84. CLONOGENICITY OF HUMAN ENDOMETRIAL EPITHELIAL CELLS

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The human endometrium regenerates from the lower basalis layer, a germinal compartment that persists after menstruation to give rise to the new upper functionalis layer. We hypothesise that epithelial stem cells (ESCs) reside in the endometrial basalis from which new endometrial glands grow with each menstrual cycle. One property of stem cells is their clonogenic ability. The aims of this study were to determine 1) the clonogenic activity of human endometrial epithelial cells, 2) which growth factors support clonogenic activity and 3) compare clonogenic activity of various endometrial states. Endometrial tissue was obtained from 21 women undergoing hysterectomy from proliferative, secretory or inactive states. The entire endometrium was dissociated with collagenase to achieve single cell suspensions. Glandular epithelial cells were selected using Ber-EP4 Dynabeads and cultured at low seeding density (500 cell/cm^2) for 15 days in serum-containing medium (SM), or serum-free medium (SFM) containing either PDGF-BB, TGF-α, EGF, IGF-1, LIF, FGF-2, HGF or SCF on mouse fibroblast feeder layers. Colonies were stained and the cloning efficiencies were determined. Two types of colonies were observed: small loosely packed colonies (SC) containing large cells (<4000 cells/colony) and large colonies (LC) with small, densely packed cells (>4000 cells/colony). In SM, the clonogenicity was 0.20 ± 0.05% (n = 21), 0.08 ± 0.02% for LC and 0.12 ± 0.03% for SC. In SFM, TGF-α, EGF and PDGF strongly supported clonogenicity of epithelial cells in the proliferative phase 0.57 ± 0.20% (n = 7), 0.36 ± 0.14% (n = 8), 0.43 ± 0.14% (n = 7) respectively and in the secretory phase 0.47 ± 0.16% (n = 5), 0.60 ± 0.29% (n = 5), 0.53 ± 0.25% (n = 5). Clonogencity was generally lower in inactive endometrium for all growth factors. HGF, SCF and FGF-2 showed no support for clonogenic activity of epithelial cells from proliferative or inactive endometrium, while LIF and IGF-1 were weakly supportive. Variation between individual samples was high, possibly masking differences in clonogenicity between the endometrial states. This study provides preliminary evidence for the existence of ESCs in the human endometrium, identifies 5 growth factors supporting the clonogenic activity of ESCs and demonstrates some differences in clonogenic activity of epithelial cells from proliferative, secretory and inactive endometrium.

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