英语学习与文献汇报

English learning & Paper reporting

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Sirtuin 6 attenuates periapical lesion propagation by modulating hypoxia-induced chemokine (C-C motif) ligand 2 production in osteoblasts

SIRT6通过调节缺氧诱导的CCL2在成骨细胞中的产生来减弱根尖周炎

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Sirtuin 6 attenuates periapical lesion propagation by modulating hypoxia-induced chemokine (C-C motif) ligand 2 production in osteoblasts

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Abstract

Lee Y-L, Lin S-K, Hou K-L, Kok S-H, Lai EH-H, Wang H-W, Chang JZ-C, Yang H, Hong C-Y. Sirtuin 6 ... of lactate dehydrogenase A (LDHA) and production of lactate ($P = 0.007$). A reciprocal effect between hypoxia-induced redox imbalance and hypoxia-enhanced glycolysis was noted which in turn aug-

Abstract and Introduction

- **Sirtuin 6** (SIRT6) is a NAD+-dependent protein deacetylase able to regulate inflammatory reactions. SIRT6 has been reported to participate in the modulation of glucose metabolism and cellular redox status.
- **Chemokine ligand 2** (CCL2) is a major chemoattractant of monocyte both in vitro and in vivo. Osteoblasts have been demonstrated as the major source of CCL2 and many pro-inflammatory cytokines have been shown to upregulate CCL2 production by human osteoblasts.

Abstract and Introduction

- **Aim** To investigate the attenuating effect of sirtuin 6 (SIRT6) on hypoxia-induced production of chemokine ligand 2 (CCL2) by osteoblasts and the relevance of this action on the pathogenesis of periapical lesions.
- **Conclusions** Sirtuin 6 has a therapeutic effect on periapical lesions through suppression of CCL2 synthesis. The anti-inflammatory action of SIRT6 is closely related to its regulatory activities in cellular metabolism and redox homoeostasis.

Materials and Methods

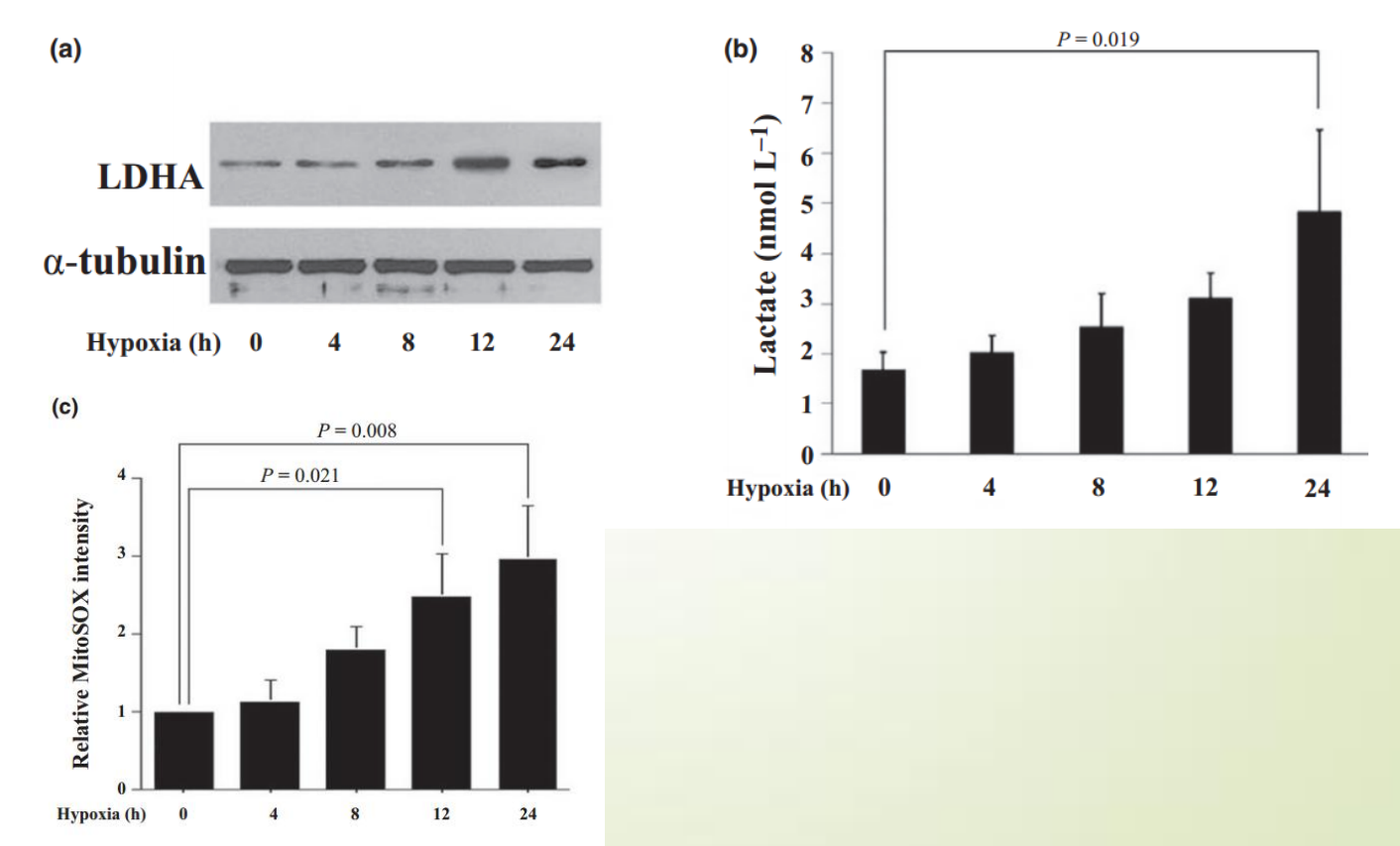
- Cell culture (MC3T3-E1 murine osteoblasts and J774 murine macrophages)
- Overexpression of SIRT6 (Sirtuin 6 was overexpressed in MC3T3-E1 cells using a lentiviral vector system) 慢病毒载体
- Western blot analysis (LDHA, a-tubulin)
- Measurement of lactate (乳酸)Colorimetric kit
- Measurement of mitochondrial ROS (线粒体活性氧) Fluorescence of MitoSOX™ Red
- Measurement of CCL2 (ELISA kit)
- Cell migration assay

