酸(SFA)、反式脂肪酸(TFA)或胆固醇的饮食,被证明会影响HCV核心基因转基因小鼠的肝脏脂肪生成和肿瘤形成。这些饮食的改变除了调节肝纤维化过程和微环境外,还调节了肝细胞的应激和增殖,从而证实了饮食习惯与脂肪变性相关的肝癌发生之间的密切联系。本文综述了HCV基因组转基因小鼠模型的研究结果,重点介绍了HCV核心基因转基因小鼠的研究结果,并讨论了HCV核心蛋白诱导脂肪变性和肝癌发生的机制,以及饮食习惯对脂肪变性所致肝癌的影响。

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1 引言

丙型肝炎病毒(HCV)感染引起的慢性肝炎是一个严重的全球性问题,因为慢性丙型肝炎是导致肝硬化和肝细胞癌(HCC)的主要原因。世界卫生组织(WHO)2017年的一份报告显示,全球约有7100万人(1%)感染HCV

[1]。大约30%的HCV感染者表现为持续性慢性肝炎,这可能会发展为肝硬化和HCC [2]。

HCV是黄病毒科中肝病毒属的一员,是一组微小的、有包膜的单链RNA病毒[3]。HCV颗粒是由结构蛋白形成的,包括HCV核心蛋白及包膜糖蛋白E1和E2。由于非结构蛋白(p7、NS2、NS3、NS4A、NS4B、NS5A和

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NS5B) 在HCV生命周期中发挥重要作用, 因此它们已成为直接作用于抗病毒药物的分子靶点。例如, NS4B调控复制复合体的形成, NS5A是HCV RNA复制和颗粒产生的主要成分, NS3~NS5B负责复制模块并催化病毒RNA基因组的扩增[3-4]。HCV含有一个9.6 kb的正链RNA基因组, 由一个带有内部核糖体进入位点(IRSE)的5'非编码区(NCR)、一个编码结构蛋白和非结构蛋白的开放阅读框以及一个3' NCR组成[5] (图1)。以前的研究表明, HCV感染与各种代谢性疾病之间存在很强的相关性, 包括脂肪肝变性、2型糖尿病(T2DM)以及铁和卟啉蓄积[6-7]。然而, 代谢紊乱在很大程度上也受到生活方式的影响, 如暴饮暴食、高脂肪和高碳水化合物饮食以及缺乏锻炼。此外, 持续的HCV感染通常伴随肝脏炎症和纤维化, 通过炎症级联和肝细胞损伤扰乱胰岛素信号和脂质代谢[8-12]。因此, 很难理解HCV对代谢性疾病的单一直接作用。为了破译HCV的致病原理, 研究人员利用这10种结构蛋白和非结构蛋白建立了多种HCV转基因小鼠模型[13-15]。事实上, 只有HCV核心蛋白转基因小鼠表现出与HCV感染患者相似的自发性葡萄糖耐受不良、脂肪肝变性和HCC, 这证实了HCV核心蛋白在HCV相关代谢紊乱中的关键作用。

本文综述了近年来HCV基因组转基因小鼠模型的研究进展, 重点介绍了HCV核心基因转基因小鼠的特征观察, 并探讨了HCV核心蛋白诱导脂肪肝细胞癌发生的机制。为了进行客观全面的综述, 我们在PubMed中输入了

几个关键词(HCV转基因小鼠、脂肪肝、HCC、脂肪酸、胆固醇、热量限制、饮食干预等), 搜索1995—2021年发表的相关文章。

2. 丙型肝炎病毒核心蛋白的功能

2.1. 丙型肝炎病毒核心蛋白的结构

HCV核心蛋白是一种21 kDa的多功能蛋白, 具有脂质和RNA结合活性, 其主要功能是构建病毒衣壳, 覆盖和保护基因组RNA, 同时将病毒从一个细胞传播到另一个细胞[4, 17]。当HCV核心蛋白的前体[191个氨基酸(aa)]从多聚蛋白中释放出来后, 由信号多肽酶进一步加工产生成熟的核心蛋白形式[4]。

HCV核心蛋白由两个结构域组成: 亲水性的D1和疏水性的D2。D1结构域由三个基本簇组成: 基本结构域1(BD1; 2~23 aa)、基本结构域2(BD2; 38~74 aa)和基本结构域3(BD3; 101~121 aa)。D1结构域与颗粒形成和RNA结合所必需的寡聚化有关[4]。D2结构域负责与内质网(ER)和脂滴(LD)联系。D2的主要结构元件由两个两亲性 α 螺旋[螺旋I(HI)和螺旋II(HII)]组成, 由疏水环隔开。这两个螺旋可以在疏水环境中折叠, 从而揭示脂质在维持结构完整性方面的相互作用。突变研究表明, 螺旋I、疏水环和螺旋II的结合与LD的形成密不可分[18] (图1)。疏水性D2结构域的大约20个末端氨基酸作为靶向包膜糖蛋白E1的信号序列[4]。

