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内皮细胞通过Hedgehog通路促进胶质瘤干细胞自我更新

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Title: Endothelial cells promote self-renewal of glioma stem cells through Hedgehog pathway

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关键词: [胶质瘤干细胞](#); [内皮细胞](#); [信号转导](#); [Hedgehog通路](#)

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摘要: 目的 探讨Hedgehog通路在内皮细胞促进胶质瘤干细胞 (glioma stem cell, GSC) 自我更新中的可能作用。 方法 实验以GL261细胞系中分离的GSC和内皮细胞系b.END3为研究材料, 采用Transwell双室细胞培养、极限稀释法成球实验、实时定量PCR、Western blot、体内移植瘤实验以及慢病毒载体基因干扰等方法, 检测内皮细胞对GSC成球、成瘤能力、干性基因表达以及Hedgehog信号通路相关的部分基因表达的影响。 结果 与对照组相比: ① GSC与内皮细胞共培养后其体外成球能力明显增强, 表现为形成干细胞球数目明显增多, 体积明显增大, 尤其在每孔5个 (24.3% vs 11.3%) 和每孔10个细胞 (39.4% vs 25.8%) 的极低浓度下更加明显 ($P<0.05$)。 ②共培养体系中, GSC的干性相关基因Oligo2、Bmi1与Hedgehog信号

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通路相关基因Gli1的mRNA和蛋白表达明显增加 ($P<0.05$)。③体内移植瘤实验发现,与内皮细胞共同注射的GSC成瘤能力明显增强,所形成的移植瘤体积显著大于对照组($P<0.05$),而且出现了部分瘤体破溃、小鼠死亡。④通过慢病毒载体基因干扰Smo基因表达抑制Hedgehog信号通路后,内皮细胞促进GSC自我更新的上述现象消失。 结论 内皮细胞可能通过激活Hedgehog信号通路促进GSC自我更新。

Abstract: **Objective** To explore the role of Hedgehog pathway in endothelial cells promoting self-renewal of glioma stem cell (GSC). **Methods** GSC derived from glioblastoma cell line GL261 and brain microvessel endothelial cell line b.END3 were used. Transwell co-culture system, limit dilution assay, real-time PCR, Western blotting, xenograft experiment and gene knock-down assay were applied to determine the self-renewal, tumorigenic ability and gene expression of Hedgehog pathway in GSC spheres. **Results** (1) More and larger tumor spheres were formed by GSC after co-culture with endothelial cells ($P<0.05$), especially under a low cell concentration of 5 cells per well (24.3% vs 11.3%) and 10 cells per well (39.4% vs 25.8%). (2) Hedgehog pathway related genes including Gli1, Oligo2 and Bmi1 were up-regulated in the co-cultured GSC spheres ($P<0.05$). (3) Larger xenografts were generated by GSC spheres mixed with endothelial cells, which also resulted in tumor rupture and model death ($P<0.05$). (4) The phenomenon above nearly disappeared when Hedgehog pathway had been inhibited by Smo gene knockdown. **Conclusion** Endothelial cells promote self-renewal of GSC through activating Hedgehog pathway.

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