Human Pathology (2017) 67, 69–77

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Clonal evolution in paired endometrial intraepithelial neoplasia/atypical hyperplasia and endometrioid adenocarcinoma☆,☆☆

Mariano Russo BS[a,b], James Broach PhD[a,b], Kathryn Sheldon PhD[a,b], Kenneth R. Houser MS[a,b], Dajiang J. Liu PhD[b,c], Joshua Kesterson MD[d], Rebecca Phaeton MD[d], Carrie Hossler MD[d], Nadine Hempel PhD[e], Maria Baker PhD[f], Jordan M. Newell MD[g], Richard Zaino MD[g], Joshua I. Warrick MD[g,*]

[a]*Department of Biochemistry, Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA 17033*
[b]*Institute for Personalized Medicine, Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA 17033*
[c]*Public Health Sciences, Penn State College of Medicine, Hershey, PA 17033*
[d]*Department of Obstetrics and Gynecology, Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA 17033*
[e]*Department of Pharmacology, Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA 17033*
[f]*Department of Medicine, Penn State College of Medicine and Milton S. Hershey Medical Center, Penn State Cancer Institute, Hershey, PA 17033*
[g]*Department of Pathology, Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA 17033*

Received 13 April 2017; revised 18 June 2017; accepted 5 July 2017

Keywords:
Endometrioid adenocarcinoma;
Endometrial hyperplasia;
Endometrial intraepithelial neoplasia;
Clonal evolution;
Next generation sequencing

Endometrial intraepithelial neoplasia (EIN) and atypical endometrial hyperplasia (AH) are histomorphologically defined precursors to endometrioid adenocarcinoma, which are unified as EIN/AH by the World Health Organization. EIN/AH harbors a constellation of molecular alterations similar to those found in endometrioid adenocarcinoma. However, the process of clonal evolution from EIN/AH to carcinoma is poorly characterized. To investigate, we performed next-generation sequencing, copy number alteration (CNA) analysis, and immunohistochemistry for mismatch repair protein expression on EIN/AH and endometrioid adenocarcinoma samples from 6 hysterectomy cases with spatially distinct EIN/AH and carcinoma. In evaluating all samples, EIN/AH and carcinoma did not differ in mutational burden, CNA burden, or specific genes mutated (all $P > .1$). All paired EIN/AH and carcinoma samples shared at least one identical somatic mutation, frequently in PI(3)K pathway members. Large CNAs (>10 genes in length) were identified in 83% of cases; paired EIN/AH and carcinoma samples shared at least one identical CNA in these cases. Mismatch repair protein expression matched in all paired EIN/AH and carcinoma samples. All paired EIN/AH and carcinoma samples had identical The Cancer Genome Atlas subtype, with 3 classified as "copy number low endometrioid" and 3 classified as "microsatellite instability hypermutated." Although paired EIN/AH and carcinoma samples were clonal, private

☆ Competing interests: The authors declare that they have no conflict of interest.
☆☆ Funding/Support: This project was funded by an Intradepartmental Research Initiation Grant from the Penn State Department of Pathology. D. J. L. is funded by 1R21DA040177-01 from the National Institute on Drug Abuse and 1R01HG008983-01 from the National Human Genome Research Institute.
* Corresponding author at: Penn State College of Medicine and Milton S. Hershey Medical Center, Department of Pathology, 500 University Dr, Hershey, PA 17033.
E-mail address: jwarrick@hmc.psu.edu (J. I. Warrick).

http://dx.doi.org/10.1016/j.humpath.2017.07.003

mutations (ie, present in only one sample) were identified in EIN/AH and carcinoma in all cases, frequently in established cancer-driving genes. These findings indicate that EIN/AH gives rise to endometrioid adenocarcinoma by a complex process of subclone evolution, not a linear accumulation of molecular events.

1. Introduction

Endometrial intraepithelial neoplasia (EIN) and atypical endometrial hyperplasia (AH) are histomorphologically defined precursors to endometrioid adenocarcinoma (EMC). Although they have somewhat different morphologic definitions [1-4], many features are shared, and these are considered the same process by the World Health Organization (WHO), named *EIN/AH* in the most recent WHO manual of gynecologic tumors [5].

