Case Report

Premalignant alterations in breast and endometrium associated with a PTEN mutation in a woman with Cowden syndrome: implications for preventive care

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Introduction

Germline mutations in the phosphatase and tensin homolog (PTEN) gene manifest in a spectrum of autosomal dominant disorders, the most common of which is Cowden syndrome (CS), but also include Bannayan–Riley–Ruvalcaba syndrome, adult Lhermitte–Duclos disease, macrocephaly, and autism spectrum disorders (Pilarski et al., 2013). CS was first described in 1963 (Lloyd and Dennis, 1963) and PTEN gene mutations were first described in patients with CS in 1997 (Nelen et al., 1997; Liaw et al., 1997). Loss of function mutations in PTEN, a tumor suppressor gene, results in oncogenesis through negative regulation of the mTOR signaling pathway. Mutations in PTEN are rare, with an estimated prevalence of 1 in 200,000 to 250,000 (Nelen et al., 1999).

Patients with PTEN mutations commonly present with hamartomatous lesions involving multiple organ systems. In 2013 Pilarski et al. proposed updated diagnostic criteria for CS, concluding that there was insufficient evidence to include uterine fibroids, benign breast disease, or genitourinary malformations in the diagnostic criteria (Pilarski et al., 2013); clinical diagnosis is suspected based on satisfying updated major and minor criteria (Table 1). Importantly, patients with CS have an estimated lifetime risk for invasive carcinomas of the breast (85%), thyroid (35%), endometrium (28%), colon (9%), and kidney (34%), and melanoma (6%) (Tan et al., 2012). The endometrial cancer risk may be underestimated as previous studies did not censor subjects for hysterectomy (Pilarski et al., 2013).

Here we report a case of incidental non-invasive endometrial neoplasia (complex atypical hyperplasia (CAH)) identified at the time of prophylactic hysterectomy. We detected loss of heterozygosity at the PTEN mutation in both the endometrial neoplasm and in the patient's atypical hyperplasia of the breast, suggesting that PTEN-driven oncogenesis in both breast and endometrial epithelium may proceed through a stage of pre-invasive intra-epithelial neoplasia.

Case report

A healthy 40 year-old nulliparous female had multiple 2–3 mm papules on the face, forehead, and chin present since 8 years old. Biopsy suggested trichilemmoma and she was referred to medical genetics for further evaluation. Physical examination showed macrocephaly, stippled café-au-lait spots, and punctated deltis involving the palms. She had recently undergone uterine artery embolization for a 12 cm uterine leiomyoma. The patient had no family history of CS. Genetic testing for PTEN mutations identified a deleterious PTEN mutation (N48K).

Endometrial biopsy (EMB) was negative for neoplasia. Screening breast MRI demonstrated a suspicious mass, and MRI-guided biopsy revealed atypical ductal hyperplasia. The patient underwent a coordinated risk-reducing total abdominal hysterectomy, bilateral salpingooophorectomy, and left breast excisional biopsy without complication. Pathology revealed multiple areas of atypical ductal hyperplasia of the left breast, but no in-situ or invasive carcinoma. The uterus weighed 438 g, including a 9 cm fundal fibroid, and pathology showed endometrial neoplasm with features of complex atypical hyperplasia. Two years later, bilateral breast MRI identified additional abnormalities, with core biopsies revealing multifocal atypical ductal hyperplasia. The patient elected for bilateral risk reducing mastectomies. Pathology revealed scattered foci of atypical ductal hyperplasia and atypical lobular hyperplasia.

