

Mechanisms of PDGF siRNA-mediated inhibition of bone cancer pain in the spinal cord

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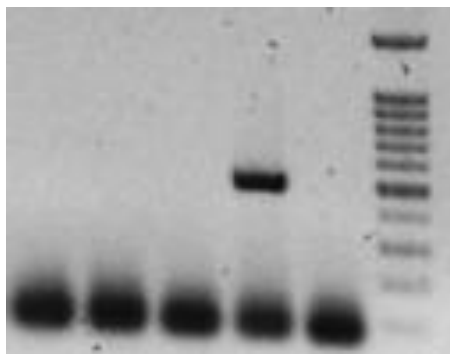
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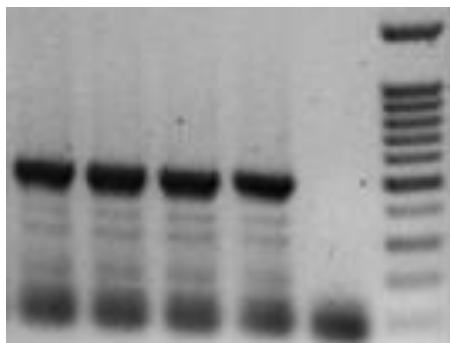
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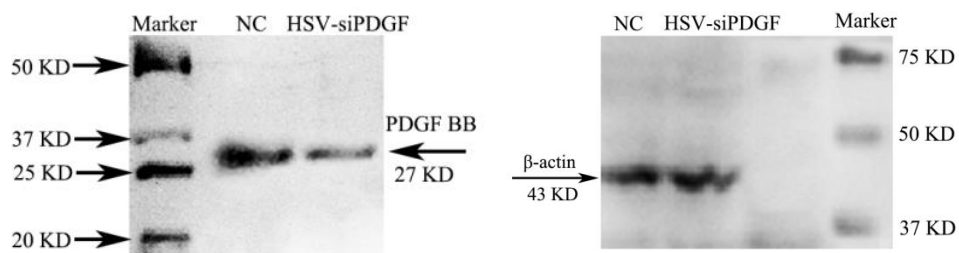
Full-length blots in Figure 1D



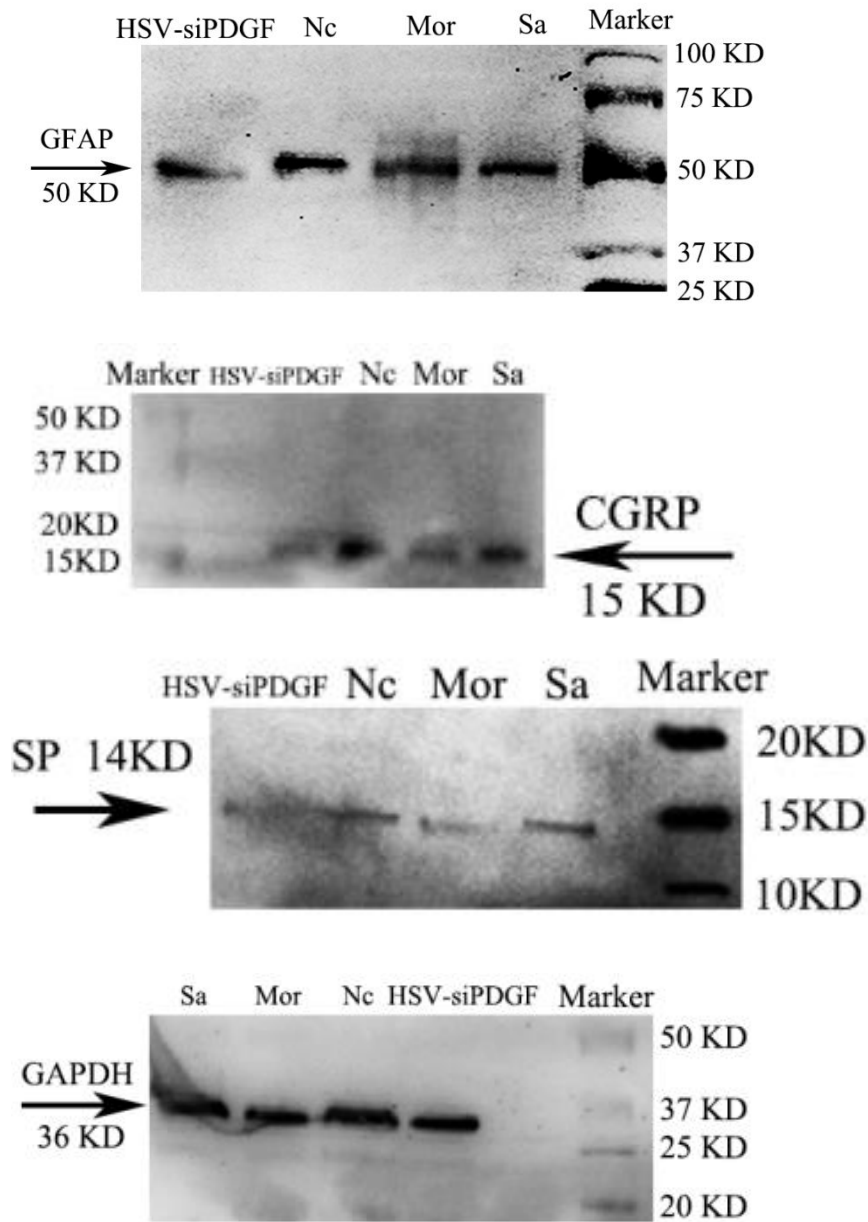
Full-length gels of detected the HSV-1-based virus using RT-PCR analysis in Figure 3C.



