·基础研究·

结核分枝杆菌Rv2647蛋白对肺组织损伤效应的初步研究

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[摘　要]　**目的**:通过噬菌体重组技术分别构建结核分枝杆菌Rv2647基因的敲除株和回补株以及过表达Rv2647的耻垢分枝杆菌(*Mycobacterium smegmatis*,Ms),评估结核分枝杆菌Rv2647蛋白对模型鼠肺组织的损伤效应。**方法**:构建同源交换位点并整合到结核分枝杆菌噬菌体基因组,获取噬菌粒并将其导入Ms,构建具有同源交换位点的重组噬菌体。体外扩增获得高滴度重组噬菌体并转染结核分枝杆菌(H37Rv),37 ℃静置培养28 d,挑取单克隆进行PCR验证,获得Rv2647基因敲除株(H37RvΔRv2647)。PCR扩增Rv2647基因并通过无缝克隆分别将其整合到载体pMV361和pMV261多克隆位点处,获得阳性质粒后分别电转化H37RvΔRv2647和Ms,获得结核分枝杆菌回补株(H37RvΔRv2647::Rv2647)及过表达Rv2647的耻垢分枝杆菌(Ms::Rv2647)。分别以H37Rv、H37RvΔRv2647、H37RvΔRv2647::Rv2647、Ms及Ms::Rv2647的菌液经气管攻击C57BL/6小鼠,分别比较H37Rv(30 d)与Ms(7 d)模型鼠的存活率、肺组织细菌负荷及肺组织病理损伤程度。**结果**:PCR结果显示,H37RvΔRv2647中Rv2647基因缺失,而H37RvΔRv2647::Rv2647及Ms::Rv2647中Rv2647基因皆存在。H37RvΔRv2647、H37Rv及H37RvΔRv2647::Rv2647组模型鼠30 d存活率分别为100.00%、83.33%及83.33%;Ms和Ms::Rv2647组模型鼠的7 d存活率分别为100.00%和83.33%;H37RvΔRv2647组模型鼠肺组织细菌负荷(lgCFU)为(3.40±0.18),显著低于H37Rv组(3.86±0.15,$P < 0.001$)和H37RvΔRv2647::Rv2647组(3.80±0.16,$P < 0.01$);H37RvΔRv2647组模型鼠肺组织炎症面积(%)为(4.37±3.06),显著低于H37Rv组(62.76±14.24,$P < 0.001$)和H37RvΔRv2647::Rv2647组(67.37±0.45,$P < 0.001$);Ms组模型鼠肺组织lgCFU为(2.53±0.16),显著低于Ms::Rv2647组(2.81±0.13,$P < 0.01$);Ms组模型鼠肺组织炎症面积(%)为(5.71±1.29),显著低于Ms::Rv2647组(33.13±13.84,$P < 0.05$)。**结论**:成功构建了结核分枝杆菌Rv2647基因敲除株(H37RvΔRv2647)及回补株(H37RvΔRv2647::Rv2647)以及过表达Rv2647的耻垢分枝杆菌(Ms::Rv2647)。Rv2647蛋白可能通过抑制宿主对结核分枝杆菌的清除,加重了模型鼠肺组织病理损伤。

[**关键词**]　结核分枝杆菌;差异区域;Rv2647蛋白;噬菌体重组技术;肺组织损伤

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Preliminary study on the effect of *Mycobacterium tuberculosis* Rv2647 protein on lung injury

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[**Abstract**]　**Objective:** To constract the Rv2647-deleted strain and Rv2647-complemented strain of *Mycobacterium tuberculosis*(*M. tb*) and Rv2647-overexpressing *Mycobacterium smegmatis*(Ms::Rv2647) by phage recombination technique, and to evaluate the effect of

