The WNT receptor FZD5 is required for the maintenance of Endometrial Mesenchymal Stem-like Cells

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Introduction

Human endometrium undergoes cyclical regeneration under the regulation of sex steroids. The existence of endometrial mesenchymal-like stem cells (eMSCs) can contribute to the remarkable regenerative capacity[1]. Myometrial cells activate the WNT/β-catenin signaling via WNT5A to maintain the self-renewal capacity of eMSCs[2]. WNT ligands operate by binding to products of the frizzled (FZD) family and co-receptor low density lipoprotein receptor-related protein 5 (LRP5) which activates WNT signaling pathway.

Objective

To investigate the expression pattern of WNT receptors in different populations of endometrial stromal cells and evaluate the importance of the WNT-FZD interaction in eMSCs self-renew and proliferation.

Methods Human endometrial stromal cells and myometrial were obtained from women undergoing hysterectomy. Single stromal cell suspensions were obtained by enzymatic digestion. Red blood cells and leukocytes were removed by ficoll-paque and CD45 dynabeads, respectively. Purified stromal cells were obtained after epithelial cells were selected using magnetic beads labeled with CD326. EMSCs were selected by magnetic beads which co-express CD140b and CD146 markers. Gene and protein expression of WNT5A-related frizzled receptors (FZD1, FZD4, FZD5 and FZD7) as well as co-receptor LRP5 were assessed by qPCR and immunofluorescent staining in different stromal subpopulations - unfractionated stromal cells, progenitor cells ($CD140b^+CD146^-$ cells) and eMSCs ($CD140b^+CD146^+$ cells). The functional activity of ligand-receptor binding was assessed with indirect co-culture of eMSCs to myometrial cells at a ratio of 1:90 or with WNT5A conditioned medium (CM) for 15 days. Inhibition of FZD5 on eMSCs was performed with the addition of anti-FZD5 antibody or gene silencing with FZD5-siRNA. The clonogenicity and phenotype expression of eMSCs were determined by counting the colonies and analyzing the co-expression of eMSC markers by flow cytometry, respectively. The WNT signal was measure with TCF/LEF luciferase assay and western blotting against active β-catenin.

Results Assessment of stromal subpopulations revealed a significantly higher level of *FZD5* (Fig 1C) gene expression when compared to unfractionated stromal and progenitor cells. While mRNA expression of other receptors (Fig 1A, B, D, E) showed no significant difference between stromal subpopulations.

