甲基转移酶在药物代谢中的研究进展

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摘要: 甲基转移酶是一种重要的代谢酶, 其主要功能是催化氮、氧和硫原子的甲基化反应。它在内外源性化合物, 包括药物的体内代谢过程中发挥重要的作用。甲基转移酶广泛分布于不同组织中, 以肝脏和肾脏分布最为丰富。此外, 该酶的结构及活性具有一定种属和个体差异。本文将介绍甲基转移酶的生物学特性及其在药物代谢中的作用。

关键词: 甲基转移酶; 组织分布; 基因多态性; 药物相互作用; 药物代谢

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Advances in the study of methyltransferase in drug metabolism

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Abstract: Methyltransferase is an important metabolic enzyme whose main function is to catalyze the methylation of nitrogen, oxygen and sulfur atoms. It plays an important role in the metabolism of exogenous and exogenous compounds, including drugs *in vivo*. Methyltransferases are widely distributed in different tissues, with the liver and kidneys being the most abundant. In addition, the structure and activity of the enzyme have certain species and individual differences. This article will describe the biological properties of methyltransferases and their role in drug metabolism.

Key words: methyltransferase; tissue distribution; gene polymorphism; drug interaction; drug metabolism

甲基转移酶 (methyltransferase, MTs) 是广泛存在于动物、植物和微生物中的重要酶系, 催化底物分子 (如DNA、RNA、蛋白质和内外源性化合物) 的甲基化反应, 在生命系统中发挥着重要的作用。MTs通过催化生物分子 (蛋白质、DNA和RNA) 的甲基化反应影响基因表达调控[1-3], 参与多种疾病的发生和发展。如MTs参与5-甲基胞嘧啶RNA修饰, 这种RNA修饰调控多种致癌基因表达, 与癌症密切相关; NSD2甲基化酶的异常活化可以正反馈促进肿瘤耐药诱导因子TIGAR的过度表达, 从而导致肿瘤细胞对治疗药物的耐药性增强; MTs介导的N6-甲基腺苷RNA修饰异常, 可影响神经功能[4-9]。此外, MTs还在机体生长发育过程中扮演重要角色, 如MTs参与DNA修复, 通过募集端粒蛋白和甲基化组蛋白至DNA双链断裂处, 抑制核酸降解, 维持基因稳定; KMT2C甲基化酶参与细胞分化过程中基因的表达, 该酶基因敲除小鼠出现胚胎致死; METTL3甲基化酶通过调控心肌细胞N6-甲基腺苷RNA修饰, 维持心脏稳态[10-12]。

MTs也是参与内源性化合物合成和代谢的关键酶, 如羟基吲哚氧甲基转移酶催化*N*-乙酰血清素甲基

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化是体内合成褪黑素的关键步骤; 苯乙醇胺甲基转移酶催化去甲肾上腺素合成肾上腺素[13]。同时, MTs还参与多种药物如硫唑嘌呤、吗啡等的代谢过程。甲基化反应使大部分药物的药理活性减弱或丧失, 但也有少数药物被活化而作用增强, 甚至产生毒性。例如, 阿扑吗啡可经MTs代谢为吗啡; MTs与其他氧化酶可协同催化含氮类药物产生致癌性代谢物[13]。

MTs的表达水平及活性与药物对机体的药效作用、毒性反应及药物间相互作用密切相关。例如, 甲氨蝶呤可以抑制MTs对硫唑嘌呤的代谢, 临床上常采用甲氨蝶呤与硫唑嘌呤联合治疗白血病, 以提高治疗效果[14]。人类已知MTs大约有300多种[15], 其中95%属于SAM依赖型MTs, 即需要SAM作为甲基供体催化底物的甲基化反应[13]。因此, 本文总结了SAM依赖型MTs的类型、作用特点、种属差异, 为开展基于甲基化代谢的先导化合物药代特性评价和新药开发提供参考。

