Correlation of KRAS Mutation with Mucinous Epithelial Lesions of the Endometrium: Implications for Biological Progression and Molecular Diagnosis

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ABSTRACT

Background: Mucinous epithelial changes are frequently encountered in endometrial biopsies. They may be classified into three morphologic categories (A, B and C) based on architectural complexity and cytological atypia to help predicting the risk of subsequent endometrial adenocarcinoma (EAC). KRAS mutations have been recently reported in atypical mucinous endometrial lesions and mucinous EAC. The aim of our study was to assess the prevalence and diagnostic and prognostic utility of KRAS mutation in the different morphological classes of mucinous endometrial changes.

Methods: Forty-six endometrial biopsies with mucinous change were retrieved from our departmental archives. All available H&E slides were reviewed and the cases were categorized into simple mucinous change without cytological atypia (Type A), complex mucinous epithelium without cytological atypia (Type B), markedly complex mucinous changes with cytological atypia (Type C) or mucinous EAC. DNA was extracted from formalin fixed paraffin embedded tissue sections, subjected to PCR amplification, followed by single strand conformation polymorphism analysis to identify the presence of KRAS exon 2 codon12/13 mutation(s). Only cases with complete histological review and informative KRAS mutation status were included in the final study. Clinical data and follow-up were recorded.

Results: Forty-four cases with informative KRAS mutation status were successfully analyzed, including 4 Type A lesions, 22 Type B lesions and 11 Type C lesions. Clinical follow-up was available for 35 patients. All Type A mucinous lesions were negative for KRAS mutation. KRAS mutation was present in 13 of 22 Type B mucinous lesions (59.1%), 50% of which had complex atypical hyperplasia and no EAC on follow up compared to patients without KRAS mutation (0% and 33%, respectively). KRAS mutation was identified in 5 of 11 (45.5%) patients with Type C mucinous lesions, and was associated with CAH (75%) and EAC (25%) on follow-up, compared to Type C cases without KRAS mutation (50% and 0%, respectively). Six of 7 mucinous EAC (85.7%) harbored KRAS mutation.

Conclusion: Corroborating the existing data, the current study confirms high incidence of KRAS mutation in complex mucinous epithelial lesions (Type B and C) and mucinous adenocarcinoma of the endometrium. Therefore, KRAS mutation may be a clinically useful molecular marker of early stage malignant transformation of mucinous lesions of the endometrium.

BACKGROUND

Among various endometrial epithelial metaplasias, mucinous change is particularly relevant since it is frequently encountered in an endometrial biopsy of peri- or post-menopausal patients and is more likely associated with additional aggressive endometrial lesions. However, the frequent disparity between the cytological atypia and architectural alterations in a mucinous lesion, especially in a small, fragmented biopsy may lead to significant diagnostic challenges of interpretation to guide patient management. Type I endometrial adenocarcinomas frequently show mucinous differentiation, a subset of which are classified as mucinous adenocarcinoma. It has been hypothesized that subtypes of endometrial mucinous metaplasia are biologically related to endometrioid adenocarcinoma as precursor lesions. KRAS is a key oncogene in the EGFR signaling cascade (RAS-MAPK pathway) and its mutation is an early oncogenic event in the development of human cancers. It is of particular inter-est that the presence of KRAS mutation correlates with mucinous differentiation in various human cancers including pancreas, colon, thyroid and lung cancer. KRAS is also frequently mutated in ovarian mucinous neoplasms and endometrial mucinous carcinomas. We systemically analyzed morphological categories of endometrial mucinous lesions in correlation with KRAS mutation status and clinical progression.

METHODS

- Retrospective text search for endometrial biopsy or curettage specimens with a final diagnosis of endometrial mucinous change or metaplasia and mucinous carcinoma from 1983 through 2011 was conducted.
- The original H.E. slides of each case were reviewed and mucinous lesions were classified into the following four categories:
- Simple mucinous change (Type A) is defined by the presence of linear or pseudo-stratified epithelial lining with minimal architectural complexity (mild epithelial tufting) and no or minimal cytological atypia (Figure 1-A).
- Complex mucinous change (Type B) consists of mucinous epithelium with more extensive papillation, the presence of microglandular or cribriform configurations with no or minimal cytological atypia. (Figure 1-B).
- Complex mucinous change (Type C) is defined as mucinous epithelium with either simple or increased architectural complexity along with the presence of moderate cytological atypia (Figure 1-C and D).
- Mucinous adenocarcinoma was defined by the presence of otherwise endometrioid adenocarcinoma with at least 50% of the tumor cells demonstrating intracytoplasmic mucin (Figure 1- E and F).
- KRAS mutation analysis was performed by polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP, Figure 2).