Full-length gels of detected of the HSV-1-based recombination using RT-PCR analysis in Figure 3B.

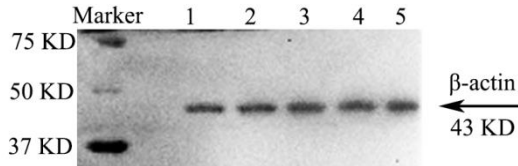


Full-length blots in Figure 4A

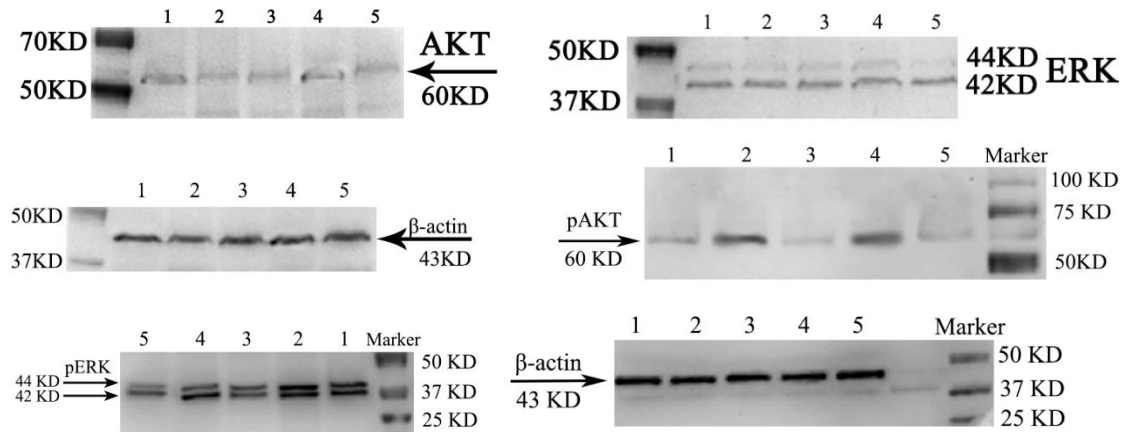


Full-length blots in Figure 4J





Full-length blots in Figure 5A. Lane 1: Normal; Lane 2: Treatment with saline; Lane 3: Treatment with morphine; Lane 4: Treatment with negative control siRNA; Lane 5: Treatment with PDGF siRNA.



Full-length blots in Figure 5C. Lane 1: Normal; Lane 2: Treatment with saline; Lane 3: Treatment with morphine; Lane 4: Treatment with negative control siRNA; Lane 5: Treatment with PDGF siRNA.

Data information for paw withdrawal thresholds in Figure 4B

Data are presented as the mean \pm SEM (0 h after surgery: 9.4 ± 0.57 in the saline group, 8.1 ± 0.99 in the morphine group, 9.0 ± 1.53 in the negative control group, 9.0 ± 1.33 in the HSV-siPDGF group; 1 week after surgery: 11.2 ± 1.49 in the saline group, 8.7 ± 1.45 in the morphine group, 9.3 ± 1.72 in the negative control group, 8.3 ± 1.22 in the HSV-siPDGF group; 2 week after surgery: 9.5 ± 3.48 in the saline group, 7.2 ± 2.01 in the morphine group, 8.0 ± 1.55 in the negative control group, 8.5 ± 1.97 in the HSV-siPDGF group; 3 week after surgery: 11.0 ± 2.13 in the saline group,

6.8±0.86 in the morphine group, 6.2±1.13 in the negative control group, 8.3±1.55 in the HSV-siPDGF group; 4 week after surgery: 6.3±1.53 in the saline group, 6.7±1.81 in the morphine group, 6.4±1.97 in the negative control group, 5.6±1.22 in the HSV-siPDGF group; 1 week after injection: 6.5±0.50 in the saline group, 10.2±0.94 in the morphine group, 8.6±3.63 in the negative control group, 8.3±1.27 in the HSV-siPDGF group; 2 week after injection: 6.6±0.48 in the saline group, 9.5±0.65 in the morphine group, 7.0±0.89 in the negative control group, 9.0±0.78 in the HSV-siPDGF group).