1 甲基转移酶的类型和催化机制

MTs具有一个保守核心结构域, 该结构域由 $(\alpha/\beta)_8$ 或者 $(\alpha/\beta)_6$TIM-barrel折叠组成, 桶状结构域的完整性和开放性决定了酶作用底物的大小 (表1)。$[4Fe\text{-}4S]^{1+}$ 位于保守结构域的N端, 其中铁离子与3个半胱氨酸残基连接, 并且与SAM配位[16]。Schubert等[17]根据结构特点将MTs分为5类 (I～V类), 每类MTs的氨基酸序列、SAM结合方式以及三级结构均具有独特性。I类MTs是最大的甲基转移酶家族, DNA甲基转移酶均为I类MTs, X射线确定该类酶结构具有显著的一致性[18]。I类MTs由3个结构域组成, 形成了一条裂缝, 以便结合DNA[19]。其中两个结构域通过铰链区相连, 最大结构域的表面具有一个空腔作为SAM结合位点[19]。II类MTs比其他类MTs催化的原子种类更多, 包括磷原子和SP_2、SP_3杂化碳原子[20]。III类MTs由两个结构域组成, SAM依然结合在这两个结构域之间形成的口袋中[21]。与其他类型的MTs不同, III类MTs的结合位点位于结构域的N端, 并且结合构象更加紧密。这种紧密的结合使得活性甲基能够暴露在外部, 从而更好地将甲基转移至大体积的底物上[21]。该类酶能催化含四吡咯类化合物的甲基化反应, 如CbiF酶参与B12的合成[21]。IV类MTs被命名为SPOUT酶超家族[22], 它们在tRNA和rRNA的转录后修饰中发挥作用。该类酶的保守结构是位于C末端的三叶草结构, 由160个氨基酸组成。这个结构提供了结合SAM和底物的位点, 并使活性位点的基团处于最佳构象, 从而促进甲基化反应的进行[23]。第V类MTs包含了SET结构域, 但其具体结构尚不完全清楚。这类酶的主要功能是甲基化组蛋白和其他在转录调控中至关重要的蛋白质[24]。

MTs的催化机制主要包括电子转移、SN2亲核反应和氧化还原反应。MTs利用$[4Fe\text{-}4S]^{1+}$提供电子, 将SAM裂解为蛋氨酸和5′-脱氧腺苷自由基[20]。随后, SAM通过SN2置换反应, 将半胱氨酸残基甲基化, 生成SAH和甲基化蛋白质[15,17,25-27]。需要指出的是, SAM分子中的磺胺离子具有手性中心, 因此甲基化反应表现出立体特异性, SAM的*S*构象为反应优势构型[27]。同时, 5′-脱氧腺苷自由基进攻甲基化半胱氨酸残基上的甲硫基团, 生成蛋白质亚甲基自由基。最终, 这个自由基失去一个电子后与底物结合[25], 导致底物结合位点附近的118位半胱氨酸催化硫醚复合物还原裂解, 生成二硫化物和甲基化代谢产物[25]。

2 甲基转移酶与药物代谢

2.1 MTs参与药物代谢的类型 人体内含有丰富的MTs, 不同种类MTs能够催化不同药物甲基化, 最终甲基化代谢物通过尿液或胆汁排出体外, 因此MTs影响药物活性、作用时长和毒性。MTs根据底物分子中被

Table 1 Methyltransferase substrate types and properties[28-31]

Substrate type	Substrate property
DNA	a. DNA is a double-stranded molecule composed of four kinds of deoxynucleotides b. Its methylation substrates include C5-cytosine, N4-cytosine, and N6-adenine, and the common reaction product is 5-methylcytosine c. In the catalytic process, DNA methylases recognize and bind to specific DNA sequences, DNA, enzyme and cofactors combine into ternary complexes, and the target base is spun out of the DNA double strand to bind to the enzyme active site
RNA	a. RNA is a double-stranded molecule composed of four kinds of ribonucleotides b. Its methylation substrates include C5-cytosine and N6-adenine, and the most common metabolite is 6-methyladenine c. 5-Methylcytosine and 6-methyladenine are associated with RNA stability and translation efficiency d. The N6-adenine methylation site is located around the stop codon and on the long chainexon
Proteins	a. Protein is a substance with a certain spatial structure formed by winding and folding polypeptide chain composed of amino acids b. Its methylation substrates include histidine, lysine, arginine and glutamine, and lysine methylation is the most common reaction
Small molecule substances	a. Small molecules substances include hormones and chemicals, containing S, N or O atoms b. Its activity changed after being catalyzed by methyltransferase

