

蚊虫血餐前后转录组分析研究进展

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摘要:吸血是蚊虫传染疾病的主要途径。吸血前后, 蚊虫体内的代谢途径会发生一系列变化。转录组分析可以从整体水平上反映细胞中基因表达情及调控规律。特对蚊虫吸血前后基因表达及代谢途径等变化进行综述。

关键词: 蚊虫; 转录组; 血餐

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Research advances on the analysis of blood-induced transcriptome changes in mosquitoes

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ABSTRACT: Hematophagy, blood-feeding, is a behaviour exhibited by most arthropod vectors of human pathogens. Hematophagous mosquitoes can transmit many diseases by blood-feeding. It causes a large number of deaths each year all over the world. The female generally feeds to repletion on a single blood meal and then proceeds to use this nutrition as the basis for the development of batch of eggs. A series of changes such as gene expression may occur after blood meal. Availability of several mosquitoes' genome sequences provides unique opportunities to study different aspects of biology, including identification of genes and pathways relevant to the developmental processes of individual life stages. However, development of novel vector control strategies requires a thorough detailed understanding of mosquito biology. Transcriptome analysis is a recently developed approach to transcriptome profiling that uses deep-sequencing technologies. It provides a comprehensive understanding of gene expression and its regulation. It also provides a far more precise measurement of levels of transcripts and their isoforms than any other methods. Transcriptome analysis has been successfully used in several mosquitoes and also achieved much useful information. Females of non-blood-feeding and blood feeding differ in many morphological, behavioural, and physiological traits, largely caused by differences in gene expression. The expression patterns of different genes are discussed in the context of female mosquitoes' physiological responses to blood feeding, including blood digestion, peritrophic matrix formation, egg development, and immunity. The changes of gene expression expose the main molecular differences between non-blood-feeding female and blood-feeding female. The present paper would summarize the current status of the transcriptome changes in mosquitoes induced by blood-feeding. It provides a valuable resource for the future studies.

KEY WORDS: mosquito; transcriptome; blood meal

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蚊虫不仅刺叮骚扰, 而且是登革热、黄热病、淋巴丝虫病、西方马脑炎、西尼罗热、疟疾等多种虫媒传染病的传播媒介。蚊虫具有兼吸人、鸟、兽等多种宿主血液的习性, 这种嗜血习性使原本储存在动物宿主中的病原体通过蚊虫的“桥梁媒介”传播到人群

中。复杂的嗜血习性正是非自育性蚊虫成为多种疾病传播媒介或潜在传播媒介的根本原因。

近年来,基因组学、蛋白质组学、转录组学等组学技术发展迅速,并且对揭示细胞生理活动规律发挥了重要作用,其中转录组学是最早发展起来且应用最为广泛的技术。转录组是指特定组织或细胞在某一发育阶段或功能状态下转录出来的所有 RNA 的总和,包括 mRNA 和非编码 RNA (non-coding RNA, ncRNA)^[1]。研究血餐前后转录组的变化,不仅可以明确蚊虫吸血前后的分子差异,量化转录本在血餐前后表达水平的变化,还可以基因功能和结构为出发点,揭示基因表达与特定代谢途径间的内在联系,从而为寻找防治蚊虫的新靶标,开辟新的蚊虫治理途径提供理论依据。