Data information for tail-flick latencies in Figure 4C

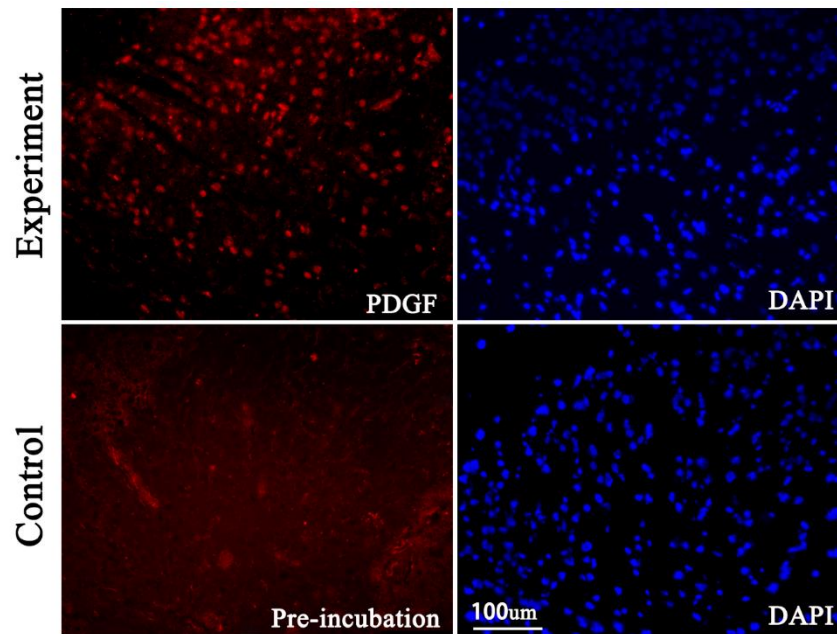
Data are presented as the mean ± SEM (0 h after surgery: 9.0±1.13 in the saline group, 8.0±0.48 in the morphine group, 9.0±1.53 in the negative control group, 9.0±1.11 in the HSV-siPDGF group; 1 week after surgery: 7.2±0.85 in the saline group, 7.3±0.78 in the morphine group, 8.2±1.55 in the negative control group, 7.9±1.21 in the HSV-siPDGF group; 2 week after surgery: 6.7±1.56 in the saline group, 6.3±1.20 in the morphine group, 7.1±0.67 in the negative control group, 7.9±0.62 in the HSV-siPDGF group; 3 week after surgery: 4.7±0.80 in the saline group, 5.9±0.63 in the morphine group, 5.9±0.88 in the negative control group, 5.9±0.94 in the HSV-siPDGF group; 4 week after surgery: 3.3±0.21 in the saline group, 4.0±0.46 in the morphine group, 3.8±0.34 in the negative

control group, 4.7 ± 3.08 in the HSV-siPDGF group; 1 week after injection: 5.2 ± 1.85 in the saline group, 9.18 ± 0.94 in the morphine group, 4.1 ± 1.02 in the negative control group, 10.0 ± 0.53 in the HSV-siPDGF group; 2 week after injection: 5.5 ± 1.71 in the saline group, 9.6 ± 0.62 in the morphine group, 4.3 ± 0.61 in the negative control group, 8.5 ± 1.65 in the HSV-siPDGF group).

Supplemental Figure Legends

Supplementary Figure 1. The specificity of the PDGF labeling is confirmed by the results of experimental controls. The immunogen of PDGF-BB antibody (Abcam, ab16829) were synthesized by HangZhou HuaAn Biotechnology Co. Ltd (Wensan road 199, Hangzhou, China. <http://old.huabio.com/en/index.asp>). The amino acid sequence of this peptide is TEVFEISRRLIDRTNA, which are corresponding to amino acids 101-116 of PDGF BB. This peptide had a purity of >95%. The characterization and purity estimation of the peptides were performed by using electrospray mass spectrometry (ESMS) and reverse phase high performance liquid chromatography (RP-HPLC). All peptides were dissolved in sterilized water to 5.6 mg/ml and stored in aliquots at 4 °C. Briefly, the PDGF-BB antibody was pre-incubated with the synthetic peptide (1:5) overnight at 4°C. The ratio was supported by the customer technical support engineer of Abcam in China. Then, the IF experiment

was done as described. The upper panel was stained with rabbit polyclonal anti-PDGF BB showing obvious PDGF immunofluorescence, whereas the images in the lower panel representing the results of the experimental control did not show any immunoreactivity.



Supplement Fig. S1