作用的基团可以分为S-、N-和O-甲基转移酶。其中O-MTs占MTs酶家族的比例最大 (54%), 其次是N-MTs (23%) 和S-MTs (3%)[24]。O-MTs中的儿茶酚氧甲基转移酶 (COMT) 是重要的二相药物代谢酶, 它利用辅因子SAM和Mg^{2+}转移电子, 将羟基基团氧化为醚基, 从而改变药物的化学结构并降低药物活性。COMT催化的底物不仅包括儿茶酚胺类神经递质如多巴胺、肾上腺素、去甲肾上腺素, 还包括阿片类药物以及儿茶酚类药物如左旋多巴、甲基多巴[32-34]。COMT存在可溶性 (S-COMT) 和膜结合型 (MB-COMT) 两种形式。MB-COMT是一种跨膜蛋白, 主要存在于脑组织中。而S-COMT则溶解于细胞质中, 广泛分布于外周组织和器官[35]。S-甲基化是含硫类化合物代谢的重要途径, S-MTs包括硫嘌呤甲基转移酶 (TPMT)、巯基甲基转移酶 (TMT)、硫醚甲基转移酶 (TEMT)。该类酶能够甲基化巯基, 将含硫药物代谢成无活性产物, 使药物毒性降低[36]。TPMT催化硫代芳香族或杂环类药物如6-巯基嘌呤、硫唑嘌呤; TMT催化硫代脂肪族类药物如卡托普利、*D*-青霉胺[36-38]; TEMT只在小鼠体内表达, 其作用为甲基化硫醚基团中的硫原子[37]。N-MTs是主要参与代谢内源性神经递质和激素的酶, 例如它在肾上腺素的体内合成中发挥重要作用[37]。砷甲基转移酶参与代谢含砷类药物, 如治疗早幼粒细胞白血病的砷剂, 以及部分中药, 如砒霜和牛黄解毒丸。砷代谢物经肾脏排泄体外, 有助于减少砷在体内的毒性积累[39]。

MTs广泛分布于各个组织, 在肝脏和肾脏中含量尤其丰富。例如, TPMT在肝脏的含量分别是小肠、肺、大脑的2、3和5倍。而COMT在肝脏的含量最高, 是肾脏的2倍。同时, COMT在大脑分布, 因而参与药物在脑部的代谢[37,40,41]。此外, MTs也在红细胞中表达, 影响药物在血液中的稳定性, 因此红细胞可用于研究MTs在药物代谢方面的作用。

2.2 影响MTs代谢活性的因素 等位基因遗传变异是影响MTs活性的直接因素。当存在缺陷基因时, 编码氨基酸发生替换, 导致翻译表达的MTs活性低于正常水平[14,42,43]。影响MTs活性的因素还包括性别和年龄。研究表明, 男性MTs活性高于女性, 而老年人的酶活性相较于年轻人更高[14]。此外, 药物也会影响酶活性。抑制TPMT的药物包括硫唑嘌呤、利尿剂、睾酮、非甾体类抗炎药、别嘌呤醇、苯甲酸衍生物等[14,44-46] (表2)。SAM类似物能够竞争性地与TPMT结合, 从而抑制其活性, 常见的类似物有SAH、西奈芬净、甲硫腺苷[15,24,45,47]。COMT抑制剂包括托卡朋 (IC_{50} = 773 nmol·L^{-1})、恩他卡朋 (IC_{50} = 151 nmol·L^{-1})、阿片卡朋等, 这类抑制剂均含有儿茶酚结构, 使其能够竞争性抑制COMT[15,48-50]。研究表明, COMT抑制剂与帕金森病治疗药物合用, 能够显著提高该类药物的生物利用度。为了获得更高效的酶抑制剂, 可以基于COMT的Mg^{2+}和SAM位点以及3,5-二硝基儿茶酚结构设计COMT双底物抑制剂, 即抑制剂占据SAM位点的同时与底物结合位点非竞争性结合[48]。

