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灵长类胚外中胚层的起源、谱系特化及分子调控机制

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摘要 胚外中胚层(extraembryonic mesoderm, EXM)是哺乳动物早期胚胎发育中形成卵黄囊、尿囊、脐带及胎盘等胚外结构的关键谱系, 参与原始造血、血管发生及母胎界面建立。小鼠中EXM源自原条, 灵长类中EXM细胞在原条形成前已出现, 起源涉及滋养外胚层、上胚层、下胚层多种来源假说, 但目前仍缺乏直接证据。该文围绕EXM的起源、特化展开讨论, 系统梳理了灵长类EXM起源争议、谱系功能特化及分子调控。现有研究表明, EXM在啮齿类和灵长类中功能保守, 但发生时序与起源路径存在物种差异, 未来仍需加强灵长类EXM体内示踪、异质性解析及体外模型验证。

关键词 胚外中胚层; 细胞起源; 谱系特化; 分子调控; 灵长类

Origin, Lineage Specification, and Molecular Regulatory Mechanisms of the Primate Extraembryonic Mesoderm

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Abstract The EXM (extraembryonic mesoderm) is a key lineage in mammalian early embryonic development that gives rise to extraembryonic structures such as the yolk sac, allantois, umbilical cord, and placenta, and is involved in primitive hematopoiesis, vasculogenesis, and the establishment of the maternal-fetal interface. In mice, EXM originates from the primitive streak, whereas in primate, EXM cells appear prior to primitive streak formation. Hypotheses regarding the origin of EXM in primate involve multiple sources, including the trophectoderm, epiblast and hypoblast, but direct evidence remains lacking. This review focuses on the origin and specification of EXM, systematically summarizing the controversies surrounding EXM origin in primate, lineage functional specification, and molecular regulation. Current studies indicate that EXM functions are conserved between rodent and primate, but the timing of its emergence and the pathways of origin exhibit species differences. Future efforts are needed to strengthen *in vivo* lineage tracing, heterogeneity analysis, and *in vitro* model validation of EXM in primate.

Keywords extraembryonic mesoderm; cellular origin; lineage specification; molecular regulation; primate

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胚胎发育(embryonic development)是指受精卵逐步发育为具有基本形态结构个体的连续过程，涵盖受精(fertilization)、卵裂(cleavage)、胚层分化(germ layer differentiation)、原肠运动(gastrulation)以及器官发生(organogenesis)等关键阶段。在胚体本体发育推进的同时，羊膜、卵黄囊、尿囊、绒毛膜、脐带和胎盘等胚外结构也同步建立，并在营养物质交换、气体运输、机械力抵抗以及维持母胎界面稳定等过程中发挥重要作用。这些胚外结构并非胚胎发育的附属产物，而是支持胚胎正常发生和持续发育不可缺少的组成部分[1-2]。

在多种胚外组织的形成及功能特化过程中，胚外中胚层(extraembryonic mesoderm, EXM)广泛参与其中，并与原始造血、血管生成及母胎界面的建立密切相关[3-6]。尽管EXM在早期发育中具有重要地位，但灵长类相关研究长期以来仍相对有限。其原因主要来自两个方面，一方面，灵长类EXM最早出现于围着床时期(peri-implantation period)，该阶段样本获取困难，研究材料十分有限。另一方面，早期研究主要依赖形态学观察，能够提供的信息多局限于组织结构层面，缺乏用于准确认定细胞身份及解析动态发育过程的分子依据。由于EXM与胚胎中胚层(embryonic mesoderm, EM)及邻近胚外谱系在形态特征、出现时序以及部分分子标志上存在一定交叠，其谱系边界和细胞身份也因此难以被清晰界定[2,4-8]。

目前，对于灵长类EXM的起源、发育路径及其分子调控机制，仍缺乏系统而深入的认识。值得注意的是，啮齿类和灵长类EXM在形成方式和功能特征上并不完全保守[4]。小鼠作为哺乳动物发育研究中最常用的模式生物，虽然为EXM研究提供了重要基础，但其相关认识并不能简单外推至灵长类。近年来，随着胚胎体外延迟培养技术、单细胞测序技术和干细胞生物学的发展，研究者已在人和非人灵长类体内及体外模型中初步描绘出EXM或胚外中胚层样细胞(extraembryonic mesoderm-like cells, EXMCs)的分子特征。其中，EXMCs通常用于指代转录组特征和部分功能层面接近体内EXM的细胞群体。这些研究为进一步解析EXM的发生过程及其动态发育轨迹提供了新的研究视角[2,9-16]。

在本综述中，我们将围绕EXM的起源、特化展开讨论，系统梳理当前关于啮齿类和灵长类EXM起源、特化路径及分子调控机制的研究进展。同时结合现有证据，讨论EXM在早期胚胎发育过程中于原始造血、母胎界面建立、生殖细胞发育和营养运输等方面所发挥的关键作用。最后，我们对建立人和非人灵长类EXM培养体系并在体外模拟EXM发育过程的研究前景进行展望。

1 胚外中胚层的起源

1.1 胚外中胚层与胚胎中胚层的区分

经典胚胎学将EXM定义为位于胚体外部且不直接构成胚体本体的中胚层细胞群，主要参与卵黄囊、尿囊、羊膜及胎盘相关结构的形成[17]。与之相对，EM位于胚体内部，是心血管、肌肉、骨骼及多种器官原基的直接来源[1,18-19]。两类中胚层在空间定位及分化轨迹上存在明显差异，但在发生时序上彼此接近，且均与原肠运动及上皮−间质转化(epithelial-mesenchymal transition, EMT)过程密切相关[18-22]。

部分研究表明，EXM与EM在发育早期可短暂共享某些分子特征，并保留一定程度的命运可塑性[23-24]。这一现象提示，在早期发育过程中两类中胚层的命运可能并非完全分离，而是在一定阶段存在发育路径上的交汇，随后在空间位置、细胞行为及组织功能需求等因素的影响下逐步走向不同的命运分化方向。

