The distribution of apoptosis and related proteins in ovarian endometriosis

Seda H. Vatansever, MD, PhD, Sevinc V. Inan, MD, Gulsen S. Giray, MPhil, Sevil Sayhan, MD, Kemal M. Ozbilgin, MD, Muzaffer Sanci, MD.

Endometriosis is a common gynecological disease, occurring when endometrial tissue normally restricted to the uterine lining implants and growths at an ectopic site.[1] The pathogenesis and mechanisms involved in development and progression of endometriosis is still controversial. Endometrial cells may exhibit abnormal proliferation and apoptotic regulation, and this may be one of the mechanisms of endometriosis. Apoptosis, which is believed to be due to programmed cell death, plays a critical role in the cyclic changes and maintenance of homeostasis in multicellular organisms.[2] There are numerous stimuli that trigger and control apoptosis. The controlling of apoptosis is regulated by several additional genes, which potentate (p53; Bax; c-Myc), or inhibit (Bcl-2; Bcl-XL; sentrin) programmed cell death.[2] The action of Bcl-2 depends on the concentration of, and interaction with a potential antagonist protein, Bax. The ratio of Bcl-2 to Bax is important in determining susceptibility to apoptosis.[2] The tumor suppressor protein, p53, mediates activation of programmed cell death, in part by up-regulation of Bax expression; in contrast Bcl-2 can block p53-mediated apoptosis.[2] The Ki-67 protein, which is a proliferative marker and expressed from proliferative cells, not resting cells, was observed in functionalis parts of the endometrium.[3] While Bcl-2 presented with higher expression until the secretory phase, expression of Bax was dramatically induced in the secretory phase.[4] Spontaneous apoptosis in single cell suspensions of eutopic and ectopic endometrium from women with endometriosis, and of eutopic endometrium from fertile control subjects without endometriosis were detected using enzyme-linked immunosorbent assay technique.[4,5] The objective of our study was to investigate the presence of apoptosis and its related proteins p53, Bax, Bcl-2, and Ki67 in ovarian endometriosis.

Formalin-fixed-paraffin embedded tissue from 15 gynecological cases was obtained from the archives of the Pathology Department of Agean Maternity Hospital, Izmir, Turkey between February 2004 and December 2006. The Ethics Committee at the Agean Maternity Hospital approved our study protocol, and all patients gave informed consents. After deparaffinization of sections, they were soaked in a decreasing series of ethanol and washed phosphate-buffered saline (PBS) for 10 minute. The 2% trypsin and then 3% H_2O_2 were incubated. After washing with PBS, the primary antibodies (anti-Bcl-2: MS-123-A1, anti-p53: MS-186-P1, anti-Bax: Rb-1486-R1, and anti-Ki67: RB-081-A1, Neomarkers Fremont, CA, USA) were added and incubated for 18 hours at 4^0C. After the washing steps, they were incubated with biotin-streptavidin secondary complex (Histostain-Plus Bulk Kit, Zymed, 85-9043, San Francisco, CA, USA), and then stained with a solution containing 3-amino-9-ethylcarbazole (AEC) for 5 minutes to visualize immunostaining. Finally, the slides were stained with Mayer's hematoxylin. The dUTP nick-end labeling (TUNEL) assay using a commercial kit (DeadEnd Colorimetric TUNEL system, Promega G7130, (Promega Inc., Madison WI, USA) was used. According to the manufacturer's instructions, briefly, after proteinase K treatment for 10 minutes, the sections were incubated at 37^0C with terminal deoxynucleotidyl transferase (TdT) for 60 minutes. The negative control for TdT was omitted during the tailing reactions. Staining was carried out with diaminobenzidine and counter staining was performed in Mayer's Hematoxylin. All staining quantitation was carried out in a blind fashion by 2 independent observers and was analyzed under a light microscope Olympus BX40, (Olympus, Tokyo, Japan). The staining intensity of immunochemistry was graded on the following scale: minimal (±), mild (+), moderate (++), and strong (+++).

After the examinations of TUNEL sections, endometriotic glands and stroma were observed on the ovarian surface (**Figure 1a**). In addition, TUNEL positive cells were detected in ovarian stroma in the endometrioma, however, there were no apoptotic cells in the epithelium lining of the endometrioma (**Figure 1a**). In the endometrial samples from patients with endometriosis, moderate immunostaining of p53 was seen in ovarian stroma in the endometrioma, there was no immunoreactivity for p53 in the epithelium lining of the endometrioma. In addition, immunoreactivity of Bax was detected in both the ovarian stroma and epithelium lining of the endometrioma (**Figure 1b**), intensities of Bax were moderate in the stromal cells and weak in the epithelial cells (**Figure 1b**). The Ki67 and Bcl-2 immunoreactivities were predominantly found in stromal and epithelial cells in patents with endometriosis. Expressions of Ki67 and Bcl-2 were similar in these sites; strong immunoreactivity was detected in epithelial cells, and minimal immunoreactivity was detected in epithelial and stromal cells.