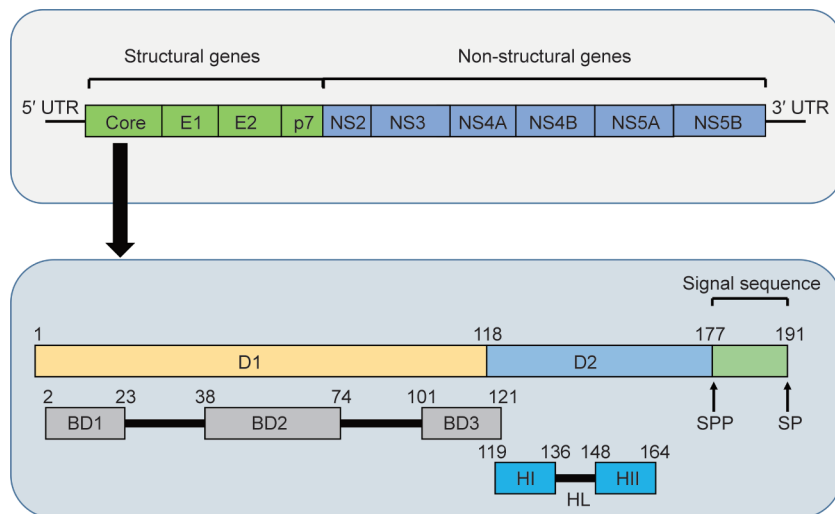


图1 丙型肝炎病毒(HCV)及其核心蛋白的结构。HCV基因组(结构基因和非结构基因)编码了一个9.6 kb的多聚蛋白, 其两端各有5'和3'非翻译区(UTR), 该多聚蛋白经细胞和病毒蛋白酶加工成10种结构蛋白和非结构蛋白(核心、E1、E2、p7、NS2、NS3、NS4A、NS4B、NS5A和NS5B)。核心蛋白由信号肽酶(SP)从多聚蛋白中释放出来。191个氨基酸的前体核心经信号肽酶(signal peptide peptidase, SPP)处理, 得到一个约177个氨基酸的成熟蛋白, 它由两个结构域(D1和D2)组成。D1域被划分为基本域1(BD1)、基本域2(BD2)和基本域3(BD3)。D2结构域包括螺旋I(HI)和螺旋II(HII), 它们被一个疏水环(HL)隔开。引用自参考文献[4], 经Springer Science Business Media New York © 2014许可, 及参考文献[5], 经Elsevier © 2011许可。

2.2. 丙型肝炎病毒核心蛋白在细胞中的作用

为了评估HCV核心蛋白在细胞中的直接功能,利用稳定过表达HCV核心蛋白的培养细胞进行体外实验是必不可少的。在QSG7701人源性非肿瘤肝细胞系中,HCV核心蛋白的表达通过干扰视网膜母细胞瘤抑制蛋白(pRb)/E2F转录因子1(E2F-1)的平衡[20]来抑制细胞凋亡[19]。相反,B细胞白血病/淋巴瘤2相关X蛋白(BAX)从细胞质到线粒体的易位、线粒体膜电位的破坏、细胞色素c的释放以及由HCV核心蛋白激活的caspase-9和caspase-3似乎促进了细胞凋亡[21]。HCV核心蛋白不仅上调核因子(NF)- κ B以抑制宿主细胞的反应,还通过催化Beclin-1的表达[19]来增强自噬。虽然自噬可以帮助消除病原体,但HCV可以通过自噬加速自我复制,导致HCV存活和持续感染[19]。

在肝癌细胞系中,HCV核心蛋白显著增强Wnt/ β -catenin信号转导活性,这是肝细胞增殖和肝癌发生的关键驱动因素。它还调节HepG2细胞中的Wnt1以促进异常增殖[23]。多项研究表明,HCV核心蛋白可促进肝癌细胞中激活蛋白(activator protein, AP)-1和血管内皮生长因子(vascular endothelial growth factor, VEGF)的表达,揭示HCV诱导的HCC肝癌血管生成可能部分是由该蛋白介导[24]。HCV核心蛋白能够在缺氧条件下上调缺氧诱导因子-1 α ,从而帮助增加血管内皮生长因子的表达[25]。一项研究还表明,HCV核心蛋白通过激活NF- κ B和上调肿瘤坏死因子(TNF)- α 的表达来促进人肝癌细胞的增殖。HCV核心蛋白通过抑制核受体亚家族4组A成员1(NR4A1)来刺激肝细胞增殖和化疗耐药性[26]。表达HCV核心蛋白的肝癌细胞通过转化生长因子 β 介导的方式激活共培养的星状细胞[27]。HCV核心蛋白与LD的相互作用在HCV感染过程中起着重要作用。HCV核心蛋白已被证明影响鞘脂和胆固醇酯的生物合成,这在一定程度上与HCV感染患者的脂代谢异常有关[28]。此外,转染HCV核心蛋白的肝细胞中,胰岛素样生长因子结合蛋白(IGFBP)-1的表达显著降低[29]。由于IGFBP-1可能是影响肝脏胰岛素敏感性和葡萄糖代谢[30]的关键因素,这种作用可能导致慢性丙型肝炎的胰岛素抵抗。

