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Research Article

# Stem Cells Expression In Endometrial Carcinoma

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**Abstract**

**Background:** The human endometrium is a dynamic remodeling tissue undergoing more than 400 cycles of regeneration, differentiation, and shedding during a woman's reproductive years. **Objective**: to evaluate stem cells expression in the endometrium. **Subjects and methods:**40 cases divided into four groups, menstrual blood (10 cases), Prolifrative endometrium (10 cases), secretory endometrium (10 cases) all formers groups are controls, and endometrial cancer group (10 cases). Two specimens from each case were obtained: The first slide was stained by haematoxline and eosin to assure the diagnosis. The second slide was collected on charged slides then stained by Oct- 4 (octamer-binding transcription factor 4) to detect stem cell expression for scoring; the Edessy stem cell score was applied by giving a score for each finding 0, 1, 2. **Results:** stem cell total score was higher for endometrial cancer group than menstrual blood and proliferative endometrium groups, but it was nearly similar to that of secretory endometrium.

**Keywords:** human endometrium, stem cells expression, Prolifrative endometrium.

## Introduction

Adult stem cells are rare, undifferentiated cells present in adult tissues and organs. They are extremely difficult to identify in tissues, as they are rare, lack distinguishing morphological features, and specific adult stem cell markers are currently unavailable. Adult stem cells are therefore defined by their functional properties: substantial self-renewal, high proliferative potential, and ability to differentiate into one or more lineages **[Shostak S. et al., 2006].** These functions are highly regulated by the stem cell niche to ensure an appropriate balance between stem cell replacement and provision of sufficient differentiated mature cells for tissue and organ function **[Schwab KE. et al ., 2012].**

There is increasing interest in the concept that endometrial stem/progenitor cells may be responsible for the highly regenerative capacity of human endometrium. It has been hypothesized that both epithelial and stromal adult stem cells exist in the basal layer of human endometrium, since regeneration occurs from this layer after the top two-thirds or functional layer is shed at menstruation, and the endometrium comprises glandular tissue supported by an extensive vascularized stroma**[Chan RWS. et al., 2004].** Initial evidence from cell cloning studies suggests that adult stem cells are likely present in human endometrium **[Schwab KE et al., 2005]**, but subsequent studies have focused on various subpopulations of epithelial and/or stromal cells rather than individual cells **[Wolff EF et al.,2007].** The pluripotency marker, Oct-4 has been observed in some cells in human endometrial stroma**[Matthai C. Et al., 2006],** but the identity and stem cell function of these cells was not examined. To date, adult stem cell

activity of individual human endometrial epithelial and stromal cells has not been investigated.

Disorders of uterine endometrial proliferation are common, leading to endometriosis, endometrial hyperplasia, and endometrial cancer despite their common occurrence and the substantial public health burden that these diseases present **[Simoens S. et al., 2007]**, little is known about their pathogenesis **[Amant F. Et al., 2005].** We hypothesize that endometrial stem or progenitor cells play key roles in the initiation of these endometrial proliferative disorders **[Gargett CE.2007].** In endometriosis, endometrial stem/progenitor cells may be shed into the pelvic cavity by retrograde menstruation to establish endometriotic growths **[Gargett CE.2007].** Endometrial epithelial progenitors or their immediate progeny may be targets of early genetic or epigenetic alterations, leading to the emergence of endometrial cancer stem cells that initiate and maintain endometrial cancer **[Di Cristofano A. Et al., 2007]**.
Stem cells are undifferentiated, "blank" cells that do not yet have a specific function. Additionally, stem cells are self-sustaining and can replicate themselves for long periods of time. **Andrews et al; (2005)**.

Stem cells are the foundation cells for every organ, tissue and cell in the body. They are like a blank microchip that can ultimately be programmed to perform any number of specialized tasks. Under proper conditions, stem cells begin to develop into specialized tissues and organs. **Stojkovic et al., (2004)**.

