Sonic Hedgehog as a Regulator of Endometrial Mesenchymal Stem/Stromal Cell Activity

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Endometrial mesenchymal stem/stromal cells (eMSCs) were first described as a clonogenic perivascular population with multilineage differentiation potential.[1] eMSCs are believed to contribute to the regenerative properties of the endometrium, and their therapeutic potential is being investigated for cell-based treatments.[2] Accordingly, there is great interest in the roles that endometrial stem/progenitor cells and signaling pathways play in endometrial dynamics. Cho et al.[3] recently published a study in *Molecular Therapy* implicating declining hedgehog (HH) signaling in the aging and senescence of eMSCs. In this issue of *Molecular Therapy*, Park et al.[4] extend their investigations to describe a role for a HH pathway ligand in promoting the regenerative properties of human eMSCs. For clarity, it should be noted that Cho et al.[3] and Park et al.[4] examine eMSC cultures derived from unsorted human endometrial stromal cells, a broader definition of eMSCs than some previous studies, including formative work in the field.[1,5]

The endometrium is the mucosal lining of the uterus that supports embryo implantation. During a woman's reproductive years, approximately 5–10 mm of endometrium is produced each month in response to hormonal stimuli. In the absence of embryo implantation, most of the endometrium is shed during menstruation. A scarless repair process ensues, followed by endometrial regeneration. Defects in endometrial repair and regeneration can result in the scar tissue and adhesions that characterize Asherman's syndrome, while failure to sufficiently regenerate the endometrial layer results in a thin endometrium that cannot support embryo implantation.[6] Proliferative diseases of the endometrium, including endometriosis, where endometrium grows outside the uterus, may involve aberrant or uncontrolled activation of endometrial regeneration programs.[6]

HH signaling is a critical determinant of vertebrate developmental patterning and also has roles in tissue homeostasis and regeneration via effects on stem/progenitor populations.[7] HH signaling in vertebrates typically involves three secreted HH ligands (Sonic hedgehog [SHH], Indian hedgehog [IHH], and Desert hedgehog), the Patched1 (PTCH1) membrane receptor, and the downstream mediator Smoothened (SMO), which controls the activity of three GLI family transcription factors (GLI1, GLI2, and GLI3). HH ligands appear to be functionally interchangeable but are expressed in a developmental- and tissue-specific manner. Signal transduction is compartmentalized to a membrane-bound microtubule-based projection called the primary cilium.[8] While this is the best characterized mechanism of HH signaling in vertebrates, GLI activation independent of SMO has also been reported, particularly in the context of cancer.[9]

Several lines of evidence point to roles for HH signaling in the adult endometrium. Cho et al.[3] previously noted a decline in SHH expression and reduced activation of HH signaling during aging in mouse endometrium and senescence in cultured human eMSCs.[3] Perivasular eMSCs from human endometrium showed evidence of activated HH signaling.[10] Other studies, while not specifically addressing stem cells, make observations that are broadly consistent with roles for HH signaling in eMSCs. IHH promotes the decidualization of endometrial stromal cells, a progesterone-driven differentiation process required for embryo implantation.[11,12] IHH expression and HH signaling are reduced in the eutopic (within the uterus) endometrium of women with endometriosis.[13] Conversely, using Kruppel-like factor 9-deficient endometrium to induce endometriosis in a mouse model results in more lesions and increased lesion SHH and HH signaling.[14] SHH-mediated HH signaling is also increased in endometrial cancer and primary cultures established from endometrial hyperplasia.[15]

In their latest study, Park et al.[4] investigated SHH during endometrial injury, repair, and regeneration. They established that cell types, including eMSCs, fibroblasts, and endothelial cells, produce SHH in response to *in vitro* injury or stress. Endometrial injury in a mouse model also induced SHH production that could be detected in serum from peripheral blood. While injury-induced production of SHH is clearly not a MSC-specific property, Park et al.[4] provide evidence that SHH enhances eMSC properties relevant to repair and regeneration. SHH treatment of human eMSCs increased proliferation and migration and promoted the expression of transcription factors frequently associated with stem cells (C-MYC, KLF4, NANOG, and SOX2). SHH treatment also increased the osteogenic differentiation of eMSCs, although this could be due to the known role of HH signaling in osteogenic differentiation[16] rather than effects related to stemness and pluripotency. Park et al.[4] also present data suggesting the effects of SHH on eMSCs are due to growth factor-induced FAK/ERK1/2 and PI3K/Akt signaling,[4] pathways they have previously shown to promote the therapeutic potential of adipose-derived MSCs in a mouse model of liver fibrosis.[17]

To examine the therapeutic potential of SHH via its action on eMSCs, Park et al.[4] delivered SHH-treated human eMSCs to an immunodeficient mouse model of endometrial injury

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by intravenous injection. SHH treatment increased homing to and/or persistence of eMSCs in the injured endometrium. The phenotype of infused eMSCs and their precise location within the endometrium were not determined. Limited qualitative assessment of histology suggested that SHH treatment enhanced the protective or reparative effects of eMSCs. Given the preliminary nature of the these data, additional studies will be required to verify the therapeutic effect of SHH- treated eMSCs and investigate the potential mechanisms of action.

Further consideration of the role of HH signaling in the endometrium is clearly warranted, particularly with respect to the regulation of stem/progenitor cells. Data presented by Park et al.[4] raise several questions. Are the endometrial effects of SHH specifically due to influences on stem/progenitor populations or are broader effects on differentiated cell types (e.g., endometrial stromal fibroblasts) also contributing? Examining a more purified eMSC population than that studied by Park et al.[4] may help answer this question. IHH also has an important role in the endometrial response to progesterone, and it is unclear whether this function has any overlap with the SHH-mediated effects described by Park et al.[4] Mechanisms of HH signal transduction in the endometrium also require clarification. Park et al.[4] describe the effects of the ligand SHH, but whether typical HH activation via PTCH1, SMO, and GLI transcription factors is involved remains unclear. Additionally, primary cilia are dynamic organelles that are central to HH transduction in other tissues but are poorly studied in the endometrium.

Understanding the role of HH signaling components in the function of endometrial stem/progenitor cells is likely to provide insight into the pathogenesis of diseases and disorders, including infertility, Asherman's syndrome, endometriosis, and endometrial cancer. HH signaling is activated in many tumors, and selective modulators targeting this pathway are being developed for cancer therapy.[7] Studies of HH signaling in the endometrium may identify new therapeutic uses for these HH modulators in the treatment of endometrial diseases and disorders.

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