1 血餐引起的代谢变化

吸血前,雌蚊一般靠吸食糖水来维持生命力。据报道,吸食糖水的雌蚊中有 28 个特异表达基因,产物以基础代谢为主,如肽酶、蛋白酶等。吸血后,雌蚊会经历一系列生理变化,饱血作为一个信号,会刺激雌蚊中肠蛋白质的水解活性,有近 80% 的蛋白质在一天内会被消化^[2-5]。这些基因表达的变化反映了蚊虫从吸食糖水到吸血过程中转录水平的转变。从糖代谢到蛋白质代谢,蚊虫需要一定的过渡时间,冈比亚按蚊(*Anopheles gambiae*)血餐后在红细胞膜中发现一定数量的糖蛋白,血餐后 6 h 编码糖蛋白的基因上调,在血餐后 12~24 h 才缓缓下调,说明血餐后一段时间还在对糖类进行代谢。不同吸血时间和吸血后不同时段冈比亚按蚊转录组数据表明,在血餐后开始的几个小时氧化磷酸化、碳水化合物代谢、蛋白质代谢、核酸代谢转运活跃,其中有 11 个基因参与蛋白质消化(包含 5 个水解蛋白基因、2 个胰蛋白酶、1 个糜蛋白酶、1 个丝氨酸蛋白酶、1 个羧肽酶)^[6]。埃及伊蚊(*Aedes aegypti*) 在血餐后上调的基因产物富集量大于 50 倍的转录本有 21 个,其中 15 个与消化酶有关。冈比亚按蚊血餐后有 40 个特异的差异表达基因,包括中肠的 2 个丝氨酸蛋白酶和胰蛋白酶(Trypsin), trypsin1 和 trypsin2 均是在血餐后被诱导出来的。实验证明, trypsin2 mRNA 在蚊虫血餐后 8, 12, 24 h 富集^[7-8]。与 trypsin1, 2 不同, trypsin3, 4, 7 在未吸血雌蚊中就表达, trypsin4 在血餐 48 h 后接近生殖营养末期才大量富集。有假说认为 trypsin3, 4, 7 的表达在某种程度上诱导了 trypsin1 和 2 的表达。在蚊虫中肠可以检测到糜蛋白酶 1, 2, 其在血餐 12 h 后表达,并

在 48 h 富集量大增,这与 trypsin3-7 类似^[9]。磷脂酶被认为在血液消化中起一定作用,埃及伊蚊在血餐 15 h 后中肠的解脂活性开始增强,但其功能还未被证实^[10]。

2 血餐引起的卵巢发育

卵巢周期分为 4 个阶段:1)卵黄生成前期,2)启动期,3)营养期,4)营养后期^[11]。对于非自育性蚊类来讲,雌蚊在血餐后 48 h 会将 20% 的营养转化为卵子成熟所需成分。事实上,雌蚊一羽化,卵子生成就随之启动,血餐时卵母细胞的发育会暂时滞缓,直到重启阶段卵巢会继续发育直到卵荚成熟被排出。成功的产卵过程不仅需要一系列卵巢发育过程和卵子成熟,还需要卵黄的合成。将冈比亚按蚊血餐后不同时期的差异表达基因分为前期基因(early gene)、中期基因(middle gene)和后期基因(late gene),中期基因在血餐后前 3 h 下调,血餐后 5 h 缓慢上调,在 12~24 h 内有高峰,而后下调至血餐后 48 h;后期基因在血餐后 12~16 h 表现出持续缓慢下调,与中期基因不同的是其在 48 h 富集量大增。有趣的是,中期基因和后期基因的表达曲线分别与蜕皮激素和保幼激素的分泌曲线类似,与卵巢发育和卵黄生成相关的基因正是属于中期和后期基因。电镜下观察致倦库蚊(*Culex quinquefasciatus*)血餐后不同时间点的卵巢切片,血餐后 12 h 才可见蛋黄素颗粒,直至 72 h 逐渐降解^[12],进一步证实卵黄生成相关的基因表达模式属于中后期基因。正是随着保幼激素和蜕皮激素的分泌,雌虫体内的代谢随之发生了变化,从而促进卵巢发育和卵黄的成熟。在冈比亚按蚊中编码激酶锚定蛋白的基因在血餐后 5 h 才上调,在 12~24 h 富集量最大,该基因在卵子生成中通过调节激酶 C 蛋白的活性调控卵子生成过程。但还存在一些功能尚不明确的基因,如冈比亚按蚊的 AS453 基因,该基因在血餐后 48 h 才大量表达,有调节卵子生成的作用,但是具体功能尚不得而知。

3 血餐诱导的免疫反应

吸血是蚊虫携带的病原体在脊椎动物间传播的最主要途径。血液作为雌蚊营养物质来源的同时,也会对雌蚊产生影响,病原体在雌蚊体内会和机体环境维持一定的平衡关系。埃及伊蚊存在两个不同的内在免疫防御机制:一是利用基因表达调控及通过小 RNA 调控途径的(Small RNA regulatory pathways, SRRPs) mRNA 降解,二是通过 Toll、

