糖原贮积性疾病(GSD-1a)相关动物模型及治疗的研究进展

王晓祎 葛梦婷 谢伊霞 闫俊艳 金立方*

(绍兴文理学院, 生命科学学院, 绍兴 312000)

摘要 糖原贮积性疾病(GSD-1a)是一种由葡萄糖-6-磷酸酶-α(G6Pase-α或G6PC)缺乏引起的常染色体隐性代谢疾病, 临床表现主要为肝肾功能失常, 死亡率高。近年研究表明, 基于新型基因编辑技术的GSD-1a大型动物模型的构建以及更为高效的基因治疗方法将成为新的研究趋势。该文着眼于近年来GSD-1a疾病的研究进展, 从分子遗传基础、动物模型的建立与应用以及药物和基因治疗研究等方面论述动物模型在GSD-1a发生机制及疾病治疗中的探索和应用。

关键词 G6PC; GSD-1a; 动物模型; 基因治疗

Research Progress on Animal Models and Treatments Related to GSD-1a (Glycogen Storage Disease)

WANG Xiaoyi, GE Mengting, XIE Yixia, YAN Junyan, JIN Lifang*

(*School of Life Sciences, Shaoxing University, Shaoxing 312000, China*)

Abstract GSD-1a (glycogen storage disease type 1a) is an autosomal recessive metabolic disease caused by glucose-6-phosphatase-α (G6Pas-α or G6PC) deficiency. The clinical manifestations are liver and kidney dysfunction with high mortality. The recent establishment of large animal models of GSD-1a based on new gene editing technology and more efficient gene therapy methods will become a new and important research trend. This article focuses on current research progress in GSD-1a as well as its underlying mechanisms and potential treatments based on molecular genetics, animal model establishment and application, and drug and gene therapy.

Keywords G6PC; GSD-1a; animal models; gene therapy

糖原贮积性疾病I型(glycogen storage disease type I, GSD-I)又称糖原累积疾病, 主要有两种亚型: 由葡萄糖-6-磷酸酶(glucose-6-phosphate catalytic enzyme, *G6PC*)基因突变失活引起的GSD-1a型和葡萄糖-6-磷酸转运体(glucose-6-phosphatase transporter, *G6PT*)基因突变失活引起的GSD-1b型[1-3]。其中, GSD-1a最为常见, 约占总发病率的百分之八十。由*G6PC*编码的G6Pase-α主要在肝脏、肾脏、小肠中表达, 其主要作用是在糖异生和糖原分解的最后阶段将葡萄糖-6-磷酸(glucose-6-phosphate, G6P)催化水解为葡萄糖[4-6]。

GSD-1a患者由于基因*G6PC*先天性异常, 无法正常翻译成G6Pase-α, 导致糖异生葡萄糖生成途径受阻。当血液中葡萄糖消耗殆尽时促进生糖激素分泌增加, 进而引发G6P的丙酮酸旁路亢进, 导致乳酸累积并引发高乳酸血症(图1), 患者出现生长迟缓及

收稿日期: 2021-04-10 接受日期: 2021-06-25

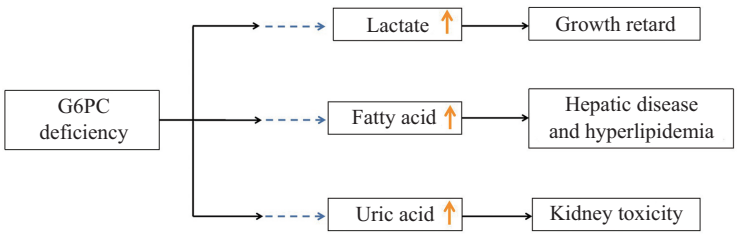
浙江省分析测试和实验动物计划(批准号: 2018C37105)资助的课题

*通讯作者。Tel: 0575-88345007, E-mail: lifangj@usx.edu.cn

Received: April 10, 2021 Accepted: June 25, 2021

This work was supported by the Analytical Testing and Laboratory Animal Foundation of Zhejiang Province (Grant No.2018C37105)

*Corresponding author. Tel: +86-575-88345007, E-mail: lifangj@usx.edu.cn



G6PC缺陷导致乳酸、脂肪酸和尿酸升高, 导致生长迟缓、高脂血症以及肾脏毒性。

G6PC deficiency induced increasement of lactic acid, fatty acid and uric acid, which resulted in growth retard, hyperlipidemia and kidney toxicity.

图1 *G6PC*缺乏引起的临床病症

Fig.1 Diseases symptoms caused by *G6PC* deficiency

骨龄落后等现象[7]。此外, 长期低血糖会促进外周脂肪分解, 提高体内游离脂肪酸含量(图1), 导致高脂血症和肝脏脂肪变性[8-9]。G6P的堆积还造成戊糖代谢旁路亢进, 产生过量嘌呤, 嘌呤分解产生大量尿酸(图1), 造成肾脏损伤[10]。在临床上, GSD-1a患者需要采取加餐的方式纠正低血糖; 生长发育受到影响的患者, 需补充高蛋白质; 尿酸指标异常的患者需服用抑制尿酸合成类药物[11]。调节饮食虽可在一定程度上缓解*G6PC*缺陷引起的代谢紊乱[12], 例如可使患者尿酸恢复至正常水平, 但仍有患者会发生慢性并发症[13-16], 包括生长迟缓、肝肿大、间歇性低血糖、高乳酸血症、高脂血症、痛风与高尿酸血症、以及肝细胞脂肪化引起的肝细胞腺瘤(hepatocellular adenoma, HCA)和肝细胞肿瘤 (hepatocellular carcinoma, HCC)等。