3. 丙型肝炎病毒核心基因转基因小鼠构建

3.1. 为什么丙型肝炎病毒核心基因转基因小鼠是必要的?

由于过表达HCV蛋白的细胞实验结果是在特定条件下获得的,因此在体内动物实验中,通过模拟人类持续感染HCV来评估HCV蛋白在全身的影响是非常有价值的。

然而,HCV不能感染啮齿类动物,啮齿类动物是大多数体内实验的常规动物。作为替代方案,人们已构建了多种HCV蛋白转基因小鼠,以了解HCV蛋白在体内的发病机制[13-14,32]。

HCV小鼠模型的建立有助于复制HCV感染患者的临床特征,以及评估慢性HCV感染的发病机制。携带HCV包膜基因E1和E2的转基因小鼠于1995年首次被报道[14]。免疫组织化学染色显示,包膜蛋白主要定位在肝中央静脉周围肝细胞的胞浆中。尽管这些动物表现出涉及唾液和泪腺的外分泌病变,但直到16个月大才能检测到肝病的证据,这表明HCV直接参与了HCV感染者涎腺炎的发病机制[33]。HCV NS5A蛋白具有许多标志性特征,包括在细胞质中隔离p53,p21蛋白的下调、激活信号转导和转录激活因子3(STAT3)的激活以及抑制肿瘤坏死因子TNF- α 介导凋亡。然而,HCV NS5A蛋白基因转基因小鼠并没有表现出明显的致病性[13],表达HCV NS3A/NS4A的转基因小鼠也是如此[32,34]。

在HCV的结构蛋白和非结构蛋白中,核心基因的突变很少,这表明核心蛋白在HCV的发病机制中发挥着关键作用[35-36]。HCV核心蛋白调节宿主体内各种信号通道的能力,如与细胞凋亡、基因转录、细胞转化和免疫反应相关的通路[37-39],也与肝癌的发生有关[16]。因此,利用1997年构建的转基因小鼠系统阐明HCV核心蛋白在体内的作用将为HCV的发病机制提供线索。

3.2. 丙型肝炎病毒核心基因转基因小鼠是如何产生的?

为了获得HCV核心基因转基因小鼠,含有乙肝病毒调控元件的表达载体pBEPBgIII被用于实验中的质粒构建过程[14]。经PstI和EcoRI双酶切,并经T4 DNA聚合酶处理和BclI连接后,从质粒pSR39[41]中切下含有核心蛋白编码区的(1 \pm 6) kb的片段。随后,将其连接到质粒pBEPBgIII的BgIII位点上。通过聚丙烯酰胺凝胶电泳(PAGE)纯化pBEP39的(1 \pm 2) kb KpnI-HindIII片段,并将其微注射到C57BL/6N小鼠胚胎中。然后通过聚合酶链反应(PCR)扩增1 ng尾部DNA以鉴定转基因小鼠[40](图2)。

在Moriya等构建的HCV核心基因转基因小鼠品系中,核心蛋白在小鼠出生时就开始在肝脏中表达。这种转基因小鼠的核心蛋白水平与感染HCV的人类相似。小鼠在出生后3个月开始在肝细胞中出现LD,肝脏脂肪变性随着动物的生长而持续。细胞质中含有脂滴的肝腺瘤大约在小鼠16个月大时出现,小鼠在17个月大时最终表现为肝癌(HCC)[16]。

