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Project Title	Ligustilide inhibits the promotion effect of prostate-cancer associated fibroblasts (CAFS) on endothelial cells
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Report

1.Ligustilide inhibits the promotion ability of CAFs supernatant on endothelial cells.

(1)Ligustilide has no direct inhibition effect on HUVEC.

In some concentration range (under 200 μM), ligustilide has no direct inhibition effect on HUVEC, which is one kind of endothelial cell line (Figure 1).

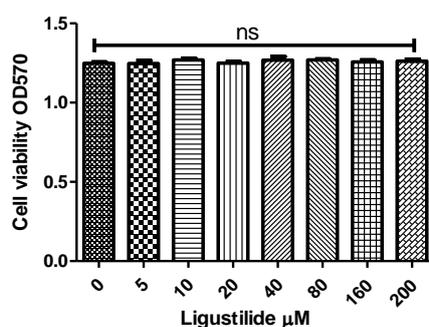


Figure 1. Ligustilide has no direct inhibition effect on HUVEC

Note: 2×10^3 HUVEC were seeded into 96-well plate, and different concentration ligustilide (0,5,10,20,40,80,160 and 200 μM) was added into culture medium, respectively. After 24h, OD₅₇₀ was detected by microplate reader.

(2)CAF's supernatant can promote the growth of HUVEC, while the effect disappear when CAF's were pretreated by Ligustilide.

CAF's were treated by different concentration ligustilide for 48h, and discarded the supernatant, and continued to culture for 24h, and the supernatant were collected (Figure 2).

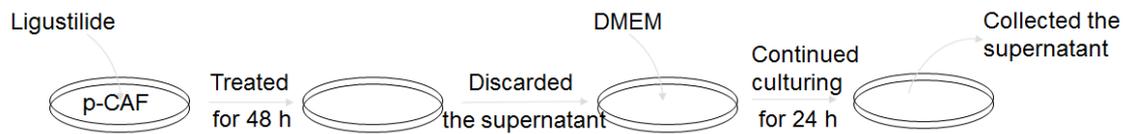


Figure 2 The protocol of ligustilide treats CAFs and the collection of supernatant of CAFs

When CAFs were treated by ligustilide for 48h, CAFs' supernatant no longer promoted the proliferation of HUVEC (Figure 3.)

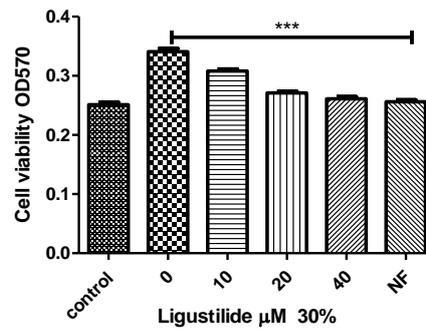


Figure 3 Ligustilide can inhibit the promotion effect of CAFs on HUVEC

(3)CAF's supernatant can significantly promote the migration ability of HUVEC, and ligustilide can inhibit the promoting effect.

CAF's supernatant can promote the migration of HUVEC. While CAFs were treated by HUVEC for 48h, the supernatant of CAFs no longer promoted the migration of HUVEC, which was similar as NF (Figure 4).

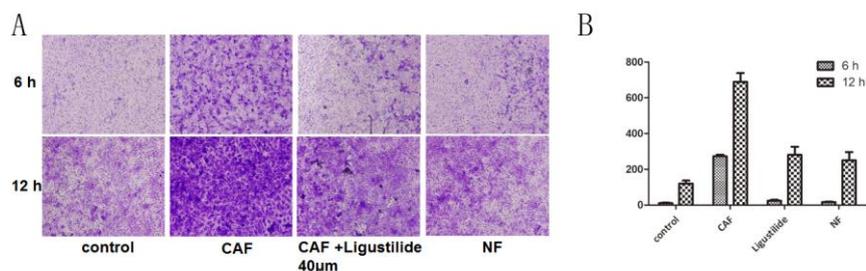


Figure 4 Ligustilide can inhibit the promotion effect of CAFs on HUVEC migration

2.Ligustilide inhibits the promoting effect of CAFs on HUVEC through TLR4.

The promotion effect of CAFs on the migration of HUVEC can be blocked by CLI-095, which is the inhibitor of TLR4(Figure 5A). Similarly, CLI-095 can block the inhibition effect of ligustilide on the promotion effect of CAFs supernatant of HUVEC (Figure 5B) or the cyclization of HUVEC (Figure 6).

