IDENTIFYING MARKERS FOR STROMAL STEM/PROGENITOR CELLS IN HUMAN ENDOMETRIUM

K. E. Schwab*, *C. E. Gargett

Centre for Women's Health Research, Dept O & G, Monash University, Clayton, VIC, Australia

The endometrium is divided into upper functionalis, which rapidly grows then differentiates before being shed, and lower basalis, from which cyclical regeneration begins. A small proportion of endometrial stromal cells have been identified with clonogenic activity, a functional property of stem cells (1). We hypothesised that stromal stem/progenitor cells expressing known stem cell markers reside in the basalis. The aims of this study were to: *(1)* investigate the clonogenic activity of human endometrial stromal cell populations enriched and depleted for known stem cell markers, and *(2)* identify a marker that will differentiate basalis from functionalis stroma. Endometrial tissue acquired from 23 ovulating women undergoing hysterectomy was digested with collagenase to produce single cell suspensions. Leukocytes and epithelial cells were removed, and stromal cells analysed by flow cytometry, FACS sorted into enriched and depleted populations, and cultured for clonal analysis as described (1). Markers analysed included stem cell markers, STRO-1, CD133, CD45 and CD34, and an endometrial stromal cell marker, CD90 (2).

Immunohistochemical analysis of CD90 was performed on full thickness human endometrial tissue. $CD45^-$ endometrial stromal cell populations contained 2.13 ± 0.65% (n = 13) $STRO\text{-}1^+$, and 5.43 ± 1.42% (n = 16) $CD133^+$ cells. Stromal cell populations enriched (0.65 ± 0.42%) and depleted (0.95 ± 0.58%) for STRO-1 showed no significant difference (P = 0.19, n = 5) for clonogenic activity. Surprisingly, clonogenicity of $CD133^+$ stromal cells (0.74 ± 0.56%) was lower than $CD133^-$ (3.89 ± 1.35%) cells (P = 0.03, n = 6). Immunohistochemical staining showed strong CD90 staining in the functionalis, with lighter staining in the basalis. These observations were confirmed by flow cytometric analysis which identified two distinct populations (n = 9), $CD90^{low}$ (19.55 ± 4.35%) and $CD90^{hi}$ (74.71 ± 5.20%). Clonogenic analysis of these two populations is underway. Interestingly, dual-colour flow cytometry showed the $CD133^+$ cells to be $CD90^{low}$ (n = 7). Further analysis suggests that the $CD90^{low}CD133^+$ population are $CD45^-CD34^+$, suggesting endothelial progenitor cells. This study identified CD90 as a marker that distinguishes basalis and functionalis stroma, and demonstrated that STRO-1 and CD133 are not functional markers for clonogenic endometrial stromal stem/progenitor cells.

(1) Chan RW, Schwab KE, Gargett CE (2004) *Biol. Reprod.* **70**, in press. (2) Fernandez-Shaw S, Shorter SC, Naish CE, Barlow DH, Starkey PM (1992) *Hum. Reprod.* **7**,156–161.

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