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M. tb Rv2647 protein on lung injury in model mice. **Methods:** A homologous exchange site was constructed and integrated into the phage genomes of *M. tb*, producing phagemids that were introduced into *Mycobacterium smegmatis* (Ms) to create a recombinant phage with a homologous exchange site. High-titer recombinant phages were amplified *in vitro* and transfected into *M. tb* (H37Rv), followed by static culture at 37 ℃ for 28 d. The single clone was selected and verified by PCR, yeilding the Rv2647 - deleted strain (H37RvΔRv2647). The Rv2647 gene was amplified by PCR and was integrated into the multiple clone sites of vector pMV361 and pMV261 through seamless cloning to obtain the positive plasmids, which were transfected into H37RvΔRv2647 and Ms to obtain the Rv2647 - complemented strain (H37RvΔRv2647: : Rv2647) and Rv2647 - overexpressing Ms (Ms: : Rv2647), respectively. The suspension of H37Rv, H37RvΔRv2647, H37RvΔRv2647: : Rv2647, Ms, and Ms: : Rv2647 were used to infect C57BL/6 mice via tracheal injection. The survival rates, lung tissue bacterial load, and pathological damage of lung tissue in model mice were compared at 7 d and 30 d, respectively. **Results:** The PCR showed that Rv2647 gene was missing in the H37RvΔRv2647, while it was present in the H37RvΔRv2647: : Rv2647 and Ms: : Rv2647. The 30-day survival rates of model mice infected with H37RvΔRv2647, H37Rv, and H37RvΔRv2647: : Rv2647 were 100.00%, 83.33%, and 83.33%, respectively; The 7-day survival rates of model mice infected with Ms and Ms: : Rv2647 were 100.00% and 83.33%, respectively. The lung bacterial load (lgCFU) of model mice in the H37RvΔRv2647 group (3.40±0.18) was significantly lower than that of the H37Rv group (3.86±0.15), $P < 0.001$) and the H37RvΔRv2647: : Rv2647 group (3.80±0.16), $P < 0.01$); The inflammation area (%) in lung tissues of the H37RvΔRv2647 group (4.37±3.06) was significantly lower than that of the H37Rv group (62.76±14.24), $P < 0.001$) and the H37RvΔRv2647: : Rv2647 group (67.37±0.45), $P < 0.001$). The lung bacterial load (lgCFU) of the Ms group (2.53±0.16) was significantly lower than that of the Ms: : Rv2647 group (2.81±0.13), $P < 0.01$); The inflammation area (%) in lung tissue of the Ms group (5.71±1.29) was significantly lower than that of the Ms: : Rv2647 group (33.13 ± 13.84), $P < 0.05$). **Conclusion:** The Rv2647 - deleted strain (H37RvΔRv2647) and Rv2647 - complementary strain (H37RvΔRv2647: : Rv2647) of *M. tb* and Rv2647-overexpressing Ms (Ms: : Rv2647) were successfully constructed. Rv2647 protein may aggravate lung injury *via* inhibiting host clearance of *M. tb*.

[**Key words**] *Mycobacterium tuberculosis*; regions of difference; Rv2647 protein; phage recombination technique; lung injury

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结核病是结核分枝杆菌(*Mycobacterium tuberculosis*, *M. tb*)导致的严重传染性疾病,目前仍在全球范围内广泛流行。在感染宿主的过程中,*M. tb*可通过进化方式逃避宿主免疫反应使之长期存活[1]。卡介苗(bacillus calmette-guerin, BCG)是目前唯一可用的*M. tb*疫苗,对儿童播散性结核病可发挥有效预防作用,但对成人的长期保护效应有限[2]。比较基因组学研究发现,与野生株相比,BCG丢失了100多个基因编码序列,即所谓差异区域(regions of difference, RD),其包含了16个区域(RD1-RD16)[3]。研究表明,RD可通过编码潜在效应蛋白在结核病发病过程中发挥重要作用[4-5]。有关RD13区Rv2647基因编码蛋白对宿主的致病性研究既往未见报道。本研究运用噬菌体介导的基因重组技术分别构建了H37Rv的Rv2647基因敲除株(H37RvΔRv2647)和回补株(H37RvΔRv2647: : Rv2647);Ms被广泛用于表达分枝杆菌蛋白,是研究*M. tb*致病机制的良好模型[6]。Rv2647基因在Ms中缺失,本研究也构建了过表达Rv2647的Ms(Ms: : Rv2647);通过构建*M. tb*和Ms攻击模型评估Rv2647蛋白对模型鼠的肺组织损伤效应,为深入研究该蛋白在*M. tb*对宿主致病性中的作用奠定基础。

1 材料和方法

1.1 材料

胰蛋白胨、酵母提取物、琼脂粉(北京索莱宝生物科技公司);氯化钠、甘油(上海生工生物工程有限公司);Middlebrook 7H9、Middlebrook 7H10 及OADC(BD公司,美国);潮霉素B(hygromycin B, HYG)、Tween-80(Sigma公司,美国);Trans2KPlusII DNA Marker、Trans15K DNA Marker(北京全式金生物技术有限公司);T4 DNA连接酶、限制性内切酶*Pac* Ⅰ、Phusion High Fidelity DNA Polymerase(Thermo Scientific公司,美国);质粒提取试剂盒(北京天根生化科技有限公司);胶回收试剂盒、细菌基因组提取试剂盒(Omega公司,美国);MaxPlax™ Lambda噬菌体包装提取物(Epicentre生物技术公司,美国)。

