KRAS Mutations in Mucinous Lesions of the Uterus

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Key Words: KRAS; Uterine mucinous lesions; Atypical mucinous proliferation; Differential diagnosis; Pathogenesis; Management

ABSTRACT

Objectives: The current study examined the KRAS mutation status in a spectrum of mucinous lesions of the uterus, including mucinous metaplasia (MM), atypical mucinous proliferation (AMP), endocervical mucosa, and microglandular hyperplasia (MGH).

Methods: Thirty-nine cases, including 15 AMPs, nine MMs, nine MGHs, and six normal endocervical mucosas, were selected from the departmental archive. All AMP cases with follow-up biopsies or hysterectomies were reviewed. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue and KRAS codons 12 and 13 sequence analyzed.

Results: KRAS codon 12 and 13 mutations were detected in 10 (67%) of the 15 AMP cases. No KRAS mutations were identified in MMs, MGHs, and endocervical mucosas (P = .002, AMP vs MM or MGH, Fisher exact test). Most women with AMP were postmenopausal (13/15 [86.7%]) and presented with dysfunctional uterine bleeding. Among the 10 cases of AMP harboring KRAS mutations, six (60%) cases were subsequently diagnosed with carcinoma, one with atypical complex hyperplasia, and two with AMP within endometrial polyps.

Conclusions: The results suggest a possible association between KRAS mutations and mucinous differentiation in endometrial carcinogenesis. KRAS status can help in assessing benign from precursor or malignant mucinous lesions as well as differentiate endometrial lesions from those of cervical origin.

In the endometrium, mucinous morphology can be found in a wide spectrum of conditions ranging from hormonal changes, metaplasia, and hyperplasia, to carcinoma.1 Endometrial epithelial mucinous metaplasia (MM) refers to the replacement of the epithelium and/or glands by cells containing intracytoplasmic periodic acid–Schiff (PAS)–positive mucin, which is resistant to diastase digestion. It resembles normal endocervical glandular cells, which appear cytologically bland and occasionally architecturally complex.2

Proliferative mucinous lesions of the endometrium exist as well and have been designated mucinous endometrial epithelial proliferation (MEEP). Based on the degree of architectural complexity, the mucinous proliferative lesions have been classified into three different categories: types A, B, and C.1 The percentage of endometrial carcinoma diagnosed following a curettage diagnosis of MEEP types A to C was
reported as 0%, 64.7%, and 100%, respectively. In type A without associated premalignant or malignant glandular proliferation, there are often small micropapillary projections. The nuclei are small and uniform, and mitoses are rare or absent. Atypical mucinous proliferation (AMP, type B and C combined) of the endometrium is defined by a spectrum of architectural atypia from mild architectural complexity to micropapillae, microglandular, or villous architecture. These lesions are considered equivalent to atypical endometrial hyperplasia (AEH). MEEP can be difficult to differentiate from MM, especially in biopsy and curettage specimens with limited material. Finally, frankly malignant mucinous lesions can develop in the endometrium. The pure example of this phenomenon is mucinous carcinoma (MC). However, a variable degree of mucinous differentiation is frequently observed in endometrioid carcinoma. Endometrial carcinoma with significant mucinous components, including MC and endometrioid carcinoma with significant mucinous differentiation (ECMD), can be diagnostically challenging to distinguish from MM and endocervical lesions, including microglandular hyperplasia (MGH) and mucinous adenocarcinoma of the cervix or minimal deviation adenocarcinoma (MDA), especially on biopsy, when a limited amount of tissue or only fragmented superficial tissues are available.3

KRAS mutations are often seen in malignancies of the lung, pancreas, colon, or ovary, displaying mucinous differentiation. Our previous study showed that KRAS mutation can be found in approximately 70% of MCs and ECMDs, significantly higher than the incidence in endometrioid carcinoma (EC) in the literature.4 According to currently accepted theories of endometrial carcinogenesis, AEH is considered a precursor to carcinoma. Progression to carcinoma can be seen in a percentage of AEH cases. In this regard, our current study examined the KRAS mutation status of a spectrum of mucinous lesions of the uterus, including MM and AMP, and normal endocervical mucosa and MGH to gain further understanding of the molecular characteristics and pathogenetic mechanisms of these lesions and investigate the utility of KRAS mutation status in the differential diagnosis workup from metaplastic and endocervical lesions.