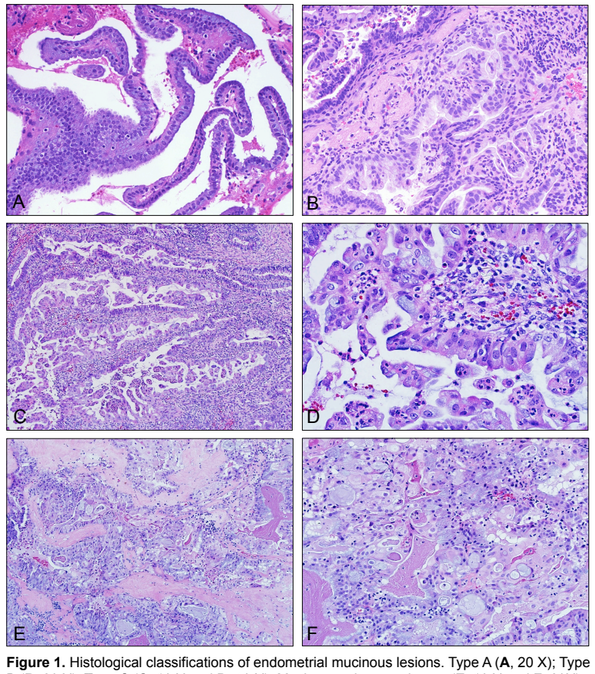

Figure 1. Histological classifications of endometrial mucinous lesions. Type A (**A**, 20 X); Type B (**B**, 20 X); Type C (**C**, 10 X and **D**, 40 X); Mucinous adenocarcinoma (**E**, 10 X and **F**, 20X).

RESULTS

- A total of 44 endometrial biopsy/curettage cases were included in the study and classified into Type A (4 cases), Type B (22 cases), Type C (11 cases) and mucinous adenocarcinoma (7 cases).
- KRAS analysis was informative in all (Table 1) and the mutation was detected in none (0/4) of Type A, 13/22 (59.1%) of Type B (Figure 2), 5/11 (45.5%) of Type C and 6/7 (85.7%) of mucinous carcinomas.
- Follow up specimens including both hysterectomy and endometrial biopsy or curettage were available in 35 cases with an average follow-up interval of 35.1 weeks. Benign endometrium was found in 2 Type A cases. CAH or EAC was found in 6 of 16 Type B cases (follow-up interval of 36.6 weeks) and 7 of 10 Type C lesions (follow-up interval of 40.2 weeks).
- When taking hysterectomy as the end point of follow-up (Table 2), 57.1% of Type B (4/7) and 71.4% Type C (5/7) cases were found to have CAH and/or EAC. In addition, 71.4% (5/7) of Type B cases and 42.9% (3/7) of Type C cases had KRAS mutation in their corresponding biopsy specimens.
- KRAS mutation has a positive predictive value (PPV) of 85.7% (7/8 cases) and a negative predictive value (NPV) of 66.7% (4/6) for CAH or EAC in the follow-up hysterectomy of patients with Type B or C mucinous lesions in their corresponding biopsy or curettage specimens.

Table 1. KRAS Mutation Status in Different Categories of Endometrial Mucinous Lesions

Total No.	Average Age	Subtype	KRAS (+)	KRAS (-)	% KRAS (+)
4	46	A	0	4	0.0%
22	40	B	13	9	59.1%
11	43	C	5	6	45.5%
7	43	Mucinous Carcinoma	6	1	85.7%

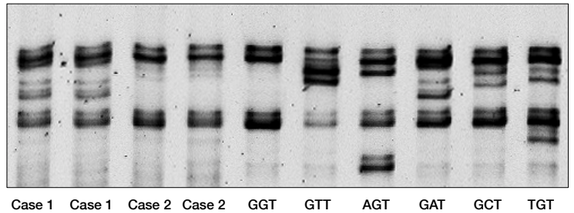

Figure 2. KRAS mutation analysis by PCR-SSCP showing mutation banding patterns found in representative Type B lesions (Case 1; GAT mutation shown in duplicates, Case 2: TGT mutation shown in duplicates). The wild type KRAS is GGT. Known positive controls show distinct banding pattern (GTT, AGT, GAT, GCT and TGT).

Table 2. Follow-up Hysterectomy Results in Correlation with KRAS Mutation

Total No.	Subtype	Mutant KRAS (%)	CAH/EAC	% CAH/EAC
1	A	0/1 (0.0%)	0/1	0.0%
7	B	5/7 (71.4%)	4/7	57.1%
7	C	3/7 (42.9%)	5/7	71.4%
7	Mucinous Carcinoma	6/7 (85.7%)	7/7	100%

CONCLUSIONS

- Our data further emphasizes the architectural complexity as an important prognostic indicator for patients with mucinous endometrial lesions.
- Diagnostic separation of endometrial mucinous metaplasia into morphologically simple and complex categories constitutes a highly sen-sitive, although not specific, approach to assess the risk of development of precancerous hyperplasia and endometrial adenocarcinoma.
- The presence of KRAS mutation in both mucinous adenocarcinoma and complex mucinous changes indicates that KRAS mutational activation is implicated in the pathogenesis of a significant subset of endometrial mucinous carcinoma.
- With a high positive predictive value, KRAS mutation analysis may offer an additional discriminatory power to refine risk stratification algorithm for patients with endometrial mucinous lesions. Additional studies are important to confirm our findings.

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