Investigating the Role of Notch Signaling in Endometrial Mesenchymal Stem-like Cells

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INTRODUCTION: Human endometrium undergoes cycles of proliferation, differentiation and shedding during the female reproductive years. Endometrial mesenchymal stem-like cells (eMSCs) contribute to this regenerative process (1). Notch signaling pathway is known to play a vital role in cell fate decisions in many somatic stem cells (2). However, its role in endometrial stem cells remains unclear. We firstly examined the gene and protein expression related to Notch signaling in subpopulations of endometrial stromal cells: endometrial stromal fibroblast (eSF, $CD140b^{+}CD146^{-}$ cells) and eMSCs ($CD140b^{+}CD146^{+}$ cells). The importance of Notch signaling by gain or loss of function approaches in eMSCs was evaluated.

METHODS: Full thickness endometrial tissues were obtained from women undergoing hysterectomy. After mechanical and enzymatic dissociation, endometrial stromal cells were purified from epithelial cells using EpCAM magnetic beads and leukocytes were removed using CD45 magnetic beads. EMSCs were then obtained using CD140b and CD146 magnetic beads. The gene and protein expression of Notch signaling in stromal subpopulations were evaluated by qPCR, western blotting and immunofluorescence. For functional assays, eMSCs (seeded at 4000 cell/cm^2) were treated with Notch activator (Jagged-1, 2ug/ml) or Notch inhibitor (DAPT, 1.25uM/ml; Notch1-siRNA) for 7 days and flow cytometry was used to assess the phenotypic expression of eMSCs. Western blotting confirmed the activation or inhibition of Notch-related proteins in eMSCs.

RESULTS: The expression of Notch target genes HES-1 (Fig 1A) and HEY-L (Fig 1B) were significantly higher in eMSCs compared with eSF (n = 12, P < 0.01). While the gene expression of HEY-1 (Fig 1C), HEY-2 (Fig 1D) were similar between these two groups (n = 10).

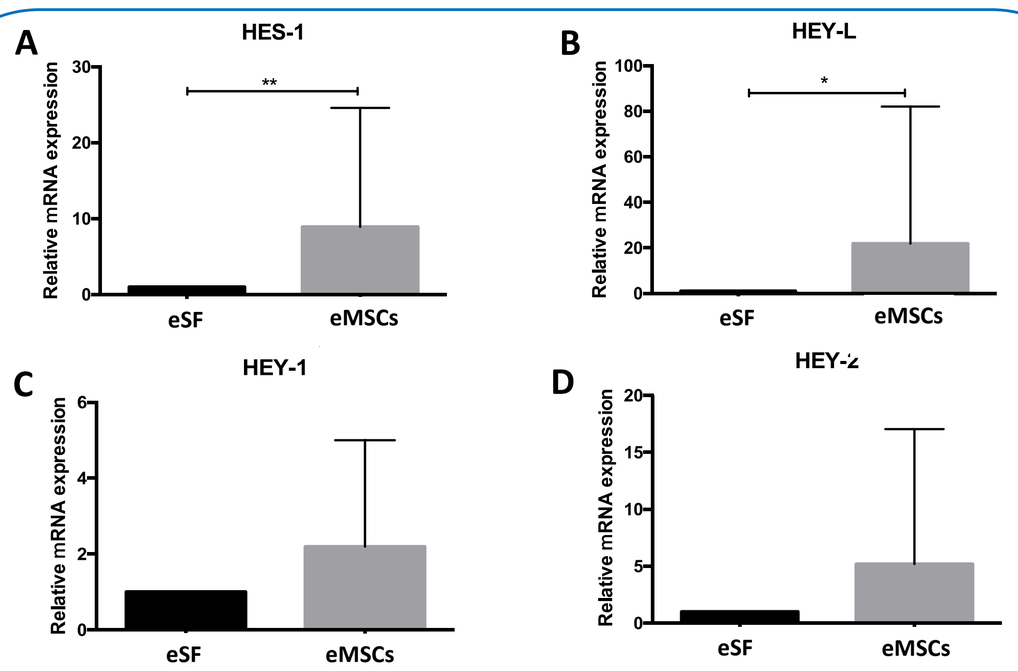

Figure 1. The expression of Notch target genes on stromal subpopulations.