Table 2 IC_{50} of different drugs inhibited thiopurine S-methyltransferase (TPMT)[44,51,52]

Drug	IC_{50}/μmol·L^{-1}
Azathioprine	430−532
Pilontanil	300−313
Furosemide	15−19
Testosterone	30−72
Mefinamine	39
Naproxen	79
Osalazine	1 474
Ketotifen	1 013
Ibuprofen	1 968
Diclofenac	1 582
Meloxicam	4 292
Paracetamol	5 168
Celecoxib	2 416
Piroxicam	2 589

2.3 MTs参与药物代谢的体内外研究模型 目前已有多种体内外模型用于研究MTs对药物的作用。体外组织和细胞研究模型均需要加入100～200 μmol·L^{-1} SAM作为甲基供体[53-58]。由于红细胞的细胞质中含有S-COMT和TPMT, 因此, 采用冰水裂解红细胞得到的红细胞裂解液是最简便的体外研究模型之一[53,55,56]。需要注意的是, 温孵体系的pH值影响红细胞中MTs的活性。Regensburger等[48]报道人红细胞体外实验最优pH值为7.5。因此, 在进行红细胞温孵实验时, 保持pH值约7.5将有利于研究MTs的作用。此外, Ca^{2+}是MTs强抑制剂, 因此温孵体系中还需要加入Chelex 100螯合剂排除Ca^{2+}的干扰[55, 57]。肝脏组织匀浆也常用于体外评估药物对MTs活性的影响。文献[58, 59]报道大鼠肝脏匀浆采用磷酸盐溶液缓冲体系, 在500 μmol·L^{-1} SAM及5 min温孵条件下, MTs的活性最佳。TPMT的代谢活性可以通过巯基嘌呤代谢产生甲基6-巯基嘌呤的反应速率进行评估, 而肾上腺素的清除率可以用于反映COMT的活性[57,58]。

MTs体内研究使用的动物模型包括小鼠、大鼠和狨猴[60-62]。C57BL/6J、AKR/J以及C58/J近交品系小鼠因肝脏中TPMT基因的沉默而表现出较低的表达水平, 因此可作为TPMT低水平表达的研究模型[63]。Liu等[61]为证明TPMT参与巯嘌呤代谢的重要性, 采用TPMT基因缺陷小鼠进行了巯嘌呤临床治疗剂量的研究, 并评估了因缺乏TPMT导致的药物毒性作用。此

外, 有学者根据小鼠或大鼠的性别、年龄及受试药物种类进行分组, 测定肝脏组织匀浆中MTs的活性, 研究影响MTs活性的因素[63-65]。COMT抑制剂可辅助左旋多巴治疗帕金森病, 相较于啮齿类动物, 狨猴与人类COMT的同源性超过90%, 是评估新型COMT抑制剂抑制能力的最佳动物模型[66]。

3 甲基转移酶的基因多态性和种属差异

人类的TPMT和COMT基因具有多态性。其中TPMT有30多种突变基因, 这些突变片段的89%位于6号染色体的18.1～18.2 Mb区域; 而COMT基因位于22号染色体的q11.1～q11.2之间[14, 67]。常见的TPMT基因突变包括TPMT*3A、TPMT*3B和TPMT*3C。TPMT*3A在白种人中较为常见, 其变异频率约为5%。该突变导致氨基酸序列第154位的Ala和第240位Tyr分别被替换为Thr和Cys。TPMT*3C则主要存在于东亚人群中, 变异频率约为2%, 其基因序列的第240位发生突变。而TPMT*3B等位基因则在基因序列的第154位发生单核苷酸变异[14,68]。白种人的COMT基因在氨基酸序列的108位和158位常发生氨基酸替换。在这两个替换位点上, 编码Met和Val的氨基酸的频率相当。而东亚人中编码Val的等位基因更为常见, 因此东亚人COMT的低活性频率低于白种人[69]。TPMT基因型与表型之间存在一致性, 基因缺陷患者在接受常规药物治疗时可能会出现危及生命的药物毒副作用[14,68]。例如, 给予泰国儿童标准剂量的巯嘌呤用于急性淋巴细胞白血病的临床治疗时, 与TPMT表达正常的患者相比, TPMT*3C基因型的患者可能出现严重的骨髓抑制。为此, FDA建议对患者进行酶基因检测, 以指导临床选择合适的药物剂量[70]。