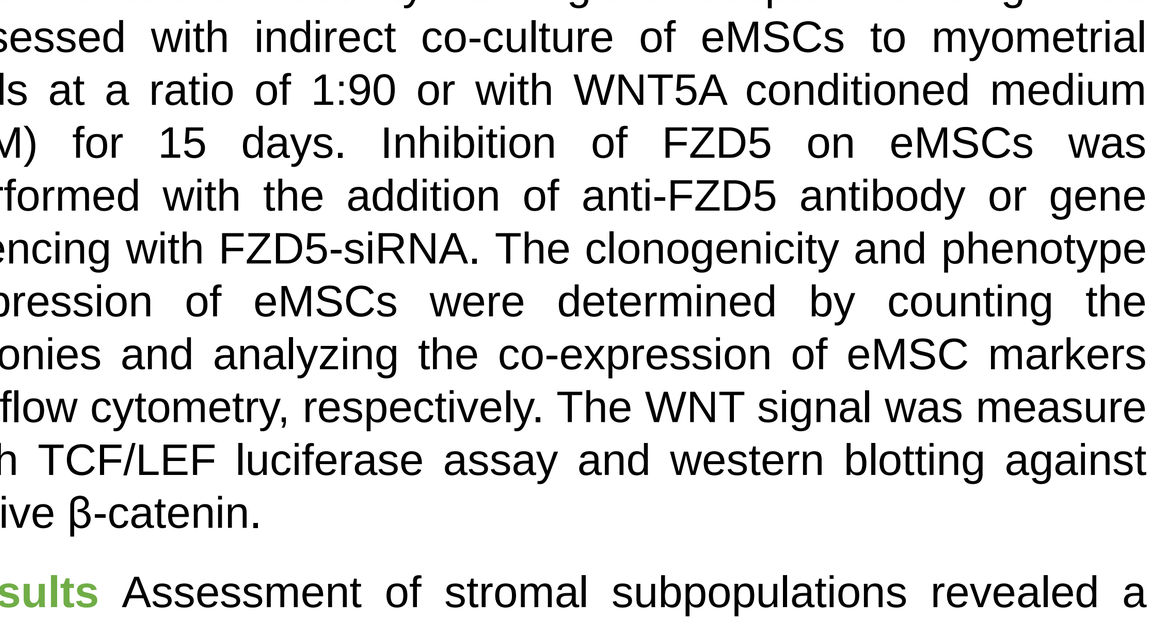
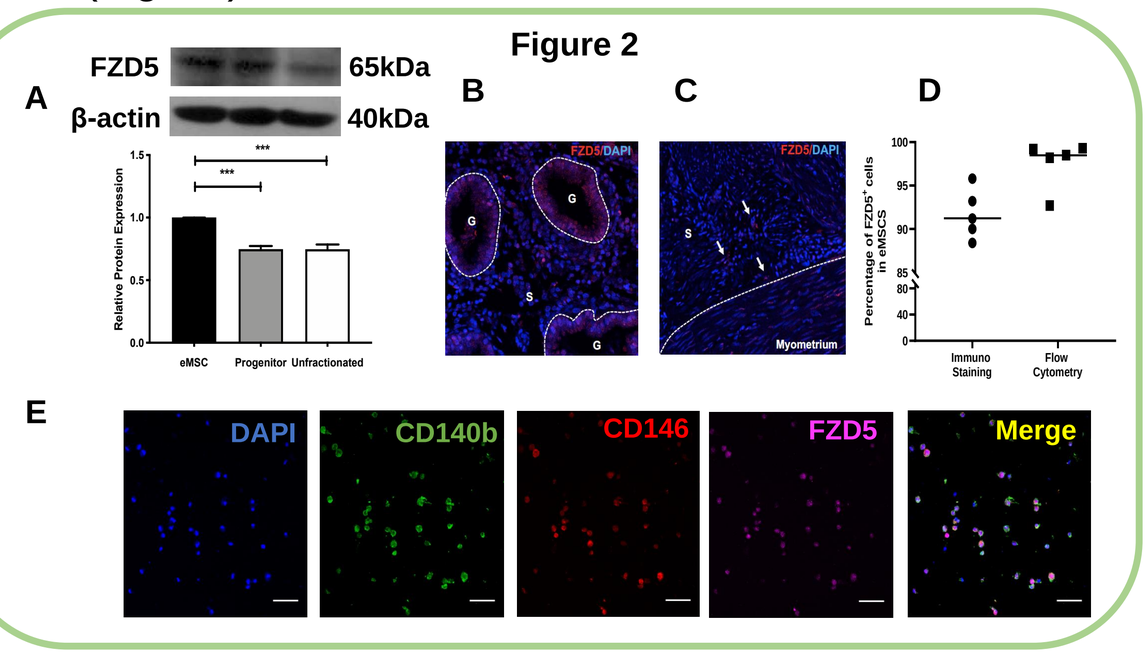
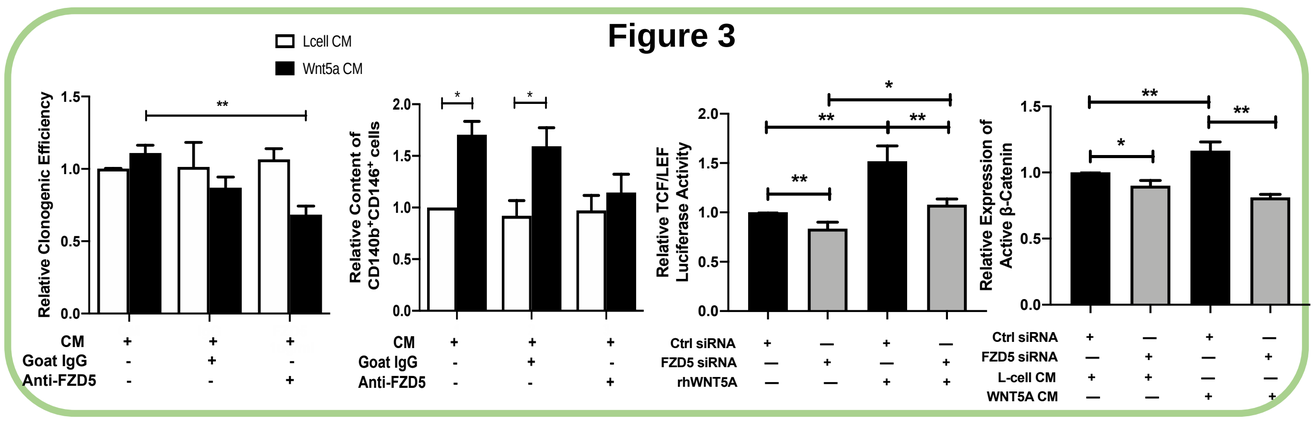