Stem cells are unspecialized cells that can differentiate into more mature ones with specialized functions. In human, they have been identified in the inner cell mass of the early embryo, in some tissues of the fetus, the umbilical cord and the placenta, and in several adult organs.

Stem cells are found in most, if not all, multi-cellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. Research in the stem cell field grew out of findings by Canadian scientists Ernest A. McCulloch and James E. Till in the 1960s .. In some adult organs , stem cells can give rise to more than one specialized cell type within that organ ( for example , neural stem cells give rise to three cell types found in the brain : neurons , glial cells and astrocytes ).

Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine. **Filip et al, (2004)**.

Stem cells were discovered from analysis of a type of cancer called a teratocarcinoma. It was noted that a single cell in teratocarcinomas could be isolated and remain undifferentiated in culture. These types of stem cells became known as embryonic carcinoma cells (EC cells)**. Tuch et al, (2006)**.

Stem cells generate an intermediate cell type or different cell types prior to acheving a mature differentiated state. The intermediate cell is called a precursor or progenitor cell. Precursor or progenitor cells in fetus or adult are partially differentiated cells and eventually divide and give rise to mature differentiated cells. These cells are often committed meaning that they tend to differentiate only along a particular cellular developmental pathway,however, some recent studies have shown that this may not be as definitive as was once thought. ***Anderson et al.,( 2001).***

**Properties of stem cells**

Stem cells have three properties that distinguish them from other types of cells in the body and make them interesting to scientists:

- Stem cells are unspecialized unlike a red blood cell, which carries oxygen through the blood stream, or a muscle cell that works with other cells to produce movement, a stem cell does not have any specialized physiological properties.
- Stem cells are able to divide and produce copies of them:Stem cells can divide and produce identical copies of them, over and over again. This process is called self-renewal and continues throughout the life of the organism. In contrast, specialized cells such as blood and muscle do not normally replicate

- themselves, which means that when they are seriously damaged by disease or injury, they cannot replace themselves.
- Stem cells have the potential to produce other cell types in the body. In addition to self-renewal, stem cells can also divide and produce cells that have the potential to become other more specialized cell types, such as blood and muscle cells. This process is called differentiation. **Tuch et al., (2006)**.

Stem cells from different tissues, and from different stages of development, vary in the number and types of cells that they can give rise to. According to the classical view, as an organism develops the potential of a stem cell to produce any cell type in the body is gradually restricted. **Pouton et al., (2007)**.

**Cancer stem cells in Endometrial Cancer**

Endometrial cancer (EC) is the most common gynecological malignancy in the industrialized world. Two different clinicopathological types can be distinguished. Estrogen-related ECs (type I) develop in both pre-and postmenopausal women, and include endometrioid type and low cellular grade. In type I EC, estrogen receptor (ER, especially ERα) is expressed. This type of EC is frequently preceded by endometrial hyperplasia and carries a good prognosis. Type II non-estrogen-related ECs occur in postmenopausal women. They are non-endometrioid types (mainly papillary serous or clear cell carcinomas), without associated hyperplasia. Type II ECs are negative for ER and progesterone receptor (PR) and have high cellular grade and poor prognosis. The most frequent genetic alteration in type I EC is phosphatase and tensin homolog (*PTEN*) inactivation, followed by microsatellite instability and mutations of KRAS and β-catenin. In type II EC, p53 mutation is the most frequent genetic alteration, followed by amplification of human epidermal growth factor receptor 2 (HER2). Some of these pathways are important determinants of stem cell activity (Wnt, β-catenin and PTEN). These suggest a stem cell contribution to endometrial cancer development.**Rossi DJ et al ., 2006.**

**Characterization of SP Cells in EC**

The endometrial cancer SP cells show CSC features, marked migratory capacity, and the potential to differentiate into the mesenchymal cell lineage **Kato K et al., 2010.**