*G6PC*基因敲除动物模型是研究人类GSD-1a发生机制和治疗方案的理想平台。本文将围绕GSD-1a近期研究进展, 从疾病分子遗传基础、动物模型的构建与应用以及药物和基因治疗研究等方面论述动物模型在GSD-1a发生机制及疾病治疗中的探索和应用。

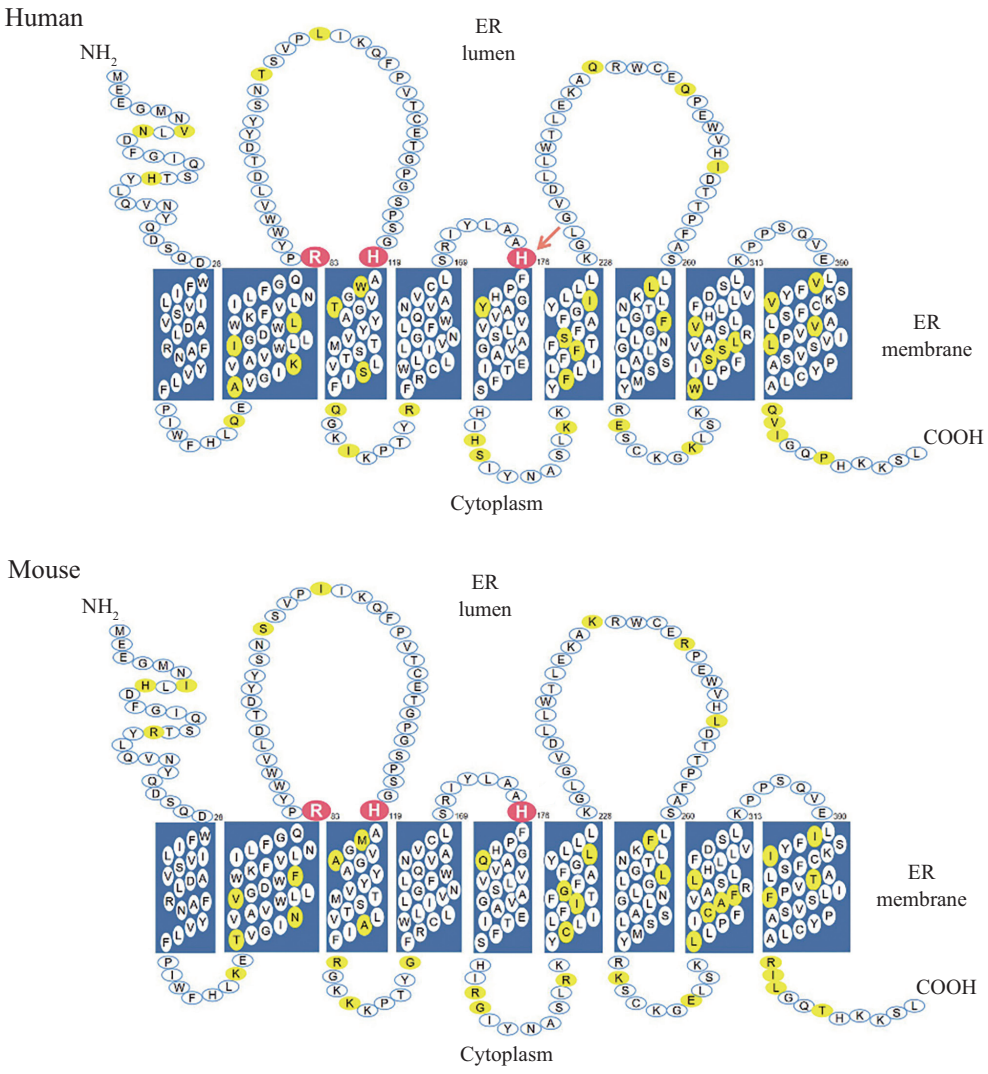
1 GSD-1a分子遗传

人类*G6PC*是一个单拷贝基因, 长度约为12.5 Kb, 由位于17q21染色体上的5个外显子组成[10]。G6Pase-α酶(糖蛋白)含有357个氨基酸, 大小为36 kDa, 通过9个跨膜螺旋单位固定在内质网膜上(图2)[18-20]。该酶的活性中心含有3个氨基酸残基, 分别是Arg83、His119和His176, 是保持跨膜完整性和酶稳定性的关键位点[19-20]。研究表明, 该酶螺旋跨膜区段及酶活性中心的突变或缺失可使G6Pase活性完全丧失, 而非螺旋跨膜区段突变或缺失可使酶活性部分缺失[21]。迄今为止, 已发现至少95个*G6PC*突变位点, 包括63个错义突变、10个无义突变、17个插入/缺失突变、4个剪接突变和1个不停止突变(http://www.hgmd.cf.ac.uk/ac/gene.php?gene=G6PC)。基于定点诱变和过表达分析技术已鉴定出55个致病性*G6PC*突变, 包括51个错义突变、2个无义突变(p.R170X和p.Q347X)和2个密码子缺失[21-23]。在51个错义突变中, 33个*G6PC*错义突变导致G6Pase-α活性完全丧失, 另外18个突变引起G6Pase-α部分失活[21-23]。与预期的结果一致, 酶活性中心的突变, 例如p.R83C/p.R83H和p.H119L位点突变导致G6Pase-α活性彻底丧失[18,21]。虽然尚未发现p.H176位点突变案例, 然而功能研究表明p.H176A突变完全抑制了G6Pase-α的活性[24-25]。