Imd 和 JAK-STAT 信号途径诱导产生抗菌多肽或促进吞噬作用、包浆和黑化作用^[13]。已有文献报道了血液中含有的不同病原体感染埃及伊蚊后上述通路中基因活性的变化^[14-20]。在侵染的数分钟内,雌蚊体内免疫基因便会开始转录,但是这些反应缺乏免疫记忆。血餐后,在病毒粒体和蚊虫中肠上皮细胞接触的 5~7 min 内,病毒就会被内化,从而开始转录,在感染后 2 d,病毒便开始自我复制增殖^[21]。有学者认为在雌蚊体内存在天然的细菌群落,并且维持一定水平的免疫应答,免疫过程与消化过程共享一些生物产物。

据统计,共有 477 个转录本是防御机制中的候选基因或假定的候选基因,167 个在血餐前后差异表达,其中 88 个下调,79 个上调。一些基因编码接收器和免疫联级效应器(清除剂受体、Clip-丝氨酸蛋白酶、肽聚糖识别蛋白、纤维蛋白原相关蛋白、C-凝集素、1,3-β-d 葡聚糖结合蛋白和抗菌多肽)^[22],这些基因在血餐后表达量超过 2 倍地下调。上调的基因包括 sRRP 成员的 MD-2 类似蛋白,MD-2 蛋白是一种分泌蛋白,包含了脂类识别域,在免疫反应中起到重要作用。G12 蛋白被认为在免疫应答中发挥重要功能。在冈比亚按蚊饱食疟原虫感染的血液 1 h 后,中肠内腔或在表面的微绒毛上可以检测到 G12 蛋白,在血餐 12 h 后其表达量达到最大。将感染西尼罗病毒的血液喂食致倦库蚊,也发现蚊虫在血餐后 G12A2 的表达量增加^[23]。

自噬是一种利用溶酶体对自身结构吞噬降解的过程,是细胞内的再循环系统,在维持体内平衡、细胞发育、成长、免疫中扮演重要角色,是一种高度调节、异化的过程。涉及到自噬、SRRP 通路和细胞凋亡的转录本在血餐后也大量富集。埃及伊蚊雌蚊血餐后 5 h,自噬作用的基因表达量大增,最大富集量可达 3.10 倍,富集基因还包括 17 个 SRRP 成员, Dicer1, Dicer2 基因, TSN 和 PiWi14, 这些基因转录本在血餐后以 2 倍富集。Dicer2 和 TSN 基因是 RNA 干扰效应器和诱导 RNA 完全沉默的共同组成成分, Dicer1 调控管家基因的表达, PIW14 是 PIWI 小 RNA 途径的成员,与抗病毒防御机制有关^[24]。但相比参与消化代谢的转录本来说,与免疫相关的转录本累积水平较低,大多以 2 倍量富集,这个发现证实了蚊虫在吸血后免疫阶段会出现一个间隔期的假说。血餐后免疫基因表达量的低下有利于病原体侵染宿主蚊虫,如登革热病毒在数分钟内便可以侵染埃及伊蚊中肠上皮细胞。

4 血餐调控的嗅觉基因

蚊虫通过嗅觉系统感受宿主呼吸以及粘液和皮肤汗液等散发的气味来寻找定位宿主,其中气味结合蛋白(odorant-binding protein, OBP)和气味受体(odorant receptor, OR)作用最关键,它们参与了蚊虫对宿主气味分子的识别过程。分析吸血和和只吸食糖水的埃及伊蚊转录组,发现有 5 个气味结合蛋白基因只在吸食糖水的雌蚊中特异性表达,这些基因可能只在寻找糖水来源才发挥作用,但其是否在寻找非血源食料中发挥作用尚不清楚。对埃及伊蚊不同发育时期的转录组进行分析,发现气味结合蛋白相关的基因只在成虫期表达,这证实了搜寻宿主等行为只发生在成虫^[25]。血餐前,嗅觉基因表达量增加,用于寻找定位宿主,吸血后,蚊虫体内消化等其他代谢途径增强,在这一生殖营养周期中完成寻找宿主和吸血后,嗅觉基因的表达量便下调。

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