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Lenti-TK介导间充质干细胞对鼻咽癌CD133+干细胞的靶向迁移及杀伤作用 点此下载全文

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摘要：

目的： 观察慢病毒-胸苷激酶（lentivirus-thymidine kinase，Lenti-TK)/间充质干细胞(mesenchymal stem cell，MSC）对鼻咽癌CD133+干细胞的靶向迁移及杀伤作用。 方法： 构建包含TK基因的重组慢病毒表达载体Lenti-TK，感染MSC后得到Lenti-TK-MSC，RT-PCR及Western blotting检测Lenti-TK-MSC中HA-TK的表达。免疫磁珠法从鼻咽癌5-8F细胞中分选CD133+细胞；Transwell小室迁移实验检测Lenti-TK-MSC对CD133+5-8F细胞的趋向性；Lenti-TK-MSC联合更昔洛韦（ganciclovir，Lenti-TK-MSC/GCV）与CD133+5-8F细胞共培养，CCK-8试剂盒检测其对细胞的杀伤作用和旁观者效应。 结果： 成功构建重组慢病毒载体Lenti-TK，其滴度为1×108UT/ml，Lenti-TK（MOI=50）感染MSC 72 h时，感染效率达（95.1±0 1）%。Lenti-TK-MSC迁移至CD133+5-8F细胞组的细胞数明显多于CD133-5-8F细胞组、未分选5-8F细胞组\[（83.0±8.7） vs （29.6±5 3）、（38.3±5.2），P=0.000\]。Lenti-TK-MSC/GCV处理组与单独GCV处理组、Lenti-TK-MSC/GCV条件培养液（即Lenti-TK-MSC加入1 mg/L GCV培养48 h的培养上清）处理组相比，CD133+5-8F细胞的存活率明显降低\[（37.2±2.3）% vs （98.5±3.1）%、（83.8±3 4）%，P=0.000\]。Lenti-TK-MSC数量达到混合细胞总数（Lenti-TK-MSC和CD133+5-8F细胞)的20%时，CD133+5-8F细胞存活率为（68.2±2.3）%，表现出明显的旁观者杀伤效应。 结论： Lenti-TK感染后MSC对鼻咽癌CD133+5-8F细胞具有靶向迁移及杀伤作用。

关键词：鼻咽癌 CD133+细胞 间充质干细胞 肿瘤干细胞 肿瘤靶向基因治疗 旁观者效应

Targeted migration and killing effect of mesenchymal stem cells infected with Lenti-TK on CD133+ cancer stem cells in nasopharyngeal carcinoma Download Fulltext

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Abstract:

Objective: To investigate the targeted migration and killing effect of mesenchymal stem cells (MSCs) infected with Lenti-TK (thymidine kinase) vector on CD133+ cancer stem cells in nasopharyngeal carcinoma. Methods: The recombinant lentiviral expression vector containing TK gene (Lenti-TK) was constructed and transduced into MSC (Lenti-TK-MSC). Fusion tag-TK (HA-TK) expression was verified by RT-PCR and Western blotting. CD133+ cells were sorted from nasopharyngeal carcinoma 5-8F cells by immunomagnetic beads. The chemotactic ability of Lenti-TK-MSC to CD133+5-8F cells was analyzed by Transwell assay. The CD133+ 5-8F cells were co-cultured with Lenti-TK-MSC and GCV to detect its killing effect on cells by CCK-8 Kit. Results: The recombinant lentivirus vector Lenti-TK was successfully constructed with titer being 1×108 UT/ml. The transduction efficiency of Lenti-TK to MSC was (95.1±0.1)%, 72 h after transduction at an MOI of 50. The migration number of Lenti-TK-MSC to CD133+5-8F cells was more than that to CD133-5-8F cells and 5-8F cells (\[83.0±8.7\] vs \[29.6±5.3\] vs \[38.3±5.2\]; P=0.000). The Lenti-TK-MSC/GCV treatment significantly inhibited the growth of the CD133+ 5-8F cells compared with the GCV group and the Lenti-TK-MSC/GCV condition medium group (the culture supernatant of Lenti-TK-MSC treated with 1 mg/L GCV for 48 h) (\[37.2±2.3\]% vs \[98.5±3.1\]% vs \[83.8±3.4\]%, P=0.000), with the cell survival being (68.2±2.3)% when the proportion of Lenti-TK-MSC was 20%, which showed a significant bystander killing effect. Conclusion: Lenti-TK infected MSC exert targeted migration and killing effects on CD133+ 5-8F cells from nasopharyngeal carcinoma.

Keywords:nasopharyngeal carcinoma CD133+ cell mesenchymal stem cell cancer stem cell tumor targeted gene therapy bystander effect

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