*G6PC*基因突变有着一定的人种和地区差异。高加索人群中, p.R83C、p.Q347X突变最为常见, 亚洲人种中c.648G>T、P.R83H突变最为常见, c.648G>T突变在中国台湾, 中国华北、华东地区, 日本和韩国患者中分别约占总突变的44.00%、80.85%、91.00%和75.00%。 p.R83H突变在上述前3个地区突变频率占3.00%~36.10%[4-5,27-28]。到目前为止, 尚未发现*G6PC*基因型与表型之间的相关性[2,29]。

2 GSD-1a动物模型

目前已建立的GSD-1a动物模型包括: 全身性*G6PC*基因敲除小鼠模型, 肝脏和肾脏特异的条件性*G6PC*基因敲除小鼠模型[30-31], 以及一种自然突变产生的全身性*G6PC*缺陷马耳他狗模型。全身性*G6PC*基因敲除小鼠和*G6PC*缺陷马耳他狗模型在青春期前死亡, 是研究GSD-1a早期致病机制的理想模型。肝脏特异的条件性*G6PC*基因敲除小鼠模型在诱导敲除前表型正常, 敲除后可在肝脏形成HCA和HCC, 模拟了GSD-1a患者肝脏病变, 是研究GSD-1a成体肝



重要酶活中心Arg-83、His-119和His-176用红色圆圈标注, 红色箭头表示尚未在人群中发现的突变。黄色圈内为人和小鼠G6PC酶的差异氨基酸。
Important enzyme active centers Arg-83, His-119, and His-176 are marked with red circles, and red arrows indicate mutations that have not yet been found in the population. The yellow circle represents the difference in amino acids between the human and mouse G6PC enzymes.

图2 人和小鼠G6PC酶的357个氨基酸序列、9次内质网(ER)跨膜图及重要酶活位点分析(根据参考文献[26]修改)

Fig.2 Human and mouse G6PC enzyme 357 amino acid sequences, 9 endogenous mesh (ER) cross-film mapping and important enzyme site analysis (modified from reference [26])

脏疾病的理想模型。

2.1 GSD-1a小鼠模型

与人类*G6PC*基因相似, 小鼠*G6PC*基因约长12 Kb, 也由5个外显子组成(图2)。1996年, LEI等[32]基于胚胎干细胞(embryonic stem cells, ESCs)同源重组技术构建了靶向第3外显子的全身性*G6PC*基因敲除小鼠模型, 第3外显子含有多个G6Pase-α酶的催化位点, 包括酶的活性中心His119。研究结果显示, *G6PC*第3外显子敲除后G6Pase-α活性完全丧失, 但不影响小鼠胚胎存活, 幼鼠生长迟缓且很少能存活超过3周[31,33-34]。表型分析结果显示, *G6PC*基因敲除小鼠模拟了人类GSD-1a大部分临床症状, 包括低血糖、肝肿大、肾肿大、高脂血症、高尿酸血症, 但由于乳酸累积水平不高, 不能反映出人类GSD-1a重要症状之一高乳酸血症[31,33-35]。全身性*G6PC*敲除小鼠早期致死, 因此不能很好模拟成年人GSD-1a疾病症状, 如HCA和HCC。

为了更好模拟GSD-1a小鼠HCA和HCC症状的发生, PENG等[36]和MUTEL等[37]分别于2009年和2011年构建了靶向*G6PC*第3外显子的*EIIa-Cre*;*G6PC*$^{flox/flox}$和*Albumin-CreERT*;*G6PC*$^{flox/flox}$肝细胞条件特异性基因敲除小鼠, 该模型小鼠在*G6PC*诱导

敲除前不影响个体发育。该研究显示, 在他莫昔芬诱导*G6PC*敲除后, 肝脏开始肿大, 并伴有糖原积累和肝脂肪变性; 在诱发敲除9个月后可检测到直径小于1 mm的肝结节, 在第12个月时, 可在30%~40%的敲除小鼠中检测到直径约为1 mm的肝结节; 到第18个月时, 所有敲除小鼠肝脏均发展为直径1~10 mm的HCA[37]。CHO等[31]的研究进一步显示, 小鼠肝脏G6Pase-α缺乏症还导致肝糖酵解和单糖己糖分流(monosaccharide hexose shunt, MHS)活动增强, 这加剧了肝癌的发生。在肾脏的研究方面, CLAR等[38]构建了*Kap-CreERT*;G*6PC*$^{flox/flox}$肾脏特异的*G6PC*条件性基因敲除小鼠, 他莫昔芬诱导处理后, *G6PC*敲除小鼠G6Pase-α活性约为野生型的一半, 进一步的研究发现, *G6PC*基因敲除小鼠肾脏肿大、糖原累积、尿液滤过屏障通透性改变、微量白蛋白和电解质失衡。GSD-1a为先天性疾病, 虽然上述模型很好地模拟了该病成体病症, 但是由于GSD-1a某些病症与时间累积有关, 因此仍需进一步寻找更合适的模型。