近年来，多组学研究和功能实验进一步加深了对二者差异的认识。在小鼠中，Saykali等[25]结合活体成像和转录组分析发现，EXM更高表达中间丝相关成分，而EM则更高表达肌动蛋白相关成分。同时，EXM的迁移过程并不依赖Rho GTPases，提示这两类中胚层可能具有彼此不同的细胞骨架调控方式[25]。在此基础上，Nahaboo等[26]利用角蛋白报告小鼠及双光子活体成像进一步揭示，角蛋白8在EXM来源的羊膜、尿囊和血岛中形成跨细胞顶端连续分布的缆索样结构，其缺失会导致胚外膜扩张受阻。这些研究表明，在小鼠中，EXM的特化与一套相对独特的细胞骨架机制密切相关。然而，目前关于这类细胞骨架差异及其功能意义的研究仍主要集中于小鼠，尚缺乏在灵长类中的系统验证。

1.2 小鼠胚外中胚层的起源

1.2.1 *原条后端来源经典模型* 目前普遍认为，小鼠EXM主要起源于原条(primitive streak, PS)，即上胚层(epiblast, EPI)。经典胚胎学观察和谱系分析发现，在E6.5至E7.0阶段，PS后端可产生T^+(brachyury)

中胚层前体细胞。这些细胞经历EMT后, 向近端及胚外方向迁移, 随后进入卵黄囊、尿囊等区域, 参与多种胚外结构的形成[24,27-31]。与此相对, PS前部产生的另一部分新生中胚层则主要向胚内迁移。

遗传学与功能研究进一步支持了这一来源路径。克隆分析显示, PS后端EPI细胞能够贡献于多个胚外结构, 其分布特征与EXM的来源范围基本一致[24,29-30,32-34]。部分影响PS形成或后端结构稳定的基因异常也可同时干扰EM和EXM的产生, 例如, *Nr5a2*异常可同时阻断两类中胚层的生成[35]。这提示小鼠胚内/外中胚层虽然共同依赖PS发生, 但其后续命运特化仍受到空间位置及局部微环境的共同影响。另有研究显示, 对EPI特异性敲除*Itpr1/Itpr2*可引起尿囊和胎盘相关缺陷, 也进一步支持EXM与EPI之间存在直接的发育联系[36]。综合现有证据, 小鼠EXM主要来源于EPI经PS后端产生的新生中胚层, 仍是当前被广泛接受的经典模型。

近年的单细胞分析进一步表明, EXM自PS后端迁出的过程并非简单的单一路径分化。PS区域的新生中胚层内部已呈现出一定的转录异质性, 其中部分细胞较早富集FOXF1等与胚外命运相关的分子[5]。在更晚阶段, EXM与EM在转录层面的分离趋势进一步增强, 前者更倾向高表达*Hand1*、*Bmp4*、*Tbx3*等胚外发育相关的基因[25,37-38]。这些结果提示, 尽管小鼠EXM在来源路径上相对集中, 但其内部细胞群的命运分支可能早于传统形态学所能分辨的时间点。

1.2.2 *其他假说及其局限* 除PS来源外, 少数研究提出小鼠原始内胚层(primitive endoderm, PrE)的特定区域可能对EXM形成存在局部贡献。谱系追踪结果显示, 内脏内胚层(visceral endoderm, VE)中与PS相接触的区段可发生EMT, 并在后续为尿囊及胎盘的动脉血管系统提供部分细胞来源, 这一过程伴随Hedgehog信号调控以及T介导的PS延伸[39-40]。不过, 这类研究结果目前更多支持PrE在局部区域参与EXM形成或相关结构特化, 而尚不足以推翻小鼠EXM主要来源于PS后端这一经典模型。

总体来看, 小鼠EXM仍主要来源于PS后端产生的新生中胚层, 但在特定解剖区域内, VE可能以协同方式参与局部结构的形成或特化。至于这一模式在灵长类中是否同样成立, 现阶段仍缺乏充分证据, 尚有待进一步验证。

