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The clinical significance of K-ras mutation in endometrial "surface epithelial changes" and their associated endometrial adenocarcinoma

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HIGHLIGHTS

- Surface epithelial changes (SECs) are neoplastic.
- SECs have the same *KRAS* mutation as the underlying endometrial carcinoma.
- *KRAS* testing may play an important role in distinguishing SECs from benign mimics.

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ABSTRACT

Objectives. The entity of 'surface epithelial changes' (SECs) was first described in 1995 [1]. Morphologically, SECs usually arise from malignant glands at the superficial aspect of well differentiated (WD) endometrioid carcinomas (ECs) and impart the appearance of a 'maturational' phenomenon at the surface of the cancer. Exhibiting a paradoxically bland histologic appearance, SECs typically show morphologic features that mimic benign entities, particularly endocervical microglandular hyperplasia (MGH). SECs have been associated with approximately half of WD endometrioid carcinomas many of which showed focal mucinous differentiation. Despite their morphologically benign histology, some have questioned whether the presence of SECs represents a 'marker' for an underlying malignancy, especially in postmenopausal women with endocervical or MGH-type SECs in their endometrial sampling. Since the biologic nature of SECs is unknown, we aimed to study the prevalence of *KRAS* gene mutations in SECs and the underlying WD endometrioid adenocarcinomas (EC) from which they directly arise.

Methods. 24 cases with biopsy proven SECs and ECs in their subsequent hysterectomy were retrieved. Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue. PCR amplification for *KRAS* codons 12 and 13 was performed, followed by sequencing using capillary electrophoresis.

Results. KRAS codons 12 and 13 mutations were detected in 19 of 24 (79%) SECs, and 19 of 24 (79%) ECs. All SECs had the same *KRAS* mutation as the underlying EC.

Conclusions. Our results suggest that SECs are of neoplastic origin and that *KRAS* mutations play an important role in the tumorigenesis of ECs and SECs.

1. Introduction

Endometrial surface epithelial changes (SECs) refer to an entity that exhibits recognizable histologic changes that differ from the underlying endometrial carcinoma from which they directly arise. First termed 'surface epithelial changes' by Jacques et al in 1995, some SECs exhibit a mucinous microglandular pattern reminiscent of endocervical microglandular hyperplasia (MGH). Other patterns of endometrial SECs include papillary, hobnail and syncytial proliferations; all showing only mild to moderate cytologic atypia [1]. These changes are present on the endometrial surface and arise from endometrioid adenocarcinomas, usually FIGO grade 1 tumors. SECs are not a well-recognized phenomena as they are commonly diagnosed as endocervical type mucinous changes, and may present a diagnostic pitfall in endometrial biopsies and curettage specimens. In a recent study from our institution, more than 50% of endometrial samplings from postmenopausal women with only SECs in a biopsy sample showed endometrial carcinoma in a subsequent hysterectomy or curettage [2]. SECs are presently considered a 'marker' or 'indicator' of underlying atypical hyperplasia, or more frequently, well differentiated adenocarcinoma.

Mutations of *KRAS*, a key oncogene in the *EGFR* signaling cascade, are an early oncogenic event in many human cancers. Most functional mutations occur in codons 12, 13 or 61. *KRAS* mutations have been

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linked to mucinous carcinomas of the pancreas and ovary, and the status of the *KRAS* gene has an important role in management of patients with colorectal cancer and non-small cell carcinomas of the lung [3–6]. Endometrial carcinomas, particularly those with mucinous differentiation, often have *KRAS* mutations [7]. As it has been reported that K-ras mutations at codon 61 are uncommon in endometrial carcinomas and ovarian epithelial carcinomas, the focus of this study was on mutations in codons 12 and 13 [8–10]. Since the biologic nature of SECs is unknown, we aimed to study the prevalence of *KRAS* gene mutations in SECs, and in the underlying endometrial carcinomas from which they arise.

2. Materials and methods

Following IRB approval, 24 cases diagnosed from 2005 to 2012 with proven SECs, and ECs in their subsequent hysterectomies, were retrieved from our institutional archives. Three cases were from our consultation files. In 10 of 24 cases, only SECs were present in the endometrial biopsy but EC was found in each uterine corpus after complete curettage or hysterectomy. 14 of 24 endometrial samplings showed concomitant SECs and EC. All tumors associated with SECs were 'Type 1' (estrogen-dependent) ECs. Selected cases were stained with mucin stains to confirm the presence of intracellular mucin.

2.1. Microdissection and DNA extraction

For this study, PCR technique with direct genomic DNA sequencing was selected to minimize potential false positive results. The technique is still considered the gold standard, although it is less sensitive and requires higher tumor cell content from tissue samples. Micro-dissection of tumor was performed to ensure study samples contained more than 80% tumor cells. Samples with positive or equivocal results by direct DNA sequencing were confirmed by re-sequencing and by Peptide Nucleic Acid (PNA) mutant enrichment PCR [11]. In the EC group, tumor foci with predominantly mucinous differentiation were selected for DNA extraction. DNA extraction, purification, and PCR amplification of all samples were performed at the Molecular Laboratory of the Department of Pathology, Rhode Island Hospital (Providence, RI). Each sample was de-identified and assigned a sequential study number per Institutional Review Board policy. All molecular analyses were performed without prior knowledge of morphologic subtypes or histological features.