Materials and Methods

- Murine model (Ten 7 to 8-week-old Sprague Dawley rats)
- Induction of apical periodontitis (not the point…pass)
- Assessment of lesion size (image analysis quantified in pixels)
- Immunohistochemical study (SIRT6, LDHA, CD68, 8-OHdG, light microscope and positive cells counting)
- Statistical analysis

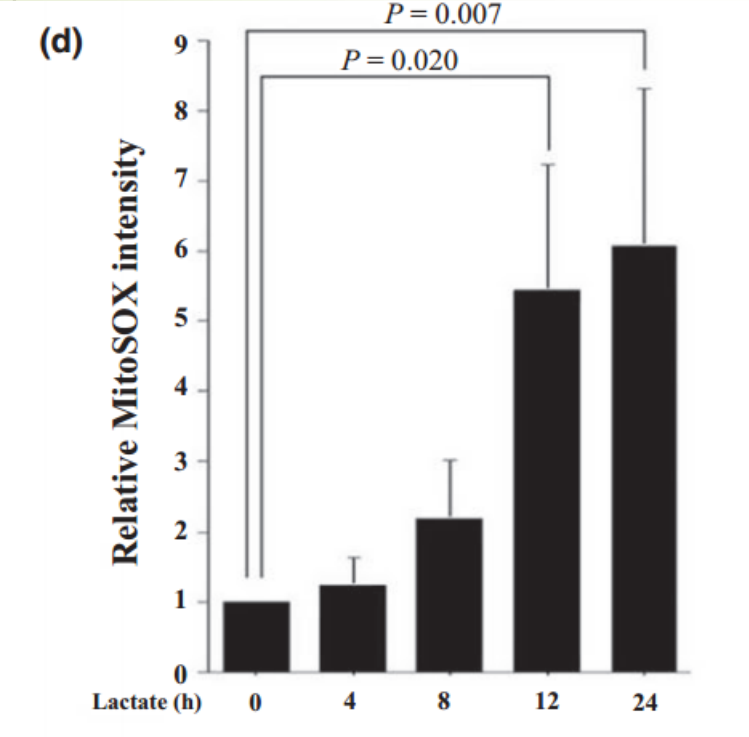
Results

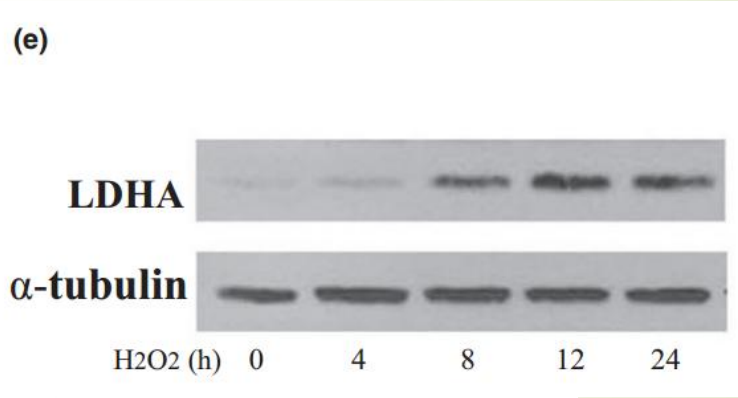
- Hypoxia increased glycolysis and ROS generation in osteoblasts

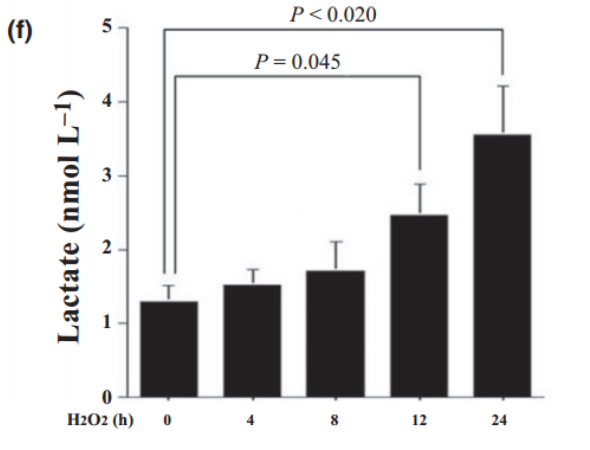


Results

- Reciprocal augmentation between glycolysis and ROS production (互惠增长，他好，我也好)