1.2 方法

1.2.1 P0004S-ΔRv2647质粒构建

以结核分枝杆菌H37Rv全基因组DNA为模板,

根据Rv2647基因序列，构建Rv2647基因的上游序列（左臂，L）及下游序列（右臂，R），分别设计左臂上、下游引物（LFP、LRP）、右臂上、下游引物（RFP、RRP），即Rv2647-LFP（5′-TTTTTTTTCACAAAGTGGACTCCCTGCCTAAGGTGCG-3′），Rv2647-LRP（5′-TTTTTTTTCACTTCGTGGCGTGTTTTCGGAGCGTT-3′），Rv2647-RFP（5′-TTTTTTTTCACAGAGTGGACAACGCAACCCGCAGC-3′），Rv2647-RRP（5′-TTTTTTTTCACCTTGTGCCGGACAGGCCGAGTTTG-3′）。通过聚合酶链反应扩增Rv2647。使用限制性内切酶*Van*91 Ⅰ酶切P0004S质粒及Rv2647的左臂、右臂，连接酶切后的片段并导入DH5α感受态细胞，挑取单克隆菌落，抽提质粒并验证。

1.2.2　PhAE159-ΔRv2647穿梭质粒构建

Pac Ⅰ酶切P0004S-ΔRv2647质粒和PhAE159质粒并连接。使用噬菌体包装试剂盒构建PhAE159-Rv2647穿梭质粒。转化HB101感受态细胞，挑取单克隆菌落抽提质粒并验证。

1.2.3　筛选噬菌斑

PhAE159-ΔRv2647穿梭质粒电转入Ms感受态细胞（电击参数：电压2.5 kV，电阻1 000 Ω，电容25 μF），加入Middlebrook 7H9（HYG，75 μg/mL），37 ℃培养过夜后，菌液与适量top agar混匀并铺于Middlebrook 7H10（HYG，75 μg/mL），30 ℃培养2~3 d后筛选噬菌斑。

1.2.4　噬菌体扩增

适量高滴度噬菌体裂解液与生长至对数期的分枝杆菌（MP buffer预先洗涤）混匀，37 ℃孵育过夜，离心弃上清，加入适量Middlebrook 7H9（HYG，75 μg/mL），37 ℃孵育过夜，离心弃上清液，收集菌体涂布于Middlebrook 7H10（HYG，75 μg/mL），37 ℃培养28~42 d。

1.2.5　Rv2647基因敲除株（H37RvΔRv2647）的构建

高滴度噬菌体感染H37Rv后，涂布于Middlebrook 7H10（HYG，75 μg/mL），37 ℃培养28 d后，挑取单克隆接种于Middlebrook 7H9（HYG，75 μg/mL），37 ℃培养28 d后，提取基因组，PCR验证（图1）。

1.2.6　Rv2647基因回补株（H37RvΔRv2647::Rv2647）的构建

PCR扩增Rv2647基因，限制性内切酶*Eco*R Ⅰ

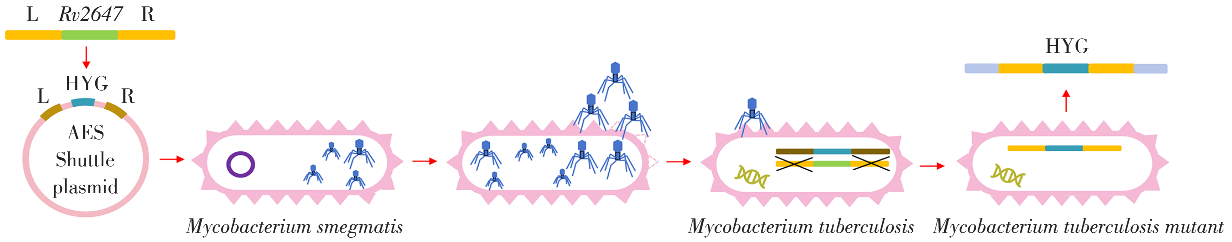


图1　*M. tb*的Rv2647基因敲除株构建示意图

Figure 1　Schematic diagram of Rv2647-deleted strain of *M. tb*

和*Hind* Ⅲ酶切Rv2647质粒和pMV361质粒，连接酶切产物，并将重组质粒导入DH5α感受态细胞，挑取单克隆测序验证pMV361-Rv2647质粒。取适量pMV361-Rv2647质粒电转化H37RvΔRv2647感受态菌株，于Middlebrook 7H10（HYG，75 μg/mL，卡那霉素，30 μg/mL）培养28 d后，挑取单克隆接种于Middlebrook 7H9液体培养基（HYG，75 μg/mL，卡那霉素，30 μg/mL），37 ℃培养28 d后，收集菌体并进行PCR验证（图2）。