2.2 GSD-1a犬类模型

犬类GSD-1a模型最早由BRIX等[39]于1995年在马耳他犬中发现, 分子遗传揭示G6Pase-α酶催化亚基位点M121I发生错义突变[41-42]。表型研究显示, 犬类GSD-1a模型与人类新生儿GSD-1a具有十分相似的临床症状: GSD-1a马耳他幼犬在出生后5~8周龄死亡, 营养不良, 生长迟缓, 肝、肾肿大, 肝肾的组织学显示存在细胞空泡化并伴有糖原累积, 肝脏糖原增加至9.4%(正常为0%~2.7%), 而肝脏和肾脏的G6Pase活性几乎没有。患病幼犬在禁食期间有低血糖和高胆固醇血症[40-41]。与人类GSD-1a相似, GSD-1a犬类模型表现出高乳酸症, 是比小鼠更佳的GSD-1a动物模型[40-41]。

3 GSD-1a的治疗

3.1 饮食疗法

GSD-1a是由低血糖引起的肝肾糖原累积疾病, 因此维持血糖平衡是治疗这一疾病的根本策略。1984年CHEN等[42]最早提出玉米淀粉的饮食疗法, 显著提高了患儿的生活质量。玉米淀粉由于分子量大, 易在肠道内停留, 口服后被缓慢消化, 逐渐释放葡萄糖等特点, 使血糖维持在一个正常水平, 减少肝脏负担[43]。GREENE等[44]的研究建议不满3岁的GSD-1a婴幼儿接受夜间鼻胃灌注葡萄糖以避免低血糖; 而3岁或3岁以上的患者可服用生玉米淀粉, 以延长餐间血糖正常的时间[45]。但淀粉服用后会出现胃胀气、腹泻等肠道不耐受症状。随后, BHATTACHARYA等[46]研究发现, 一种新型经化学修饰的淀粉能更长时间维持血糖浓度, 减少用餐次数, 且能减轻胃肠道不耐受症状。

3.2 药物疗法

即使饮食疗法非常到位, 部分并发症例如肾脏毒性, 仍无法有效纠正, 故需采用药物治疗改善症状[47]。研究显示, 高脂血症的降脂药、低硝酸的柠檬酸钾和肾功能不全的血管紧张素转换酶(angiotensin converting enzyme, ACE)抑制剂都可有效改善患者的肾脏毒性症状[48-49]。MELIS等[48]在一项对95例GSD-1a患者的10年回顾性研究中发现, ACE抑制剂明显减弱了肾小球白蛋白的滤过作用。这一发现得到了MARTENS等[49]的研究支持, 该研究报道了ACE抑制剂处理可显著降低GSD-1a患者肾小球滤过率。这两项研究均表明, 早期应用血管紧张素转换酶抑制剂干预可预防GSD-1a的肾脏损害。然而, MELIS等[48]的研究表明, ACE抑制剂并不能减缓从微量白蛋白尿向蛋白尿的进展。此外, 也有研究报道抗氧化剂处理能促进GSD-1a小鼠模型肾功能的恢复, 延缓肾脏损害和纤维化[50]。

GSD-1a易发生肝细胞脂肪化, 肝脂肪化持续存在将发展成肝纤维化, 因此降脂方法也是近年来GSD-1a药物治疗的研究方向。LAUREN等[51]的研究显示, 苯扎贝特(Bezafibrate)处理可诱导肝脏细胞自噬, 同时增加*G6PC*基因敲除小鼠的脂肪酸氧化并减少脂肪生成。ZHOU等[52]的研究显示, 一种肝特异性甲状腺激素VK2809可通过肝脏细胞自噬、线粒体的生物合成以及脂肪酸的的β-氧化作用, 降低GSD-1a小鼠的肝甘油三酸酯水平, 减轻肝脏的脂肪化。此外, YAVAROW等[53]的研究发现, 非诺贝特(Fenofibrate)处理可迅速降低GSD-1a新生小鼠的肝脂质和糖原贮积。但这些药物处理是否有助于减轻GSD-1a小鼠的肝纤维化仍有待进一步的研究。

3.3 基因治疗

尽管饮食疗法和辅助疗法可改善GSD-1a患者的临床症状, 但潜在的病理过程仍未得到有效治疗, 患者仍然会出现长期的并发症, 如HCA, 并伴有恶性肿瘤和肾衰竭的风险。因此, 通过饮食疗法和药物治疗仍无法有效解决导致GSD-1a患者发病和死亡

的两大主要原因: 肾脏疾病和肝腺瘤[1-3,54-55]。

G6Pase-α是一种疏水性极强的跨膜蛋白, 很难被纯化, 因此无法实现蛋白替代治疗[16-17]。近年来, 基因治疗成为研究热点[56-57]。迄今已建立多个*G6PC*基因转染载体, 包括腺病毒载体[58]、辅助病毒依赖型腺病毒载体[59]、慢病毒载体[60-61]以及重组腺病毒相关载体(rAAV)[62-66], 研究发现, rAAV载体的有效性最好, 毒性最低, 被普遍接受和使用[67]。常用的*G6PC*过表达rAAV载体由人*G6PC*启动子/增强子(*G6PC* promoter/enhancer, GPE)驱动, 可分为两种类型: 微GPE(rAAV-miGPE)和全长GPE(rAAV-GPE), 序列长度分别是382 bp和3 000 bp[10]。研究结果表明, 两种rAAV介导的基因治疗均可长时间有效改善小鼠或犬 类GSD-1a的代谢异常, 且未发现明显毒性[62-66]。基于CBA/CMV启动子驱动的rAAV载体是另一种*G6PC*过表达载体, 这一载体虽能纠正GSD-1a代谢异常, 但容易引发$CD8^+$淋巴细胞介导的细胞免疫反应, 而这一反应并未在rAAV-GPE载体上观察到。ZHANG等[68]比较了人类突变型*G6PC*(Ser-298 to Cys-298; S298C)和野生型*G6PC*的基因治疗效果, 他们的研究结果显示, S298C突变型G6Pase-α活性效率是野生型的3倍, 这为G6Pase-α疾病的高效治疗提供了新的思路。

