Embryologic Origin of Endometriosis: Analysis of 101 Human Female Fetuses

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The etiology of endometriosis, a gynecological disease characterized by the presence of endometrial glands and stroma outside the uterine cavity, is still unknown. Our research group has recently demonstrated the presence of ectopic endometrium in human female fetuses at different gestational ages. In this manuscript we describe four new cases of fetal endometriosis found among a series of 52 female fetuses analyzed at autopsy. The anatomical localization of this ectopic endometrium, and its histological and immunohistochemical characteristics are depicted. We suggest that endometriosis is caused by dislocation of primitive endometrial tissue outside the uterine cavity during organogenesis. The clinical and pathological implications of these findings are discussed.
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Endometriosis is defined as the growth of endometrial glands and stroma at extra-uterine sites (Giudice and Kao, 2004; Baldi et al., 2008; Signorile et al., 2009a). The prevalence in the general female population is 6–10%; the frequency increases to 35–60% in women with pain, infertility, or both, (Wheeler, 1992). Endometriosis is responsible for more than 100,000 hysterectomies each year in the United States alone (Carlson et al., 1994). Despite the fact that this disease is quite common among women, it is frequently misdiagnosed, the pathogenesis is unknown and the diagnostic and therapeutic protocols are still not fully adequate (Bulun, 2009).

The most widely accepted theory for the pathogenesis of endometriosis is the retrograde menstruation/transplantation (Sampson, 1927). However, this theory fails to explain the presence of endometriosis under the peritoneum (the so-called deep endometriosis) and in remote areas outside the peritoneal cavity (Signorile and Baldi, 2010a). Indeed, in the last years new pathogenetic mechanisms have been proposed, such as the coelomic metaplasia hypothesis or the involvement of circulating stem cells originating from bone marrow (Nisolle and Donnez, 1997; Sasson and Taylor, 2008). Interestingly, it has been postulated by pioneer scientists of this disease in the late 19th and early 20th century, that endometriosis is caused by small defects of embryogenesis (Knapp, 1999; Benagiano and Brosens, 2006). The Müllerian ducts, indeed, give rise to the female reproductive tract, and this organogenesis is controlled by complex molecular pathways including the anti-Müllerian hormone signaling (Klattig and Englert, 2007). Aberrant differentiation or migration of the Müllerian ducts during embryogenesis could cause spreading of cells or tracts of cells in the migratory pathway of fetal organogenesis across the posterior pelvic floor, thus explaining the observation that endometriosis is commonly found in the cul-de-sac, uterosacral ligaments, and medial broad ligaments (Mai et al., 1998). Recently, our research group has demonstrated the presence of ectopic endometrium in a significant number of human female fetuses (5 over 49 cases) analyzed by autopsy in two different works (Signorile et al., 2009b; Signorile et al., 2010b).

Goal of this study was to significantly increase the number of fetuses analyzed in order to better determine the real biological impact of this phenomenon.

Materials and Methods

We collected at autopsy from three different institutions, a series of 101 human female fetuses who died at different times of gestation. The first 49 cases have been already described in two precedent works (Signorile et al., 2009b; Signorile et al., 2010b). The other 52 fetuses were analyzed essentially as previously described. Briefly, pelvic organs were collected en-block, fixed in paraformaldeyde and included in paraffin. Histological analysis of the pelvic organs of the fetus was performed using Hematoxylin/Eosin and Hematoxylin/Van Gieson staining. For immunohistochemistry 5–7 μm specimen sections embedded in paraffin, were cut, mounted on glass and dried overnight at 37°C. Tissue sections were quenched sequentially in 3% hydrogen peroxide in aqueous solution and blocked with PBS-6% non-fat dry milk (Biorad, Hercules, CA) for 1 h at room temperature. Slides were then incubated at 4°C overnight at 1:100 dilution with the following antibodies: The affinity-purified rabbit antibody ERα for the estrogen receptor (Santa Cruz, Santa Cruz, CA; cat. # sc-542), the mouse monoclonal antibody for CD10 (clone M7308) (Dako Laboratories, Carpinteria, CA). After three washes in PBS to remove the excess of antiserum, the slides were incubated with

