NF-κB and let-7f-5p form positive feedback loop to regulate epidermal dendritic T cells in wound healing

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Introduction:
Dendritic epidermal T cells (DETCs) are the only source of insulin-like growth factor-1 (IGF-1) in epidermal tissue, which can promote re-epithelialization and wound healing. In refractory wounds, IL-1β activated NF-κB and inhibited the expression of IGF-1 in DETCs. However, whether and how NF-κB regulates IGF-1 expression in DETCs is unclear.

Methodology:
The mechanism was explored by RT-qPCR, western blot, ChIP analysis, methylation-specific PCR, HE, and immunohistochemistry.

Results: ChIP analysis revealed that NF-κB p65 directly bound to the IGF-1 promoter, implying that NF-κB p65 may compete with a "positive regulatory transcription factor" for binding sites in the promoter of IGF-1, or that NF-κB p65 may induce the expression of regulatory genes such as microRNAs (miRNAs) to inhibit IGF-1 mRNA translation. Let-7f-5p, miR-1a-3p, and miR-98-5p have been identified as IGF-1 specific miRNAs that can bind directly to the 3'UTR of IGF-1 mRNA, and dysregulate IGF-1 expression level. In this study, let-7f-5p was upregulated in IL-1β-treated DETCs in vitro or epidermis around wounds. Pre-let-7f-5p, let-7f-1 and let-7f-2 are respectively located in the 3'UTR of LOC118568094 and the intron of Huwe1. ChIP analysis revealed that NF-κB p65 bound to the promoters of LOC118568094 and HUWE1 to accelerate let-7f-5p expression. A20 is one of NF-κB pathway inhibitors. Let-7f-5p mimic suppressed A20 expression, but let-7f-5p inhibitor enhanced A20 expression and hence diminished NF-κB activation. Let-7f-5p antagomir significantly restored IGF-1 secretion in DETCs and promoted healing of refractory wounds such as IL-1β-treated and ischemic wounds. In addition, let-7f-5p antagomir could improve A20 expression, decrease NF-κB activation, declined IL-17A and IL-1β production to alleviate inflammation.

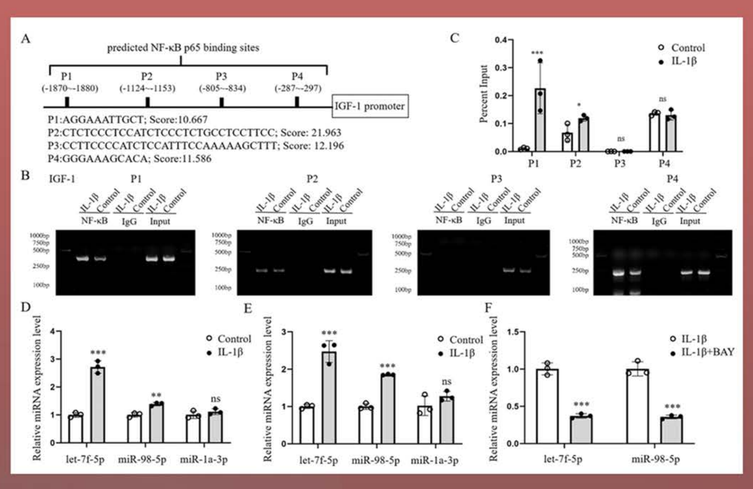

Figure 1. NF-κB did not directly inhibit DETCs to express IGF-1 but promoted the production of let-7f-5p and miR-98-5p.

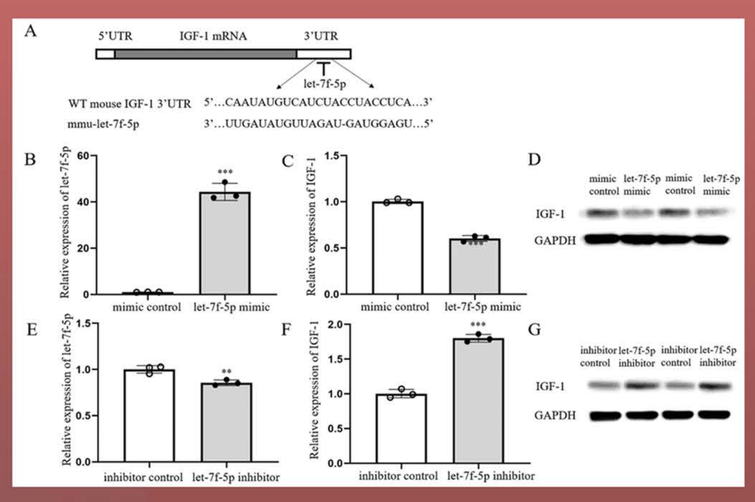

Figure 2. Let-7f-5p downregulated its target IGF-1 in DETCs.