Molecular testing of tissue specimens

The patient participated in an IRB approved tissue bank providing for collection and molecular evaluation of surgical specimens. DNA was
Table 1
Revised Cowden syndrome/PTEN hamartoma tumor syndrome clinical diagnostic criteria (adapted from Pilarski et al., 2013).

| Major criteria | Minor criteria |
|----------------|---------------|
| Breast cancer  |               |
| Endometrial cancer (epithelial) |               |
| Thyroid cancer (follicular) |               |
| Gastrointestinal hamartomas (including ganglioneuromas, but excluding hyperplastic polyps; ≥ 3) |               |
| Lhermitte–Duclos disease (adult) |               |
| Macromecephaly (≥ 97th percentile: 58 cm for females, 60 cm for males) |               |
| Macular pigmentation of the pinnae |               |
| Multiple mucocutaneous lesions (any of the following): |               |
| Multiple trichilemmomas (≥3, at least one biopsy proven) |               |
| Acral keratoses (≥3 palmoplantar keratotic pits and/or acral hyperkeratotic papules) |               |
| Mucocutaneous nevi (≥3) |               |
| Oral papillomas (particularly on tongue and gingiva), multiple (≥3) OR biopsy proven OR dermatologist diagnosed |               |
| Minor criteria |               |
| Autism spectrum disorder |               |
| Colon cancer |               |
| Esophageal glycogenic acanthosis (≥3) |               |
| Lipomas (≥3) |               |
| Mental retardation (i.e., IQ ≤ 75) |               |
| Renal cell carcinoma |               |
| Testicular lipomatosis |               |
| Thyroid cancer (papillary or follicular variant of papillary) |               |
| Thyroid structural lesions (e.g., adenoma, multinodular goiter) |               |
| Vascular anomalies (including multiple intracranial developmental venous anomalies) |               |

Operational diagnosis in a family where one individual meets revised PTEN hamartoma tumor syndrome clinical diagnostic criteria or has a PTEN mutation:
1. Any two major criteria with or without minor criteria; or
2. One major and two minor criteria; or
3. Three minor criteria

PTEN mutation: The loss of the wildtype allele could be used to confirm the neoplastic nature of hyperplastic lesions in individuals with PTEN mutations in cases of pathological uncertainty. Given the finding of incidental endometrial hyperplasia/neoplasia at hysterectomy, we propose that prophylactic hysterectomy specimens from women at high-risk should be evaluated by thorough pathological sampling of the endometrium to exclude the presence of an occult carcinoma which might require further evaluation or treatment. Patients presenting with clinical features of CS and complex atypical hyperplasia/endometrial cancer should be offered germline testing for PTEN mutations rather than tissue testing, as somatic PTEN mutations are common in the pathogenesis of endometrial cancer. Identification of a germline mutation carries significant implications for counseling, surveillance, and recommendations for prophylactic surgery.

The National Comprehensive Cancer Network recommends educating patients with CS about breast and endometrial cancer screening (Table 2). Patients should undergo clinical breast exams and annual mammography, as well as breast MRI starting at age 30–35, or 5–10 years before the earliest age of familial breast cancer (Daly et al., 2014). Endometrial cancer screening guidelines encourage patient awareness and prompt recognition of symptoms. In addition, providers may consider annual ultrasound and/or EMB. However, the sensitivity of random EMB in asymptomatic women is not well defined. Several studies have investigated the role of surveillance for gynecologic cancers in women with Lynch syndrome (LS) summarized in a recent review (Auranen and Joutsiniemi, 2011). Women with LS also have an elevated lifetime risk of endometrial cancer of approximately 40–60% (Schmeler and Lu, 2008). Of the five included studies, only one was prospective and none were randomized; studies that performed routine endometrial sampling at screening visits had the highest rate (5–6.5%) of detecting pathology (Auranen and Joutsiniemi, 2011). However, random sampling did not prevent the occurrence of all interval cancers. At present, there is no data demonstrating that routine EMB in women with LS or CS syndrome impacts survival.

Given the high rate of endometrial cancer in women with CS, it is reasonable to offer annual screening for endometrial cancer in women with CS using annual ultrasound and/or EMB, similar to LS. Interestingly, our patient had a negative preoperative EMB but surgical pathology revealed endometrial neoplasm (CAH), supporting the hypothesis that the sensitivity of an EMB for detecting neoplasia in asymptomatic women is less than that in women experiencing abnormal vaginal bleeding. Similarly, endometrial screening in asymptomatic breast cancer patients taking tamoxifen with routine transvaginal ultrasound, EMB, or both was not more effective than evaluation at the time of abnormal bleeding (Bertelli et al., 1998; Fung et al., 2003). Given the uncertainty over the efficacy of endometrial cancer screening in asymptomatic women, a cornerstone of preventive gynecologic care for women with CS and LS should be careful patient education on the recognition of abnormal uterine bleeding and the need for prompt evaluation of new symptoms with EMB.