LEE等[65]比较了rAAV-miGPE和rAAV-GPE在*G6PC*基因敲除小鼠GSD-1a疾病治疗上的长期有效性和安全性, 结果表明, 与rAAV8-miGPE载体相比, rAAV8-GPE载体可更高水平诱导肝G6Pase-α的表达, 更大程度减少肝糖原累积, 并带来更好的禁食耐受性, 表明rAAV8-GPE载体是GSD-1a临床试验的较好载体。在剂量的探索方面, LEE等[66]的研究显示, 经rAAV8-GPE转基因治疗的*G6PC*敲除小鼠G6Pase-α酶表达活性达到正常活性的3%(5个单位)以上水平时就足以维持小鼠的血糖水平, 经治疗的*G6PC*纯合敲除小鼠肝脏脂肪存储正常, 能够耐受24 h禁食, 且在缺乏饮食的情况下肝脏通过G6Pase-α催化的糖原分解和糖异生来维持血糖能力, 空腹血胰岛素水平降低, 没有肝异常或HCA的证据。这一结果得到KIM等[69]的研究支持, 他们对载体剂量进行了梯度评估, 研究结果表明rAAV8-GPE载体表达的G6Pase-α活性低于正常小鼠肝活性的2%时就存在HCA发生的风险, 在这一研究中, 3只酶活性为野生型小鼠0.9%~1.3%的小鼠自发形成了HCA/HCC。这说明, rAAV8-GPE的基因治疗提高了G6Pase-α酶表达活性。LEE等[64]的研究发现, AAV8-GPE表达载体治疗的小鼠, *G6PT* mRNA表达水平升高, 以及微粒体G6P摄取活性增强, 提示肝脏糖原分解和糖异生活动加强。

GSD-1a的肾脏疾病研究较少。rAAV2/8-GPE介导的基因转染在肾脏中几乎不表达, 导致G6Pase-α活性不足, 肾脏异常病理持续存在。研究显示, 不同的AAV血清型具有不同的组织转导效率[70-73], 最近的研究结果表明, rAAV9可能是肾脏基因传递的首选, 例如ROCCA等[73]采用肾静脉逆行注射法可提高rAAV9肾脏感染效率, 但是AAV9介导的基因转染在肾脏中的表达效率仍然显著低于肝脏。因此, 仍需持续探索高效的肾脏转染病毒。值得注意的是, 血清型在不同物种中具有非常不同的靶向效率。迄今为止, 只有少数血清型被用于临床试验, 而了解灵长类病毒转染特性将有助于解决这一问题, 为临床试验提供切实的解决方案。

4 结论及展望

GSD-1a是由G6Pase-α功能缺失导致的以肝肾异常为主的代谢性疾病, 饮食和药物干预治疗能在一定程度上缓解GSD-1a临床症状, 但严重的肾脏疾病和HCA/HCC的并发症依然长期存在。基于rAAV2/8-GPE的基因治疗不仅能改善*G6PC*基因敲除小鼠肝肾功能失常, 且可预防HCA/HCC的发生和发展, 但应用在临床上仍需要做大量的研究。总体来说, 基因治疗具有一定的应用前景。

在动物模型上, 不同基因编辑工具的小鼠GSD-1a模型和犬类GSD-1a模型在很大程度上满足GSD-1a疾病研究的需要。全身性*G6PC*基因敲除/突变小鼠和犬类模型能很好地模拟人类早期GSD-1a临床症状; 而条件性基因敲除小鼠能实现对GSD-1a的长期研究, 尤其是HCA/HCC发生的理想动物模型。但基于条件性*G6PC*基因敲除小鼠的HCA/HCC模型不能完全模拟人类GSD-1a肝病表型, 因此构建全身性*G6PC*基因敲除成体模型仍有必要。

未来对GSD-1a的研究或应将着眼: 基于新型基因编辑技术的GSD-1a大型动物模型(猕猴、猪等)的构建, 更为高效和特异的基因表达方法的建立, 尤其是注重发展高效的肾脏*G6PC*基因转染方法, 促进GSD-1a肾脏疾病的研究; 建立更为全面的基因治疗安全性和有效性评估。特别是大型动物模型的构建

或可为GSD-1a的基因治疗奠定坚实的理论基础, 为临床试验提供切实的解决方案。

参考文献 (References)

[1] CHOU J Y, MATERN D, MANSFIELD B C, et al. Type I glycogen storage diseases: disorders of the glucose-6- phosphatase complex [J]. Curr Mol Med , 2002, 2(2): 121-43.

[2] CHOU J Y, JUN H S, MANSFIELD B C. Glycogen storage disease type I and G6Pase-βdeficiency: etiology and therapy [J]. Nat Rev Endocrinol, 2010, 6: 676-88.

[3] CHOU J Y, JUN H S, MANSFIELD B C. Type I glycogen storage diseases: disorders of the glucose-6-phosphatase/glucose-6-phosphate transporter complexes [J]. J Inherit Metab Dis, 2015, 38(3): 511-9.

[4] 王霞, 邱文娟. 糖原累积病I型研究进展[J]. 国际儿科学杂志(WANG X, QIU W J. Research progress of glycogen accumulation disease type I [J]. Int J Pediatr), 2008, 35(5): 436-8.