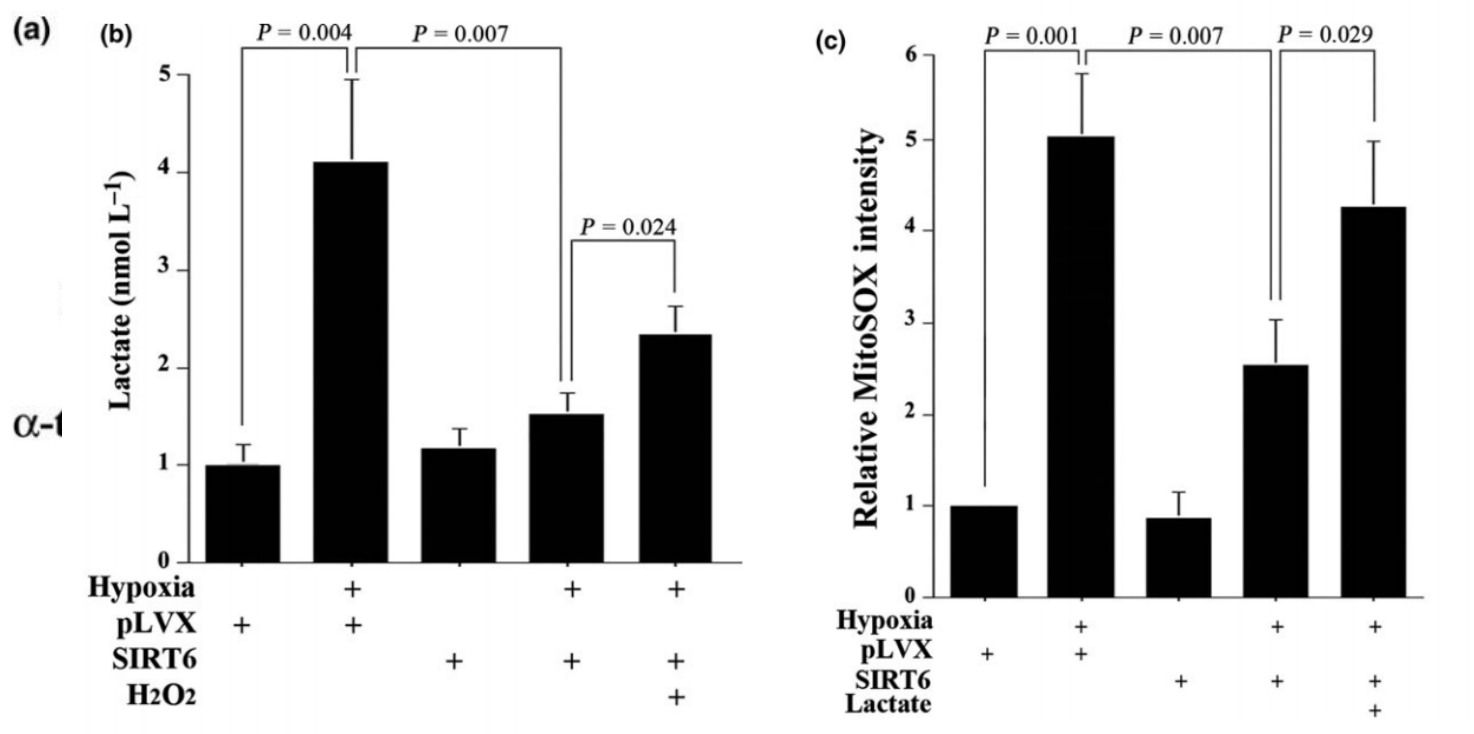






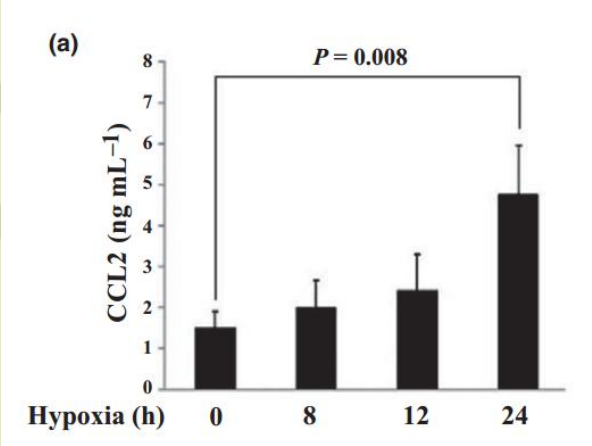
Results

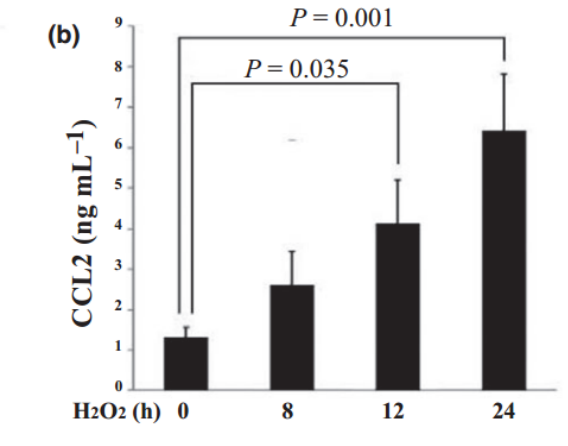
- SIRT6 suppressed hypoxia-enhanced glycolysis and ROS generation

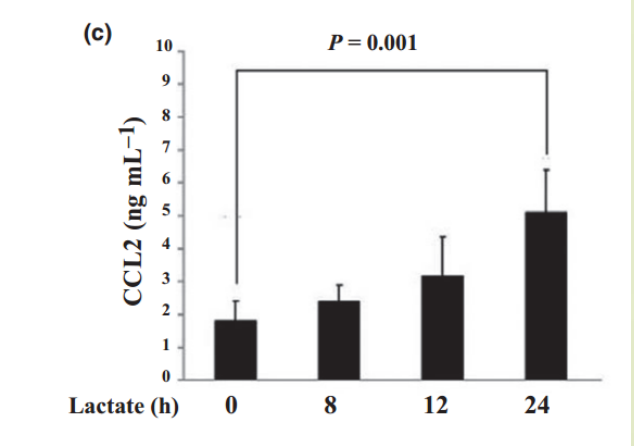


Results

- Antiglycolytic and antioxidative activities of SIRT6 contributed to the suppression of hypoxia-enhanced CCL2 synthesis







