

# 肿瘤移植裸鼠粪类圆线虫感染病原和分子诊断

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**【摘要】 目的** 肿瘤移植裸鼠粪类圆虫病诊断。**方法** 肿瘤移植裸鼠的尸体解剖显微镜检查发现类圆线虫作形态学鉴定并采用二重 PCR 进行分子诊断。**结果** 尸检发现肿瘤移植裸鼠样本中出现大量粪类圆线虫, 初步确诊粪类圆线虫病, 二重 PCR 检出样本中粪类圆线虫特定 DNA 进一步确诊。**结论** 防止这种严重后果是早期诊断。肿瘤移植受体和供体应进行寄生虫感染包括类圆线虫病筛查。本研究第一个广泛报道了肿瘤移植裸鼠粪类圆虫病诊断。

**【关键词】** 粪类圆线虫; 裸鼠; 诊断

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## Pathogenic and molecular diagnoses of *Strongyloides stercoralis* infection in tumor transplanted nude mice

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**【Abstract】 Objective** To diagnosis tumor transplanted nude mice Strongyloidiasis. **Methods** Postmortem microscopic examination of the tumor transplanted nude mice detected *Strongyloides stercoralis* for morphological identification and double polymerase chain reaction (PCR) assay for molecular diagnosis of *Strongyloides stercoralis* infection in tumor transplanted nude mice. **Results** Presence of numerous *S. stercoralis* in autopsy in tumor transplanted nude mice samples preliminary determined the diagnosis of strongyloidiasis. Confirmed diagnosis of *Strongyloides stercoralis* infection by double PCR detection of specific DNA in tumor transplanted nude mice samples. **Conclusion** The most important clue to prevent such serious consequences is early diagnosis. Tumor transplanted recipients and donors should be screened for parasitic infections including strongyloidiasis. To the authors' knowledge, this study is the first extensive report on diagnosis tumor transplanted nude mice Strongyloidiasis.

**【Key words】** *Strongyloides stercoralis*; Nude mice; Diagnosis

粪类圆线虫 (*Strongyloides stercoralis*) 是一种自生和寄生世代交替的兼性寄生虫, 也是一种机会致病性肠道线虫。机体感染后, 成虫寄生于消化道, 当宿主免疫力正常时, 该虫经自身感染方式造成长

期寄生可达数十年之久而不表现明显致病症状, 但当宿主免疫功能低下时, 幼虫可侵入肺、肝、脑、肾等器官, 引起粪类圆线虫病 (strongyloidiasis), 甚至死亡<sup>[1-4]</sup>。至今尚未见肿瘤移植裸鼠粪类圆线虫感

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染的报道。本文首次对肿瘤移植裸鼠粪类圆线虫感染进行了病原和分子诊断。现报告如下。

## 1 材料和方法

### 1.1 样本来源

肿瘤移植裸鼠(不明原因突发死亡)来自中国某药物研究机构。

### 1.2 病原诊断

将肿瘤移植裸鼠剖检发现:结肠水肿,收集水肿液多达 2 mL。肉眼可见空肠内有数条细小线虫在内容物中蜿蜒游动。取结肠水肿液、空肠内容物涂片,实时动态显微视频摄录结果。