[5] 刘璐, 孙梅. 肝糖原累积病研究进展[J]. 国际儿科学杂志(LIU L, SUN M. Research progress of hepatic glycogen accumulation disease [J]. Int J Pediatr), 2011, 38(1): 62-5.

[6] LU Y L, WANG L Y, LI J, et al. Molecular genetic analysis and phenotypic characteristics of a consanguineousfamily with glycogen storage disease type Ia [J]. Mol Med Rep, 2016, 14(4): 3251-4.

[7] XU N, HUANG X M, FANG W G, et al. Glycogen storage disease type Ia: a rare cause of gout in adolescent and young adult patients [J]. Zhonghua nei ke za zhi, 2018, 57(4): 264-9.

[8] FANG L J, ABUDUXIKUER K, YAN X M, et al. An infant presenting with extreme hypertriglyceridemia diagnosed as glycogen storage disease type Ia [J]. J Pediatr Endocrinol Metab, 2020, 33(6): 803-8.

[9] BALLAVENUTO J M A, OLIVEIRA J D Ó D DE, ALVES R J. Glycogen storage disease type I (Von Gierke disease): report of two cases with severe dyslipidemia [J]. Arq Bras Cardiol, 2020, 114(4 Suppl 1): 23-6.

[10] CHOU J Y, KIM G Y, CHO J H. Recent development and gene therapy for glycogen storage disease type Ia [J]. Liver Res, 2017, 1(3): 174-80.

[11] 吴唯诚, 王建设. 糖原贮积症I型肾脏病变诊断及治疗进展[J]. 中华肝脏病杂志(WU W C, WANG J S. Progress in diagnosis and treatment of glycogen storage type I kidney lesions [J]. Zhonghua Gan Zang Bing Za Zhi), 2021, 29(1): 75-8.

[12] ALESSANDRO R, CHIARA S, MARIACAROLINA S, et al. Imbalanced cortisol concentrations in glycogen storage disease type I: evidence for a possible link between endocrine regulation and metabolic derangement [J]. Orphanet J Rare Dis, 2020, 15(1): 99.

[13] WIKROM K, STACEY G, KATHRYN S, et al. Obesity and reversed growth retardation in a child with type Ia glycogen storage disease [J]. J Pediatr Endocrinol Metab, 2010, 23(5): 507-12.

[14] FERNANDES J, ALAUPOVIC P, WIT J M. Gastric drip feeding in patients with glycogen storage disease type I: its effects on growth and plasma lipids and apolipoproteins [J]. Pediatr Res, 1989, 25(4): 327-31.

[15] RAKE J P, VISSER G, LABRUNE P, et al. Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European study on glycogen storage disease type I (ESGSD I) [J]. Eur J Pediatr, 2002, 161(1): S20-34.

[16] FRANCO L M, KRISHNAMURTHY V, BALI D, et al. Hepatocellular carcinoma in glycogen storage disease type Ia: a case series [J]. J Inherit Metab Dis, 2005, 28(2): 153-62.

[17] GHOSH A, SHIEH J J, PAN C J, et al. The catalytic center of glucose-6-phosphatase. HIS176 is the nucleophile forming the phosphohistidine-enzyme intermediate during catalysis [J]. J Biol Chem, 2002, 277: 32837-42.

[18] LEI K J, SHELLY L L, PAN C J, et al. Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a [J]. Science, 1993, 262(5133): 580-3.

[19] PAN C J, LEI K J, ANNABI B, et al. Transmembrane topology of glucose-6-phosphatase [J]. J Biol Chem, 1998, 273(11): 6144-8.

[20] PAN C J, LEI K J, CHOU J Y. Asparagine-linked oligosaccharides are localized to a luminal hydrophilic loop in human glucose-6-phosphatase [J]. J Biol Chem, 1998, 273(34): 21658-62.

[21] SHIEH J J, TERZIOGLU M, HIRAIWA H, et al. The molecular basis of glycogen storage disease type 1a: structure and function analysis of mutations in glucose-6-phosphatase [J]. J Biol Chem, 2002, 277(7): 5047-53.

[22] CHOU J Y, MANSFIELD B C. Mutations in the glucose-6-phosphatase-alpha (G6PC) gene that cause type Ia glycogen storage disease [J]. Hum Mutat, 2008, 29(7): 921-30.

[23] SHIEH J J, LU Y H, HUANG S W, et al. Misdiagnosis as steatohepatitis in a family with mild glycogen storage disease type 1a [J]. Gene, 2012, 509(1): 154-7.

[24] AKANUMA J, NISHIGAKI T, FUJII K, et al. Glycogen storage disease type Ia: molecular diagnosis of 51 Japanese patients and characterization of splicing mutations by analysis of ectopically transcribed mRNA from lymphoblastoid cells [J]. Am J Med Genet, 2000, 91(2): 107-12.

[25] KAJIHARA S, MATSUHASHI S, YAMAMOTO K, et al. Exon redefinition by a point mutation within exon 5 of the glucose-6-phosphatase gene is the major cause of glycogen storage disease type 1a in Japan [J]. Am J Hum Genet, 1995, 57(3): 549-55.

[26] CHOU J Y, KIM G Y, CHO J H. Recent development and gene therapy for glycogen storage disease type Ia [J]. Liver Res, 2017, 1(3): 174-80.

[27] OKUBO M, AOYAMA Y, KISHIMOTO M, et al. Identification of a point mutation (G727T) in the glucose-6-phosphatase gene in Japanese patients with glycogen storage disease type 1a, and carrier screening in healthy volunteers [J]. Clin Genet, 1997, 51(3): 179-83.