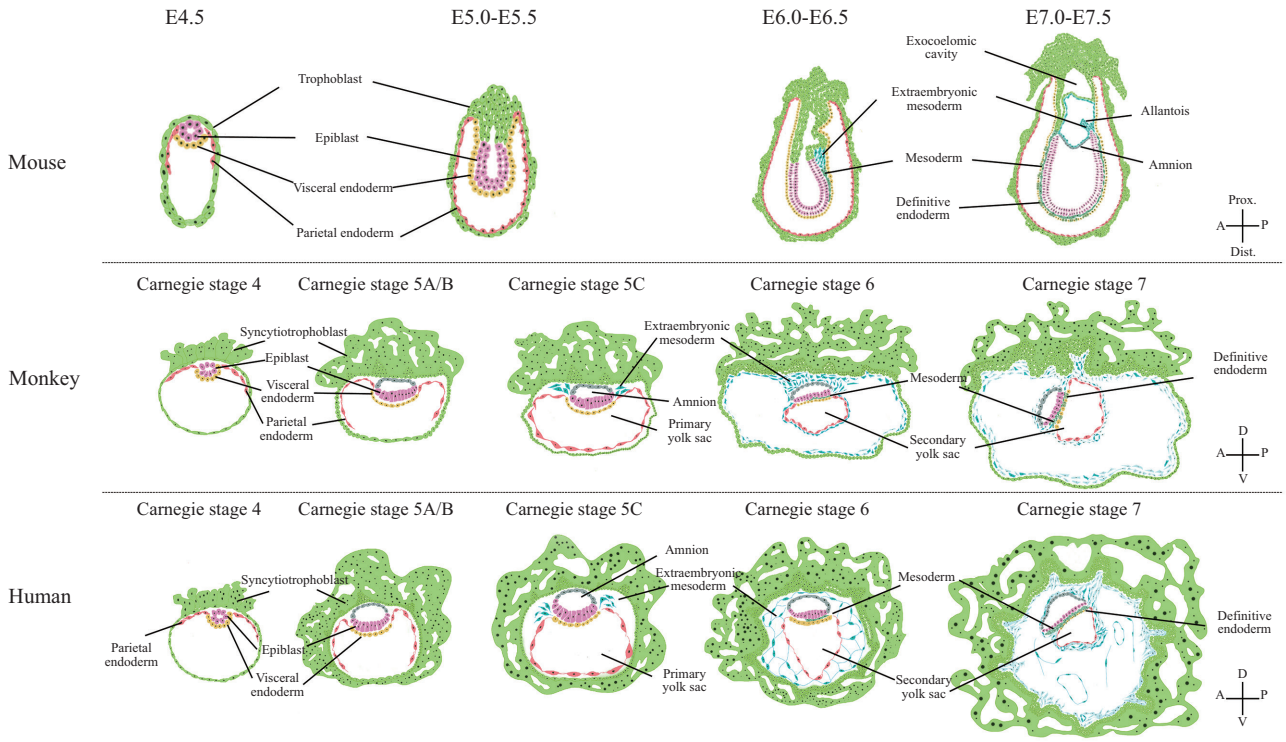
1.3 灵长类胚外中胚层的起源

与小鼠EXM主要来源于PS后端不同, 灵长类EXM的起源问题更为复杂。早期组织学研究报道, 在人类及非人灵长类胚胎卡耐基第5阶段(Carnegie stage 5, CS5)即可观察到EXM细胞, 此时PS尚未形成, 明显早于小鼠EXM的出现时序[3-4,6](图1)。这一时间特征提示, 小鼠EXM的形成模式不能简单外推至灵长类, 其细胞来源也仍有待进一步厘清。基于早期形态学观察, 灵长类EXM细胞多出现于EPI、下胚层(hypoblast, HYP)与滋养外胚层(trophectoderm, TE)交界区域(图1), 因此目前主要存在三类来源假说(图2), 即TE来源、EPI来源和HYP来源[3-5,41]。

1.3.1 *滋养外胚层来源假说及其证据局限* 早期研究曾提出, TE内层的细胞滋养层(cytotrophoblast, CTB)可能参与EXM形成[3,42]。这一观点主要建立在胚胎切片中两类细胞空间位置相邻以及早期形态学观察的基础上。后续超微结构研究发现, CTB与EXM细胞之间存在较为完整的基膜分隔, 二者并未显示出清晰的细胞连续性或直接过渡关系[3,42]。由于缺乏支持TE直接贡献于EXM的连续形态学证据, 这一假说在目前的主流讨论中总体仍被视为证据不足。不过, 近期也有研究提出, EXM样细胞与TE谱系之间可能拥有共同祖细胞, 从而使TE相关来源路径再次受到关注[43]。

1.3.2 *下胚层来源假说及其支持证据* 部分组织学研究发现, HYP衍生的壁内胚层(parietal endoderm, PE)在特定区域可见细胞分层现象, 少量细胞侵入滋养层与PE之间的间隙[1,4-5]。随后, 这群细胞进一步扩增, 并与PE细胞连续排列于初级卵黄囊内表面, 在形态上与PE较难明确区分。早期体内样本及延时培养胚胎的单细胞转录组分析显示, 部分EXM细胞表达*GATA4*、*GATA6*等HYP相关标志物, 而不表达典型EM标志物*T*、TE标志物*CDX2*以及EPI标志物*OCT4*, 提示EXM细胞群体基因表达特征与HYP群体更为接近[7,14]。基于体细胞突变的谱系追踪研究也为HYP来源假说提供了支持[44]。

在类囊胚延迟培养体系中, 研究者发现的EXMCs经单细胞测序分析后被归入HYP相关亚群, 提示其可能与HYP谱系有关。另外, 在naïve人多能性干细胞(pluripotent stem cells, PSCs)与过表达GATA6的primed PSCs共培养模型中, 同样可以观察到EXMCs的形成, 且这些细胞能够进一步分化为内



图中描绘了小鼠、猴、人胚胎植入后的结构形态和细胞类型空间分布。A: 前端; P: 后端; D: 背侧; V: 腹侧; Prox.: 近端; Dist.: 远端。

This figure depicts the structural morphology and spatial distribution of cell types after implantation of mouse, monkey and human embryos. A: anterior; P: posterior; D: dorsal; V: ventral; Pro.: proximal; Dist.: distal.

图1 小鼠、猴和人早期发育过程中关键细胞类型与胚胎结构模式图(根据参考文献[4,6]改绘)

Fig.1 Schematic diagram of key cell types and embryonic structures during early development of mouse, monkey, and human (adapted from references [4,6])

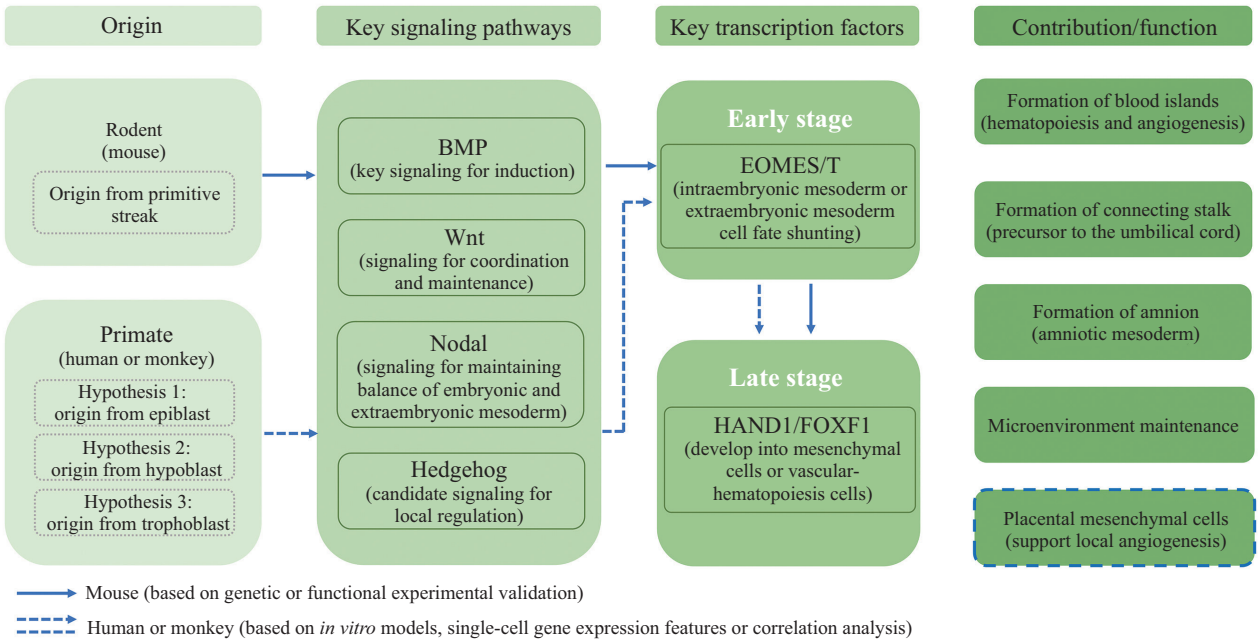
皮细胞及造血祖细胞, 从而为研究人类卵黄囊原始造血过程提供体外模型[45-46]。