Our result demonstrated that spontaneous apoptosis was induced in the stromal part of endometrioma with expresions of both p53 and Bax, while the epithelial part of endometrioma was rescued from apoptosis with

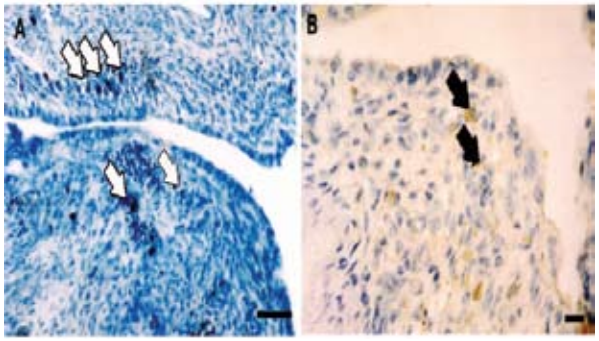

Figure 1 - Positive immunoreactivity of Bax was detected in the endometriotic area of ovarium. **a**) Tunel staining and **b**) Bax immunoreactivity of ovarian endometriosis. Tunel positive cell were indicated with white arrows. Scale bars: A - 25 μm, B - 150 μm.

expression of Bcl-2. This suggested that endometriosis may proliferate from surface epithelium, via rescue of cell death, although there might be only apoptosis in the stromal site of the endometrioma.

In healthy women, multiple and redundant intracellular and extracellular controls prevent implantation of misplaced ectopic cells, and dysregulation in that system results in initiation, promotion, and progression of endometriosis.[1] It has been suggested that the ectopic cell accumulation could result from either increased proliferation or the deficiency of cells to undergo apoptosis. Different expression of apoptosis-related proteins in endometriosis was reported and underlined the pathophysiology of endometriosis.[5] Previous studies suggest that the development of endometriosis may be associated with anomalies of the apoptotic system.[4,5] Harada et al[4] did not found any correlation between the apoptosis rate of Bcl-2 and Bax proteins and the stage of endometriosis. However, in our study, while expression of Bax was detected in the stroma of endometrioma, expression of Bcl-2 was observed in the cytoplasm of epithelial linings of the endometrioma. Differentiation and apoptosis in the endometrial epithelium and stroma appear to be linked to the ability of endogenous and exogenous increase of Bax expression and aberrant Bcl-2 expression in the endometrioma, thus increasing the Bax/Bcl-2 ratio is also suggested to play a role during progression of endometrioma.

In conclusion, our data demonstrated that expression of p53 was only detected in the stroma of endometrioma. This expression was correlated with the expression of Bax. In addition, the expression of Ki67 was observed in the cytoplasm of epithelial linings of the endometrioma. While the epithelial cells of endometrioma were rescued from apoptosis, the proliferation of cells may control expression of Ki67. However, stromal cells of ovarian endometriosis could not be rescued from cell death and the apoptotic pathway of these cells may control expression of p53 and Bax proteins. A decrease in endometrial cell apoptosis might thus lead to the survival and implantation of misplaced cells. The Bcl-2 and Bax expression in ovarian endometriosis may have important implications for the survival and proliferation of ectopic endometrial tissue. Control of apoptosis in the uterine endometrium may be disrupted in women with endometriosis. Therefore, the induction of apoptosis might control the implantation and survival of ectopic endometrial cells and prevent endometriosis.

Received 28th February 2009. Accepted 10th May 2009.

From the Department of Histology and Embryology (Vatansever, Inan, Giray, Ozbilgin), Faculty of Medicine, Celal Bayar University, Manisa, and the Departments of Pathology (Sayhan), Obstetrics and Gynecology (Sanci), Agean Maternity Hospital, Izmir, Turkey. Address correspondence and reprint requests to: Dr. Seda H. Vatansever, 198 Sok. No: 7/4, Bornova 35040, Izmir, Turkey. Tel. +90 (532) 5730820. Fax. +90 (236) 2331466. E-mail: hsedav@yahoo.com

References

1. Sampson J. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the pelvic cavity. ***Am J Obstet Gynecol*** 1927; 14: 422-469.
2. Schwartzman RA, Cidlowski JA. Apoptosis: the biochemistry and molecular biology of programmed cell death. ***Endocr Rev*** 1993; 14: 133-151.
3. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. ***J Immunol*** 1984; 133: 1710-1715.
4. Harada T, Kaponis A, Iwabe T, Taniguchi F, Makrydimas G, Sofikitis N, et al. Apoptosis in human endometrium and endometriosis ***Hum Reprod Update*** 2004; 10: 29-38.
5. Dufournet C, Uzan C, Fauvet R, Cortez A, Siffroi JP, Daraï E. Expression of apoptosis-related proteins in peritoneal, ovarian and colorectal endometriosis. ***J Reprod Immunol*** 2006; 70: 151-162.