MTs广泛存在于各种动物和细菌中。比较甲基化酶的种属差异不仅为非临床药效学和安全性评价提供选择动物种属和给药方案的依据, 还有助于正确利用动物实验数据预测药物的临床有效性和安全性, 为药物从动物实验走向人体试验提供科学依据。通过对细菌DNA MTs的氨基酸序列进行研究, 发现了一组保守序列, 这组序列在哺乳动物中也存在[14]。保守的序列和蛋白质结构揭示了SAM结合位点的保守性[14,71-75]。细菌的MTs主要与限制性内切酶系统和细菌耐药有关。MTs甲基化回文序列中的腺嘌呤可以保护DNA免受限制性内切酶的消解作用[14]。16S rRNA MTs及Erms酶能够甲基化细菌核糖体上特定的核苷酸, 改变药物作用的靶点, 使抗生素无法高效地结合于靶点发挥作用, 从而产生抗生素耐药性[71,73,75]。细菌耐药是抗感染治疗领域的重点关注内容, 通过研究细菌MTs的种类以及其参与耐药的机制, 可以为抗生素抗耐药研发提供理论基础。

啮齿动物, 包括小鼠和大鼠, 其TPMT和COMT的基因序列与人类相似[67,76]。具体而言, 小鼠TPMT基因序列与人类相似度80%, 并且具有与人类相同的起始和终止密码子[77]。不同品系的小鼠可能具有不同的TPMT活性, 而TPMT基因缺陷的小鼠则可能表现出TPMT活性降低或丧失。例如, C57/BL6小鼠的TPMT基因序列较野生型小鼠多出一段基因片段, 导致表达的TPMT不具有生物活性[63,77-79]。另外, 小鼠COMT并不存在Met突变, 但与野生型小鼠相比, Met-COMT转基因小鼠的酶活性较低[80]。这些发现为应用小鼠模型研究TPMT和COMT基因功能提供了重要依据。

狨猴MTs的基因序列和组织分布与人类高度相似, 因此可用于MTs药物代谢研究的模型构建[62,66,81]。人类和狨猴的TPMT和COMT基因序列相似度达到90%。狨猴TPMT基因位点与人类相似, 分别位于狨猴第4号染色体短臂和人类第6号染色体上的KDM1B和KIF13A基因组之间。另外, 狨猴COMT的第148位氨基酸为Leu, 与人类和恒河猴编码的Ile不同。这种序列差异可能导致COMT底物选择性存在种属差异[81]。与人类和恒河猴不同, 狨猴缺乏砷甲基转移酶, 无法对砷原子进行甲基化并将砷化物通过尿液排出体外。因此, 狨猴不适合作为砷甲基转移酶的研究模型[82,83]。在药物代谢研究中, 正确选择动物模型至关重要, 以便更准确地预测人体的药物代谢过程。

4 结语

MTs作为体内广泛分布的II相药物代谢酶, 对经其代谢的药物, 包括阿片类、儿茶酚类、含硫类及含氮类药物的体内过程和药理作用具有重要影响。尽管目前关于甲基化酶的研究不断增加, 但是仍需要进一步深入研究甲基化酶种属差异、药物与甲基化酶之间的相互作用以及疾病对甲基化酶活性的影响, 以便准确预测药物在临床的有效性和安全性, 并为药物从动物实验走向人体试验提供科学依据。

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