Among premenopausal women with CS who elect for risk-reducing hysterectomy after completing childbearing, how should they be counseled regarding oophorectomy? Patients with CS are not at elevated risk of ovarian cancer and the ovaries may be safely retained. However, among women who decline mastectomy in favor of intensive breast cancer surveillance, oophorectomy likely reduces breast cancer risk. Prior large prospective studies in women with BRCA1 and BRCA2 mutations, who have a similarly high breast cancer risk, demonstrated the benefit of oophorectomy on breast cancer incidence and mortality (Rebeck et al., 2002; Kauff et al., 2002; Domchek et al., 2006). Given the lack of specific prevention data in CS, discussion of oophorectomy should be an

Discussion

Cowden syndrome is characterized by a significantly increased risk of multiple cancers, but the natural history of cancer development has not been documented. Our patient’s deleterious germline mutation in PTEN (N48K) is a missense mutation that impairs the ability of the mutant PTEN protein to inhibit PKB/Akt activation (Vega et al., 2003). In our analysis of endometrium and breast tissues, we identified loss of the wildtype PTEN allele in the atypical hyperplasia of both sites (Fig. 1). These data support the hypotheses that N48K is pathogenic and that aberrant PTEN function drove the development of atypical hyperplasia/intraepithelial neoplasm in both breast and endometrium. We believe that this is the first demonstration of the in vivo process of neoplastic transformation in breast and endometrial epithelium in women with PTEN mutations, which, in at least some cases, appears to progress through an identifiable pre-invasive precursor.

Identification of loss of the wildtype allele could be used to confirm the neoplastic nature of hyperplastic lesions in individuals with PTEN mutations in cases of pathological uncertainty. Given the finding of incidental endometrial hyperplasia/neoplasia at hysterectomy, we propose that prophylactic hysterectomy specimens from women at high-risk should be evaluated by thorough pathological sampling of the endometrium to exclude the presence of an occult carcinoma which might require further evaluation or treatment. Patients presenting with clinical features of CS and complex atypical hyperplasia/endometrial cancer should be offered germline testing for PTEN mutations rather than tissue testing, as somatic PTEN mutations are common in the pathogenesis of endometrial cancer. Identification of a germline mutation carries significant implications for counseling, surveillance, and recommendations for prophylactic surgery.

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individualized part of preoperative counseling. At the time of hysterectomy, our patient had a new diagnosis of breast atypical hyperplasia, further elevating her breast cancer risk. She was not interested in bilateral mastectomy, but wished to otherwise maximize her breast cancer risk reduction and thus elected bilateral salpingo-oophorectomy. Tamoxifen is often advocated for chemoprevention in women with atypical ductal hyperplasia of the breast but should probably be avoided in CS patients with intact uteri, given the already elevated risk of endometrial cancer.

In summary, we present an informative case demonstrating the in vivo process of malignant transformation in a patient with Cowden syndrome. Loss of heterozygosity at the PTEN mutation in endometrium and breast suggests that PTEN-driven oncogenesis may proceed through a stage of pre-invasive intra-epithelial neoplasia. Thorough pathological sampling of breast and endometrium is indicated at the time of prophylactic surgery.

Table 2
Recommendations for breast and endometrial cancer risk management in Cowden Syndrome (adapted from Daly et al., 2014).

| Breast | Endometrium |
|--------|-------------|
| Annual mammography and breast MRI beginning age 30–35 or 5–10 years before earliest breast cancer diagnosis in family | Educate patient on symptoms of endometrial neoplasia and need for prompt evaluation of abnormal bleeding |
| Consideration of bilateral risk reducing mastectomy | Consideration of annual transvaginal ultrasound and/or endometrial biopsy |

Conflict of interest
The authors have nothing to disclose.

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