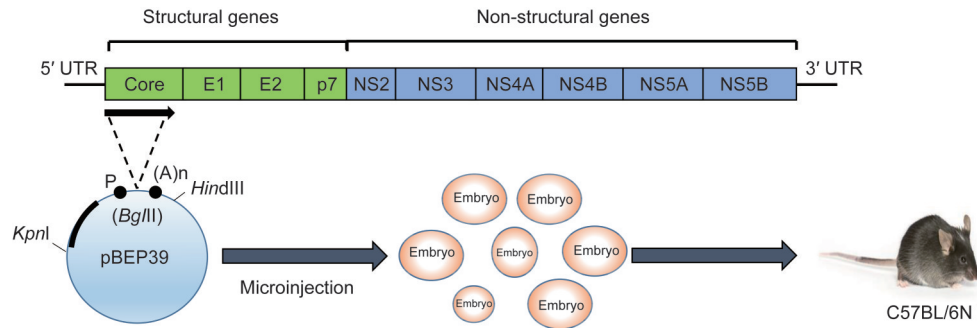


图2. 丙型肝炎病毒核心基因转基因小鼠构建。pBEP39是一个包含乙肝病毒调控元件的表达载体。将(±2) kb *KpnI*-*HindIII*片段显微注射到小鼠胚胎中,然后将小鼠胚胎导入C57BL/6N小鼠。P: 启动子; (A)_n: 多聚腺苷化信号。摘自参考文献[5], 经Elsevier © 2011许可; 摘自参考文献[40], 经Society for General Microbiology © 1997许可。

3.3. 丙型肝炎病毒核心基因转基因小鼠的肝脏胰岛素抵抗

II型糖尿病T2DM是一种复杂的多系统疾病,涉及胰岛素分泌缺陷,导致肝脏葡萄糖产生增加和胰岛素抵抗[42-43]。虽然流行病学研究已报道T2DM和慢性丙型肝炎之间的正相关关系,但尚不清楚HCV导致II型糖尿病(T2DM)产生的机制。利用HCV核心基因转基因小鼠[40],研究者首次证实了胰岛素信号与HCV之间的显著和直接的机制联系。转基因小鼠的体重与对照组小鼠相似,但它们的血清胰岛素水平和胰腺β细胞质量均显著高于对照组。在转基因小鼠中,研究者早在1月龄时就观察到高胰岛素血症,这一情况先于肝脏脂肪变性出现,这表明HCV核心蛋白在胰岛素抵抗中发挥着直接和最初的作用。肿瘤坏死因子TNF-α是胰岛素抵抗的关键因素。事实上,阻断TNF-α的作用恢复了转基因小鼠的肝胰岛素敏感性,这证实了HCV核心蛋白对肝脏胰岛素信号的干扰是由肿瘤坏死因子TNF-α介导的[44]。HCV核心蛋白还可能刺激蛋白酶体激活物28γ(PA28γ)的激活,从而影响胰岛素信号转导[45]。虽然胰岛素抵抗的机制是多因素的[42-43, 46-47],但上述研究结果支持HCV核心蛋白在肝脏胰岛素抵抗和T2DM发展中的直接作用,这些疾病通常伴随慢性丙型肝炎的发生[12](图3)。

3.4. 丙型肝炎病毒核心基因转基因小鼠的肝脏脂肪变性

肝脏脂肪变性在慢性丙型肝炎中比在慢性乙型肝炎中更常见[40,48]。事实上,在72%的慢性丙型肝炎患者中观察到脂肪变性,而在自身免疫性慢性活动性肝炎患者中仅为19%[49]。据报道,肝脂肪变性是诱发慢性丙型肝炎肝细胞癌的危险因素之一。由于肝脏脂肪变性的机制复杂,涉及多条代谢途径和与肝外器官的相互连接,因此构建携带HCV蛋白的转基因小鼠有助于评估HCV在肝骨病发生中的直接作用。

在已经产生的针对HCV蛋白的转基因小鼠中,只有

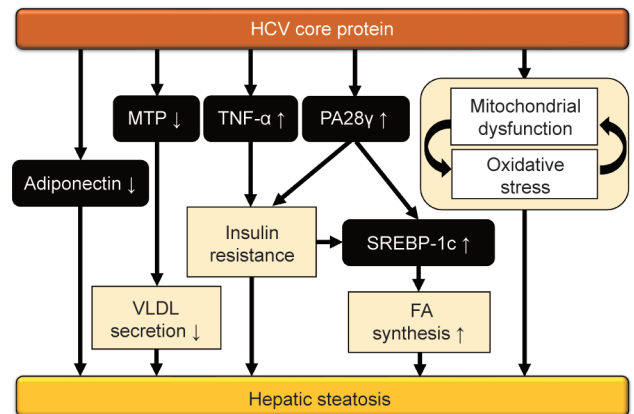


图3. 丙型肝炎病毒核心蛋白诱导肝脂肪变性的机制。HCV核心蛋白通过多种途径诱导转基因小鼠肝脏脂肪变性。TNF-α和PA28γ引起胰岛素抵抗,上调固醇调节元件结合蛋白(SREBP)-1c,并刺激脂肪生成。脂联素和微粒体甘油三酯转移蛋白(MTP)表达减少也会导致极低密度脂蛋白(VLDL)分泌减少和肝脏脂肪变性。HCV核心蛋白导致的线粒体功能障碍会减少FA分解代谢,增强氧化应激,从而加重肝脏脂肪变性。