综合来看, HYP来源假说同时获得了形态连续性、分子标志物相近性以及体细胞突变谱系关系等多方面证据的支持。尽管这些结果仍不能等同于体内直接谱系示踪, 但在现阶段的研究中, HYP来源假说具有较强的解释力。

1.3.3 上胚层来源假说及其支持证据 早期组织学研究曾将与PS后端相关的区域视为灵长类EXM候选来源之一, 并推测在PS形态出现之前, 胚盘相应区域可能已经形成一定的空间预分区或信号预设, 使部分EPI细胞获得EXM相关命运[5-6,47]。不过, 这一观点与灵长类EXM在PS形态出现前已大量存在的现象相悖, 因此其解释范围受到一定限制。

近年的体外模型研究为EXM起源于EPI提供了新的重要线索。naïve PSCs在转录组特征上对应植入前胚胎的上胚层, 在激活Wnt并抑制转化生长因子-β(transforming growth factor-β, TGF-β)及组蛋白去乙酰化酶的条件下, 可被诱导形成EXMCs, 且这些细胞与灵长类体内EXM表现出相似的转录特征[12,48-53]。primed PSCs则对应植入后的上胚层, 在同时激活BMP和Wnt信号的条件下, 同样可以被诱导形成EXMCs[54-55]。此外, Niu等[10]建立的CB(CHIR+BMP4)/CBA(CHIR+BMP4+Activin-A)培养体系可高效将naïve和primed状态的人PSCs诱导为EXMCs, 其分子表型与灵长类EXM高度接近。在狨猴naïve PSCs中, 研究者也在特定培养条件下获得了EXMCs[56]。这些结果共同表明, 不同发育阶段的EPI细胞均具备形成EXMCs的潜能, 从而为EXM起源于EPI的观点提供了较强的体外研究支持。

除此之外, 在非人灵长类体外延时培养胚胎中也可观察到少量EXMCs, 拟时序分析提示其可能来源于EPI[9]。嵌合体实验进一步发现, 胚盘周围存在一群COL6A1$^+$/OCT4$^+$双阳性细胞, 同时, 在naïve人PSCs与滋养层干细胞共培养形成的组装体中, 同样可产生EXMCs, 这些结果均提示EXM可能与上胚层来源相关[13,16]。另有研究表明, 通过激活人naïve PSCs中的Wnt、JAK/STAT3、BMP及mTOR通路获



图中概括了EXM潜在来源、关键信号通路、关键转录因子及其功能特化之间的关系。小鼠EXM主要来源于原条后端, 灵长类EXM则可能涉及上胚层、下胚层和滋养外胚层相关来源假说。BMP、Wnt、Nodal和Hedgehog等信号参与EXM命运建立与调控, 其中BMP更接近核心诱导信号, Wnt主要发挥协同和维持作用, Nodal更偏向谱系边界调节, Hedgehog目前更多体现为候选局部调控信号。EOMES/T与HAND1/FOXF1等转录因子可能参与中胚层进入、胚内/胚外分流、EXM身份塑造及功能特化过程。

The figure summarizes the relationships among the potential origin of EXM, key signaling pathways, key transcription factors, and their functional specialization. In mice, EXM mainly originates from the posterior primitive streak, whereas in primate, EXM may involve hypotheses related to epiblast, hypoblast and trophoblast origins. Signals such as BMP, Wnt, Nodal, and Hedgehog participate in the establishment and regulation of EXM fate. Among them, BMP acts more as a core inductive signal; Wnt primarily exerts synergistic and maintenance effects; Nodal tends to regulate lineage boundaries; and Hedgehog currently appears more as a candidate local regulatory signal. Transcription factors including EOMES/T and HAND1/FOXF1 may be involved in mesoderm entry, embryonic/extraembryonic segregation, EXM identity establishment, and functional specialization.

图2 EXM命运建立与功能特化的分子调控模式图

Fig.2 Molecular regulatory model of EXM fate establishment and functional specialization

得的EXMCs, 在与滋养层干细胞、下胚层干细胞及PSCs共培养后, 可形成模拟人植入后胚胎的三维结构, 这也进一步体现了上胚层来源的EXMCs在体外胚胎模型构建中的应用价值[48,57-58]。

总体来看, 与HYP来源假说相比, EPI来源目前更依赖于体外诱导体系、延时培养胚胎及嵌合体模型等间接证据。这些结果至少表明, EPI具备产生EXMCs的发育潜能, 因此现阶段EPI仍作为灵长类EXM的潜在来源之一。

1.4 起源路径的跨物种比较

从演化角度看, 灵长类与小鼠EXM的发育特化具有一定可比性, 但其具体形成路径受到物种特异性发育机制的影响, 因而呈现出明显的时序差异(图1)。

两者的EXM最终均承担支撑胚外膜、促进血管形成和原始造血及参与母胎界面建立等重要功能, 因此其形成过程都与中胚层相关程序、EMT过程以及胚外环境信号密切相关。不过, 在具体发生时序和来源路径上, 两者并不一致。小鼠EXM的出现与PS形成高度同步, 目前普遍认为其主要来源于PS后端。灵长类EXM则最早可见于PS形成之前, 其来源至今仍存在较大争议。与此同时, 关于EXM起源的研究证据在两类物种中的性质也并不相同, 小鼠相关认识主要建立在遗传学和功能实验基础上, 灵长类则更多依赖形态学观察、转录组学分析以及体外模型推断, 因此二者在证据强度和解释范围上存在明显差异。