EMSCs showed an increasing trend on the gene expression of *Notch1* on eMSCs compared with eSF (Fig 2A). Interestingly, western blotting and immunofluorescence revealed that Notch1 protein was abundantly expressed by eMSCs when compared to eSF (n = 5, P < 0.01, Fig 2B and 2C).

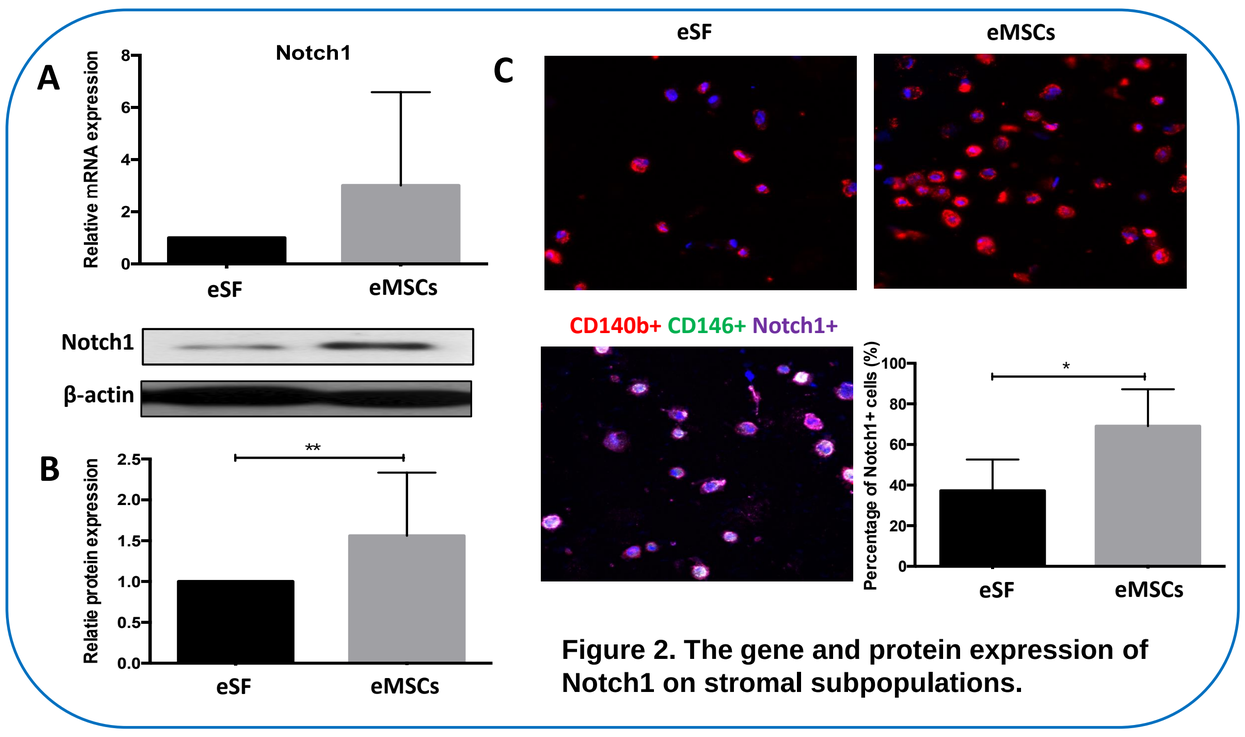

Figure 2. The gene and protein expression of Notch1 on stromal subpopulations.

The relative percentage of the cells co-expressing CD140b and CD146 evidently increased when the Notch was activated by Jagged-1 (n = 6, P < 0.01, Fig 3A). Inhibition of Notch with DAPT significantly reduced the proportion of eMSCs at high seeding density when compare to untreated cells (n = 6, P < 0.01, Fig 3A). Similarly, knock down with Notch1-siRNA, reduced the stimulatory effect of Jagged1 protein on the eMSC phenotypic expression compared to siRNA control (n = 7 , P < 0.01, Fig 3B).