diluted goat anti-rabbit or anti-mouse biotinylated antibodies (Vector Laboratories, Burlingame, CA) at 1:200 dilution in PBS-3% non-fat dry milk (Biorad, Milan, Italy) for 1 h. All the slides were then processed by the ABC method (Vector Laboratories) for 30 min at room temperature. Diaminobenzidine (Vector Laboratories) was used as the final chromogen and haematoxylin was used as the nuclear counterstaining. Negative controls for each tissue section were prepared by leaving out the primary antiserum. Positive controls of breast, intestinal, and uterine tumor tissues expressing each of the antigens analyzed, were run at the same time. All samples were processed under the same conditions. Experiments were performed in compliance with the Helsinki Declaration and the protocols were approved by the ethics committee of the Italian Endometriosis Foundation.

Results

Pelvic organs were analyzed in their entirety. To this end, four sections were taken every 150 microns and stained for histology and for immunohistochemistry, as described in the methods section. Indeed, we did not find any evidence of macroscopical defects of the genital system in the fetus analyzed (data not shown). We found in four out of 52 fetuses (7.7% of cases), the presence of glandular structures outside the uterine cavity, clearly resembling the structure of the primitive endometrium and expressing estrogen receptor. Moreover, the stroma surrounding these glandular structures expressed both CD10 and estrogen receptor. The anatomical locations of these endometrial structures were: Two cases in the recto-vaginal septum, one case in the proximity of the Douglas pouch, and one case in the mesenchimal tissue close to the posterior wall of the uterus. To note, these anatomical sites are common location for endometriosis in women (Baldi et al., 2008). To note, the four fetuses were also screened for the most common chromosomal abnormalities and found to have a normal cariotype. Table 1 summarizes the characteristics of the four fetuses and the anatomical locations of the glands. The histological and immunohistochemical appearances of these epithelial structures are depicted in detail in Figure 1. We conclude that these structures must be ascribed to endometrial tissue, misplaced outside the uterine cavity during the earlier steps of organogenesis and displaying identical phenotype to the endometrium present in the uterus.

Discussion

The data of this manuscript give evidences for an embryological origin of endometriosis, suggesting alterations in the fine tuning of female genital structures organogenesis. Indeed, considering all the 101 human female fetuses analyzed in this and in previous works form our research group (Signorile et al., 2009b; Signorile et al., 2010b), we found a total of nine cases of ectopic endometrium (9% of the total). This incidence is very close to the one found in the adult female population (Bulun, 2009). Nevertheless, the existence of choristoma composed of müllerian remains in adult has been codified and named müllerianosis, even if this phenomenon has been interpreted, but not demonstrated, as different from endometriosis (Batt

et al., 2007). In particular, we have carefully analyzed the molecular phenotype of this ectopic endometrium, showing that it expresses characteristic markers of the epithelium and of the stroma of the genital tract, such as CA125, estrogen receptor, and CD10. The histological and immunohistochemical analysis of the eutopic and ectopic endometrium shows a very similar phenotype, as already described in our previous works (Signorile et al., 2009b; Signorile et al., 2010b). This observation argues against the hypothesis that this ectopic endometrium could disappear during the final steps of organogenesis. We propose that this ectopic endometrium would remain quiescent and asymptomatic until puberty, when the hormonal inputs, would cause its growth and, consequently, the onset of the symptoms of endometriosis.

Interestingly, several data from the scientific literature underline the fact that the uterus of women with endometriosis displays some congenital alterations. Parker et al. (2006), indeed, described alterations in the muscular characteristics of the innermost myometrium, such as the thickness and fiber orientation and abnormal JZ morphology, securely due to the congenital alteration of uterine wall in patients with endometriosis. Similarly, Kunz et al. (2000) have described that infertile women with endometriosis show alterations of the myometrial wall with an archimetral significantly expanded. These alterations, moreover, are identical in patients with adenomyosis, thus supporting the concept that endometriosis and adenomyosis are the same diseases and that the defect is primarily at uterine level. Nevertheless, it has also been shown that endometriosis is more frequent in patients with Müllerian anomalies (Nawroth et al., 2006) and other genital anomalies (Acién, 1986).