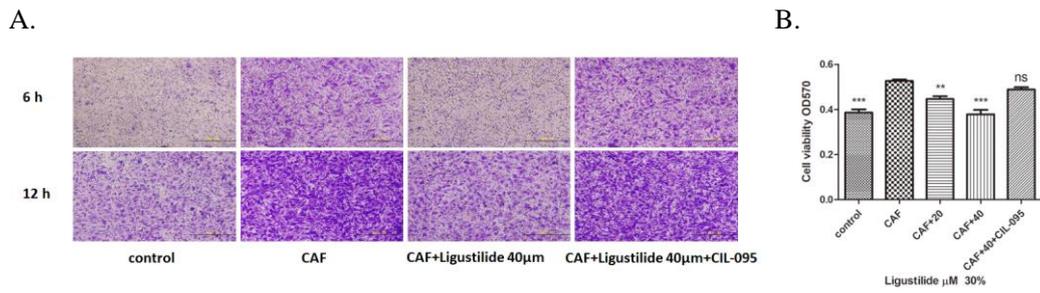


Figure 5 The promotion of CAFs on HUVEC migration was blocked by TLR4 inhibitor (CIL-095) A. The images of transwell assay results of CAFs in bottom chamber and HUVEC in top chamber. B. The statistics results of cell viability detected by MTT.

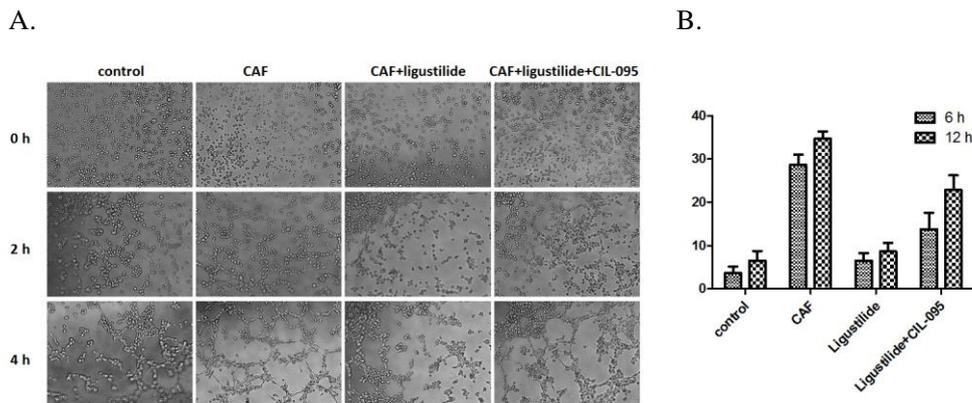


Figure 6 TLR4 inhibitor can block the inhibition effect of ligustilide on the CAFs promotion effect of HUVEC cyclization. A. The cyclization results of HUVEC. B. The statistics results of A.

3. Ligustilide reduces VEGFA derived from CAFs through TLR4.

Ligustilide reduces VEGFA of CAFs, and the effect can be blocked by CIL-095. Moreover, HGF, α -SMA and S100A4 has the similar change as VEGFA (Figure 7).

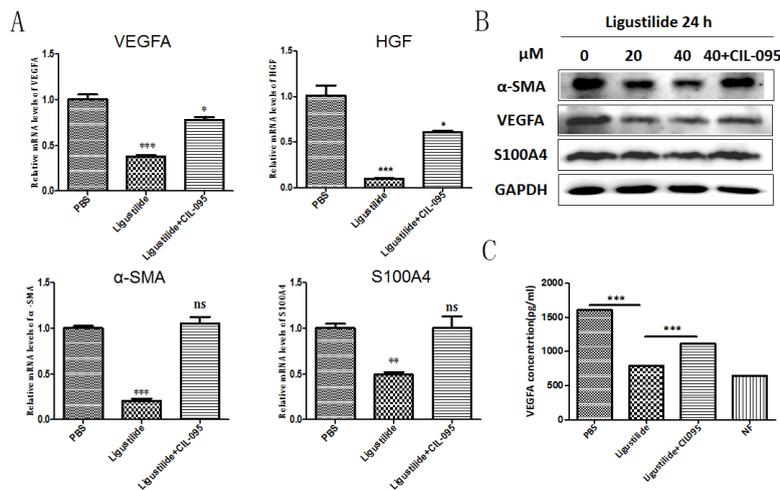


Figure 7 Ligustilide reduces VEGFA derived from CAFs through TLR4. A. The qPCR results of VEGFA, HGF, α -SMA and S100A4. B. The western blot results of α -SMA, VEGFA and S100A4. C. The ELISA results of VEGFA derived from four groups: CAFs, CAFs treated by ligustilide, CAFs treated by ligustilide and CLI-095, and NF.

4. Ligustilide-induced CAFs supernatant inhibits AKT, p38 and Jun in HUVEC.

When HUVEC was cultured by the supernatant of CAFs treated by ligustilide, the p-AKT, p-p38 and p-Jun protein level and mRNA level both decreased. The results indicated that AKT, p38 and Jun involves in the effect of ligustilide on CAFs to HUVEC.