Results

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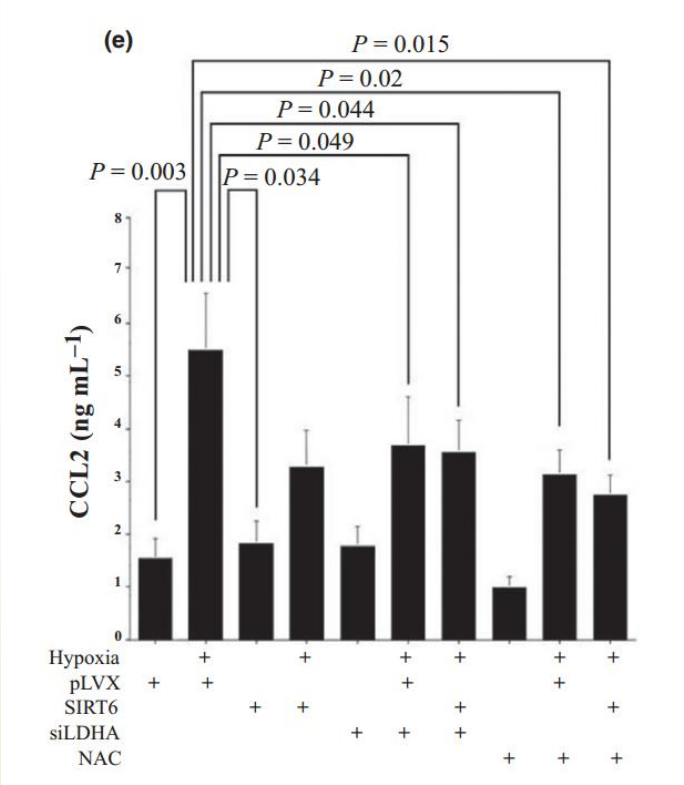
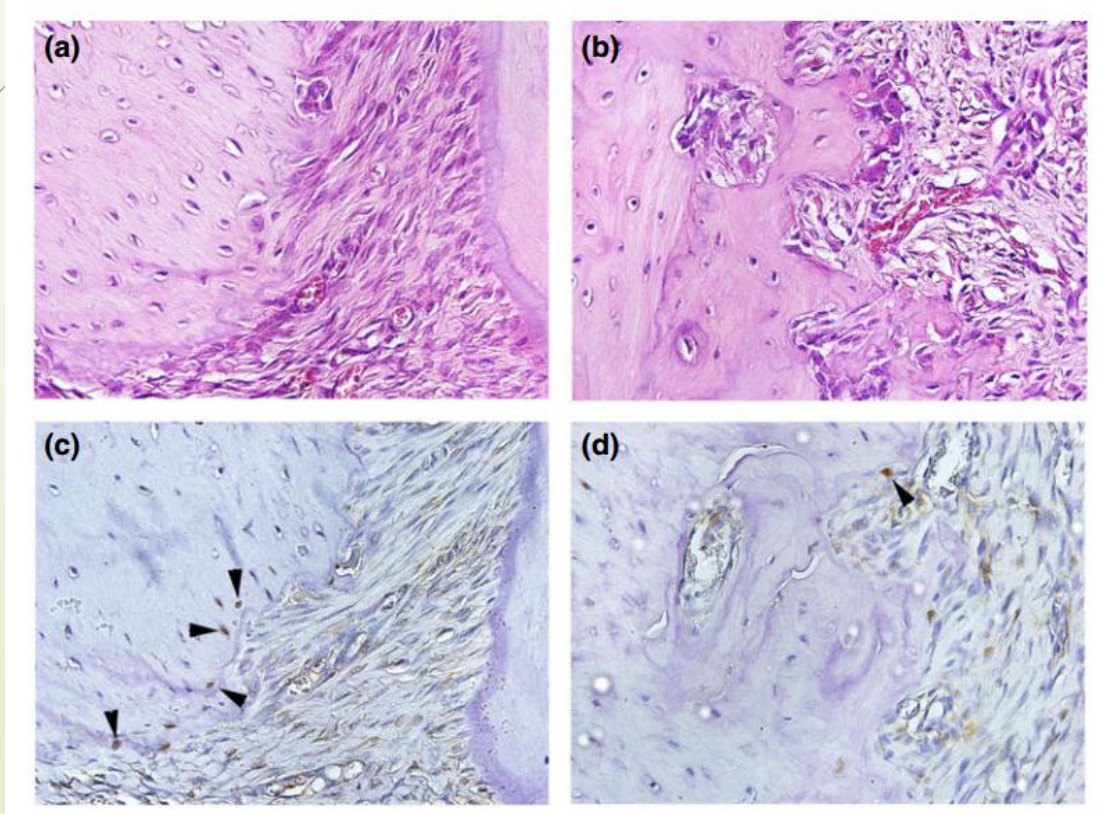