### 1.3 二重 PCR 分子诊断

针对粪类圆线虫 *act-2* 和 *btub* 基因设计引物。SSact-2F: 5'-GATTATCGTAGTGAGAGGACGTGTAGAGATAATAAAA-3'; SSact-2R: 5'-CGGCAACCTCTTCGTCACACATTTTCTATAGCAAGT-3'; 片段长度 1183 bp。SSbtubF: 5'-ATGAGAGAAATTGTTTCACGTCCAAACCGGCAATGT-3'; SSbtubR: 5'-TTTTTTTTTTTTTTTTGCAAGATAATAGTTTAAT-3'; 片段长度 1460 bp。收集肿瘤移植裸鼠结肠水肿液、空肠内容物中粪类圆线虫,用德国 Qiagen 公司的 QIAamp DNA Mini Kit 试剂盒,按说明书操作抽提 DNA。以抽提的 DNA 为模板,同时用刚地弓形虫、贾第鞭毛虫、蠕形住肠线虫 DNA 作为粪类圆线虫二重 PCR 扩增时的阴性对照。PCR 总反应体系为 50  $\mu$ L,包括 10  $\times$  Buffer (Mg<sup>2+</sup> + Plus) 5.0  $\mu$ L、dNTP Mixture 4.0  $\mu$ L、正反向引物(20 pmol/ $\mu$ L)各 1.0  $\mu$ L、EX Taq (5U/ $\mu$ L) 0.5  $\mu$ L、模板 DNA 1  $\mu$ L、nuclease-free water 37.5  $\mu$ L。循环参数:95 $^{\circ}$ C 1 min 1;95 $^{\circ}$ C 15 s,55 $^{\circ}$ C 15 s,72 $^{\circ}$ C 30 s,35 个循环;72 $^{\circ}$ C 7 min 1 个循环。琼脂糖凝胶电泳检测 PCR 扩增产物。

## 2 结果

### 2.1 病原学诊断结果

2.1.1 肿瘤移植裸鼠结肠水肿液涂片镜检结果:采集肿瘤移植裸鼠结肠水肿液低速离心,取适量沉淀用生理盐水直接涂片,实时动态显微视频摄录结果:可见数条异常活跃、呈蛇形浮游的线虫幼虫,呈 C 型或反 S 型,虫体外观无色透明,头端钝圆,尾部尖细,长约 0.3 mm,具有双球型咽管(图 1)。依据虫体形态,鉴定为粪类圆线虫杆状蚴<sup>[5-6]</sup>。

2.1.2 肿瘤移植裸鼠空肠内容物涂片镜检结果:取

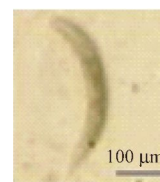


图 1 肿瘤移植裸鼠结肠水肿液中粪类圆线虫杆状蚴

Fig. 1 Rhabditoid larva of *Strongyloides stercoralis* in tumor transplanted nude mouse's colon edema fluid

肿瘤移植裸鼠空肠内容物,用生理盐水直接涂片,实时动态显微视频摄录结果:可见大量无色透明、运动活泼、呈蛇形游走样线虫虫体,长约 2.2 mm,宽 0.03 mm,虫体半透明,体表具细横纹,口腔短,咽管细长,约为体长的 1/3(图 2),尾端尖细,末端略呈锥形,生殖器官为双管型,肛门位于距尾端 1/3 处的腹面(图 3)。依据虫体形态,鉴定为粪类圆线虫寄生世代雌虫<sup>[5-6]</sup>。



图 2 肿瘤移植裸鼠空肠内容物中粪类圆线虫寄生世代雌虫头端

Fig. 2 The head end of parasitic female of *Strongyloides stercoralis* in tumor transplanted nude mouse's jejunum contents

### 2.2 分子生物学诊断结果

在粪类圆线虫感染肿瘤移植裸鼠结肠水肿液、空肠内容物样本中均检测出粪类圆线虫 *act-2* 和 *btub* 基因特异性 DNA 片段(大小分别为 1183 bp 和 1460 bp),而作为阴性对照的刚地弓形虫、贾第鞭毛虫、蠕形住肠线虫却未观察到目的基因片段扩增。肿瘤移植裸鼠粪类圆线虫二重 PCR 扩增产物琼脂糖凝胶电泳结果见图 4。粪类圆线虫二重 PCR 扩增获得了清晰特异的 *act-2* 和 *btub* 目的条带,分子诊断结果确诊了肿瘤移植裸鼠粪类圆线虫感染。