Materials and Methods

Case Selection

With institutional review board approval, endometrial mucinous lesions were searched in the archival files of the Department of Pathology and Laboratory Medicine, Women and Infants Hospital (Providence, RI) during the study period from January 2005 through December 2010. A total of 39 cases, including 15 AMPs, nine MMs, nine MGHs, and six normal endocervical mucosas, were selected. All tissue samples were taken from endometrial and endocervical biopsy specimens or curettings, except two samples taken from hysterectomy. All AMP cases with follow-up biopsy specimens or hysterectomies were reviewed. Previous MC and ECMD cases with mutations served as positive controls. Six normal cervical samples from hysterectomies were used as negative controls. H&E-stained slides were reviewed in all cases to confirm the diagnosis. PAS stains with and without diastase were performed in the selected cases to confirm diastase-resistant intracytoplasmic mucin.

Microdissection, DNA Extraction, and KRAS Mutation Analysis

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue sections that were macrodissected to ensure more than 80% of lesional cells. Samples were assigned a sequential study number so that KRAS analysis was carried out blinded from the clinical information and diagnosis. DNA extraction, purification, and polymerase chain reaction (PCR) amplification were performed at the Clinical Molecular Laboratory, Department of Pathology, Rhode Island Hospital (Providence, RI). PCR amplification for KRAS codons 12 and 13 was performed and followed by sequencing using capillary electrophoresis as previously described.4 The sequencing results were analyzed by the Sequence Scanner v1.0 program (Applied Biosystems, Grand Island, NY).

Statistical Analysis

Statistical analyses were performed using the Fisher exact test. P < .05 was considered statistically significant.

Results

The search from the departmental archive identified nine endometrial MMs and nine endometrial AMPs, 15 endometrial AMPS and MGHs, and nine cases of MGH of the cervix. Patient’s age ranged from 44 to 84 years (median, 59 years) for MM, from 31 to 83 years (median, 57 years) for AMP, and from 22 to 50 years (median, 47 years) for MGH.

The clinical information, follow-up, and KRAS codon 12 and 13 mutation analysis results of the 15 AMP cases are summarized in Table II. Most women were postmenopausal (13/15 [86.7%]). All except one patient, the youngest in the study (31 years old), had dysfunctional uterine bleeding. Most patients were not receiving hormonal therapy (13/15 [86.7%]; two unknown). KRAS mutations were detected in 10 (67%) of the 15 cases. Mutations detected include three G12V, two G12D, two G12A, two G13D, one G12S, and one G12A. Most mutations were at codon 12 (8/10 [80%]).
Endometrial and endocervical mucinous lesions. A and B, Endometrial mucinous metaplasia (H&E; A, ×4; B, ×20). C and D, Endometrial atypical mucinous hyperplasia (H&E; C, ×4; D, ×20). E and F, Endocervical microglandular hyperplasia (H&E; E, ×4; F, ×20).
Among the 10 cases of AMP harboring KRAS mutations, six (60%) cases were subsequently diagnosed with carcinoma, one with atypical complex hyperplasia, and two with AMP within endometrial polyps; one was lost to follow-up. The two cases of endometrial polyps with AMP were found without underlying carcinoma in subsequent biopsy or curettage specimens. Worth noting is that the age of these two polyp cases represents the youngest (31 and 50 years, respectively) in the AMP group.

No KRAS mutations were identified in the nine cases of MM, nine cases of MGH, and six cases of normal endocervical mucosa. Comparing the KRAS mutational status of AMP with MM or MGH, the differences are statistically significant ($P = .002$, Fisher exact test).

### Table 1: Clinical Information, Follow-up, and KRAS Codon 12 and 13 Status of Atypical Mucinous Proliferation

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y</th>
<th>PM</th>
<th>DUB</th>
<th>HT</th>
<th>FU</th>
<th>Grade</th>
<th>INV, %</th>
<th>LVI</th>
<th>CER</th>
<th>KRAS</th>
<th>LN</th>
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<tr>
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<td>74</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Ca</td>
<td>1</td>
<td>73</td>
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<td>N</td>
<td>WT</td>
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<tr>
<td>2</td>
<td>54</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Ca</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>WT</td>
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<tr>
<td>3</td>
<td>57</td>
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<td>No</td>
<td>Ca</td>
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<td>54</td>
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<td>N</td>
<td>G12 V</td>
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<td>4</td>
<td>59</td>
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<td>No</td>
<td>Ca</td>
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<td>N</td>
<td>N</td>
<td>G12 D</td>
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<td>N</td>
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<td>NA</td>
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<td>No</td>
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<td>NA</td>
<td>NA</td>
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ACH, atypical complex hyperplasia; Ca, cancer; CER, cervical stroma involvement; DUB, dysfunctional uterine bleeding; FU, follow-up; HT, hormone therapy; INV, depth of invasion; LFU, lost to follow-up; LN, lymph node; LVI, lymphovascular invasion; N, none; NA, not applicable; PM, postmenopausal; WT, wild-type.