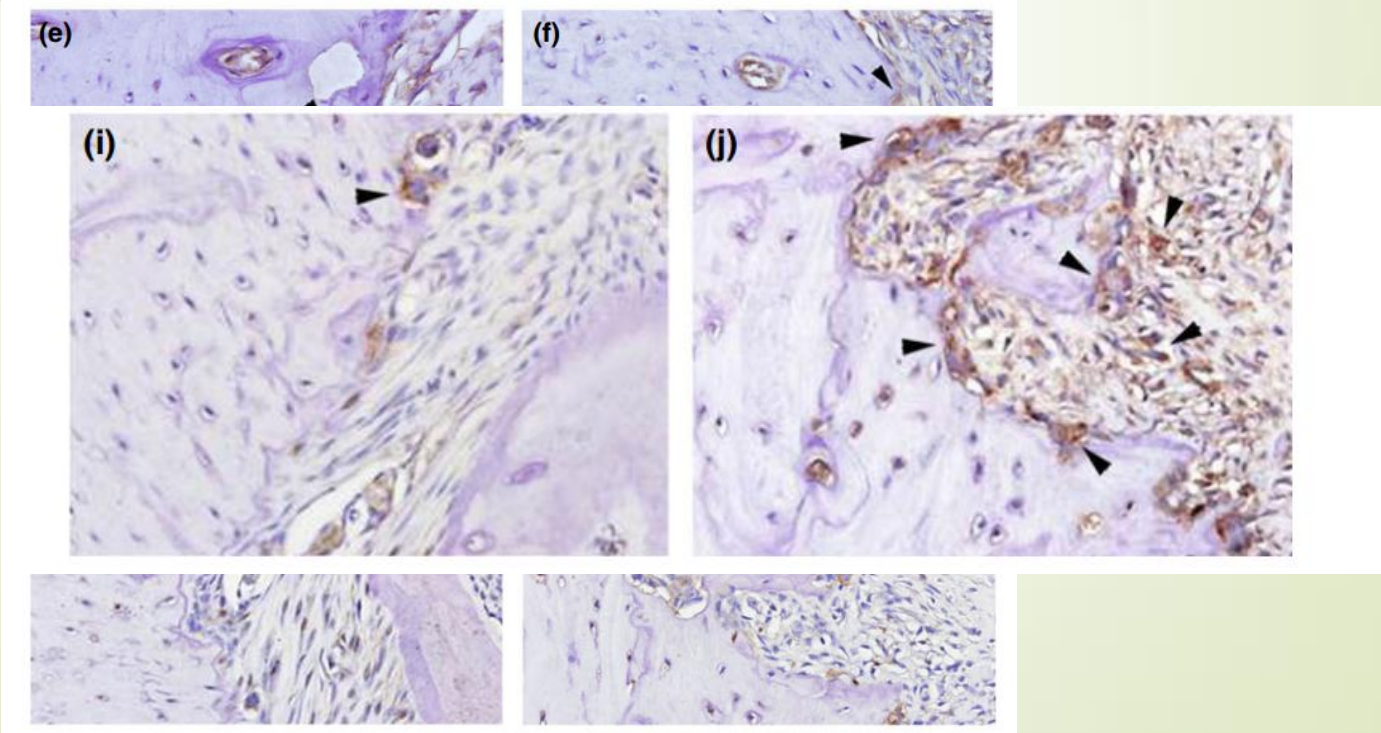
Results

- SIRT6 expression was suppressed in osteoblasts in apical periodontitis



Results

- Osteoblasts in apical periodontitis had higher amounts of oxidative lesions and LDHA



Discussion

- 1. In the present study, hypoxic stress initiated a vicious cycle of redox imbalance and enhanced glycolysis in osteoblasts which in turn augmented the secretion of CCL2 and contributed to periapical bone resorption.
- 2. Through its antiglycolytic and antioxidative effects, SIRT6 is capable of breaking the vicious cycle. The findings suggested that enhancement of SIRT6 activity is beneficial for alleviation of the progress of apical periodontitis.