核心基因小鼠表现出自发性肝脂肪变性。LD最初在年轻小鼠中很小,随着年龄的增长,大液滴成为主要的形态,这类似于慢性丙型肝炎患者的肝脏。所有雄性转基因小鼠和大约一半的雌性转基因小鼠在6个月大时出现肝脏脂肪变性。肝胰岛素抵抗和脂肪变性是HCV感染患者和HCV核心基因转基因小鼠的共同特征,而仅在HCV感染患者中观察到肝实质内淋巴细胞浸润和胆管损伤[48]。

HCV核心蛋白诱导的非肥胖性肝骨病与低脂联素血症相关,可以通过给予脂联素改善[51]。PA28γ不仅通过过量产生TNF-α与胰岛素抵抗产生相关性,而且还参与HCV核心基因转基因小鼠的FA合成,因为HCV核心蛋白激活肝脏X受体α/视黄素X受体α(RXRα)中的固醇调节元件结合蛋白1启动子和PA28γ依赖方式[52-55]。

HCV基因1b和3a型核心蛋白对FA合成酶(FAS)启动子的影响以及这一过程背后的分子机制已被阐述。微粒体甘油三酯转运蛋白(microsomal triglyceride transfer

protein, MTP)可以促进极低密度脂蛋白(VLDL)的合成。由于HCV核心蛋白降低MTP活性[56],核心蛋白对MTP的这种作用可能导致甘油三酯的积累[57]。研究还表明,HCV核心蛋白可以直接结合RXR α -过氧化物酶体增殖物激活受体 α (PPAR α)复合物,这是一种控制FA β 氧化的核受体,从而激活信号转导。HCV核心蛋白持续激活PPAR α -RXR α 和肝细胞中过量FA已被证明可增强氧化应激[58–60]。此外,一项研究表明,HCV感染可抑制肝细胞自噬[61]。PA28 γ 的激活导致微管相关蛋白1A/1B轻链3B(LC3)-I蛋白酶体的降解,从而阻止自噬并增强脂质储存[62–63]。由于HCV核心基因转基因小鼠有线粒体功能障碍,推测可能是由于HCV核心蛋白的直接作用[64],这种恶性循环加剧了肝脏线粒体损伤和FA的积累[57,60,65]。图3总结了HCV核心蛋白导致脂肪生成的可能机制。

3.5. 丙型肝炎病毒核心基因转基因小鼠的肝细胞癌研究

HCV核心蛋白在由慢性HCV感染引起的HCC的发展中也起着重要作用。HCV核心蛋白转基因小鼠(C21和C49)的HCC发病率显著高于正常对照组[16]。HCV核心蛋白转基因小鼠表现出与慢性HCV感染患者相似的临床病理特征,包括伴有高频率的脂肪变性[48],增加肝脏中C18单不饱和脂肪酸的积累[66],线粒体功能障碍[38],胰岛素抵抗和氧化应激增加[67],以及多中心HCC发生[58,68–69]。在转基因雌性小鼠中,HCC的发病率较低,这与流行病学数据显示的慢性丙型肝炎患者中男性更有可能发生HCC相一致[2,16,70]。在16月龄的HCV核心转基因小鼠体内发现了具有肝细胞腺瘤特征的肝脏结节。肝细胞腺瘤细胞胞浆内有丰富的脂滴,非肿瘤性肝细胞胞浆内脂滴较多,而肝癌细胞内脂滴较少。HCV核心基因转基因小鼠的这一现象与慢性丙型肝炎患者中发现的现象非常相似,即癌前病变和分化良好的HCC病变在恶性转化过程中偶尔会出现显著的脂肪变性和脂肪含量降低[16]。然而,与慢性HCV感染患者相比,HCV核心转基因小鼠在肝癌发生过程中并未出现肝脏炎症或纤维化[40,71]。

HCV核心蛋白通过多种途径调控HCC的发生。例如,在啮齿动物中,长期的PPAR α 激活可上调多种致癌因子,包括c-Fos、c-Myc、细胞周期蛋白D1、细胞周期蛋白依赖激酶4、增殖细胞核抗原和磷酸化的细胞外信号调节激酶(ERK)[58,72–74]。HCV核心蛋白可以结构性地激活激活蛋白-1(AP-1),而AP-1与c-Jun氨基末端激酶(JNK)和丝裂原活化蛋白激酶(MAPK)的激活有关[53,75–76],同时下调肿瘤抑制基因p53和p21的活性[77]。

氧化应激和内质网应激是导致细胞损伤和恶性转化的

重要因素。PPAR α 的激活增加了活性氧(ROS)生成酶,如酰基辅酶A氧化酶和细胞色素P450 4A1,可能导致核DNA损伤[58]。据报道,HCV核心蛋白引发内质网钙缺乏和应激,导致caspase和BAX的激活[78–79]。持续的内质网应激也可能导致基因组的不稳定性和突变以及对细胞死亡的抵抗[80]。这些事件可能促进细胞恶性转化和异常细胞异常,从而加速HCC的进展[81](图4)。