We isolated and characterized SP cells from human EC cells (Hec1 cells) and rat endometrial cells expressing oncogenic human K-Ras protein (RK12V cells) (Figure 24). SP cells exhibited a reduction in the expression levels of differentiation markers, long-term proliferative capacity in cell culture, self-renewal capacity *in vitro*, enhanced migration, formation of lamellipodia and uropodia, and enhanced tumorigenicity (Figure 25). When SP cells and non-SP (NSP) cells formed in nude mice, they differed in their biological properties. Tumors generated from SP cells (but not NSP cells) consisted of tumor tissues and an extracellular matrix (ECM) enriched with stromal-like components. Evidence exists that stromal cells, such as inflammatory cells, vascular cells, and fibroblasts from the bone marrow give rise to a tumor matrix in response to growth factors or cytokines secreted from tumor cells or activated fibroblasts **Grunewald M et al., 2006.**

Alternatively, stromal cells may be derived from tumor cells that have undergone an epithelial-mesenchymal transition (EMT). We showed that stroma-like tissues stained positively for vimentin, CD13, and α smooth muscle actin (SMA), and contained human *KRAS* DNA sequences. FISH studies demonstrated that both human genomic and mouse genomic signals were detected in the stroma-like tissues with enriched ECM (human 76%, mouse 24%), showing that most of these stromal-like cells were derived from the inoculated SP cells. Additionally, Hec1-SP cells had the potential to differentiate into αSMA-expressing cells when seeded into Matrigel and incubated with a differentiation medium (Figure 26). These results suggest that EC SP cells are capable of undergoing EMT. This feature of endometrial SP cells is putatively involved in the development of endometrial stromal sarcoma or carcinosarcoma of the uterus **Orimo A et al., 2005.**

The effect of cisplatin, paclitaxel and doxorubicin (clinically used for chemotherapy of EC) on the proliferation of RK12V both SP and NSP cells (Figure 27). Incubation of RK12V-NSP cells with medium containing these chemotherapeutic drugs for 96 h-inhibited proliferation compared to untreated controls. Relative to the controls, the extent of inhibition was 61% in 1 μMcisplatin, 51% in10 nM paclitaxel and

56% in 1 μM doxorubicin. All drugs inhibited the proliferation of RK12V-NSP cells significantly compared to the control ($p$<0.001). In contrast, none of these drugs had an inhibitory effect on the growth of RK12V-SP cells. These results clearly demonstrate that RK12V-SP cells have a higher resistance to conventional chemotherapeutic drugs, indicating a requirement for new targets for the treatment of CSCs. To develop new approaches in molecular cancer therapy, we performed microarray assays to identify overexpressed genes in RK12V-SP cells compared to those in RK12V-NSP cells. The expression of a number of genes including cytokines and growth factors was enhanced in RK12V-SP cells, suggesting that multiple signaling pathways maintain the phenotype of SP cells. It would be difficult to identify a single selective molecular target for SP cells.

**Sodium Butyrate (NaB), an inhibitor of Histone Deacetylase (HDAC), Aleters the properties of Endometrial SP Cells**

HDAC inhibitors have multiple biological effects, including growth arrest, apoptosis, senescence, reactive oxygen-species facilitated cell death, mitotic cell death and antiangiogenesis . The regulation of histone acetylation is a vital mechanism controlling cellular differentiation and the biological phenotype of cancer cells **Marks PA et al., 2009.** HDACs and histone acetyl transferases are enzymes that ensure a proper level of histone acetylation. Dysregulated HDAC activity has been found in certain types of human cancer **Blackwell L et al., 2008.**

Several studies have demonstrated the antiproliferative or the proapoptotic effects of HDAC inhibitors on EC cells **Blackwell L et al., 2008.**