[28] 邱文娟, 张雅芬, 潘骏, 等. 葡萄糖6磷酸酶基因热点突变检测结合1176多态位点连锁分析快速产前诊断Ia型糖原累积病[J]. 中华医学遗传学杂志(QIU W J, ZHANG Y F, PAN J, et al. Detection of hotspot mutations in glucose 6 phosphatase gene combined with 1176 polymorphic site linkage analysis for rapid prenatal diagnosis of type Ia glycogen accumulation disease [J]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi), 2005, 22(1): 44-8.

[29] MATERN D, SEYDEWITZ H H, BALI D, et al. Glycogen storage disease type I: diagnosis and phenotype/genotype correlation [J]. Eur J Pediatr, 2002, 161(1): S10-9.

[30] 王薇, 宋红梅, 张胜海, 等. CRISPR/Cas9结合loxP-Cre技术

制备肝特异性G6PC基因缺陷小鼠的实验研究[J]. 北京医学 (WANG W, SONG H M, ZHANG S H, et al. Experimental study of CRISPR/Cas9 combined with loxP-Cre technology to prepare liver-specific G6PC gene-deficient mice [J]. Chin Med J), 2020, 42(11): 1127-31.

[31] CHO J H, KIM G Y, MANSFIELD B C, et al. Hepatic glucose-6-phosphatase-α deficiency leads to metabolic reprogramming in glycogen storage disease type Ia [J]. Biochem Biophys Res Commun, 2018, 498(4): 925-31.

[32] LEI K J, CHEN H, PAN C J, et al. Glucose-6-phosphatase dependent substrate transport in the glycogen storage disease type-1a mouse [J]. Nat Genet, 1996, 13(2): 203-9.

[33] SALGANIK S V, WEINSTEIN D A, SHUPE T D, et al. A detailed characterization of the adult mouse model of glycogen storage disease Ia [J]. Lab Invest, 2009, 89(9): 1032-42.

[34] BROOKS E D, KOEBERL D D. Large animal models and new therapies for glycogen storage disease [J]. J Inherit Metab Dis, 2015, 38(3): 505-9.

[35] SEVER S, WEINSTEIN D A, WOLFSDORF J I, et al. Glycogen storage disease type Ia: linkage of glucose, glycogen, lactic acid, triglyceride, and uric acid metabolism [J]. J Clin Lipidol, 2012, 6(6): 596-600.

[36] PENG W T, PAN C J, LEE E J, et al. Generation of mice with a conditional allele for G6pc [J]. Genesis, 2009, 47(9): 590-4.

[37] MUTEL E, ABDUL-WAHED A, RAMAMONJISOA N, et al. Targeted deletion of liver glucose-6 phosphatase mimics glycogen storage disease type 1a including development of multiple adenomas [J]. J Hepatol, 2011, 54(3): 529-37.

[38] CLAR J, GRI B, CALDERARO J, et al. Targeted deletion of kidney glucose-6 phosphatase leads to nephropathy [J]. Kidney Int, 2014, 86(4): 747-56.

[39] Brix A E, HOWERTH E W, MCCONKIE-ROSELL A, et al. Glycogen storage disease type Ia in two littermate maltese puppies [J]. Vet Pathol, 1995, 32(5): 460-5.

[40] KISHNANI P S, FAULKNER E, VANCAMP S, et al. Canine model and genomic structural organization of glycogen storage disease type Ia (GSD Ia) [J]. Vet Pathol, 2001, 38(1): 83-91.

[41] SPECHTA, FISKE L, ERGER K, et al. Glycogen storage disease type Ia in canines: a model for human metabolic and genetic liver disease [J]. J Biomed Biotechnol, 2011, 2011: 646257.

[42] CHEN Y T, CORNBLATH M, SIDBURY J B. Cornstarch therapy in type I glycogen-storage disease [J]. N Engl J Med, 1984, 310(3): 171-5.

[43] ROSS K M, BROWN L M, CORRADO M M, et al. Safety and efficacy of chronic extended release cornstarch therapy for glycogen storage disease type I [J]. JIMD Rep, 2016, 26: 85-90.

[44] GREENE H L, SLONIM A E, O'NEILL J A, et al. Continuous nocturnal intragastric feeding for management of type 1 glycogen-storage disease [J]. N Engl J Med, 1976, 294(8): 423-5.

[45] WEINSTEIN D A, WOLFSDORF J I. Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type 1a glycogen storage disease [J]. Eur J Pediatr, 2002, 161(1): S35-9.

[46] BHATTACHARYA K, ORLON R C, MUNDY H, et al. A novel starch for the treatment of glycogen storage diseases [J]. J Inherit Metab Dis, 2007, 30(3): 350-7.

[47] KOEBERL D D, KISHNANI P S, BALI D, et al. Emerging therapies for glycogen storage disease type I [J]. Trends Endocrinol Metab, 2009, 20(5): 252-8.

[48] MELIS D, PARENTI G, GATTI R, et al. Efficacy of ACE-inhibitor therapy on renal disease in glycogen storage disease type 1: a multicentre retrospective study [J]. Clin Endocrinol, 2005, 63(1): 19-25.

[49] MARTENS D H, RAKE J P, NAVIS G, et al. Renal function in glycogen storage disease type I, natural course, and renopreservative effects of ACE inhibition [J]. Clin J Am Soc Nephrol, 2009, 4(11): 1741-6.

[50] YIU W H, MEAD P A, JUN H S, et al. Oxidative stress mediates nephropathy in type Ia glycogen storage disease [J]. Lab Invest, 2010, 90(4): 620-9.

