Suicide Gene Therapy Approach with a Dual Expression LV Vector Enhances the Safety of Ex Vivo Gene Therapy for Bone Repair

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Introduction

"Ex vivo" gene therapy has been successfully used to heal critical sized defects in rodent models1-3. Previously, we transduced rat BM cells using a lentiviral vectors to over express BMP-2. The transduced cells were implanted in a rat critical sized femoral defect and achieved complete union4. Insertional mutagenesis and emergence of replication competent viral particles remain areas of concern with respect to safety of the lentiviral vectors. In this study we developed a "suicide approach" using a dual gene expression lentiviral vector encoding BMP-2 or Luciferase and a truncated form of Herpes Simplex Virus Thymidine Kinase (ΔTK) to enhance the safety of "ex vivo" gene therapy. Thymidine Kinase phosphorylates a number of antiviral pro-drugs such as Ganciclovir (GCV) to toxic metabolites that can kill viral infected cells. We hypothesized that GCV administration would result in the killing of transduced cells after the defect was healed.

Methods:

Vector construction: The dual gene expression vectors (LV-ΔTK-T2A-BMP-2 and LV-ΔTK-T2A-Luc) were constructed. The ΔTK-BMP2 or ΔTK-Luc cDNA was created by overlap PCR and cloned into pCR®II-H-TOPO® (Invitrogen, Carlsbad, CA). Two transgenes were linked by T2A sequence that allows for post-translational splicing.

Viral Transduction: Mouse bone marrow cells (MBMCs) of 8 week old male BL/6 mice were cultured for 1 week. Overnight transduction of MBMCs was carried out at MOI of 25 as previously described 4.

In vivo studies: Two sets of experiments were performed to evaluate the safety of the vector. To determine bone formation by the dual expression vector and the effects of early GCV administration, 2x10⁶ LV-ΔTK-T2A-BMP-2 transduced MBMCs were implanted in a 2 mm femoral defects of 14 week-old male BL/6 mice. The mice received GCV (100 mg/kg i.p) or PBS from day 0-14. X-ray and micro CT was used to evaluate bone formation. In the second experiment 5x10⁶ LV-ΔTK-T2A-Luc transduced MBMCs were implanted into the femoral defects to evaluate cell killing by delayed administration of GCV. The mice were treated with either GCV or PBS from day 14-28. In vivo bioluminescent imaging by a Xenogen IVIS CCD optical system was done to detect Luciferase expression.

Statistical Analysis: Data was expressed as Mean ± SEM and student t-test was used for group comparison with significance at P<0.05.

Results

In vivo cell killing effect of GCV administration

After 12 days of treatment with GCV at doses ranging from 0.01 to 1000 μg/ml, LV-ΔTK-T2A-BMP-2 transduced MBMCs were entirely killed by 10, 100 and 1000 μg/ml of GCV and 95% were killed by 1 μg/ml. At 0.01 and 0.1 μg/ml 70% and 25% of the transduced MBMCs survived, respectively. Non-transduced MBMCs treated with 10 μg/ml of GCV showed a 50% cell death and higher doses were lethal to the vast majority of the non-transduced cells (Fig1). Similar results were obtained with the LV-ΔTK-T2A-Luc transduced cells (data not shown).

In vivo cell killing effect of GCV administration

Early GCV administration (from day 0-14): Implantation of 2x10⁶ MBMCs transduced with LV-ΔTK-T2A-BMP-2 in the defect resulted in the healing of the defect at 4 weeks post-op; however, the GCV-treated animals at 4 weeks post-op, (3.06±0.66 mm³ vs. 0.95±0.12 mm³, P=0.05). The GCV was clearly toxic to the transduced cells.

Delayed GCV administration (from day 14-28): 5x10⁶ LV-ΔTK-T2A-Luc transduced MBMCs were implanted in the 2mm femoral defect. There was no significant difference in Luc expression (Total flux, photon/s) detected in the femoral defect prior to starting GCV treatment between the two groups of mice (9.93E+05 ± 1.22E+05 photon/s in the GCV group vs. 8.48E+05 ± 1.43E+05 photon/s in the PBS group, P=0.4). 2 weeks of treatment with GCV resulted in about 60% decrease in the signal intensity at 4 weeks after the surgery (3.82E+05 ± 4.29E+04 photon/s in the GCV group vs. 8.60E+05 ± 2.27E+05 photon/s in the PBS group, P=0.08) (Fig3).

Discussion

We demonstrated successful use of post-translational splicing by T2A sequence to over express BMP-2 or Luciferase and ΔTK. The BMP-2 levels were adequate for defect healing. Early administration of GCV significantly decreased bone healing in the femoral defect indicating a biological response. The clinical application of this strategy requires the elimination of transduced cells after the process of bone healing is completed. Since the defects implanted with BMP-2 over expressing cells already demonstrated bone formation at 2 weeks, we used Luciferase expression for assessment of delayed GCV treatment in a second experiment. There was a trend towards diminished Luc expression after 2 weeks of GCV treatment, but the signal was not completely eradicated. The reasons for different responses to early and late GCV administration are not clear but may include the different number of cells used in each experiment, differences in the biological environment and tissue availability of GCV in the early vs. the delayed period.

In conclusion, early GCV successfully inhibited bone formation by the transduced cells demonstrating proof of concept that TK expression by the vector could result in cell death. When administration of GCV was delayed for 2 weeks after implantation of the transduced cells, 60% of the cells were eradicated. This approach clearly has clinical potential and further experiments are necessary to enhance the suicide gene therapy and to achieve an appropriate level of eradication of the transduced cells.

Significance

We addressed the safety issue involved in using lentiviral vector based regional gene therapy for bone repair and our results suggested that a dual expression vector over expressing BMP-2/Luc and ΔTK could reduce the population of transduced cells after bone healing has occurred.

References