Discussion

- 3. The experiments confirmed the increase in ROS and lactate synthesis under hypoxia.Moreover, it was demonstrated that both ROS and lactate were capable of stimulating CCL2 synthesis in osteoblasts.
- 4. On the other hand, this study is the first to establish a link between hypoxia-enhanced lactate synthesis and CCL2-induced chemotaxis of monocytes/macrophages in inflammatory bone resorption.

Discussion

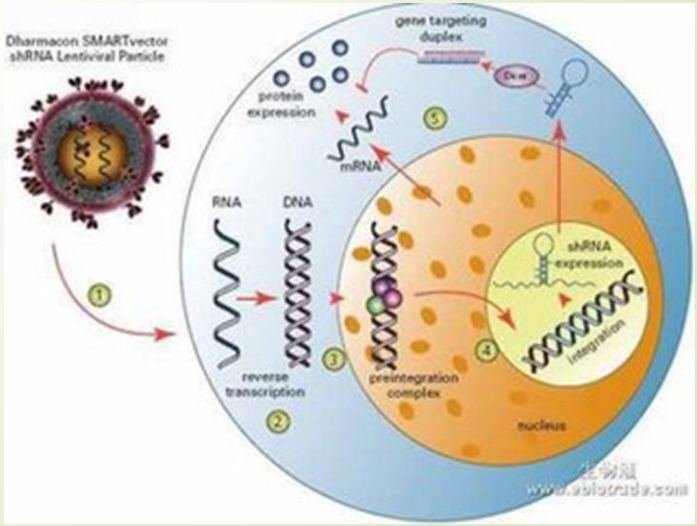
- 5. In the present study, antiglycolytic and antioxidative activities of SIRT6 were also responsible for its protective action against inflammation. SIRT6 also suppressed ROS generation by inhibition of anaerobic glycolysis, representing a novel antioxidative effect of SIRT6. Certainly, the beneficial effects of SIRT6 in the maintenance of redox homoeostasis deserve further investigations.

Conclusions

- 1. Hypoxic stress associated with apical periodontitis initiates a vicious cycle of redox imbalance and enhanced glycolysis which helps to perpetuate the inflammatory state and induce bone resorption.
- 2. By inhibition of glycolysis and ROS generation, SIRT6 was capable of restoring cellular homoeostasis of the redox system and glucose metabolism.
- 3. The data suggest that enhancement of SIRT6 may have a beneficial effect on the prognosis of apical periodontitis.

慢病毒（Lentivirus）

- 基本概论
- 慢病毒（Lentivirus）载体是以HIV-1（人类免疫缺陷I型病毒）为基础发展起来的基因治疗载体。区别一般的逆转录病毒载体，它对分裂细胞和非分裂细胞均具有感染能力。