[51] WASKOWICZ L R, ZHOU J, LANDAU D J, et al. Bezafibrate induces autophagy and improves hepatic lipid metabolism in glycogen storage disease type Ia [J]. Hum Mol Genet, 2019, 28(1): 143-54.

[52] ZHOU J, WASKOWICZ L R, LIM A, et al. A liver-specific thyromimetic, VK2809, decreases hepatosteatosis in glycogen storage disease type Ia [J]. Thyroid, 2019, 29(8): 1158-67.

[53] YAVAROW Z A, KANG H R, WASKOWICZ L R, et al. Fenofibrate rapidly decreases hepatic lipid and glycogen storage in neonatal mice with glycogen storage disease type Ia [J]. Hum Mol Genet, 2020, 29(2): 286-94.

[54] JAUZE L, MONTEILLET L, MITHIEUX G, et al. Challenges of gene therapy for the treatment of glycogen storage diseases type I and type III [J]. Hum Gene Ther, 2019, 30(10): 1263-73.

[55] KISHNANI P S, SUN B, KOEBERL D D. Gene therapy for glycogen storage diseases [J]. Hum Mol Genet, 2019, 28(R1): R31-41.

[56] CHO J H, LEE Y M, STAROST M F, et al. Gene therapy prevents hepatic tumor initiation in murine glycogen storage disease type Ia at the tumor-developing stage [J]. J Inherit Metab Dis, 2019, 42(3): 459-69.

[57] KANG H R, GJORGJIEVA M, SMITH S N, et al. Pathogenesis of hepatic tumors following gene therapy in murine and canine models of glycogen storage disease [J]. Mol Ther Methods Clin Dev, 2019, 15: 383-91.

[58] ZINGONE A, HIRAIWA H, PAN C J, et al. Correction of glycogen storage disease type 1a in a mouse model by gene therapy [J]. J Biol Chem, 2000, 275(2): 828-32.

[59] CRANE B, LUO X, DEMASTER A, et al. Rescue administration of a helper-dependent adenovirus vector with long-term efficacy in dogs with glycogen storage disease type Ia [J]. Gene Ther, 2012, 19(4): 443-52.

[60] SALANI B, DAMONTE P, ZINGONE A, et al. Newborn liver gene transfer by an HIV-2-based lentiviral vector [J].Gene Ther, 2005, 12(10): 803-14.

[61] GRINSHPUN A, CONDIOTTI R, WADDINGTON S N, et al. Neonatal gene therapy of glycogen storage disease type Ia using a feline immunodeficiency virus-based vector [J]. Mol Ther, 2010, 18(9): 1592-8.

[62] KOEBERL D D, PINTO C, SUN B, et al. AAV vector-mediated reversal of hypoglycemia in canine and murine glycogen storage disease type Ia [J]. Mol Ther, 2008, 16(4): 665-72.

[63] YIU W H, LEE Y M, PENG W T, et al. Complete normalization of hepatic G6PC deficiency in murine glycogen storage disease

type Ia using gene therapy [J]. Mol Ther, 2010, 18(6): 1076-84.

[64] LEE Y M, JUN H S, PAN C J, et al. Prevention of hepatocellular adenoma and correction of metabolic abnormalities in murine glycogen storage disease type Ia by gene therapy [J]. Hepatology, 2012, 56(5): 1719-29.

[65] LEE Y M, PAN C J, KOEBERL D D, et al. The upstream enhancer elements of the G6PC promoter are critical for optimal G6PC expression in murine glycogen storage disease type Ia [J]. Mol Genet Metab, 2013, 110(3): 275-80.

[66] LEE Y M, KIM G Y, PAN C J, et al. Minimal hepatic glucose-6-phosphatase-α activity required to sustain survival and prevent hepatocellular adenoma formation in murine glycogen storage disease type Ia [J]. Mol Genet Metab Rep, 2015, 3: 28-32.

[67] CHOU J Y, MANSFIELD B C. Recombinant AAV-directed gene therapy for type I glycogen storage diseases [J]. Expert Opin Biol Ther, 2011, 11(8): 1011-24.

[68] ZHANG L, CHO J H, ARNAOUTOVA I, et al. An evolutionary approach to optimizing glucose-6-phosphatase-α enzymatic activity for gene therapy of glycogen storage disease type Ia [J]. J Inherit Metab Dis, 2019, 42(3): 470-9.

[69] KIM G Y, LEE Y M, KWON J H, et al. Glycogen storage disease type Ia mice with less than 2% of normal hepatic glucose-6-phosphatase-α activity restored are at risk of developing hepatic tumors [J]. Mol Genet Metab, 2017, 120(3): 229-34.

[70] MICHELFELDER S, TREPEL M. Adeno-associated viral vectors and their redirection to cell-type specific receptors [J]. Adv Genet, 2009, 67: 29-60.

[71] ZINCARELLI C, SOLTYS S, RENGO G, et al. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection [J]. Mol Ther, 2008, 16(6): 1073-80.

[72] SCHIEVENBUSCH S, STRACK I, SCHEFFLER M, et al. Combined paracrine and endocrine AAV9 mediated expression of hepatocyte growth factor for the treatment of renal fibrosis [J]. Mol Ther, 2010, 18(7): 1302-9.

[73] ROCCA C J, UR S N, HARRISON F, et al. rAAV9 combined with renal vein injection is optimal for kidney-targeted gene delivery: conclusion of a comparative study [J]. Gene Ther, 2014, 21(6): 618-28.