*Polyp with atypical mucinous proliferation.

### Discussion

**Potential Role of KRAS Mutation Status in Differential of Mucinous Lesions**

AMP presents diagnostic challenges, especially when only scant diagnostic material is available or presented at the lower uterine segment. The major differential diagnoses for AMP includes MM and MC of the endometrium and MGH and MDA of the uterine cervix.

No KRAS mutations were detected in the MM group. This finding is consistent with what the original morphologic study revealed—that true metaplastic changes, with no neoplastic implications, can be seen in the endometrium. When judgment of architectural complexity is difficult due to scant...
material, KRAS mutation analysis may aid in the differentiation since testing can be performed from very scant material, including formalin-fixed, paraffin-embedded tissue.

A similar high prevalence of KRAS mutations is seen in both MC and AMP. Endometrial MC often presents with deceptively bland cytology and locally aggressive clinical behavior. It relies heavily on morphologic features such as architectural complexity with mild cytologic atypia to differentiate it from AMP. Our finding of almost 86% of AMP associated with either carcinoma or atypical complex hyperplasia (ACH) suggests a similar management approach, especially in postmenopausal patients.

The high prevalence of KRAS mutations in endometrial mucinous neoplasms compared with the lack of mutations in endocervical MGH and normal mucosa indicates that positive KRAS mutation status could alert the pathologist of a possible endometrial origin. This could be helpful for the differential diagnosis of uterine mucinous lesions from endocervical lesions.

MDA of the uterine cervix is a well-differentiated variant of cervical mucinous adenocarcinoma, including 1% to 3% of all cervical glandular malignancies. A diagnosis of MDA is problematic because of its bland histology. A panel of estrogen receptor, progesterone receptor, p16, and vimentin immunohistochemical (IHC) stains can be helpful in distinguishing endometrial versus endocervical origin, but sometimes the results are equivocal. Furthermore, immunostaining results may be hard to read in scanty mucinous material. KRAS mutations have not been reported in MDA. Recently, several publications suggested a link between lobular endocervical glandular hyperplasia (LEGH) and MDA by IHC and molecular studies. Common chromosomal imbalances in MDA were also seen in a subset of LEGH. X chromosome inactivation clonality analysis in MDA with and without LEGH demonstrated an identical clonality pattern between the two, further suggesting that a subset of LEGH could be the precursor to MDA. While no KRAS mutation study has examined MDA, so far, mutational analysis found a KRAS mutation in only one (5%) of 19 LEGH cases. Therefore, KRAS testing could be very useful to distinguish AMP from MDA.

MGH of the cervix is a benign disease with a highly variable degree of complex glandular proliferations. It can be problematic in some cases to differentiate from AMP or MC using morphology alone. A panel of IHC markers can be used to differentiate MGH from AMP or MC. Our results showed that KRAS mutation testing could be an additional aid for differentiation, since no KRAS mutations were detected in MGH.

**KRAS as Driver Mutation on Pathogenetic Pathway of Uterine Mucinous Neoplasm**

Although MC comprises only a relatively small percentage of endometrial carcinoma, ECMD (mucinous component >10%) is much more common and has a higher rate of nodal metastasis. Findings from this study further indicated that mucinous differentiation in endometrial epithelial neoplasms deserves more investigation. Compared with other types of endometrial carcinoma, there are very few published studies investigating the molecular characteristics and pathogenic mechanisms of mucinous endometrial carcinoma and other uterine mucinous changes and lesions. This is also true for KRAS mutation studies. While KRAS mutations are known to be a common genetic alteration in other carcinomas displaying mucinous morphology such as carcinoma of the pancreas, lung, and colon, much fewer studies have investigated KRAS (codon 12 and 13) mutation status in the endometrial mucinous lesion spectrum.

Our previous study of KRAS codon 12 and 13 status detected mutations in 80% of MCs and more than two-thirds of ECMDs, significantly higher than ECs without mucinous components. Our current study revealed KRAS mutations in 67% of AMPs but in none (0%) of the MMs. A recent study of KRAS mutations in endometrial mucinous epithelial lesion detected mutations in 55%
of complex mucinous changes but in none of the simple mucinous changes.\textsuperscript{11} These results support our findings. Our results not only help gain more understanding of the pathogenic pathways of endometrial mucinous lesions but also carry important differential diagnostic and management value.