1.2.7　过表达Rv2647的耻垢分枝杆菌（Ms::Rv2647）的构建

PCR扩增Rv2647基因，回收目的DNA片段与线性化的双酶切pMV261质粒（*Eco*RI和*Hind* Ⅲ）重组并转化DH5α感受态细胞，挑取单克隆测序验证pMV261-Rv2647质粒。取适量pMV261-Rv2647质粒电转至Ms感受态细胞，涂布于7H10固体培养基（卡那霉素，30 μg/mL），37 ℃培养3~4 d，挑取单克隆接种至7H9液体培养基（卡那霉素，30 μg/mL），37 ℃培养3~4 d，收集菌体，PCR验证。

1.2.8　Rv2647蛋白对肺组织病理损伤效应的体内研究

分别用H37Rv、H37RvΔRv2647和H37RvΔRv2647::Rv2647（$2×10^6$ CFU/只）以及Ms和Ms::Rv2647（$1×10^7$ CFU/只）气管攻击C57BL/6小鼠[7-8]，腹腔注射三溴乙醇麻醉小鼠并固定于操作台，充分消毒后切开颈部皮肤，钝性分离气管旁肌肉组织，暴露气管，使用1 mL注射器通过气管注射上述菌液，注射结束后立即竖直放置小鼠，轻轻摇晃，缝合颈部皮肤后再次消毒。监测模型鼠30 d（H37Rv、H37RvΔRv2647及H37RvΔRv2647::Rv2647）和7 d（Ms和Ms::

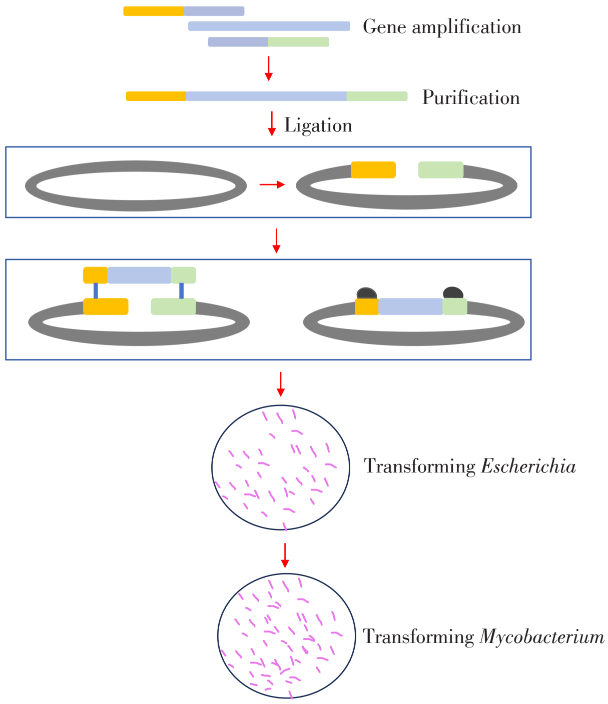


图2　*M. tb* 的Rv2647基因回补株构建示意图

Figure 2　Schematic diagram of Rv2647-complementary strain of *M. tb*

Rv2647)的存活情况,分别于30 d和7 d后,颈椎脱臼法处死模型鼠,无菌条件下取出模型鼠肺组织,无菌PBS漂洗后匀浆,梯度稀释后涂布于Middlebrook 7H10,分别于培养28 d和5 d后进行菌落计数,评估模型鼠肺组织细菌负荷。肺组织固定于4%多聚甲醛,常规脱水后石蜡包埋,5 μm连续切片。二甲苯脱蜡后利用梯度酒精水化,苏木精染色后流水冲洗,用1%盐酸乙醇进行分化,伊红复染,流水冲洗,梯度酒精脱水,二甲苯透明,树脂封片,镜下观察,使用Image-Pro Plus软件进行肺组织炎症评分,评估肺组织病理损伤程度[9-10]。

1.3　统计学方法

采用Graphpad prism 9.0进行统计分析,数据表示为均值±标准差($\bar{x} \pm s$)。两组间差异比较采用独立样本t检验。多组间差异分析采用单因素方差分析。$P < 0.05$为差异有统计学意义。