HDAC inhibitors include short-chain fatty acids [*e.g.* butyrates and valproic acid (VPA)], organic hydroxamic acids [trichostatinA(TSA) and suberoylanilide*bis*-hydroxamine (SAHA)], cyclic tetra peptides (*e.g.* Trapoxin), and benzamides (*e.g.* MS-275). TSA, NaB, VPA and SAHA can inhibit malignant cells *in vitro* and *in vivo* **Takai N et al., 2004** Have previously demonstrated that NaB induces p21 expression, resulting in growth arrest and cell death **Terao Y et al., 2001.**Therefore, we investigated the effect of NaB on the properties of RK12V-SP cells **Kato K et al., 2011.**

Treatment with 2 or 5 mMNaB for 96 h significantly inhibited the proliferation of RK12V-SP cells as well as RK12V-NSP cells (Figure 28A, $p$<0.01). RK12V-SP cells have the potential to regenerate SP cells after incubation, which is an important characteristic of stem-like cells. Treatment with NaB for 24 h significantly inhibited the proportion of SP cells regenerated (control, 15%; 2 mMNaB, 1.5%, $p$<0.02; 5 mMNaB, 0.023%, $p$<0.01; n=4, Figure 28B). The primary colony-forming potential of RK12V-SP cells was completely suppressed by treatment with 2 mMNaB (Figure 28C). NaB treatment completely suppressed colony formation of RK12V-SP cells in soft agar cultures. These results demonstrated that treatment with NaB reduced self-renewal capacity and tumorigenicity of RK12V-SP cells.**Inoue T et al., 2009**investigated the molecular mechanism underlying the inhibitory effect of NaB treatment of RK12V-SP cells. They reported that treatment with NaB induced cell death in several cancer cell lines mediated by enhanced reactive oxygen species (ROS) levels, DNA damage response (DDR) signals and up-regulation of p21 . Thus, we examined the change of these signal levels in RK12V-SP and -NSP cells.

**Objectives**

To evaluate stem cells in endometrial carcinoma and normal endometrium.

**Subjects and methods**

This prospective controlled study was conducted at Al Azhar university hospital in Assiut and Cairo and in Cancer institute in Assiut and Cairo in the interval from January 2013 to August 2014. The number of subjects was40 and divided into 4 groups:

- The first group: Menstrual blood group and included 10 patients.
- The second group: Proliferative endometrium group and included 10 patients.
- The third group: secretory endometrium group and included 10 patients.
- The fourth group: Endometrial carcinoma group and include 10 patient

Two specimens from each case were obtained:The first slide was stained by haematoxline and eosin to assure the diagnosis and the second slide was collected on charged slides then stained by Oct- 4 (octamer-

binding transcription factor 4) to detect stem cell expression.

**Inclusion criteria:**

- Endometrial stem cell candidate.
- Endometrial carcinoma for the patient group

**Exclusion criteria:**

- Hormonal Treatment within 3 months.
- Another malignanacy
- Preoperative chemotherapy or radiotherapy.

**A written consent was obtained from all patients who were thensubjected to:**

Complete history taking with special emphasis on gynecological history( age of menarche, D/C, regularity of the cycle, amount of the menstrual blood, age of menopause if present, intermenstrual bleeding, dysmenorrhea, postciotal spotting, pelviabdominal distension, heaviness, and mass, dyspareunia either superficial or deep and Contraceptive methods, period of amenorrhea, infertility, galactorrhea, hisutism). Past history for ovulation induction medications, hypertension, diabetes, exposure to radiation, surgical history, previous blood transfusion, allergy to drugs.Family history for consanguinity, another genital tumors, breast lesions.

- General and abdominal examinations as well as local examination.
- Ultrasound examination: Trans abdominal and Transvaginal to detect masses: site, size, shape, homogenous or heterogeneous, contents, unilateral or bilateral, and to detect fluid: its amount, nature, and its site.
- Computed tomography or MRI evaluation to confirm what seen by U/S, give the suggestion for the nature of the mass, and Staging of the disease.
- Tumor markersthat strongly help in the diagnosis and the protocol of the treatment
- Routine investigations to prepare the patients for operation if indicated
- Intravenousurography to detect ureteric pathway and any distortion.
- Immunohistochemistry evaluation for stem cell scoring.

