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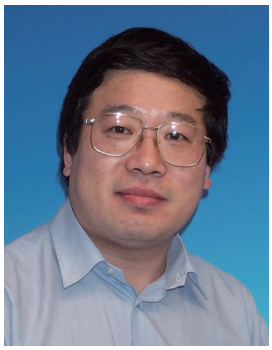

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哺乳动物与肠道菌群的共代谢相互作用

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摘　要：哺乳动物的消化道居住着数以万亿的，数千种共生微生物或者肠道菌群。肠道菌群与动物宿主通过共进化而共生并经过相互作用而影响其生理与病理生理过程。胆汁酸、胆碱、膳食纤维及芳香类代谢物等多种物质的共代谢是该相互作用的关键分子过程，肝肠循环是上述物质交换与调控相互作用的主要途径。肠道菌群组成与代谢组的关系及其功能的精确刻画是急需解决的科学问题。

关键词：肠道菌群；哺乳动物；肝肠循环；共代谢组；代谢组学
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Co-metabolism：vital interactions between mammals and symbiotic gut microbiota

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Abstract: Trillions of microbes also known as gut microbiota live in the gastrointestinal tract (GIT) of mammals with more than two thousands species. These gut microbiota and their mammalian hosts undergo close interactions to form symbiotic "superorganisms" through co-evolution, contributing towards the physiological and pathophysiological processes of mammals. Co-metabolisms of bile acids, choline and carnitines, dietary fibers and aromatic compounds are the major host-microbial interactive exchanges of bioactive metabolites whilst enterohepatic recirculation is the major route for such exchanges and regulations. Accurate definition of the gut microbiota, metabolite composition and their functional relationships is urgently required for further understanding the host-micriobiota interactions and implication in mammalian health.

Key words: gut microbiota; mammals; enterohepatic recirculation; co-metabolism; metabolomics

1 哺乳动物是“超级生物”

正常的哺乳动物都是“超级生物”，是由宿主与数千种微生物组成[1-3]并通过共进化而形成的共生体[3-5]。因此，哺乳动物的生理与病理生理过程均需要考虑宿主本身、共生微生物群及二者的相互作用[1-4]。宿主的消化道居住着数以万亿的共生微生物群，它们被称作肠道菌群或者微生物组[2-7]。成年人肠道中有将近 1.5 kg 的数千种微生物，它们通过与宿主的协同发育而影响宿主健康的各个方面[3-7]。肠道菌群被认为是动物宿主的又一必需“器官”而构成动物的拓展基因组、转录组、蛋白质组与代谢组[4,7-8]。由于肠道菌群中多数物种尚无法进行体外培养，目前还无法像 (人类与啮齿动物等) 宿主一样对肠道菌群的基因组、转录组、蛋白质组及代谢组进行精准测定。

肠道菌群的结构 (或者微生物组成) 既依赖于宿主的基因型、年龄与生理状态等内因，也依赖于宿主的饮食与生活条件等环境因素[2-10]。肠道菌群的功能包括对宿主免疫系统的塑造[11-15]、食物消化与营养吸收[16-24]及对药物与胆汁酸等代谢的调控等多个方面[25-29]。越来越多的证据表明肠道菌群与大肠癌[30-31]、炎症性肠病[12]、代谢性疾病[16-23]、动脉粥样硬化[32]以及神经性疾病[33-34]等多种病理过程相关。因此，哺乳动物肠道菌群的结构与功能已成为微生态学[2-10]、病理生理学[16-23,30-36]、药物代谢与疗效[37-39]及宿主与肠道菌群作用[3-10,24,40-41]等诸多生物医学领域的研究热点。