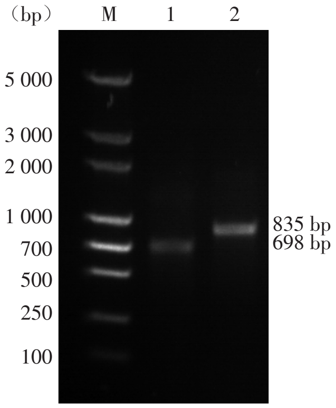
2　结　果

2.1　PCR扩增Rv2647基因左、右臂DNA片段

使用高保真DNA聚合酶扩增H37Rv的Rv2647基因左臂DNA片段(698 bp),右臂DNA片段(835 bp),PCR可见明显条带(图3)。

2.2　构建H37RvΔRv2647

酶切并回收Rv2647基因左、右臂PCR产物,与

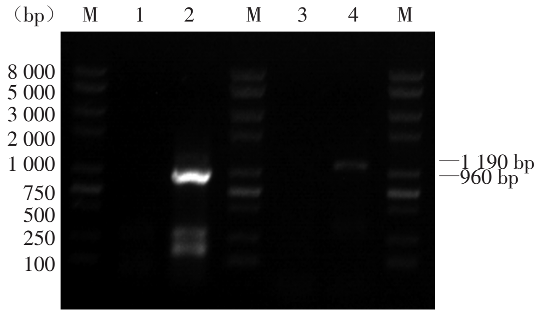


M: DNA marker; 1: Left arm of DNA fragment; 2: Right arm of DNA fragment.

图3　PCR扩增Rv2647基因左、右臂片段

Figure 3　The left and right arm fragments of Rv2647 gene were amplified by PCR

*Van*91 Ⅰ酶切的质粒p0004S转化DH5α感受态细胞。筛选阳性克隆子并测序。*Pac* Ⅰ酶切并回收,与*Pac* Ⅰ酶切的质粒phAE159连接、包装并转化HB101感受态细胞,筛选阳性噬菌粒并电转化感受态Ms,涂板,培养3 d后筛选噬菌斑,扩增制备高滴度噬菌体并感染H37Rv,涂板,37 ℃培养28 d后,挑取单克隆,接种于Middlebrook 7H9,37 ℃培养28 d后,提取基因组,PCR验证。以H37RvΔRv2647基因组为模板,可见大小约960 bp和1 190 bp的DNA片段。以H37Rv基因组为模板,未见目的DNA片段(图4)。



M: DNA marker; 1 and 3: wild type strain (H37Rv), primer pair LYZFP/LYZRP (1) and RYZFP/RYZRP (3); 2 and 4: Rv2647-deleted strain (H37RvΔRv2647), primer pair LYZFP/LYZRP (2) and RYZFP/RYZRP(4).

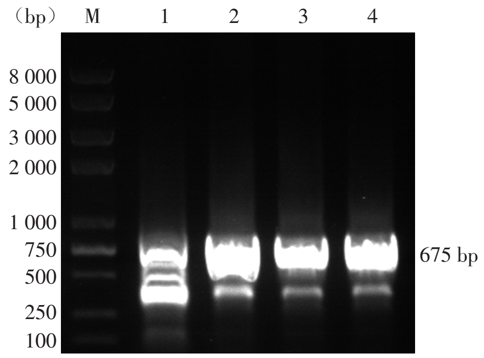
图4　PCR验证H37RvΔRv2647

Figure 4　Verification of H37RvΔRv2647 by PCR

2.3　构建H37RvΔRv2647::Rv2647

PCR扩增Rv2647基因左、右臂并回收,与*Eco*R Ⅰ和*Hind* Ⅲ双酶切的pMV361质粒重组并转化DH5α感受态细胞,挑取单克隆测序验证pMV361-Rv2647

质粒。pMV361-Rv2647质粒电转化H37RvΔRv2647感受态菌株，涂板，37 ℃培养28 d后，挑取单克隆接种于Middlebrook 7H9，37 ℃培养28 d后，收集菌体提取基因组，PCR验证，可见大小约675 bp的条带（图5）。



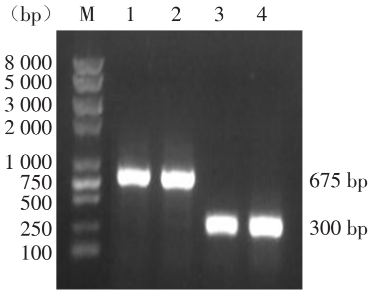
M，DNA marker；1，2，3，and 4：DNA fragment of Rv2647 gene.