综上, 灵长类EXM的发生路径与小鼠并不完全重合。现阶段, 转录谱相似性和基因组层面的遗传示踪尚不足以判定其谱系来源。EPI、HYP与TE相关发育路径在灵长类EXM形成中的具体贡献, 仍缺乏直接而有力的证据。

2 胚外中胚层的谱系特化与功能

EXM形成后, 并不会停留于均一的细胞状态, 而

是在不同时空信号的调控下进一步分化为多个功能相关亚群，参与卵黄囊、尿囊、绒毛膜等连接结构、羊膜及胎盘绒毛等胚外组织的建立[3-4,6,25-26]。从功能角度看，EXM的谱系特化包括：驱动卵黄囊形态发生和原始造血；参与母胎界面建立和结构连接；影响羊膜和原始生殖细胞(primordial germ cells, PGCs)的特化，最终为胚外膜系统与母胎界面稳定提供支架基础[43,59-61]。因此，EXM在早期发育中既是胚外结构的重要细胞来源，也是局部微环境和谱系互作网络中的关键参与者。

2.1 卵黄囊的形态发生与功能特化

啮齿类具有翻转卵黄囊，其并非短暂存在的过渡性结构，而是在整个妊娠过程中与尿囊绒毛膜胎盘协同发挥作用，共同构成母胎物质交换体系[4,62]。灵长类的情况有所不同，其早期可见初级卵黄囊，随后在胚外腔室重塑过程中被次级卵黄囊取代，而这一过程与EXM的形成和扩展密切相关[63]。尽管两类动物的卵黄囊在形态发生路径上存在明显差异，但EXM均直接参与了相关结构的建立，并对其后续功能分化具有重要作用。

卵黄囊是哺乳动物早期原始造血和血管发生的重要场所。既有研究表明，EXM衍生细胞可在此形成血岛，并进一步分化出内皮及造血相关成分[59,64-65]。在小鼠中，VEGFR2^{+}的EXM细胞可响应邻近内胚层提供的VEGF信号，从而支持早期血管网络和造血程序启动[66-67]。与此同时，*Flk1*、*Tie2*等基因的缺失均可导致血岛形成障碍，但具体表型并不完全相同。*Flk1*缺失更接近早期内皮分化程序的阻断，而*Tie2*缺失则更偏向血管成熟和稳定阶段的受损[66,68]。EPOR相关通路在原始红细胞的初始增殖与分化过程中具有功能性活性，*BMP*相关突变可导致EXM减少并伴随血岛缺陷[69-70]。同时，血管网络仅在同时含有EXM与VE的体系中才能稳定形成，这表明卵黄囊的形成和功能依赖于EXM的协同作用[67,71]。*Tal1*的增强子在EXM及造血位点中活性特征趋于一致，进一步提示原始造血与EXM之间具有紧密的协同性[72-73]。这些差异提示，EXM衍生细胞进入造血/血管形成相关谱系后，其后续分化可能受到不同层级的调控。

灵长类与小鼠跨物种单细胞比较进一步提示，在循环系统尚未建立之前，灵长类已出现相较于小鼠更早分化的髓系、红系和巨核系细胞群[74]。这些结果说明，卵黄囊相关EXM在不同物种中都承担血管和造血支持功能，但其内部细胞组成和发育阶段并不相同。这一差异提示，EXM在不同物种间可能指向相近的功能终点，却通过不完全相同的发育路径实现。

2.2 母胎界面相关结构中的EXM贡献

在小鼠发育过程中，PS后端产生的EXM向胚外区域迁移，形成尿囊，即未来连接胚胎与母体进行物质交换的脐带前体[24,30]。在E8.0~E8.5阶段，尿囊向外延伸并与绒毛膜融合，形成尿囊绒毛膜胎盘，标志着母胎循环系统的建立[75-76]。从尿囊形成到与绒毛膜融合，EXM构成了母胎界面连接结构的基本细胞来源。

在上述连接结构形成过程中，EXM的分子调控程序直接影响结构稳定性与血管网络建立。CUT&Tag分析揭示，*Isl1*在EXM与尿囊祖细胞中表达，*Isl1*缺失导致尿囊生长缺陷、尿囊与绒毛膜融合障碍及胎盘血管形态异常[60]。在尿囊与绒毛膜融合建立胎盘血管网络时，ZFP36L1精确控制EXM相关组织中VEGF-A蛋白产量，过量VEGF-A会扰乱血管生成信号，造成血管难以有效长入滋养层，导致胎盘构建失败与胚胎死亡[77]。EPI中特异性敲除*Itpr1*与*Itpr2*后出现尿囊绒毛膜胎盘缺陷与胚胎致死表型，进一步提示EXM对母胎界面建立的贡献不局限于连接结构本身，还体现在胎盘间质形成及血管整合过程中[36]。这些研究共同说明，EXM不仅提供结构支架，还通过调节血管生成与信号稳态确保母胎循环的功能建立。

在灵长类中，后期发育为脐带的体蒂，其间质成分主要来源于EXM[1]。这一结构对应小鼠尿囊的功能位置，承担胎儿来源血管与胎盘之间的连接任务。灵长类单细胞图谱研究进一步提示，EXM相关谱系与胎盘中的间质细胞、胎儿来源内皮细胞以及部分免疫微环境成分存在密切联系，霍夫鲍尔细胞、胎儿来源内皮细胞以及绒毛间质的形成，都依赖EXM衍生或其相关微环境的支撑[61]。相较于小鼠，灵长类母胎界面具有更高的结构复杂度，其滋养层侵袭方式和绒毛组织化程度也更高，因此EXM在灵长类中可能承担更复杂的间质整合与信号协调任务。这种差异并不意味着EXM基本功能的改变，而更可能反映同一谱系在不同物种中的适应性扩展。

总体而言，从小鼠到灵长类，EXM均参与连接结构建立与母胎界面血管整合过程，在胎盘间质形成、局部血管发育及界面稳态维持中发挥重要作用。但值得引起重视的是，不同物种中EXM的具体贡献