2 哺乳动物与肠道菌群的共代谢相互作用

共代谢是哺乳动物宿主与肠道菌群相互作用的一个重要方面，共代谢通常经过肝肠循环而进行宿主与肠道菌群之间的代谢物交换[25-28]。其中，绝大多数在体内循环的胆汁酸[25-28]、尿液中的芳香类代谢物及胆碱的代谢物均是典型共代谢产物[35-37,42-47]。譬如，植物多酚与苯丙氨酸等芳香类物质由肠道菌群代谢产生苯乙酸与苯甲酸，而后在肠道吸收并经肝门静脉进入宿主肝脏，再通过甘氨酸等复合代谢而产生苯乙酸甘氨酸与马尿酸，最后由尿液中排出[35-36,42-46]。尿液中的吲哚与对甲基苯酚等芳香类代谢物也通过相似的共代谢途径而形成[42-46]。尿液中的甲胺、二甲胺、三甲胺及其代谢物氧化三甲胺则源于肠道菌群对胆碱与肉碱的代谢[35-36,44-46]。这些共代谢物对宿主的健康有深远的影响。

胆汁酸是宿主 - 肠道菌群之间“合作”最为紧密的一类共代谢物。大约 95% 的胆汁酸由宿主肝脏及肠道菌群之间进行转化并在体内循环使用[24-28]。哺乳动物肝脏通过羟基化与异构化将从头 (*de novo*) 合成及食物来源的胆固醇转化为胆酸与鹅胆酸，再通过羟基化、与甘氨酸及牛磺酸等结合而形成数十种初级胆汁酸[25-28]。之后，它们通过胆管被排入肠道而实现其乳化并协助脂肪酸吸收的功能。同时，

肠道内居住的菌群会对这些胆汁酸进行去结合 (甘氨酸与牛磺酸等)、去羟基化等代谢而再转化为初级胆汁酸，还会对初级胆汁酸进行代谢而形成脱氧胆酸、石胆酸等一系列次级胆汁酸 [25]。上述胆汁酸通过肠吸收而经肝门静脉进入肝脏，进行第二轮肝肠循环。它们在亲脂性维生素吸收、宿主的内分泌功能、避免肠道微生物的错误移位等方面有重要作用 [25-26]。因而，肠道菌群对宿主生理过程的调控有其不可或缺的功能 [25-26]。正常生理条件下，大约 80% 的胆汁酸在小肠的空肠与近端回肠完成吸收，5% 由回肠中段及末端完成吸收，10% 胆汁酸通过大肠吸收，这些吸收的胆汁酸通过肝肠循环而进入肝脏 [24]。 只有 5% 的胆汁酸经大便排出体外 [24]。尽管大肠所吸收的胆汁酸较少，但这些却与大肠癌、炎症性肠病及肝癌等重大疾病的发生发展密切相关，相关详细的机制尚需深入研究。

事实上，肝肠循环也是宿主与肠道菌群对药物等外源性物质进行共代谢的关键过程。譬如，多种药物的吸收、(共) 代谢及排泄均与肠道菌群对药物的活化和解毒等功能密切相关 [25-26]。食源性胆碱对哺乳动物健康的影响很可能与肠道菌群降解产物二甲胺、三甲胺及其共代谢物氧化三甲胺有关 [48]。肝肠循环还是宿主与肠道菌群协同代谢芳香类氨基酸 (苯丙氨酸、酪氨酸与色氨酸)、食物 (或植物药) 来源的多酚类物质、膳食纤维的关键过程。这个循环过程为宿主肝脏及肠道菌群之间的共生相互作用提供了有效且严密调控的特异途径。宿主大肠黏膜上皮细胞与肠道菌群之间也有密切的合作，肠道菌群水解膳食纤维 (多糖) 产生单糖并发酵产生短链脂肪酸，为宿主上皮细胞提供能量、信号调控等功能物质 [49-51]。显而易见，对这些共代谢过程的深入认识有利于揭示肠道菌群对宿主代谢表型的贡献与机制。

3　宿主-菌群的共代谢与哺乳动物的代谢表型

哺乳动物的代谢表型包含宿主、肠道菌群及二者共代谢的综合信息，是其分子表型的重要组成部分。当肠道菌群的基因组、转录组及蛋白质组无法进行准确定量测量时，代谢表型的定量能够提供动物生理及病理生理 [52-57]、宿主与肠道菌群共生相互作用及肠道菌群功能的关键分子信息 [8,25,58-60]。动物体液及组织等中的代谢物的组成 (代谢组) 重要而复杂，其所含代谢物的种类繁多、浓度范围大、物理化学性质及所在基质各异 [43,61]。定量分析动物体液、细胞、组织及活体的全部代谢物组成与变化规律却是一个极具挑战的世界性难题。