图5　PCR验证H37RvΔRv2647：：Rv2647

Figure 5　Verification of H37RvΔRv2647：：Rv2647 by PCR

2.4　构建Ms：：Rv2647

PCR扩增Rv2647基因，与pMV261质粒重组并转化DH5α感受态细胞，挑取pMV261-Rv2647质粒电转化Ms感受态细胞，涂板培养，挑取单克隆接种至7H9液体培养基，37 ℃培养3~4 d，收集菌体，提取基因组，PCR验证，电转成功的菌体基因组可见大小约675 bp的条带（图6）。



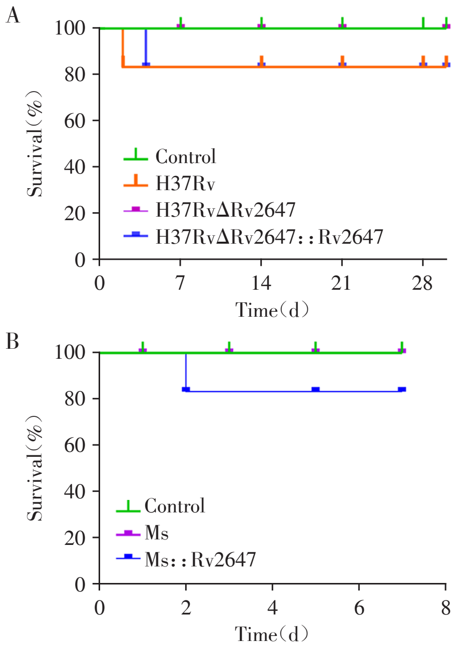
M，DNA marker；1 and 2，DNA of the pMV261-Rv2647 plasmid；3 and 4，DNA of the pMV261 plasmid.

图6　PCR验证Ms：：Rv2647

Figure 6　Verification of Ms：：Rv2647 by PCR

2.5　Rv2647蛋白对模型鼠生存率的影响

分别用H37Rv、H37RvΔRv2647及H37RvΔRv2647：：Rv2647经气管攻击C57BL/6小鼠（2×10^6 CFU/只），感染后30 d内连续监测模型鼠存活情况，结果示H37RvΔRv2647组模型鼠的30 d存活率为100.00%，高于H37Rv组（83.33%）和H37RvΔRv2647：：Rv2647组（83.33%）（图7A）；用Ms和Ms：：Rv2647经气管攻击C57BL/6小鼠（1×10^7 CFU/只），感染后7 d内连续监测模型鼠存活情况，结果示Ms组模型鼠的7 d存活率为100.00%，高于Ms：：Rv2647组（83.33%）（图7B）。



A：Survival of C57BL/6 mice 30 days after tracheal infection with H37Rv，H37RvΔRv2647，and H37RvΔRv2647：：Rv2647（2×10^6 CFU/mouse）（n=6）. B：Survival of C57BL/6 mice 7 days after tracheal infection with Ms and Ms：：Rv2647（1×10^7 CFU/mouse）（n=6）.

图7　模型鼠生存曲线

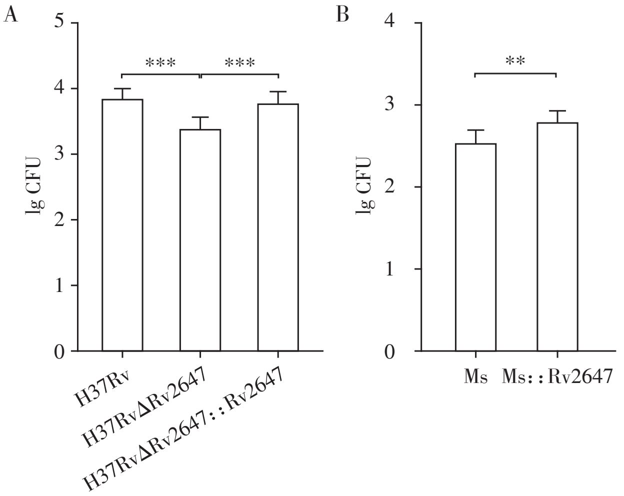
Figure 7　Survival curve of model mice

2.6　Rv2647蛋白抑制模型鼠对*M. tb*的清除

分别取感染H37Rv、H37RvΔRv2647、H37RvΔRv2647：：Rv2647以及Ms、Ms：：Rv2647的模型鼠肺组织，匀浆后梯度稀释并涂布于Middlebrook 7H10，37 ℃静置培养，进行菌落计数。结果显示H37RvΔRv2647组模型鼠肺组织细菌负荷（lgCFU）为（3.40±0.18），显著低于H37Rv组（3.86±0.15，$P < 0.001$）和H37RvΔRv2647：：Rv2647组（3.80±0.16，$P < 0.01$，图8A）；Ms组模型鼠肺组织细菌负荷（lgCFU）为（2.53±0.16），显著低于Ms：：Rv2647组（2.81±0.13，$P < 0.01$，图8B）。

