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Title: Changes and roles of mTOR and its downstream signaling passway in mouse bone marrow stem cells with oxidative stress injury

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关键词: 氧化应激; 骨髓间充质干细胞; 哺乳动物雷帕霉素靶蛋白; 细胞凋亡

Keywords: oxidative stress; bone marrow stem cells; mammaliatargetofrapamycin; apoptosis

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摘要: 目的　研究小鼠骨髓间充质干细胞（mouse bone marrow stem cells，mBMSCs）在氧化应激损伤中哺乳动物雷帕霉素靶蛋白（mammaliatargetofrapamycin，mTOR）及其下游信号通路的变化及其作用。　方法　从30只雄性健康昆明小鼠的股骨中分离、培养和扩增BMSCs。H_2O_2刺激 mBMSCs 建立氧化应激损伤模型。实验分为对照组（不予H_2O_2处理）、不同浓度H_2O_2（100、200、300、400、500、800、1 000 μmol/L处理mBMSCs）损伤组（n=5）。采用MTT法检测24、48、72 h各组的细胞活力；倒置显微镜观察BMSCs的形态学改变；采用细胞核Hoechst33342染色观察凋亡细胞核形态；Western blot检测各组Bcl-2、Bax、mTOR及其下游蛋白以及蛋白的磷酸化的表达。　结果　100～1 000 μmol/L 浓度的H_2O_2作用mBMSCs 24 h 后，其形态学和病理学发生浓度依赖性的改变。H_2O_2浓度在100～300 μmol /L 时，随着H_2O_2浓度的增高，mBMSCs的mTOR、p70S6K、S6的表达水平有增高的趋势，磷酸化水平明显增

高（P<0.01），抗凋亡蛋白Bcl-2表达增高（P<0.01），凋亡蛋白 Bax表达不明显（P>0.05）。 H_2O_2浓度在400 μmol /L以上时，随着H_2O_2浓度的增高，p-mTOR、p-p70S6K、p-S6、BCL-2的表达水平降低（P<0.05，P<0.01），而mTOR、p70S6K、S6蛋白变化不明显，同时Bax的表达水平明显增高（P<0.01）。 结论 一定强度的氧化应激可以降低mBMSCs存活率，促进细胞凋亡，其机理可能与抑制mTOR及其下游信号通路的活性和抗凋亡蛋白Bcl-2表达，促进凋亡蛋白Bax表达有关。

Abstract: Objective To investigate the possible effects and changes of mammalian target of rapamycin (mTOR) and its downstream signaling pathway in mouse bone marrow stem cells (mBMSCs) induced by oxidative stress. Methods mBMSCs were isolated from bone marrows from 30 healthy Kunming adult male mice, cultured and expanded. An oxidative stress model of mBMSCs was established by different concentrations of H_2O_2 (100, 200, 300, 400, 500, 800 and 1 000 μmol/L). Cell viability was detected by MTT assay, and morphological changes of BMSCs were observed by inverted microscopy. The nucleus apotosis were accessed by Hoechst 33342 staining. Western blotting was employed to evaluate the expression of Bcl-2, Bax, mTOR, p70S6K and S6, as well as phosphorylated mTOR, p70S6K and S6. Results The mBMSCs had pathophysiologic changes after 100 to 1 000 μmol/L H_2O_2 treatment in a dose-dependent manner. When H_2O_2 was given at concentrations of 100 to 300 μmol/L, the protein expression of mTOR, p70S6K and S6 in mBMSCs tended to be increased in a dose-dependent fashion, while the expression of their phosphorylated forms and anti-apoptosis protein Bcl-2 were significantly increased (P<0.01). But, the expression of apoptosis protein Bax was not obviously changed (P>0.05). However, when H_2O_2 was given at concentrations over 400 μmol/L, the expression of Bcl-2, p-mTOR, p-p70S6K and p-S6 proteins were in a dose-dependent decrease in mBMSCs (P<0.05, P<0.01), while the expression of mTOR, p70S6K and S6 protein was not visibly altered, whereas the expression of was obviously increased (P<0.01). Conclusion Oxidative stress to some extent causes reduced survival and increased apoptosis in BMSCs. The underlying mechanisms may be partly due to suppression of mTOR and its downstream signaling, decreased expression of Bcl-2, and enhanced expression of Bax.

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