目前，核磁共振波谱 (NMR) 与质谱技术是优势与信息互补的两大主流代谢表型定量分析手段 [56,62-65]。前者具有优异的重现性，在无需破坏样品的条件下即可对体液、细胞、组织及活体的代谢表型进行原位无创的定量刻画。NMR 技术能提供的物质的原子间连接关系便于代谢物的定性，是准确鉴定微量代谢物绝对结构的独一无二手段。NMR 对不同代谢物的响应 (系数) 相同，且所获信号的强度与代谢物浓度成线性正比关系，定量简单而便捷。基于滤波技术的谱编辑实验方法丰富而灵活，可以在不分离样品的前提下对代谢物信号进行选择性检测，便于目标代谢物的原位无创定量分析。高分辨魔角旋转核磁共振 (high-resolution magic-angle spinning NMR, HRMAS NMR) 是细胞 [66] 与组织 [67-70] 代谢组原位无创定量分析的关键技术手段；核磁共振活体谱 (MRS *in vivo*) 则是适合临床应用的活体代谢表型的原位无创分析核心技术。

质谱技术灵敏而重现性好。代谢组质谱分析常需要与色谱分离技术进行结合，以提高灵敏度、同分异构体分辨与定性能力。常用于代谢组分析的质谱包括四级杆飞行时间质谱 (QTOF-MS)、三重串联四级杆质谱、傅里叶变换质谱 (FT-ICR) 等，而色谱 - 质谱联用技术包括液相色谱 - 质谱 (LC-MS)、气相色谱 - 质谱 (GC-MS) 及毛细管电泳 - 质谱 (CE-MS)，上述技术已广泛用于体液、细胞与组织提取物的非原位分析 [56,63-65]。质谱的直接进样分析虽具有速度快等特点，但常因样品中盐的存在而受到制约 [71]。近年来，基于电喷雾解吸电离 (DESI) 与基质辅助激光解吸电离 (MALDI) 等技术的质谱成像方法得到了快速发展，该技术可分析组织切片表面代谢物的丰度及空间分布，在分子组织病理学研究等方面表现出了良好的临床应用潜力 [72-75]。

然而，实践中需要特别注意上述两大主流技术各有的不足。核磁共振技术对小分子代谢物的分析灵敏度在纳克或微摩尔浓度量级，代谢物的定性或准确鉴定需要系列二维谱的验证。质谱技术对不同代谢物的响应相差较大而影响同步定量，对代谢物的准确定性需要参考色谱保留时间、母离子质荷比及子离子 (或中性碎片) 的相对丰度，同时需要使用标准品进行进一步的验证；对于完全未知代谢物难以使用质谱进行结构的准确鉴定。在实践中，上述两大主流技术的选择必须考虑其各自的优势和不

足、科学问题和研究目的与技术的匹配性、数据分析方法对上述技术所得数据的适用性、数据处理所得结果的物理含义等多种因素。事实上，上述两大技术的有机结合与优势集成是代谢表型精密测量与定量分析的理想方法[76,77]。这种结合包括两种技术所获数据的逻辑结合、基于运算的统计相关结合[78-84]与基于物理连接的联用技术。已有的 LC-DAD-SPE-CryoNMR-MS 联用技术就可以在色谱分离的同时采集代谢物的紫外可见光谱、核磁共振谱及质谱数据[85-91]，进而进行定性定量分析。此联用技术已成为新代谢物的发现与结构准确鉴定的重要手段。其中，低丰度代谢物可以通过固相萃取微柱进行多次富集后进行详尽的谱学分析，进而实现对微量完全未知代谢物绝对结构的准确鉴定。

多变量统计分析是广泛用于代谢组数据挖掘的技术[92-94]，所获数据是阐明发育和基因改变等内因及应激和药物等环境因素对代谢表型影响规律的基础。代谢物信号的归一化与标准化是此数据分析的必需预处理步骤，鉴于不同代谢物的核磁共振响应相似但质谱响应差别较大，相关数据预处理方法的选用必须考虑数据的来源与处理后数据的物理意义。此外，PLS-DA 与 OPLS-DA 等监督性分析模型无一例外地需要严格的验证[95-97]。