方式及其对应细胞谱系关系中存在的差异仍待进一步解析。

2.3 羊膜形成

羊膜由EPI来源的外胚层与EXM共同构成，EXM形成异常会影响羊膜的正常建成[78]。小鼠与灵长类在羊膜形成的形态学路径上存在差异。在小鼠的原肠胚形成早期，EXM与相邻外胚层共同形成后部褶皱结构，即羊膜绒毛膜褶[78-79]。在灵长类中，靠近极性滋养外胚层(polar trophectoderm, pTE)一侧的EPI与pTE间出现腔隙，并扩展为羊膜腔[80]。羊膜形成伴随着背腹轴对称性的打破，是早期形态发生的重要节点[78-79]。

*BMP2*基因在羊膜相关区域表达水平较高，其异常可能影响羊膜及相关胚外结构的正常发育。羊膜相关EXM中检测到*Tie2*而非*Tie1*的表达，提示羊膜相关的EXM分子特征与部分血管/造血相关细胞不同[81]。灵长类单细胞相关研究显示，EXM细胞中富集胰岛素样生长因子(insulin-like growth factor, IGF)相关转录本，而PS区域表达IGF受体，提示EXM可能通过生长因子相关信号影响PS区域的增殖状态或信号响应阈值，从而参与轴向模式建立[80-81]。在羊膜分化过程中，EXM不仅参与保护性膜结构的构建，也可能通过调节局部信号环境维持形态发生过程的稳态[78-79,81]。

2.4 微环境的建立及潜在影响

EXM的作用可能并不局限于其直接衍生结构，还延伸到对邻近谱系分化环境的塑造。小鼠PGCs最早在E7.25左右出现在PS后端附近区域，接近尿囊发育区[82-86]。在PGCs特化阶段，胚外区域表达BMP4、BMP8b等诱导因子，并受VE来源的BMP2协同增强。较高的BMP信号可诱导邻近上胚层细胞启动生殖系特化转录程序[87-89]。*BMP4*基因纯合缺失会导致胚胎缺乏PGCs，说明胚外信号在PGCs形成中具有重要作用[37,88-90]。

EXM本身并不直接转化为PGCs，但它通过维持诱导窗口期的信号环境和黏附机制，为PGCs生成提供支持，并影响其形成效率与空间定位[91-92]。与此不同，食蟹猴PGCs更倾向于起源于原肠前的新生羊膜细胞，而人类PGCs的发育起源仍未完全明确[93]。这一差异提示，EXM与邻近谱系的关系在不同物种中可能并不相同，其作用方式更接近间接支持和空间协同，而非单一的直接诱导场所。

在卵黄囊发育中，EXM不仅参与血岛形成和早期血管相关细胞分化，也可能构成支持造血和血管发生的局部微环境。EXM来源的间质细胞与邻近内胚层、血岛及新生内皮结构在空间上紧密相邻，这种排布为内皮前体和造血前体的聚集、迁移与分化提供了间质支撑。同时，卵黄囊血管网络的形成依赖EXM与内胚层之间的相互作用，涉及VEGF、FLK1、TIE2、BMP及TAL1等分子调控轴，这些通路与血岛形成、内皮分化和原始造血启动密切相关[59,64-73]。因此推测，卵黄囊EXM可能通过提供结构支架、调节局部细胞黏附状态以及整合内胚层来源的旁分泌信号，共同塑造血液−血管发生的微环境，不过这仍需空间组学、谱系示踪和功能干预实验进一步验证。

就现有证据而言，EXM对羊膜、卵黄囊、PGCs及其他邻近谱系的影响更倾向于微环境塑造和信号诱导，而不是直接起决定作用。从卵黄囊间质、尿囊组织到胎盘间质等多个功能亚群来看，EXM谱系特化并非单一路径，而是在与TE、HYP、EPI谱系衍生物的持续互作中，对不同微环境作出响应，逐步完成功能特化[60-61,94]。同时，由EXM分泌的信号分子所构成的局部微环境，也会进一步影响邻近谱系的特化。

3 命运决定和功能特化的分子调控机制

小鼠的遗传和功能分析为EXM的机制调控的解析提供了重要支持。相比之下，目前灵长类EXM起源仍缺乏直接的体内谱系追踪证据。现有认识主要建立在形态学观察、标志物表达分析、体细胞突变谱系推断、转录组轨迹分析以及体外模型模拟(表1)等研究基础之上[9-10,13,16,45-46,48,54-57,95-102]。这也提示不同信号通路对EXM命运的调控具有较高复杂性，而体外模型可在一定程度上弥补体内证据的不足(图2)。

3.1 核心信号通路

3.1.1 BMP信号：EXM诱导与命运建立的核心通路

BMP信号是早期胚胎发育中的关键通路之一，参与前后轴(anterior-posterior axis, AP)建立、中胚层诱导及胚外组织特化[87,103]。在小鼠原肠运动过程中，BMP4表达于PS后部及EXM相关区域，并参与EM与EXM命运边界的建立。

在EXM研究中，BMP信号是证据最为充分的上游通路之一。在小鼠中，BMP4在PS后部和胚外相关区

表1 胚外中胚层相关主要体外模型概览

Table 1 Overview of major *in vitro* models for extraembryonic mesoderm

模型类别/具体模型 Types of model/specific model	种属 Species	细胞初始状态 Ground state of cell line	方式 Method
2D model	Mouse	EpiLCs	Direct induction[95]
	Mouse	EpiSCs	Direct induction[96]
	Human	primed PSCs	Direct induction[10,54,55,97]
	Human	naïve PSCs	Generation during conversion from naïve hPSCs to hTSCs[48]
	Human	naïve PSCs	Direct induction[10]
	Non-human primate	naïve PSCs	Direct induction[56]
Embryo-like assembloids	Mouse	PSCs	Derivatives of stem cell-derived embryo models[98]
	Mouse	PSCs, TSCs, Gata4-expressing PSCs	Derivative of embryo-like assembloids[15,99]
	Human	naïve PSCs, EXMCs, hypoSCs, TSCs	Derivative of embryo-like assembloids[13,45,57]
Embryoid bodies	Mouse	naïve PSCs, induced PSCs	Derivative of embryoid bodies[100]
	Human	primed PSCs	Direct induction[101]
Inducible embryoid bodies	Human	PSCs, iPSCs, GATA6-SOX17 inducible cells, GATA3-AP2γ inducible cells, GATA6-overexpressed PSCs	Derivative of inducible embryoid bodies[46,102]
Chimera	Human	EPSCs	Derivative of human-primate chimeric embryos[16]
Long-term *in vitro* culture	Non-human primate	Cynomolgus monkey embryos	Long-term *in vitro* culture[9]

EpiLCs: 上胚层样细胞; EpiSCs: 上胚层干细胞; PSCs: 多能性干细胞; TSCs: 滋养层干细胞; EPSCs: 扩展多能性干细胞; EXMCs: 胚外中胚层样干细胞; hypoSCs: 下胚层干细胞; iPSCs: 诱导多能性干细胞。

EpiLCs: epiblast-like cells; EpiSCs: epiblast stem cells; PSCs: pluripotent stem cells; TSCs: trophoblast stem cells; EPSCs: extended pluripotent stem cells; EXMCs: extraembryonic mesoderm-like cells; hypoSCs: hypoblast stem cells; iPSCs: induced pluripotent stem cells.