Moreover, the presence of the disease in early puberty and exceptionally also in newborns (Diez Garcia et al., 1996; Batt and Mitwally, 2003; Marsh and Laufer 2005; Ebert et al., 2009), as well as in women affected by the Mayer–Rokitansky–Küster–Hauser, a syndrome characterized by congenital aplasia of the uterus and the upper part of the vagina (Enatsu et al., 2000; Yan and Mok, 2002; Balci et al., 2008), further supports the validity of an embryological origin for endometriosis.

Increasing experimental evidences are showing that exposure to toxicants during critical periods of pre- and peri-natal development can have long-lasting effects. In particular, the ability of endocrine disruptors to alter reproductive function and health in females are quite well characterized, thanks especially to the numerous works on the effects of endocrine disruptors exposition in utero (Newbold et al., 2009). Interestingly enough, there are several studies in humans linking exposition to endocrine disruptors with insurgence of endometriosis (Foster, 2008). In particular, a robust epidemiological study on a wide cohort of patients with endometriosis has shown that the rate of endometriosis is 80% greater among women exposed to the endocrine disruptor diethylstilbestrol in utero (Missmer et al., 2004). In very recent works, indeed, we have described in mice exposed in utero to the endocrine disruptor bisphenol A the presence of endometriosis-like structures and premature ovarian failure (Signorile et al., 2010c; Signorile et al., 2011), a phenotype, that strictly recapitulates the clinical picture seen in women suffering of endometriosis. This observation is in agreement with the work by Huseby and Thurlow (1982), that have described a similar phenotype in mice exposed prenatally to low dose of diethylstilbestrol: Alterations in the genital tract consisting of adenomyosis and enlargement of the cervix, and reduced fecundity.

In conclusion, it is possible to claim that endometriosis is a multi-factorial disease with multifaceted features. Nevertheless, the demonstration of the presence of ectopic endometrium in the female fetus in same anatomical locations

TABLE 1. Characteristics of the fetuses with endometriosis

No.	Gestational age	Cause of death	Location of ectopic endometrium
1	21 weeks	Placental pathology	Recto-vaginal septum
2	23 weeks	Placental pathology	Recto-vaginal septum
3	24 weeks	Placental pathology	Douglas pouch
4	24 weeks	Placental pathology	Posterior wall of the uterus