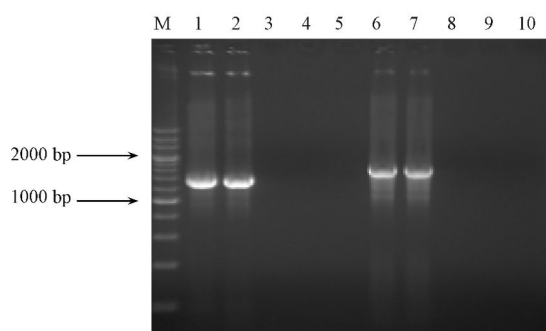
## 3 讨论

粪类圆线虫最先由 Normand 于 1876 年在越南



图 3 肿瘤移植裸鼠空肠内容物中粪类圆线虫寄生世代雌虫尾端

Fig. 3 The tail end of parasitic female of *Strongyloides stercoralis* in tumor transplanted nude mouse's jejunum contents



注:泳道 1-2:粪类圆线虫感染裸鼠结肠水肿液样本 act-2 基因特异性 PCR 产物;泳道 6-7:粪类圆线虫感染裸鼠空肠内容物样本 btub 基因特异性 PCR 产物;泳道 3-5 和泳道 8-10:阴性对照:刚地弓形虫、贾第鞭毛虫、蠕形住肠线虫。

图 4 粪类圆线虫感染裸鼠结肠水肿液和空肠内容物样本二重 PCR 扩增 act-2

和 btub 基因特异性 DNA 产物琼脂糖凝胶电泳

Note: Lanes 1-2: The act-2 gene specific PCR products of tumor transplanted nude mice colon edema fluid samples infected with *Strongyloides stercoralis*; Lanes 6-7: The btub gene specific PCR products of tumor transplanted nude mice jejunum contents samples infected with *Strongyloides stercoralis*; Lanes 3-5 and Lanes 8-10: negative control: *Toxoplasma gondii*, *Giardia lamblia*, *Enterobius vermicularis*; M: 200 bp DNA marker ladder.

Fig. 4 Agarose-gel electrophoresis of the double PCR products amplified by primers targeted the act-2 and btub genes specific DNA of genomic DNA extracted from tumor transplanted nude mice colon edema fluid and jejunum contents samples infected with *Strongyloides stercoralis*

时又在肠道、胆管、胰管中发现许多线虫,但形态与粪便中的虫体不同。后经众多学者的不断研究后认为其为同一虫种的不同发育阶段,并对其生活史及侵入人体的途径和在人体内的移行有了进一步的研究。恶性肿瘤、器官移植、造血干细胞移植、HIV 感染、使用皮质类固醇药物增加粪类圆线虫机会性感染的风险,未及时发现治疗者死亡率较高<sup>[7-12]</sup>。

本研究首次对肿瘤移植裸鼠粪类圆线虫感染进行了病原和分子诊断。作者首先在肿瘤移植裸鼠结肠水肿液和空肠内容物直接涂片中发现了粪类圆线虫的幼虫(杆状蚴)和成虫(雌虫),此类报道实属罕见。首次应用实时动态显微视频摄录技术跟踪监测寄生虫,获得了粪类圆线虫幼虫和成虫典型特征形态及独特运动方式的宝贵视频资料。应用二重 PCR 技术,从分子水平上对肿瘤移植裸鼠感染的粪类圆线虫进行多基因特征确认,为快速准确鉴别诊断粪类圆线虫感染提供新的思路。

裸鼠先天性无胸腺,缺乏免疫反应,是免疫缺陷动物,类圆线虫病的发生可能没有任何症状。本研究中的肿瘤移植裸鼠,可能是因为肿瘤的移植加剧了免疫功能的受损,从而激发了潜在的粪类圆线虫自体加速传染扩散传播,也可能是因为移植了污染有粪类圆线虫的肿瘤而高度感染。因此,在肿瘤移植手术前期,一定要对供体肿瘤和受体裸鼠进行粪类圆线虫等病原体筛查检测,以保障质量和安全。

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