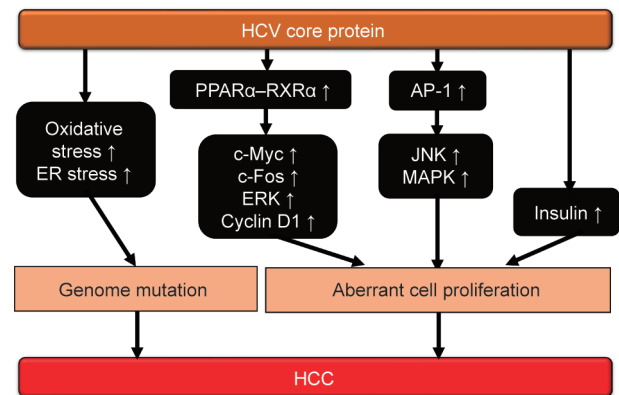


图4. 丙型肝炎病毒核心蛋白诱导肝癌的机制。HCV核心蛋白通过多种途径诱导转基因小鼠发生肝癌。氧化应激和内质网应激驱动恶性转化。PPAR α -RXR α 的激活会上调c-Myc、c-Fos、ERK和cyclin D1,诱导细胞异常增殖。此外,NF- κ B、AP-1、JNK、STAT和MAPK通路的激活,以及高胰岛素血症都会影响细胞增殖。上述因素共同促进了肝癌的发生。

4. 生活方式和饮食习惯对丙型肝炎病毒核心基因转基因小鼠脂肪变性源性肝癌发展的影响

一些流行病学研究表明,生活方式和饮食因素可以影响慢性丙型肝炎患者的临床病程[82–84]。在对9221例慢性丙型肝炎患者为期13.3年的随访中发现,高蛋白饮食的参与者在调整了潜在的混杂因素后,因肝硬化或肝癌住院或死亡的风险会显著升高[82]。尽管总脂肪摄入量与肝硬化或肝癌的发病风险没有显著关联,但胆固醇摄入量与此存在显著相关性[82]。然而,由于生活方式和饮食习惯的复杂性,人们仍难以阐明哪些饮食特征会降低患病风险以及其中涉及的具体作用机制。为了解决这些问题,在HCV核心基因转基因小鼠中比较正常喂养和饮食干预方案是有用的[8–11]。与此同时,非酒精性脂肪性肝病(NAFLD)引起的HCC在世界范围内呈上升趋势,一些HCC病例发生在没有晚期纤维化或肝硬化的脂肪变性肝脏中[85–86]。作为脂肪变性HCC模型的HCV核心基因转基因小鼠的研究将为评估饮食干预对与NAFLD相关的HCC的影响提供线索。研究HCV核心基因转基因小鼠作为脂肪变性源性HCC模型,将为评估饮食干预对NAFLD相关HCC的影响提供线索。在本节中,我们总结了高脂

饮食、高铁饮食、酒精和饮食限制对脂肪变性源性肝癌发生的影响。

4.1. 富含饱和脂肪酸的饮食

饱和脂肪酸（SFA）是一种典型的脂肪酸，广泛存在于动物产品（如红肉和乳制品）和植物产品（如棕榈油和椰子油）中[87]。过量的SFA摄入与肥胖、胰岛素抵抗、NAFLD、非酒精性脂肪性肝炎（NASH）和HCC相关[88–90]。当用对照饮食或等卡路里的SFA高热量饮食治疗15个月的HCV核心蛋白转基因小鼠时，虽然两组摄入的热量类似[9]，但SFA治疗组的大泡状LD数量更多。富含SFA的膳食加重肝脏脂肪变性的主要原因是脂肪生成酶乙酰辅酶A羧化酶（ACC） α 和 β 、FAS和硬脂酰辅酶A去饱和酶1（SCD1）促进了肝细胞中脂肪酸的合成。肝脏肿瘤的患病率随着富含SFA的饮食的增加而显著增加。虽然SFA处理的转基因小鼠没有明显的肝纤维化[9]（表1[8–11,91–92]），但因Toll样受体4（TLR4）和炎性体信号转导而激活的NF- κ B、JNK/AP-1激活、细胞周期蛋白D1的诱导和p62-核因子红细胞2相关因子2（NRF2）轴的上调可能与促进肝肿瘤的发生有关[9]。