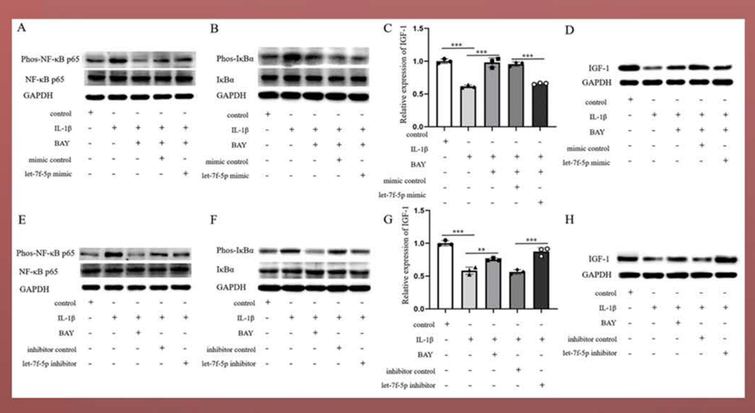

Figure 3. NF-κB impeded IGF-1 expression via let-7f-5p.

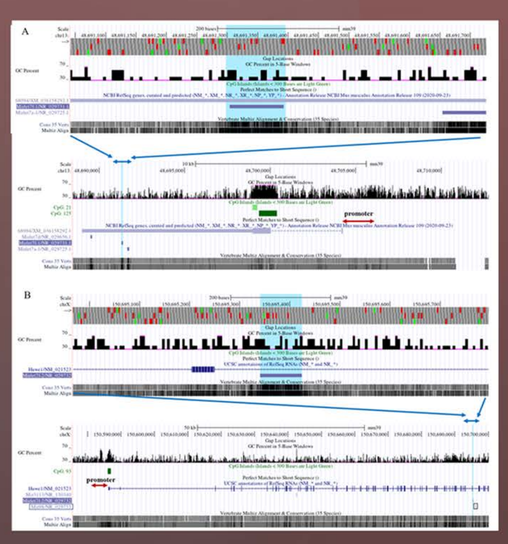

Figure 4. The locations of pre-miRNAs in Genome Reference Consortium Mouse Build 39 (GCA_000001635.9) GRCm39 were obtained from UCSC.

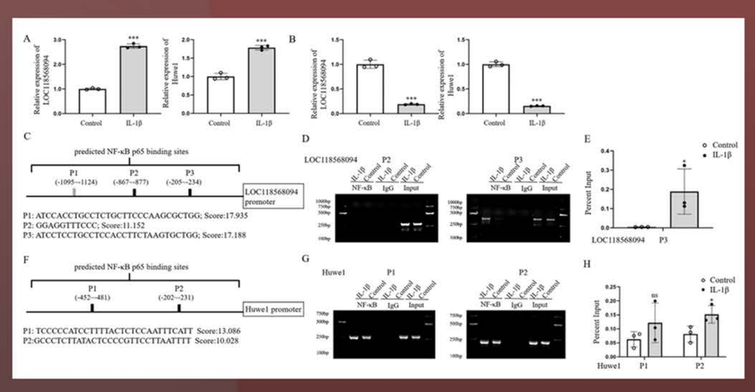

Figure 5. NF-κB p65 could directly bind the promoter of LOC118568094 and facilitate the transcription of pre-let-7f-5p.

Figure 6. NF-κB did not influence the methylation levels of CpG islands in LOC118568094 and HUWE1.

