

蛔虫全发育阶段单细胞图谱揭示了调控蛔虫行为的关键的温度感受器 *Twk-24a*

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摘 要:【目的】蛔虫的宿主范围涵盖人类在内的绝大多数脊椎动物，其卵及幼虫能在适宜温度的刺激下迅速启动发育随后感染宿主，环境温度 ($\geq 25\text{ }^{\circ}\text{C}$) 是诱导幼虫发育的关键因素，但蛔虫感知温度信号并调控幼虫行为的分子机制尚不清楚，阐明该机理可为建立针对蛔虫早期传播的温度防控策略提供新的分子靶点。

【方法】本研究通过单细胞测序技术构建蛔虫休眠期、八个发育阶段（一细胞至 L2/L3 期幼虫）、体内感染阶段（L4 期）以及成虫全组织的发育图谱。通过注释得到胚胎各细胞群及分化细胞类型。从典型的温度变化期前后，即由休眠期 ($< 15\text{ }^{\circ}\text{C}$) 至发育早期 ($\geq 28\text{ }^{\circ}\text{C}$) 阶段的细胞类型中鉴定差异表达的基因集，利用电生理实验测定目标基因温度敏感性，识别蛔虫温度感受器。使用 RNAi 等手段使用饲喂法对 L2 期幼虫进行功能实验验证，明确在不同温度条件下温度感受器基因沉默是否会影响二期幼虫对不同温度的反应，其活力及发育速率是否发生显著变化。

【结果】本研究过滤得到共计 233,826 个细胞，注释得到 58 种不同的细胞类型或状态，并通过拟时序分析绘制得到蛔虫早期胚胎的分化轨迹表明原肠胚和 L2 幼虫期是器官发生和感染的关键时期。根据温度变化期 ($4\text{ }^{\circ}\text{C} - 30\text{ }^{\circ}\text{C}$) 差异表达分析，基因功能富集和电生理实验验证，鉴定得到蛔虫温度敏感型离子通道基因 *Twk-24a*，其温度敏感范围为 $25\text{ }^{\circ}\text{C} - 37\text{ }^{\circ}\text{C}$ 。通过设计 *Twk-24a* 基因的 dsRNA 探针对 L2 期幼虫进行 RNAi 实验表明 *Twk-24a* 抑制能够有效降低幼虫活力。

【结论】本研究通过构建蛔虫单细胞全发育图谱阐明蛔虫各发育阶段的基因转录谱，筛选与鉴定得到蛔虫温度敏感型离子通道基因 *Twk-24a* 并揭示了其在高温条件下对蛔虫发育的重要意义。进一步实验表明，抑制该基因可导致幼虫对温度变化的敏感性显著降低，同时伴随着生存能力下降和潜在感染能力的丧失，阐明了蛔虫“环境信号-感受器-幼虫行为”发育过程具体的生物学意义，对研究蛔虫的环境适应性具有重要价值。本研究也为以温度为手段防控蛔虫感染提供了一个潜在有效的分子靶点，为全球范围内控制土源性寄生虫感染提供了重要参考。

关键词: 土源性寄生虫；蛔虫；单细胞测序；温度感受器

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Single-Cell Atlas of the Whole Developmental Stages of *Ascaris suum* Unveils Critical Thermosensory *Twk-24a* Regulating Worm Behavior

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Abstract: 【Objective】The host range of *Ascaris*, encompasses most vertebrates, including humans. Their eggs and larvae can rapidly initiate development and subsequently infect hosts upon stimulation by appropriate temperatures. Environmental temperature ($\geq 25\text{ }^{\circ}\text{C}$) serves as a crucial factor in inducing larval development. However, the molecular mechanisms underlying how *Ascaris* sense temperature signals and regulate larval behavior remain unclear. Elucidating these mechanisms could provide novel molecular targets for establishing temperature-

based control strategies aimed at preventing early transmission of *Ascaris*.

【Method】 This study employs single-cell sequencing technology to construct a developmental atlas of the *Ascaris suum*, including its dormant phase, eight developmental stages (from one-cell to L2/L3 larvae), the in vivo infection stage (L4 larvae), and adult tissues. Through annotation, various cell populations and differentiated cell types in the embryo were identified. A set of differentially expressed genes was determined by comparing cell types before and after typical temperature fluctuations, specifically from the dormant phase ($< 15\text{ }^{\circ}\text{C}$) to the early developmental stage ($\geq 28\text{ }^{\circ}\text{C}$). Electrophysiological experiments were conducted to assess the temperature sensitivity of target genes, leading to the identification of *A. suum* temperature sensor. Functional experiments using feeding methods and RNA interference (RNAi) on L2 larvae were performed to verify whether silencing temperature sensor affects the response of L2 larvae to different temperatures, as well as their vitality and developmental rate under varying thermal conditions.

【Result】 In this study, a total of 233,826 cells were filtered and annotated, resulting in the identification of 58 distinct cell types or states. Pseudotime analysis was conducted to map the differentiation trajectories of the early embryonic development in *A. suum*, revealing that the gastrula and L2 larval stages are crucial periods for organogenesis and infectivity. Differential expression analysis across a temperature between $4\text{ }^{\circ}\text{C}$ and $30\text{ }^{\circ}\text{C}$, combined with gene function enrichment and electrophysiological experiments, led to the identification of the temperature-sensitive ion channel gene *Twk-24a*, which exhibits temperature sensitivity within the range of $25\text{ }^{\circ}\text{C}$ to $37\text{ }^{\circ}\text{C}$. RNA interference (RNAi) experiments targeting the L2 larval stage using dsRNA probes designed for the *Twk-24a* demonstrated that inhibition of *Twk-24a* effectively reduced larval viability.

【Conclusion】 This study elucidates the gene transcription profiles of whole developmental stages of *A. suum* by constructing a comprehensive single-cell developmental atlas. Through screening and identification, the temperature-sensitive ion channel gene *Twk-24a* in *A. suum* was isolated and its crucial role in *A. suum* development under high temperature conditions was revealed. Further experiments demonstrated that inhibiting *Twk-24a* significantly reduced the larvae's sensitivity to temperature changes, accompanied by decreased survival ability and loss of potential infectivity. It elucidates the specific biological significance of the *A. suum*'s developmental process involving "temperature-sensor-larval behavior" which holds significant value for studying the environmental adaptability of *Ascaris*. This study also provides a potentially effective molecular target for the prevention and control of ascariasis through temperature manipulation, offering crucial insights for the global management of soil-transmitted helminth infections.

Key words: Soil-transmitted helminth; *Ascaris suum*; Single-Cell sequencing; temperature sensor.