4.2. 富含反式脂肪酸的饮食

反式脂肪酸（TFA）天然存在于乳制品和动物肉类中，含量较低，而工业生产的TFA则存在于人造黄油和起

酥油等硬化植物脂肪中，以及休闲食品和油炸食品中。世界卫生组织估计，过量摄入TFA每年导致超过50万人死于心血管疾病，并呼吁各国政府推动替代项目，以剔除饮食中的TFA[93]。过量摄入TFA不仅与心血管疾病有关，还与预期寿命缩短、NAFLD和认知障碍有关[94–96]。早些时候，我们研究了富含TFA的饮食对HCV核心基因转基因小鼠肝脏肿瘤发生的影响[10]。与对照组相比，采用富含TFA的饮食喂养的小鼠肿瘤患病率显著升高。富含TFA的饮食显著增加了氧化和内质网应激，4-HNE、烟酰胺腺嘌呤二核苷酸/烟酰胺腺嘌呤二核苷酸磷酸脱氢酶1（NQO1）和CCAAT/增强子结合蛋白同源蛋白（CHOP）水平的升高证明了这一点。氧化和内质网应激增加会损伤DNA并促进肝脏肿瘤的发生。富含TFA的饮食也刺激了TLR2和炎性体信号，激活NF- κ B和p62-NRF2，促进纤维化的发生。随后，饮食上调了cyclin D1、ERK、c-Myc、Wnt/ β -catenin信号通路和增殖细胞核抗原（PCNA），推动转化细胞的不可控增殖[10]（表1）。

4.3. 高胆固醇饮食

动物源性食品含有各种各样的胆固醇。胆固醇的主要饮食来源包括红肉和蛋黄[97]。虽然胆固醇是构建细胞膜所必需的[98]，但其过量摄入不仅与动脉粥样硬化和脑血管疾病有关，还与慢性丙型肝炎的形成有关[83,98–100]。

表1 生活方式干预对丙型肝炎病毒核心基因转基因小鼠肝脏肿瘤发生的影响

Factors associated with hepatic tumorigenesis	SFA-rich diet [9]	TFA-rich diet [10]	Cholesterol-rich diet [8]	Iron-rich diet [91]	Ethanol intake [92]	Dietary restriction [11]
Lipid metabolism	FAS \uparrow , ACC \uparrow , SCD1 \uparrow	CD36 mRNA \uparrow , ACC mRNA \uparrow , SCD1 mRNA \uparrow	CD36 mRNA \uparrow	?	?	FAS mRNA \downarrow , ACC mRNA \downarrow , SCD1 mRNA \downarrow
Inflammasomes	\uparrow	\uparrow	\uparrow	?	?	\downarrow
TLR	TLR4 \uparrow	TLR2 \uparrow	TLR2 \uparrow	?	?	~
NF- κ B	\uparrow	\uparrow	\uparrow	~	?	\downarrow
Oxidative and ER stress	NQO1 \uparrow	4-HNE \uparrow , NQO1 \uparrow , CHOP \uparrow	4-HNE \uparrow , CHOP \uparrow	HO-1 \uparrow , ROS \uparrow , NQO1 \uparrow		4-HNE \downarrow , CHOP \downarrow
Apoptosis	DR5 \uparrow , BAX \uparrow	~	DR5 \uparrow , BAX \uparrow	?	?	~
p62-NRF2	\uparrow	\uparrow	\uparrow	~	?	\downarrow
Cell proliferation	Cyclin D1 \uparrow , JNK \uparrow	Cyclin D1 \uparrow , ERK \uparrow , PCNA \uparrow , c-Myc \uparrow , Wnt/ β -catenin \uparrow	PCNA \uparrow	?	ERK \uparrow , MAPK \uparrow	Cyclin D1 \downarrow , STAT3 \downarrow , STAT5 \downarrow , ERK \downarrow
Fibrogenesis	~	α SMA \uparrow , CTGF \uparrow , OPN \uparrow	α SMA \uparrow	?	?	~
Autophagy	~	?	?	?	?	LC3 \uparrow , Atg5-Atg12 \uparrow
Senescence	?	?	?	?	?	PGC1 α \uparrow , AMPK α \uparrow , SASP \downarrow

\uparrow : Upregulated; \downarrow : downregulated; ~: unchanged; ?: undetermined. HNE: hydroxynonenal; SMA: smooth muscle actin; AMPK: adenosine 5'-monophosphate-activated protein kinase; Atg: autophagy related; CD36: cluster of differentiation 36; CHOP: CCAAT/enhancer binding protein homologous protein; CTGF: connective tissue growth factor; DR: death receptor; HO: heme oxygenase; NQO: nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphoric acid dehydrogenase; NRF: nuclear factor erythroid 2-related factor; OPN: osteopontin; PCNA: proliferating cell nuclear antigen; PGC: PPAR γ coactivator; SASP: senescence-associated secretory phenotype; TLR: Toll-like receptor.