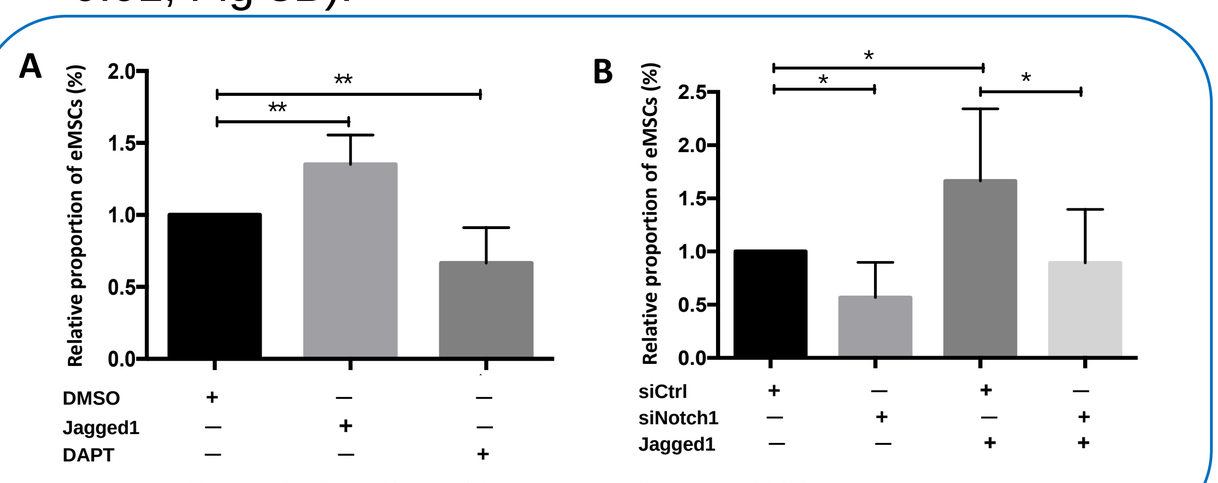

Figure 3. The effect of Notch signaling on eMSCs maintenance.

Western blotting confirmed that Jagged1 upregulated the expression of Notch-related proteins NICD, HES-1 and HEY-2 in eMSCs, while DAPT showed the opposite effect (n = 5, P < 0.05, Fig 4).

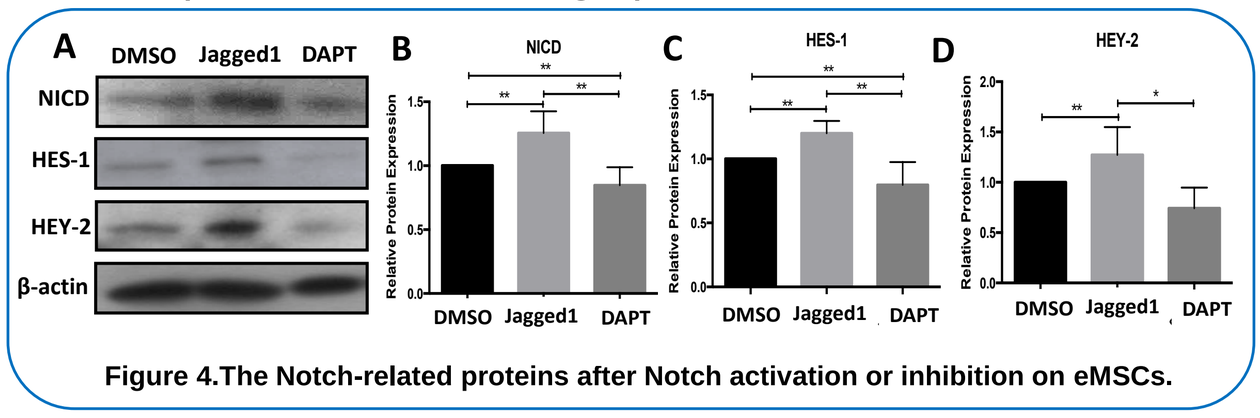

Figure 4.The Notch-related proteins after Notch activation or inhibition on eMSCs.

CONCLUSION: Our findings suggest that eMSCs can activate Notch signaling and may have a role in cell-fate specification of stem cells.

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