Pathological evaluation of the specimen: Samples were obtained from the Endometrial tissue; via laparotomy and D&C procedures. Biopsy samples were fixed in 10% neutral-buffered formalin at 4_C overnight and were subsequently paraffin embedded. Before performing immunohistochemistry, sections of the tissues were stained with hematoxyline- eosin (H&E) to select tissues that we want. Serial sections of the same selected samples, 5-mm thick, were used for immunohistochemistry. Commercially available monoclonal antibodies (m Ab) were used for the detection of Oct- 4. Oct- 4 is a transcription factor, molecular marker for pleuripotent cells and plays an essential role in maintaining the undifferentiated state needed for cell pleuripotency**(Matthai et al., 2006)**. It is well known that Oct- 4 is expressed in embryonic stem cells, germ cells, and in the embryo at various stages of development. Also expressed in several cancers such as osteosarcoma, prostate cancer, cervical carcinoma, and lung cancer **(Cervello et al., 2007).** Oct- 4 has been found in the epithelial cells of normal endometrium **.**Tissue sections were dewaxed and rehydrated conventionally and the quenching of the endogenous peroxidase was achieved by incubation with 0.3% hydrogen peroxidase in menthanol for 30 minutes at room temperature. All tissue sections were exposed to anonimmune block with normal horse serum for 30 minutes at room temperature. Incubations with the first antibody were carried at 4_C overnight with a dilution of 1:100 for the monoclonal mouse anti human Fas-L and with the dilution of 1:50 for the monoclinal mouse anti human Fas antigen. Thereafter tissue sections were labeled with avidin-biotin-peroxidase detection system Vectastain (Vector Laboratories, Burlington, VT). Each step was followed by meticulous washing with phosphate-buffered saline (PBS). Finally 3, 30-diaminobenzidine was used as a chromogen. Conterstaining was performed with Meyer hematixyline. The positive controls were Endometrial tissue that showed expression of Oct- 4. a semi quantitative analysis of specific stainig was performed using the histochemical score (HSCORE) system according to score the immunohistochemistry slides and perform statistical analysis. The HSCORE was calculated using the following eqution: HSCORE 1/4 SPi (ib1), where is the intensity of the staining with the value of 1, 2, 3 (weak, strong, or very strong) and pi is the percentage of stained cells for each intensity varying from 0% to 100%.For scoring, the Edessy stem cell score was applied by giving a score for each finding 0, 1, 2 as shown in table (1).

**Table (1) Edessy stem cell score (Edessy et al., 2014)**

| Score factor | 0 | 1 | 2 |
|---|---|---|---|
| **Intensity Of SC Marker** | Negative to mild | moderate | strong |
| **Percentage Of Stained Cells** | 0 | 0-50% | > 50-100% |
| **Focality** | None | focal | diffuse |
| **Distribution** | None | Epithelial or mesenchmal | both |
| **Localization Of The Stain** | None | Cytoplasmic or nuclear | both |

**Statistical Analysis**

The collected data was organized, categorized, tabulated, and analyzed by using the computer software (Statistical Package for Social Science {SPSS} version 12). Suitable statistics was used for quantitative data. Yates corrected chi-square ($\chi^2$) and Fisher exact (FE) were used as tests of significance. The significance level for them was accepted if P-value <0.05. If P-value <0.01was considered highly significant.