域高表达, 其缺失可导致PS后部结构异常、EXM减少、血岛形成受阻, 以及尿囊和PGCs相关缺陷[70,87,89]。这说明BMP不仅与EXM形成相关, 也影响其后续功能特化。体外研究显示, 在人PSCs分化体系中, BMP4处理可高效诱导EXMCs形成, 并在与Wnt信号协同作用时进一步提高诱导效率[10,48]。总体来看, BMP在小鼠和灵长类EXM中均处于其命运诱导的上游位置, 但其具体作用仍受到培养条件、其他信号诱导及物种背景的共同影响。

3.1.2 Wnt信号: 命运维持 Wnt信号通路在早期胚胎中参与细胞增殖、极性建立和中胚层诱导[104-106]。在EXM调控中, Wnt在小鼠PS后端与中胚层产生密切相关, 在人EXMCs体系中可单独或与BMP共同提高EXM诱导效率[10,13,101]。这提示Wnt可能进一步增强中胚层向胚外方向发育的潜能, 但并非单独决定EXM命运。

3.1.3 Nodal信号: 谱系边界调节 Nodal是TGF-β超家族成员, 在原肠运动中参与维持PS、诱导三胚层形成, 并参与胚胎左右不对称性的建立[107-110]。在EXM研究中, Nodal信号更像一个谱系边界调节器。小鼠实验表明, Nodal活性异常并不简单阻断EXM, 而是通过改变PS形成、局部信号阈值及胚内−胚外分支平衡, 间接影响EXM发生[111-112]。在体外分化体系中, 当BMP存在时, 较低Nodal活性更利于胚外偏向, 而较高Nodal活性则更倾向推动胚胎中胚层分化[107]。在人EXMCs模型中, 特定条件下抑制Nodal并联合激活mTOR、Wnt和BMP, 有助于EXMCs身份建立与维持, 而在激活BMP和Wnt信号通路时, 激活Nodal信号又可抑制其向羊膜样细胞(amnion-like cells)分化, 将其命运推向EXM[10,13,48]。因此, Nodal并非单向的促进或抑制信号, 而是通过限定谱系发生窗口参与EXM命运决定过程。

3.1.4 其他潜在相关信号 Hedgehog信号在脊椎动物胚胎中主要参与前后轴模式形成、神经管背腹分化及肢体发育等过程, 其经典作用是通过浓度梯度调控细胞命运决定的[113-116]。近年来, 该通路也被纳入EXM调控的讨论之中。

小鼠相关的研究提示, VE局部区域可发生Hedgehog依赖的EMT, 并与母胎界面局部结构形成有关[39]。*Zfp568*相关突变也会同时影响*Ihh*表达和EXM形态发生[117]。在灵长类方面, 也有研究提出VE来源的Ihh信号可能与EXMCs特化相关[118]。这些结果表明, Hedgehog通路并非与EXM毫无关联, 但现阶段更适合将其视为候选调控信号, 而不是与BMP、Nodal处于同等证据层级的核心通路。

总体来看, 调控EXM命运的核心信号通路存在层级关系。BMP信号更靠近上游, 在EXM诱导和胚外命运建立中起核心作用, 这一点在小鼠体内和灵长类体外模型中均已反复验证。Wnt信号主要起协同和稳定作用, 可能推动中胚层程序向胚外方向倾斜。Nodal信号则偏向调节谱系边界, 其活性变化影响胚内与胚外中胚层分支的平衡, 但这种作用受BMP、Wnt及具体培养体系的共同影响。Hedgehog信号的证据目前相对有限, 更倾向于与局部EMT相关。因此, EXM命运的建立是多信号共同作用下的动态过程, 而非单一通路的线性决定。需要指出的是, 上述信号调控在小鼠和灵长类之间可能存在一定保守性, 其中灵长类证据主要来自体外诱导体系、单细胞表达特征和相关性推断, 体内因果验证仍需加强(图2)。

3.2 核心转录因子网络

EXM命运建立并非由单一转录因子线性驱动, 而更像是由不同层级因子连续接力完成的过程。部分因子主要作用于进入中胚层程序和完成胚外分支初步分流的早期阶段, 另一些因子则更多参与EXM身份塑造、亚群特化及后续功能维持。

3.2.1 EOMES与T: 中胚层进入及早期命运分化 EOMES和T是新生中胚层发生的重要转录因子[23,119]。结合小鼠体内与体外模型证据, EOMES对卵黄囊相关EXM的形成具有明显调控作用, 但对尿囊相关EXM的形成并非绝对必需, 这提示不同EXM衍生结构对同一因子的依赖程度存在差异[119]。T突变可造成体蒂发育异常和迁移障碍, 而EOMES与T同时缺失则可显著阻断卵黄囊与尿囊中EXM的特化[119]。单细胞轨迹分析还显示, 部分EXM前体在短暂表达T后会转入HAND1、FOXF1高表达状态, 提示T参与决定细胞进入中胚层程序及后续胚外分支选择的早期过程节点[23]。总体来看, EOMES和T在小鼠EXM中的表达和功能已较为明确, 但其在灵长类EXM中的作用仍缺乏系统研究。