事实上，一项使用丙型肝炎肝硬化的抗病毒长期治疗（HAL T-C）项目数据的队列研究显示，胆固醇摄入量增加与临床结果（死亡、静脉曲张出血、肝功能失代偿、腹膜炎和HCC）减少相关[83]。当用高胆固醇饮食喂养HCV核心蛋白基因转基因小鼠15个月时，所有小鼠都发生了HCC。在HCV核心蛋白转基因小鼠中，分化簇36的表达可能与胆固醇引起的脂肪变性有关。此外，富含胆固醇的饮食诱导NASH细胞周围纤维化，促进肝细胞分裂，上调细胞应激，激活NF- κ B和p62-NRF2，最终加速肝脏肿瘤的发生[8]（表1）。这些结果有助于理解HAL T-C的结果：在HCV感染的患者中，较高的胆固醇摄入与疾病发展相关。

4.4. 富含铁的饮食

铁是生命必不可少的辅助因子[101]。由于该元素可能通过Fenton反应产生自由基，因此有理由推测，铁的过度积累可能会增加细胞损伤[91,102–103]。事实上，肝脏的铁积累与慢性丙型肝炎的进展有关，而去铁疗法，如静脉切断术可减轻与疾病相关的肝细胞损伤[103–104]。正常饮食喂养小鼠15个月后，HCV核心基因转基因小鼠肝脏铁含量明显高于正常小鼠[91]。在进行高铁元素饮食3个月后，HCV核心基因转基因小鼠与对照组小鼠相比，肝内ROS水平显著升高。在转基因小鼠中，血红素加氧酶-1（HO-1）和NQO1等抗氧化酶的诱导受到抑制，而在表达核心蛋白的HepG2细胞中，铁诱导的HO-1的增加作用被证实是减弱的。这种衰减不依赖于NRF2。由于增强的氧化应激可能导致核DNA损伤并促进HCC的发生[91]（表1），因此，未来评估长期饮食铁元素超标是否促进该转基因小鼠的肝脏肿瘤发生将是重要的。

4.5. 酒精

酒精是慢性肝病发病的主要原因，最终导致脂肪变性、脂肪性肝炎、纤维化和HCC [105]。酒精的氧化代谢扰乱了信号转导通路，阻碍了某些基因的转录控制[106]。在HCV感染的患者中，研究发现酒精摄入与患HCC的风险显著相关[107]。当给予含有5%酒精的饮食饲养HCV核心蛋白转基因小鼠3周后，其肝内ROS水平显著增加，ERK和p38 MAPK被激活[92]（表1）。这些发现有力地表明，持续的酒精摄入促进了这些小鼠肝脏肿瘤的发生。这需要进一步的研究来验证这一假设。

4.6. 饮食限制

近年来，限制热量摄入在促进健康和控制疾病方面受到了广泛的关注。该方法已被证明对肥胖、代谢综合征、

T2DM、NAFLD/NASH的进展具有预防作用，并有望在未来用于癌症的预防和治疗[108–111]。早些时候，我们调查了限制饮食是否可以避免HCV核心蛋白转基因小鼠脂肪变性相关的肝脏肿瘤的发生[11]。将食物摄入量限制在正常饮食的70%以内可以改善肝脏脂肪变性，并显著降低肝脏肿瘤的发病率。饮食限制还可显著降低肝脏氧化应激和内质网应激；显著抑制NF- κ B活性、促炎细胞因子表达和衰老相关分泌型的表达，通过STAT3、STAT5、ERK和insulin-Akt途径下调生长信号，并激活自噬[11]（表1）。事实上，限制饮食可能是一种有前景的干预措施，通过多种有益效果来预防NAFLD相关的肝癌发生。

5. 结论

通过部分复制HCV感染患者的表型并揭示HCV蛋白在体内的直接致病性，产生表达HCV蛋白的转基因小鼠的技术已经在肝病学和病毒学领域开辟了新的的大门。只有HCV核心基因转基因小鼠表现出肝脏胰岛素抵抗、肝脂肪变性和HCC，因此其可作为确定脂肪变性相关肝癌发生中的分子事件的良好模型。该小鼠品系说明了代谢方法在肝脏疾病病理分析中的重要性。此外，使用HCV核心基因转基因小鼠进行的序贯研究揭示了饮食干预对脂肪变源性HCC的影响，这可能适用于人类NAFLD相关的HCC。HCV核心基因转基因小鼠将有助于针对脂肪变性衍生的肝癌预防药物的发现。这种有价值的动物模型跨越了病毒学、肝病学、代谢和营养学的交叉领域，并强调了新的工程发展给医学研究带来创新发现的重要性。

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Compliance with ethics guidelines

Pan Diao, Fangping Jia, Xiaojing Wang, Xiao Hu, Takefumi Kimura, and Takero Nakajima declare that they have no conflict of interest or financial conflicts to disclose.

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