对哺乳动物的代谢表型分析而言，宿主代谢与宿主 - 菌群共代谢是两个关键贡献源。宿主消化道是多数肠道菌群的居所，消化道不同区段的代谢特征及其发育依赖性值得关注。HRMAS-NMR 技术对大鼠[98]、小鼠[60]和人类[81,99]肠道组织代谢表型的无创原位分析已有较为详细的报道。这些研究发现模式动物[60,98]与人类[81,99]肠道代谢表型具有鲜明的区段特异性，小肠和大肠组织的代谢表型差异尤为突出；模式动物的肠道区域代谢特征有显著地发育依赖性[98]。事实上，这些不同肠段内的肠道菌群结构及内容物代谢物组成均有显著差异，并同时存在发育依赖性[60,100]。定量分析肠道菌群的结构(及组成)、代谢表型及肠段依赖性是阐明其功能的关键，也是阐明肠道菌群对宿主生理与病理生理过程的影响规律，发展有效干预策略措施与维护健康的重点。然而，肠道菌群中绝大多数微生物尚无法在体外得到有效培养，这成为其功能研究的一个瓶颈问题，也必将成为微生物学及肠道微生态学领域的一个重要任务。

鉴于此，肠道内容物代谢物组成分析已成为研究宿主 - 肠道菌群共代谢相互作用的重要途径。多数肠道微生物的功能会以其代谢物影响宿主生理过程而得以体现，肠道菌群整体的代谢表型携带着肠道菌群代谢及宿主 - 肠道菌群共代谢的分子信息。因此，多种实验参数优化型的哺乳动物粪样代谢组提取与检测分析方法得到了发展建立[24,101]，多项研究工作已见报道[21,30,101-106]。结果表明，人类及啮齿动物粪样代谢组之间存在显著的物种依赖性[102]；大鼠消化道内容物的代谢组具有明显的肠段与发育依赖性，该代谢表型能清晰地反映大鼠肠段的营养吸收等功能[24]。粪样代谢组分析也已用于对炎症性肠病[21,103]、肝硬化与肝细胞肿瘤[104]、慢性胰腺炎[105]及大肠癌[30,106]等多种重大疾病的诊断研究；肠道菌群功能发生改变时，其典型代谢物甲胺、三甲胺与短链脂肪酸(即膳食纤维的细菌发酵产物)均会发现显著变化[24,59,107]。

尿液代谢组分析是研究宿主 - 肠道菌群共代谢相互作用的又一有效途径。已有研究表明，哺乳动物尿液中既有宿主与肠道菌群各自的代谢信息，也包含丰富的宿主 - 肠道菌群共代谢信息[40]，人类尿液代谢表型与其肠道菌群之间还存在广泛而密切的相关性[40]。一系列尿液代谢组分析表明，肠道菌群与宿主的共代谢相互作用既对宿主的正常生理过程有其贡献，也对宿主的病理生理与药物等有效干预过程有显著影响[8,42,108-110]。这些共代谢过程与营养及植物药的作用[111]、寄生虫病[44-45,112-113]及胰岛素抵抗等代谢异常[35]密切相关。尿液代谢组与肠道菌群宏基因组的整合分析有利于发现代谢与微生物之间的相关性，是研究共生相互作用的一个重要思路[40]，是认识肠道菌群与疾病防治关系的途径。然而，肠道菌群对宿主尿液代谢表型直接贡献的详细内涵依然需要深入研究。

4 展望

宿主与肠道菌群共代谢研究需要代谢组技术的进一步突破。目前，代谢表型组的核磁共振与质谱分析方法虽已成型但远未成熟[114-117]，新技术与新方法及新装置的发展依然是核心任务，低丰度代谢物的更高覆盖分析是代谢表型定性与定量刻画的根本需求。代谢物对宿主、肠道菌群及宿主 - 肠道菌群相互作用等方面的功能与机制需要深入研究。肠道微生物的体外培养技术是肠道菌群结构及功能研究中亟待解决的问题。

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