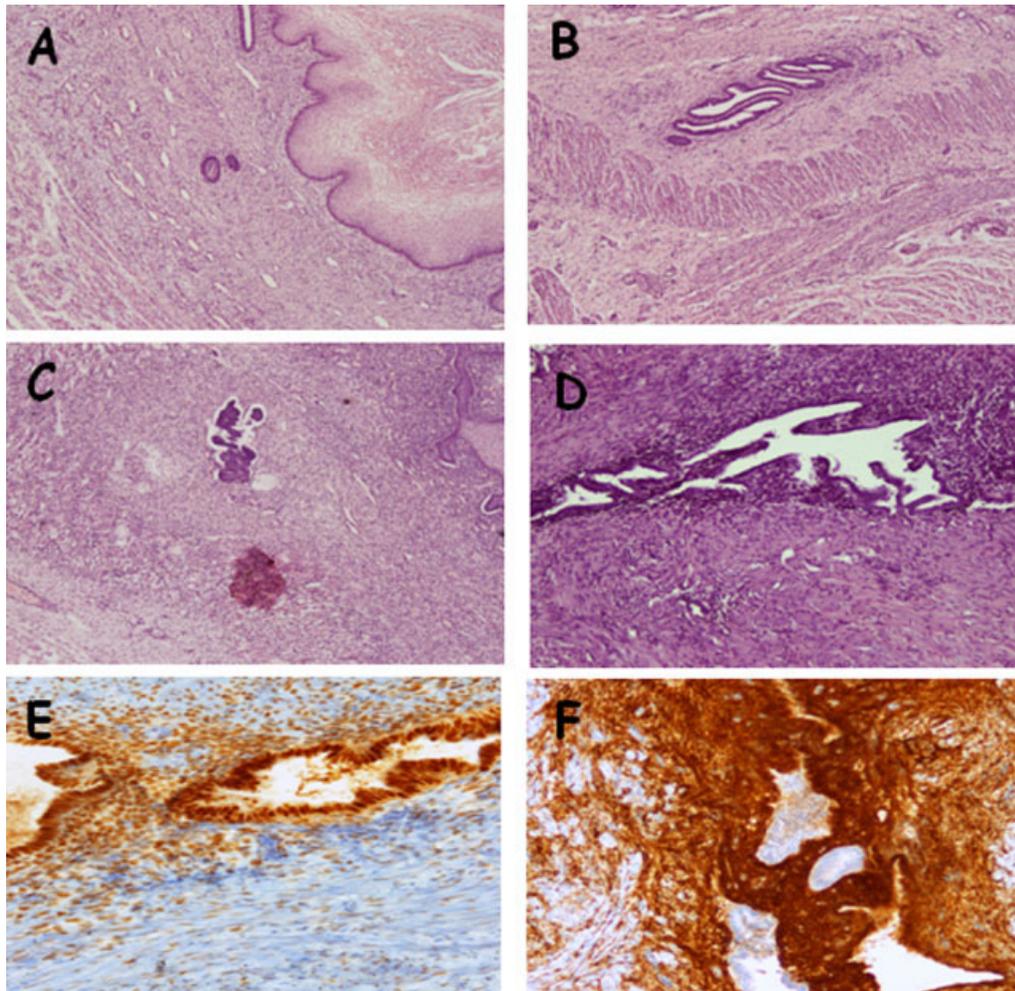**Fig. 1.** Histological and immunohistochemical appearance of ectopic endometrium. Panel A: Case 1: Endometriosis in the recto-vaginal septum. Histological appearance of the endometriotic gland; the vagina is visible in the upper right corner of the picture (Haematoxylin and Eosin, original magnification ×20). Panel B: Case 2: Endometriosis in the recto-vaginal septum. Histological appearance of the endometriotic gland (Haematoxylin and Eosin, original magnification ×20). Panel C: Case 3: Endometriosis in the proximity of the Douglas pouch. Histological appearance of the endometriotic gland (Haematoxylin and Eosin, original magnification ×20). Panel D: Case 4: Endometriosis in the mesenchimal tissue close to the posterior wall of the uterus (Haematoxylin and Eosin, original magnification ×20). Panel E: Higher magnification of one of the glandular structures displaying strong immunohistochemical expression for estrogen receptor. The stromal cells also expressed estrogen receptor (ABC, original magnification ×40). Panel F: Higher magnification of one of the glandular structures displaying strong immunohistochemical expression for CD10 in the stroma (ABC, original magnification ×40).

found in the adult patients affected by endometriosis and with a frequency very similar, makes the embryogenetic theory on endometriosis the only one scientifically proved and suggests that this pathogenetic mechanism is prevalent in the genesis of this disease. Several epidemiological and animal studies, finally, suggest an important role for an abnormal estrogenic signaling during embryogenesis in causing the endometriosis phenotype. The clinical and therapeutic implications are clear-cut. Endometriosis could still be regarded as a recurrent disease; nevertheless recurrence could not be ascribed to the retrograde menstruation, but to an incomplete surgical intervention, since it is demonstrated that endometriosis lesions could be also made up of microscopic foci (Redwine, 2003), and or to different timing of growth of the lesions in the same patient, probably due to individual susceptibility that is a typical phenomenon of the diseases inducted by endocrine disruptors (Mori et al., 2003). Therefore surgery, if complete in exhausted growth disease can be considered curative.

Contrarily, exposition to endocrine disruptors such as synthetic estrogens or SERM chemical compounds, though reducing the symptoms, could increase the growth of endometriosis. Such studies shed new light on the pathogenesis of this disease and, possibly, suggest suitable therapeutic targets for both the typical phenotypes of endometriosis: Ectopic endometrial tissue and infertility.

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