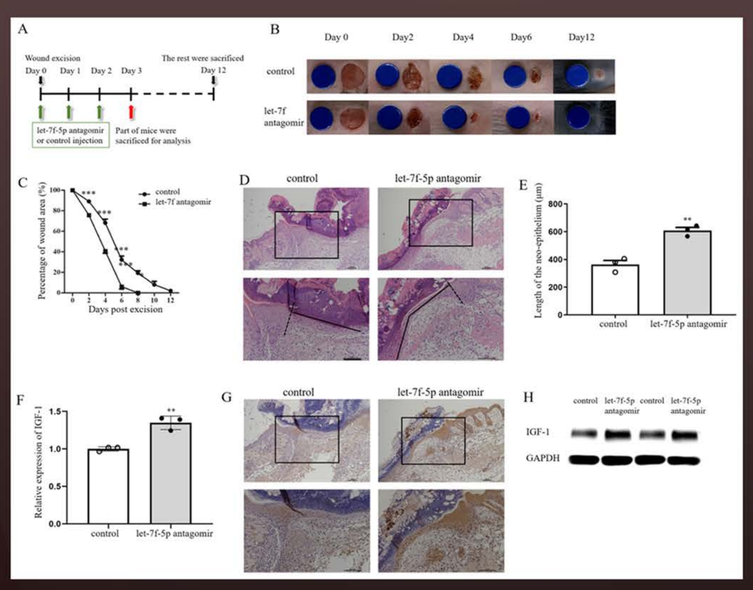

Figure 7. Let-7f-5p antagomir facilitated wound healing in IL-1β-treated mice.

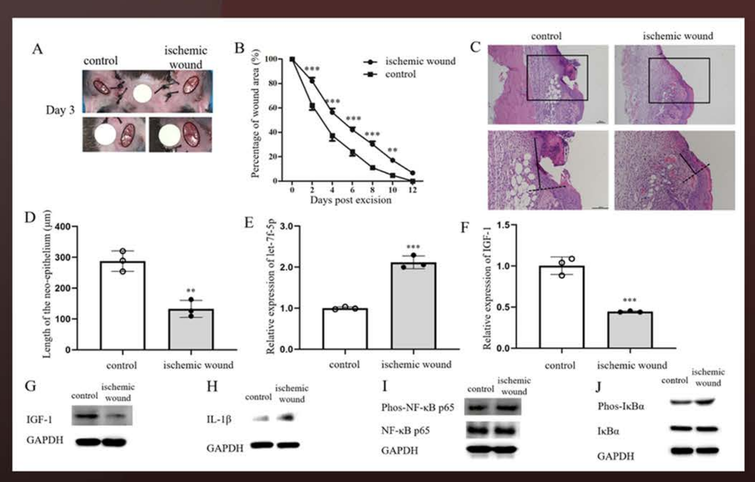

Figure 8. The expression of let-7f-5p was increased in ischemic wound margin.

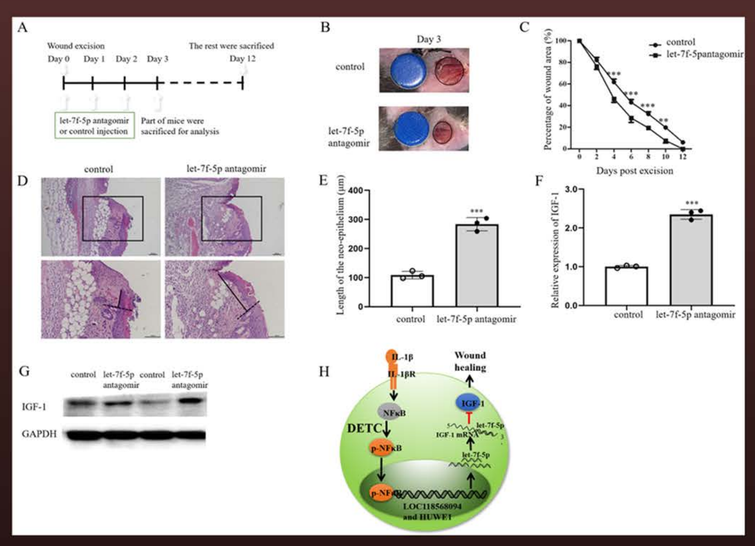

Figure 9. Wound healing in ischemic model was promoted by let-7f-5p antagomir injection in wound margin.

Conclusion: NF-κB and let-7f-5p may form positive feedback loop to diminish IGF-1 production by DETCs and amplify inflammation in wound healing.

Applicability to Clinical Practice: Let-7f-5p antagomir utilized in wound margin could effectively promote refractory wound healing.