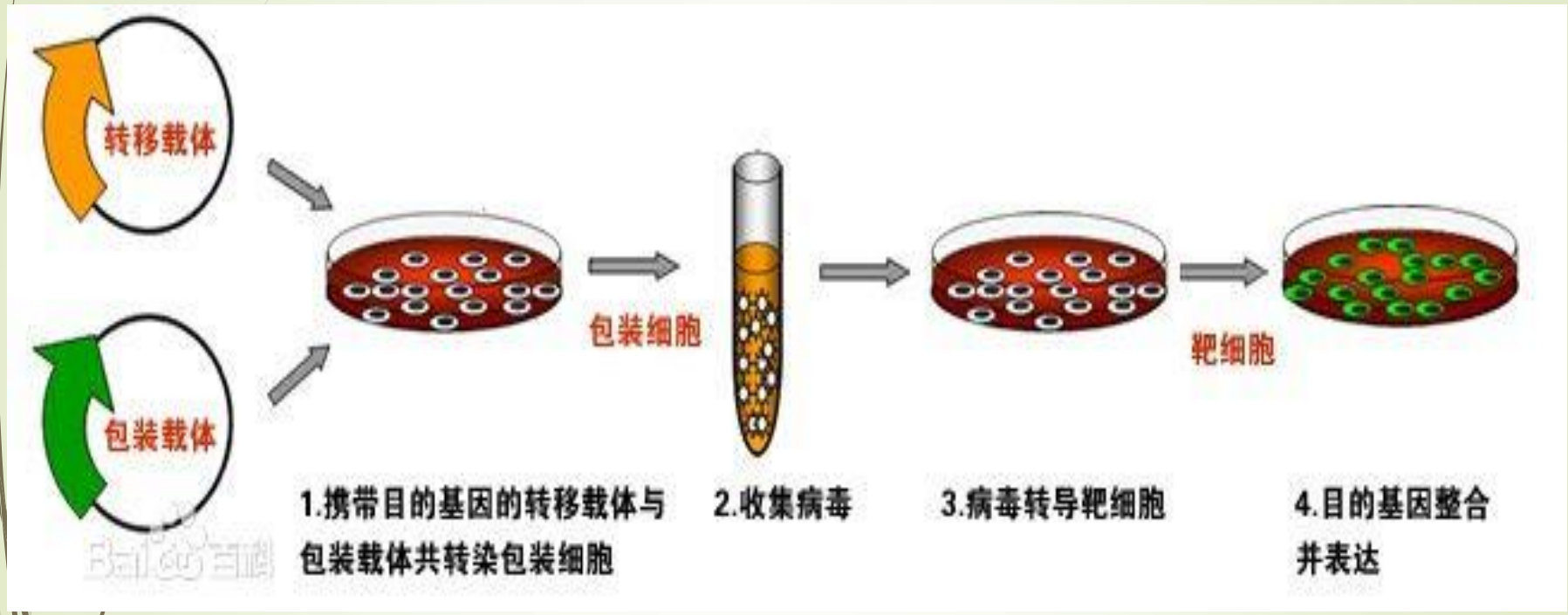
慢病毒（Lentivirus）

- 慢病毒载体是指以人类免疫缺陷病毒-1 (H IV-1) 来源的一种病毒载体，慢病毒载体包含了包装、转染、稳定整合的必需遗传信息。
- 携带有外源基因的慢病毒载体在慢病毒包装质粒、细胞系的辅助下，经过病毒包装成为有感染力的病毒颗粒，通过感染细胞或活体组织，实现外源基因在细胞或活体组织中表达
- 慢病毒载体可以将外源基因或外源的shRNA有效地整合到宿主染色体上，从而达到持久性表达目的序列的效果。

慢病毒载体与其它病毒载体的特征比较

病毒表达系统	腺病毒表达系统	慢病毒表达系统	逆转录病毒
病毒基因组	双链 DNA 病毒	RNA 病毒	RNA 病毒
是否整合	病毒基因组游离于宿主基因组外，瞬时表达外源基因	病毒基因组整合于宿主基因组，长时间、稳定表达外源基因	病毒基因组整合于宿主基因组，
感染细胞类型	感染分裂和不分裂细胞	感染分裂和不分裂细胞	感染分裂细胞，但在干细胞中表
表达丰度	高水平表达	高水平表达	高水平表达
表达时间	快（1-2 天）	慢（2-4 天）	快（1-2 天）
滴度	滴度高达 1012pfu/ml	最高可达 109－10TU/ml	最高可达 109－10TU/ml
克隆容量	可插入高达 8kb 的外源片段，滴度随插入片段长度增加而降低	可插入不超过 8kb 的外源片段，滴度随插入片段长度增加而降低	可插入不超过 6kb 的外源片段
免疫原性	高免疫原性	低免疫原性	低免疫原性
动物模型	不能得到转基因动物	可产生转基因动物，效率达 50 以上	可以，但很难
启动子	可以更换特异性启动子	可以更换特异性启动子	不需要启动子
能否用于 MIRCORNA	可以	可以	不可以
能否用于四环素诱	不可以	TET-ON ， TET-OFF	不可以

操作基本过程



病毒质粒构建8-12 天

包装病毒5-10天

包装细胞感染筛选14天

靶细胞过表达或干扰3天

慢病毒的应用

- 获得性免疫缺陷综合征(AIDS)的基因治疗
- 神经系统疾病的基因治疗
- 血液系统疾病的基因治疗
- 其他疾病的基因治疗，应用于基因传递系统，将治疗基因运送到目标部位

感谢各位老师、师兄弟的收听，欢迎提问！