The detection rate of \textit{KRAS} mutations is much higher in AMP than in AEH (Table 2).\textsuperscript{12,13} Previously, a study reported \textit{KRAS} mutation in two (18.2\%) of 11 cases of complex atypical hyperplasia,\textsuperscript{13} while another study reported that none (0\%) of the nine endometrial hyperplasias had \textit{KRAS} mutations.\textsuperscript{12} Combined with our previous study, in which significantly more \textit{KRAS} mutations were identified in endometrial carcinoma with mucinous components compared with those without, our data demonstrate a significantly higher prevalence of \textit{KRAS} mutations in the mucinous neoplastic spectrum from AMP to ECMD and MC, compared with the spectrum without mucinous components from atypical hyperplasia to endometrioid carcinoma. These results suggest that \textit{KRAS} codon 12 and 13 mutations may play an important role in carcinogenesis of endometrial carcinoma with significant mucinous components.

The finding of no significant differences in \textit{KRAS} mutations between AMP associated with or without carcinoma (see Table 1) suggests that somatic \textit{KRAS} mutations might occur at an earlier stage of mucinous carcinogenesis. Previously, Cohn et al\textsuperscript{14} found discordant \textit{KRAS} mutations between atypical hyperplasia and the associated cancer, thus suggesting possible genetic heterogeneity in endometrial hyperplasia and concomitant cancer. A similar study in endometrial mucinous neoplastic lesions is needed.

### Potential Implication of \textit{KRAS} Mutation Status for Management of AMP and Endometrial Carcinoma

The ages of the 15 patients with AMP ranged from 31 to 83 years (mean, 57 years). All patients were postmenopausal with dysfunctional uterine bleeding, except for the two cases of the youngest age (31 and 50 years, respectively) (see Table 1). These two cases showed atypical mucinous proliferation confined within an endometrial polyp. Follow-up biopsies or hysterectomy in this AMP group showed 71\% of cases (10/14 cases with known follow-up) associated with underlying well-differentiated endometrioid carcinoma (Table 1), all with stages Ia and Ib. The rate of carcinoma detected by follow-up in the current study is consistent with a previous report.\textsuperscript{1} Overall, almost 86\% (12/14) of AMPs was associated with carcinoma or ACH. These results suggest that in postmenopausal patients with dysfunctional uterine bleeding without hormonal therapy, if the endometrial biopsy specimen shows atypical mucinous proliferation, further workup is needed to rule out an underlying adenocarcinoma.

From the \textit{KRAS} status prospective, six (60\%) of 10 AMPs with \textit{KRAS} mutations were associated with carcinoma, one (10\%) with ACH, one (10\%) unknown due to loss of follow-up, and two (20\%) with endometrial polyp without underlying carcinoma. These two polyps with AMP might be representatives of an early and localized AMP within the polyps, which were cured by endometrial biopsy. Age appears to be another important factor in the diagnosis and management in addition to morphology and \textit{KRAS} mutation status.

Recently, baseline \textit{KRAS} status has been used as a biomarker in clinical randomized phase II trials to assess the antitumor efficacy of the MEK inhibitor alone or in combination with an AKT inhibitor and safety in patients with recurrent or persistent endometrial cancer.\textsuperscript{15} The seemingly important role of \textit{KRAS} mutation as a driving force in the carcinogenesis of carcinoma with significant mucinous components implies that the testing could be used as a biomarker included in the clinical testing of endometrial carcinoma and as a prognostic marker for targeted therapies, especially for those tumors with significant mucinous components.

### Conclusions

In the spectrum of endometrial mucinous lesions, higher prevalence of \textit{KRAS} mutations was found in the AMP group, comparable to previously reported incidence in MC and ECMD. No \textit{KRAS} mutation was detected in MM and MGH. These findings indicate a close association between \textit{KRAS} mutations and mucinous differentiation in endometrial carcinogenesis and suggest a possible unique pathogenetic pathway for MC and ECMD. \textit{KRAS} status plays an important role in assessing benign from precursor or malignant mucinous lesions of the endometrium (ie, MM from AMP or MC). \textit{KRAS} status can aid in the distinction of primary endometrial lesions from those of cervical origin and is particularly useful in cases with limited diagnostic material.

In postmenopausal patients with dysfunctional uterine bleeding without a history of hormone replacement therapy, if endometrial biopsy shows atypical mucinous proliferation, further workup to rule out an underlying adenocarcinoma is warranted.

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