2.7　Rv2647蛋白加重模型鼠肺组织病理损伤

分别取感染H37Rv、H37RvΔRv2647、H37RvΔRv2647：：Rv2647以及Ms、Ms：：Rv2647的模型鼠的肺组织进行HE染色，H37RvΔRv2647组模型鼠肺组织结构相对完整，肺泡腔结构较清晰，少量炎性细胞浸润，炎症面积为（4.37±3.06）%；H37Rv组和H37RvΔRv2647：：Rv2647组模型鼠肺组织结构破坏明显，肺泡腔塌陷，肺泡间隔增厚或破坏，大量炎性细



A: C57BL/6 mice were intratracheally infected with the H37Rv, H37RvΔRv2647, and H37RvΔRv2647::Rv2647 (2×10^6 CFU/mouse), and CFU in lung of model mice in each group were evaluated on day 30 (n=6). B: C57BL/6 mice were intratracheally infected with Ms and Ms::Rv2647 (1×10^7 CFU/mouse), and CFU in lung of model mice in each group were evaluated on day 7 (n=6). $^{**}P < 0.01$, $^{***}P < 0.001$.

图8　模型鼠肺组织细菌负荷

Figure 8　Lung bacterial load of model mice

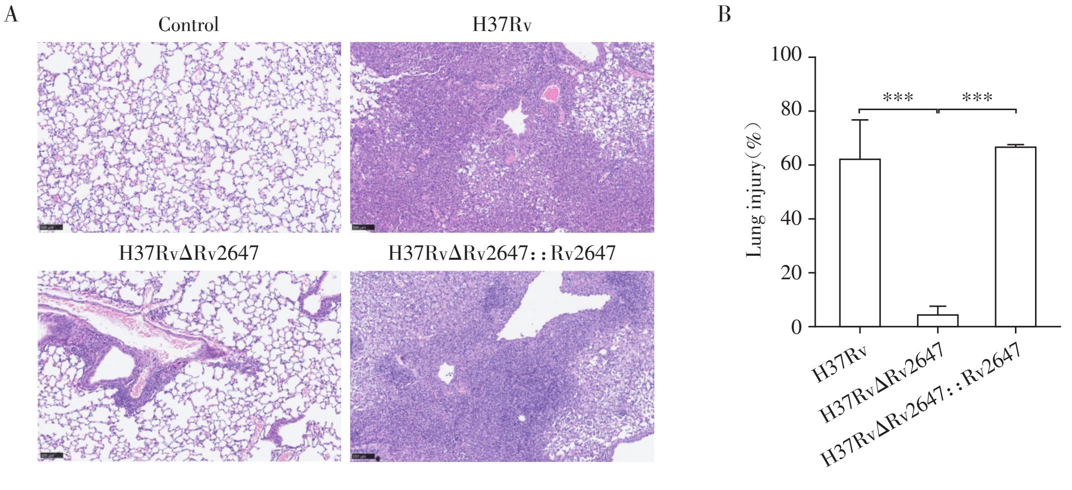
胞浸润，炎症面积[H37Rv：(62.76 ± 14.24)%，P < 0.001；H37RvΔRv2 647::Rv2 647：(67.37±0.45)%，P < 0.001]均显著高于H37RvΔRv2647组(图9)。同样地，Ms组模型鼠肺组织结构较完整，肺泡腔结构相对清晰，炎症面积为(5.71±1.29)%；Ms::Rv2647组模型鼠肺组织结构破坏明显，肺泡间隔破坏，炎症面积为(33.13±13.84)%(P < 0.05，图10)。

3　讨　论

结核病是除新冠肺炎外全球第一大因单一病原感染导致的致死性传染病[11]。目前，BCG的预防效果不佳，且多耐药菌株及泛耐药菌株的流行进一步加剧了结核病的防治形势。因此，迫切需要深入研究*M. tb*的关键致病机制以开发新型疫苗与药物。大量研究表明，*M. tb*的RD区含有众多毒力因子及特异性抗原，在结核病的发生发展中发挥重要作用[12-16]。

Rv2647基因位于RD13区，该区域共包含16个阅读框(Rv2645-Rv2660c)。研究显示，该区Rv2645基因编码的假想蛋白，可诱导强烈的抗*M. tb*特异性IFN-γ应答，与BCG联合可显著增强免疫保护效应[17]；另有研究发现，Rv2654c和Rv2659c分别编码噬菌体蛋白和噬菌体整合酶，可诱导结核病特异性的T细胞应答，显著提高结核病诊断的特异性和敏感性[18-19]；此外，Rv2660c编码的相关蛋白，在*M. tb*潜伏感染状态下表达显著上调，可通过TLR2介导TNF-α、IFN-γ等炎症因子的分泌，利于*M. tb*潜伏感染[20]。Rv2647基因编码蛋白在*M. tb*感染及致病中的作用尚不清楚。