**Results:**

**Table 2: Stem cell score formenstrual blood and endometrial carcinoma groups**

| | Menstrual blood | | Endometrial Carcinoma | | P. value |
|---|---|---|---|---|---|
| | Range | Mean + SD | Range | Mean + SD | |
| **Intencity** | 0 - 2 | 0.7 + 0.9 | 2 - 2 | 2 + 0 | 0.000 |
| **Percentage** | 0 - 1 | 0.8 + 0.4 | 1 - 2 | 1.4 + 0.5 | 0.001 |
| **Focality** | 0 - 1 | 0.8 + 0.4 | 1 - 2 | 1.1 + 0.3 | 0.039 |
| **Distribution** | 0 - 1 | 0.5 + 0.5 | 1 - 2 | 1.5 + 0.5 | 0.000 |
| **Localization** | 0 - 2 | 0.9 + 0.6 | 1 - 2 | 1.6 + 0.5 | 0.000 |
| **Total** | 0 - 6 | 3.7 + 2.2 | 6 - 10 | 7.6 + 1.4 | 0.000 |

**Table 3: Stem cell score for proliferative endometrium & endometrial carcinoma groups**

| | Proliferative end | | Endometrial Carcinoma | | P. value |
|---|---|---|---|---|---|
| | Range | Mean + SD | Range | Mean + SD | |
| **Intencity** | 1 - 2 | 1.4 + 0.5 | 2 - 2 | 2 + 0 | 0.006 |
| **Percentage** | 1 - 2 | 1.1 + 0.3 | 1 - 2 | 1.4 + 0.5 | 0.096 |
| **Focality** | 1 - 1 | 1 + 0 | 1 - 2 | 1.1 + 0.3 | 0.485 |
| **Distribution** | 1 - 2 | 1.3 + 0.5 | 1 - 2 | 1.5 + 0.5 | 0.243 |
| **Localization** | 1 - 1 | 1 + 0 | 1 - 2 | 1.6 + 0.5 | 0.000 |
| **Total** | 5 - 8 | 5.9 + 1.1 | 6 - 10 | 7.6 + 1.4 | 0.006 |

**Table 4:Stem cell score for secretory endometrium and endometrial carcinoma groups**

| | Secretory end | | Endometrial Carcinoma | | P. value |
|---|---|---|---|---|---|
| | Range | Mean + SD | Range | Mean + SD | |
| **Intencity** | 1 - 2 | 1.9 + 0.3 | 2 - 2 | 2 + 0 | 0.640 |
| **Percentage** | 1 - 2 | 1.6 + 0.5 | 1 - 2 | 1.4 + 0.5 | 0.265 |
| **Focality** | 1 - 2 | 1.6 + 0.5 | 1 - 2 | 1.1 + 0.3 | 0.001 |
| **Distribution** | 1 - 2 | 1.9 + 0.3 | 1 - 2 | 1.5 + 0.5 | 0.021 |
| **Localization** | 1 - 2 | 1.9 + 0.3 | 1 - 2 | 1.6 + 0.5 | 0.036 |
| **Total** | 5 - 10 | 8.9 + 1.7 | 6 - 10 | 7.6 + 1.4 | 0.035 |

## Discussion

Endometrial carcinoma by definition, CSCs have similar properties to ASCs. CSCs may not necessarily acquire the first genetic mutation that initiates tumourigenesis, but they are the cells that maintain the tumour over time. Subsequent mutations within a cancer may create new CSCs that overtake or coexist with the older CSCs. Whatever the mutation; the CSCs must retain the capacity to self-renew. To date, the best *in vivo* indicator of CSC self-renewal is the serial transplantation of CSC-enriched populations into immunocompromised mice, where the tumour is re-established, with a similar phenotype to the original tumour, with each serial xenografting. ***Visvader&Lindeman( 2008).*** In our research we also found that stem cell score were increased markedly in all cases to be 8-10 and this suggest its role in malignancy.

## Conclusion and Recommendation

- Endometrial carcinoma show increase in stem cell expression score 8-10 and this indicate the role of stem cells in carcinoma so treatment should be mediated though stem cell therapy especially in radiotherapy and chemotherapy resistant cases.
- More researches to study the characteristics of CSCs are recommended.

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