DNA was purified using a standard phenol:chloroform procedure. Briefly, dissected tissue was deparaffinized in xylene and washed with ethanol. The sample was incubated overnight in lysis solution containing proteinase K. DNA was purified in a series of phenol:chloroform washes, eluted in 100% ethanol containing ammonium acetate, and washed with ice cold 70% ethanol. The DNA pellet was dried and resuspended in TE. DNA purity and concentration were evaluated with a Nanodrop 2000c spectrophotometer (Thermo Scientific Waltham, MA).

2.2. Sequencing PCR

A 224 base pair fragment encompassing codons 12 and 13 of exon 2 was PCR amplified using forward and reverse primers 5′-GTGTGACATG TTCTAATATAGTCA-3′ and 5′-CTGTATCAAAGAATGGTCCTGCAC-3′ (Integrate DNA Technologies), respectively. Each sample was amplified in triplicate 25 μl reactions containing 0.5 μM of each primer, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.7 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and 5 μl (100–500 ng per reaction) genomic DNA. Following amplification, triplicate reactions were purified using Qiaquick PCR purification kit (Qiagen) and were confirmed by electrophoresis using 2% agarose gel.

2.3. DNA sequencing and analysis

Direct DNA sequencing was performed by Keck DNA Sequencing Laboratory at Yale University, New Haven, CT and Rhode Island Hospital, Providence, RI. Automated fluorescent sequencing was carried out on an ABI 3730xL (Applied Biosystems) using the Big Dye Terminator v3.1 sequencing kit, following a standardized variation of the kit insert protocol. Sequencing data were analyzed by Sequence Scanner v1.0 software (Applied Biosystems). Electrographs were visually inspected for a mutant *KRAS* peak. Samples that were determined as positive or equivocal by direct DNA sequencing were confirmed by re-sequencing following a Peptide Nucleic Acid (PNA) mutant enrichment PCR. The PNA was designed to inhibit *KRAS* wild type amplification, thereby enriching the mutant sequence.

3. Results

3.1. Clinical findings

Table 1 shows that patient age ranged from 49 to 84 years old (mean 59.6). 23 of 24 were postmenopausal and all presented with dysfunctional uterine bleeding. There was no history of hormone replacement therapy in 22 of 24 cases. *KRAS* codons 12 and 13 mutations were detected in 19 of 24 (79%) SECs, and 19 of 24 (79%) ECs. All SECs had the same *KRAS* mutation as the underlying EC. 18 of 24 showed focal or significant (>10% of tumor cells) mucinous differentiation. 11 of 24 cases were accompanied by lymph node dissection, of which 4 showed lymph node metastases (16.7%). There were 3 (12.5%) cases of recurrent disease, including 1 vaginal and recurrence and 2 inguinal metastases. A total of 8 cases were stage II or higher at the time of surgery, and 6 of these cases were FIGO grade 1 EC. In addition, 1 of the FIGO 1 Stage II cases subsequently developed recurrent disease (Table 1).

3.2. Microscopic features

Architecturally, SECs revealed varying proportions of a microglandular pattern simulating cervical MGH, complex mucinous proliferations, and syncytial aggregates of relatively bland appearing eosinophilic cells, frequently with ciliary, papillary and squamoid differentiation (Fig. 1). SECS were characterized by both better cellular differentiation and an architectural growth pattern that was different from the underlying endometrial carcinoma. (Figs. 2–4).

3.3. KRAS mutational analysis

KRAS codons 12 and 13 mutations were detected in 19 of 24 (79%) SECs, and 19 of 24 (79%) EC. 14 of 19 point mutations were identified at codon 12 with the most prevalent mutation being G12D (codon 12, GGT > GAT, from glycine to aspartic acid) (5/14). Only 5 point mutations were seen at codon 13, all being G13D. All SECs had the same *KRAS* mutation as underlying EC. All 12D *KRAS* mutations were present in advanced stage and recurrent cases.

4. Discussion

The entity that we term "surface epithelial changes" (SECs), a term coined by Jacques et al., has been studied by several investigators who have referred to them as mucinous endometrial epithelial proliferations, endocervical-like epithelial proliferations, endometrial carcinoma with surface metaplastic change, and endometrial (mucinous) metaplasia associated with endometrial carcinoma [12–15]. SECs most commonly exist at the superficial aspect of well differentiated endometrioid carcinomas, arise directly from malignant glands, but exhibit a bland cytologic appearance. SEC's typically show mucinous, syncytial, squamoid and papillary features that mimic benign entities, particularly microglandular hyperplasia. In their study of 116