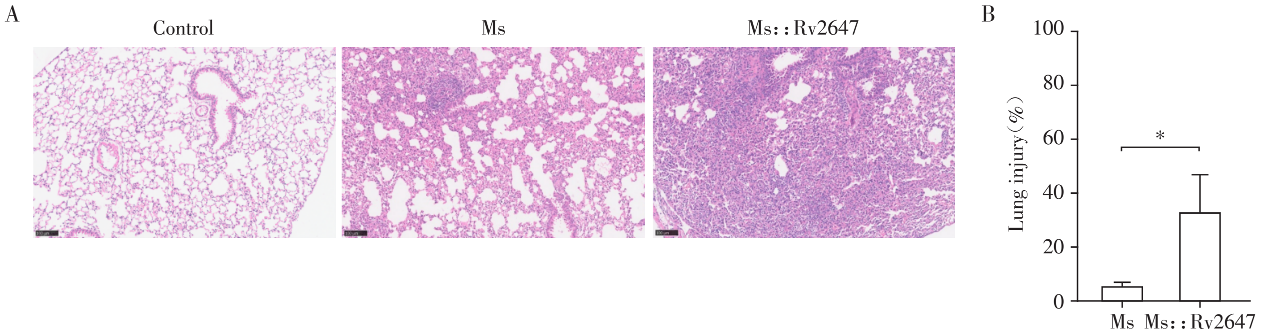
基因编辑技术是研究*M. tb*基因功能的有效手段。目前，应用于*M. tb*的基因敲除技术主要有依赖于同源重组的基因敲除技术和不依赖于同源重组的基因沉默技术。其中，噬菌体介导的同源重组技术可显著提高转染效率及同源重组效率而被广泛应用。本研究采用具有高DNA传递效率的含温敏



A: The HE staining of lung tissues were observed under microscope 30 days after tracheal infection with the H37Rv, H37RvΔRv2647, and H37RvΔRv2647::Rv2647 (2×10^6 CFU/mouse) in C57BL/6 mice (scale bar=100 μm). B: Comparison of lung tissue inflammation area (%) 30 days after tracheal infection with the H37Rv, H37RvΔRv2647, and H37RvΔRv2647::Rv2647 (2×10^6 CFU/mouse) in C57BL/6 mice. $^{***}P < 0.001$ (n=3).

图9　感染*M. tb*后模型鼠肺组织损伤

Figure 9　Lung injury of model mice infected with *M. tb*



A：The HE staining of lung tissues were observed under microscope 30 days after tracheal infection with the Ms and Ms::Rv2647（1×10^7 CFU/mouse）in C57BL/6 mice（scale bar=100 μm）. B：Comparison of lung tissue inflammation area（%）7 days after tracheal infection with the Ms and Ms::Rv2647（1×10^7 CFU/mouse）in C57BL/6 mice. $^*P < 0.05$（n=3）.

图10 感染Ms后模型鼠肺组织损伤

Figure 10 Lung injury of model mice infected with Ms

型噬菌体元件的穿梭质粒phAE159，构建了phAE159-ΔRv2647噬菌粒，在Ms内扩增后导入H37Rv，获得H37RvΔRv2647，并进一步构建H37RvΔRv2647::Rv2647和Ms::Rv2647，为深入研究Rv2647基因的功能奠定了基础。

本研究中分别以H37Rv、H37RvΔRv2647、H37RvΔRv2647::Rv2647、Ms及Ms::Rv2647经气管攻击C57BL/6小鼠，H37RvΔRv2647组模型鼠30 d生存率高于H37Rv组和H37RvΔRv2647::Rv2647组；Ms组模型鼠7 d生存率也高于Ms::Rv2647组；H37RvΔRv2647组模型鼠的肺组织细菌负荷和肺组织病理损伤程度均显著低于H37Rv组和H37RvΔRv2647::Rv2647组；同样地，Ms组模型鼠的肺组织细菌负荷和肺组织病理损伤程度亦显著低于Ms::Rv2647组。本研究提示，Rv2647蛋白在*M. tb*感染的致病过程中可能通过削弱模型鼠对*M. tb*的清除效应而加重了肺组织的病理损伤，其具体致病机制值得进一步探究。

本研究成功构建了*M. tb*的Rv2647基因敲除株（H37RvΔRv2647）和回补株（H37RvΔRv2647::Rv2647）以及过表达Rv2647的Ms（Ms::Rv2647）；初步探明了Rv2647蛋白可能通过抑制模型鼠对*M. tb*的清除，加重肺组织病理损伤。

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