Figure 1

Consistently, protein expression of FZD5 was the highest in eMSCs among stromal populations (Fig 2A). Endometrial glands and stromal cells express FZD5 (Fig 2B) and $FZD5^+$ cells resided near the endometrial-myometrial junction (Fig 2C). EMSCs express FZD5 (Fig 2E) and the expression level is ~90% (Fig 2D).



Addition of neutralizing anti-FZD5 antibody reduced the stimulatory effect of WNT5A CM on clonogenicity (Fig 3A) and phenotypic expression (Fig 3B) in eMSCs. After knocked down of FZD5 expression using FZD5-siRNA, the induced effect of recombinant WNT5A protein (Fig 3C)/WNT5A CM (Fig 3D) on TCF/LEF luciferase activity and expression of active β-catenin reduced when compared to siRNA control.



FZD5 and LRP5 co-expressed in eMSCs (Fig 4A). Blocking WNT co-receptor LRP5 with recombinant DKK1 protein reduced the stimulatory effect of recombinant WNT5A protein on clonogenicity (Fig 4B) and phenotypic expression (Fig 4C) in eMSCs.

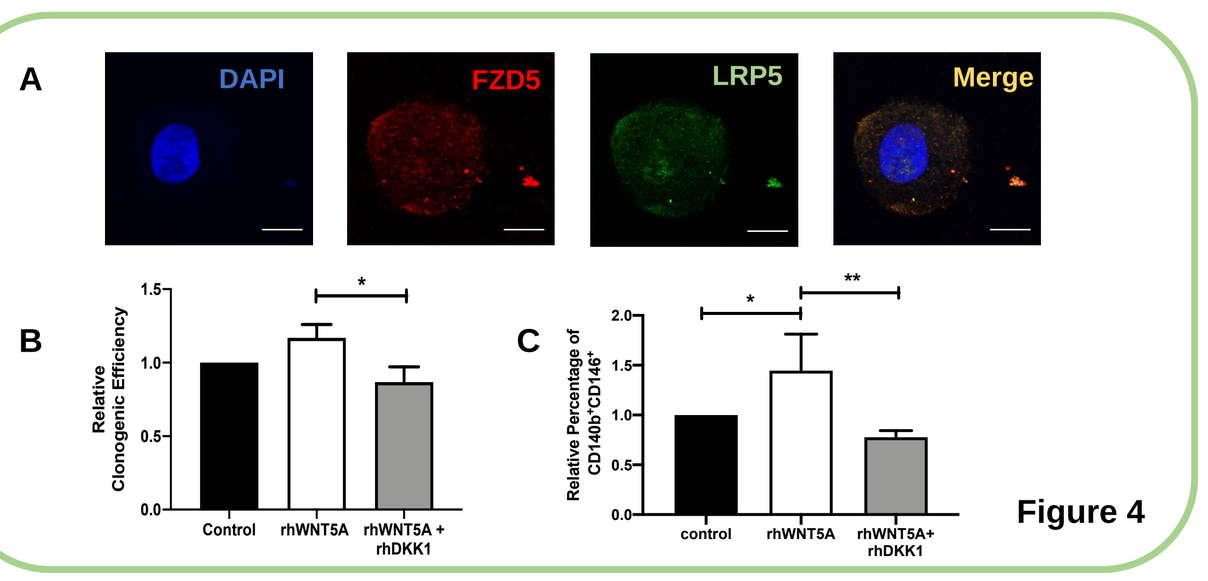


Figure 4

Conclusion: Our findings suggest that WNT5A secreted by myometrial cells can interact with WNT receptor FZD5 and co-receptor LRP5 to modulate the self-renewal of eMSCs by the activation of WNT/β-catenin signaling pathway.

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References:

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