Several lines of evidence support that EIN/AH is a direct precursor to EMC. First, women with a diagnosis of EIN/AH have a greatly increased risk of developing EMC [3]. Second, uteri containing EIN/AH sampled on biopsy often have EMC upon examination of the uterus at hysterectomy [6]. Third, and perhaps most compelling, several studies have shown that EIN/AH harbors a constellation of molecular alterations similar to those seen in endometrioid EMC, including microsatellite instability and somatic mutations in *PTEN*, *PIK3CA*, *KRAS*, and *CTNNB1*, among others [7-14].

Early evidence indicates that cancer precursors evolve to invasive carcinoma via complex and dynamic processes. For example, histologically identifiable Barrett mucosa progresses to invasive adenocarcinoma through a complex set of subclonal evolutions, not a linear accumulation of mutational events. This is evidenced by Barrett mucosa and adjacent adenocarcinoma harboring both public mutations (present in both lesions) and private mutations (present in only one lesion), frequently in established cancer-driving genes [15].

In the present study, we performed next-generation sequencing on paired EIN/AH and EMC from a series of hysterectomy specimens. Our results show that progression from EIN/AH to EMC occurs through a complex process of clonal evolution, not linear accumulation of mutational events, similar to evolution from Barrett mucosa to esophageal adenocarcinoma.

2. Materials and methods

2.1. Patient selection and slide review

This study was performed with approval from the Penn State College of Medicine Human Subjects Protection Office (Institutional Review Board). Hysterectomy specimens performed for EMC were identified. Cases were selected if, and only if, (1) distinct EIN/AH and fully developed EMC were present in spatially distinct locations in the endometrium (ie, EIN/AH and EMC were not intermixed); (2) the specimen was well fixed; and (3) 2 separate subspecialized gynecological pathologists (J. I. W. and R. Z.; see below) agreed on the diagnosis of EIN/AH and EMC in the selected foci, blinded to the others' diagnoses. Patient demographic and staging information were obtained by medical record review. Staging information was determined per The American Joint Committee on Cancer manual, seventh edition [16].

2.2. Definition of EIN/AH in the present study

The histomorphologic criteria for EIN and AH are distinct. *EIN* is defined as a focus of crowded endometrial glands with glandular area greater than stromal area and linear dimension at least 1 mm. The nuclei of EIN must be distinct from the background endometrium, and carcinoma and benign mimics, such as tubal metaplasia, must also be excluded [1,2,4]. *AH* is less precisely defined as crowded endometrial glands with atypical nuclei, described as enlarged and round with loss of polarity [5]. Strictly applying criteria for EIN places approximately 60% of cases of AH in this category [2]. Examples are presented in Fig. 1.

Although histomorphologic criteria differ between EIN and AH, the most recent edition of the WHO manual considers EIN and AH to represent the same disease process [5]. Thus, for the present study, we considered a focus as EIN/AH if it met histomorphologic criteria for either EIN or AH as agreed upon by 2 subspecialized gynecological pathologists.

2.3. DNA extraction

Distinct areas of EIN/AH and EMC, as well as normal control tissues (lymph node, ovary, or myometrium), were extracted directly form paraffin blocks by punch biopsy in the specific areas of interest. To ensure against contamination from different tissues deeper in the block, exhaustive deeper sections were obtained on all cases after punches were taken, and the hematoxylin and eosin (H&E) slides from these deeper sections were reviewed. Cases were excluded if different neoplastic tissue was present on deeper sections. DNA was extracted from each sample using the QIAamp DNA formalin-fixed, paraffin-embedded tissue kit (#56404; Qiagen, Hilden, Germany). The extraction procedure was performed as outlined by the manufacturer. DNA concentration and quality for all samples were measured using the Agilent 2200 Tapestation Instrument (Agilent Technologies, Santa Clara, CA).