3.2.2 HAND1、FOXF1: 胚外命运塑造与功能分化 如果说EOMES与T主要参与中胚层程序的启动和早期分流, 那么HAND1和FOXF1则更接近EXM身份塑造与功能分化阶段。HAND1在胎盘与卵黄囊间质中高表达, 其在EXM中的功能缺失被认为是HAND1完全敲除致死的重要原因之一[38,120]。在人干细胞模型中, HAND1是EXMCs特化的重要调控因子, 缺失HAND1表达的细胞更倾向于滞留在滋养层谱系[43]。在小鼠中, FOXF1参与侧板中胚层及EXM分化, 并调控血管生成相关基因, 其缺失可导致卵黄囊与尿囊血管发生受损、绒毛尿囊融合失败以及羊膜表达血管与造血标志物异常[121-122]。在灵长类单细胞数据中, EXM或EXMCs特异表达*HAND1*、*FOXF1*相关基因, 提示灵长类EXM在基因表达层面与小鼠EXM保持一致, 但HAND1、FOXF1在灵长类EXM中发挥的调控作用是否具有保守性仍需实验来验证[16,48,96,98]。

从现有证据看, EXM相关的转录因子网络存在层级性和功能分工(图2)。EOMES和T参与中胚层程序的早期启动, 并影响胚内与胚外中胚层命运决定。HAND1和FOXF1则更靠近EXM身份塑造、间质特化及血管发生相关功能的维持阶段。小鼠遗传学为这些因子功能提供了较强的因果证据, 灵长类EXM转录因子网络的全局结构及上下游调控关系, 目前仍主要基于表达相关性和体外模型推断, 有待高时空分辨率数据和功能实验进一步验证。

4 总结与展望

综合现有研究, 从啮齿类到灵长类, EXM均为支撑胚外系统建立的动态谱系, 而非均一、静态的细胞群。从功能层面看, 小鼠和灵长类EXM均与多个谱系互作, 参与卵黄囊建成、血管与原始造血启动、连接结构形成以及母胎界面整合, 提示其核心生物学任务具有相当程度的保守性[2,25]。从调控层面看, BMP、Nodal、Wnt等信号, 以及EOMES、T、HAND1、FOXF1等转录因子, 也在两类动物或其体

表2 啮齿类与灵长类胚外中胚层的多维度比较

Table 2 Multidimensional comparison of extraembryonic mesoderm between rodent and primate

比较维度 Dimension of comparison	啮齿类 Rodent	灵长类 Primate
The first occurrence	Accompanied by PS formation[18,21,24,30]	Before PS formation; around CS5[3-4,6]
origin	Posterior of PS[24,27-31]	Controversy still exists, and no direct lineage-tracing evidence; originate from epiblast, hypoblast or trophoblast have each been put forward[3-5,7,14,41,44,48-56]
Contribution and function	Involved in extraembryonic structure formation; promotes blood island formation, primitive hematopoiesis, and vasculogenesis; modulates the formation of adjacent lineages, including PGCs, via local signals and microenvironmental cues[24,30,36,59-60,67,75-77,82-92]	Involved in extraembryonic structure formation; promotes blood island formation, primitive hematopoiesis, vasculogenesis, and placental mesenchymal integration; exert a stronger coordinating function in orchestrating the complex villous architecture and the local microenvironment[4,43,61,74,93-94]
Regulatory mechanism	The regulatory activities of signaling pathways (e.g., BMP, Wnt, Nodal) and transcription factors (e.g., EOMES, T) have received support from studies conducted at the embryonic sta ge[23,38,70,87,89,107,111-112,119-122]	BMP, Wnt, and Nodal signaling are repeatedly validated in EXMC *in vitro* induction systems and are closely linked to EXM fate specification; HAND1 and FOXF1 are also found in EXM/EXMCs *in vivo*. Nevertheless, most current data rely on *in vitro* systems, single-cell expression, and correlations, leaving *in vivo* causal evidence notably lacking[10,13,16,43,48,54-58,101]
Maturity of *in vitro* model	Genetic manipulation of embryos *in vivo*, live imaging, 2D differentiation, embryoid bodies, and stem cell-based embryo models are now relatively mature, enabling a robust combination of *in vivo* phenotypes and mechanistic investigations[15,25,95-96,98-100]	A range of models has been established, such as time-lapse cultured embryos, naïve/primed PSC-derived EXMCs, assembloids, induced embryoid bodies, and human-monkey chimeras. Although the repertoire of available models is now more varied, the direct correspondence to *in vivo* EXM, lineage fidelity, and functional integrity remain to be rigorously validated[9-10,13,16,45-46,48,54-58,101-102]

外模型中反复出现，提示EXM命运决定机制中存在一定的共同分子框架[10,13,23,38,43,48,70,89,101,111-112,117,119-120]。不过，目前两类动物EXM的机制研究主要围绕信号通路和转录因子展开，表观遗传修饰层面存大量空白等待解析。但在细胞起源层面，功能和调控层面的保守性并不意味着完全相同的路径。小鼠EXM更多表现为PS后端新生中胚层向胚外区域分配的结果，而灵长类EXM则更早出现，可能存在更复杂的起源，体外模型的细胞来源也同样更为复杂和多样[1-4,6,18,21,123]。

目前两个物种EXM的研究，小鼠主要基于胚胎，灵长类往往局限于体外模型，灵长类EXM在不同类型证据之间尚未形成足够清晰的因果链。小鼠EXM的起源、迁移与功能特化已有较好的体内研究支撑。灵长类缺乏直接的体内谱系示踪和高时空分辨率验证。现有形态学、单细胞和体外模型结果只可作为进一步探索EXM的提示，小鼠的研究也不可完全推至灵长类中。灵长类EXM来源、细胞亚群仍需直接示踪和多组学技术的结合进行验证，体外EXMCs与体内EXM对应关系需要真实的体内数据作为对照，从而构建更高保真度的体外模型，进一步用于EXM调控机制的验证。同时，高保真的EXMCs模型的发展也将为胚胎模型的胚外谱系正确建立提供新的线索(表2)。

EXM与胎盘发育、血管生成、原始造血等过程密切相关，深入理解EXM的形成机制、功能特化及其精细调控机制，可能为多种发育性疾病提供潜在的治疗靶点，并为早期诊断和治疗提供新思路[36]。此外，EXM的DNA修复能力与其他谱系存在差异，这为部分先天异常与DNA修复缺陷病提供了新的研究视角[124]。

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