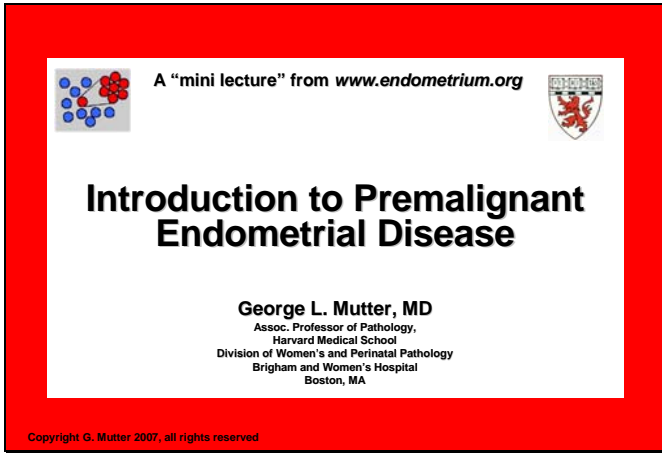


Slide 1



Introduction

This is Dr. George Mutter, I am a gynecologic pathologist at Harvard Medical School and the Division of Women's and Perinatal Pathology at Brigham and Women's Hospital in Boston. Today's mini lecture is entitled "Introduction to Premalignant Endometrial Disease".

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Differences in Type I/II Endometrial Carcinomas

Feature	Endometrioid	Non-Endometrioid
p53 mutation	5-10%	80-90%
PTEN inactivation	55%	11%
Histotype	endometrioid, mucinous, secretory, squamous	papillary serous, clear cell, carcinosarcoma
Grades	I-III	not applicable
Behavior	Indolent	Aggressive
Precursor	EIN	?serous EIC
Risk factors	hormonal	not significant

Types of Endometrial Cancer

Today we will be talking about precursor lesions to the endometrioid type of endometrial adenocarcinoma. In fact, there are two major classes of glandular malignancies in the endometrium. These are commonly described as Type I and Type II cancers, which respectively correspond to endometrioid and non-endometrioid histologic types. Non-endometrioid tumors, which include papillary serous carcinomas, have not been associated with established risk factors, nor have true premalignant lesions been identified. An early stage of serous carcinoma called serous endometrial intraepithelial carcinoma, or "serous EIC" has in fact been described. This probably represents a pre-invasive form of disease, which is otherwise identical to that of the invasive stage of clinical progression.

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Differences in Type I/II Endometrial Carcinomas

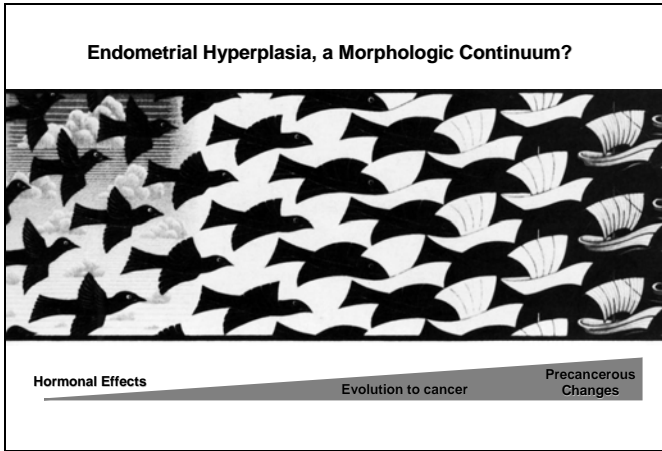
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Precursor of Endometrioid Type Cancers

Let us set aside the non-endometrioid tumors for the rest of this talk, and concentrate upon endometrioid class precancers. These carcinomas may have an endometrioid, mucinous, or secretory histology, and sometimes have admixed squamous elements. The most common genetic change is inactivation of a tumor suppressor gene, called PTEN. Later you will be seeing images of loss of this tumor suppressor protein within some affected neoplastic glands. Unopposed estrogen exposure has been associated with an increased risk for this type of carcinoma. Many examples of endometrioid endometrial adenocarcinoma are preceded by a morphologically distinctive precursor lesion, which we now know as endometrial intraepithelial neoplasia, or EIN.

Lecture: Introduction to EIN
Speaker: George L. Mutter, MD

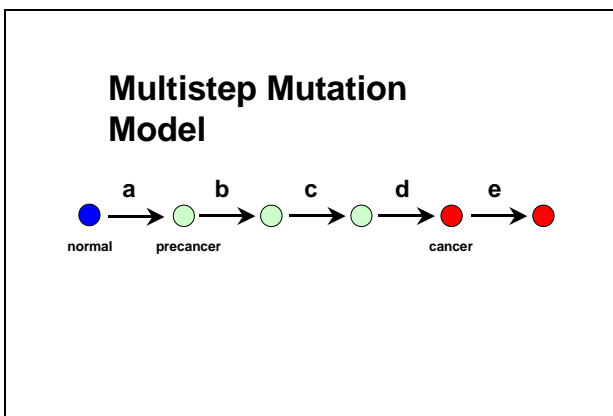
Slide 4

**Traditional Hyperplasias**

The concept of endometrial intraepithelial neoplasia is a relatively new one, having arisen just since the late Nineties, as a direct result of new data generated on the pathogenesis of this disease. Prior to definition of EIN as an entity, premalignant lesions were a subset of endometrial hyperplasias. Traditionally, endometrial hyperplasias have been envisioned as a gradual and sequenced evolution of the endometrial tissue field, getting progressively closer and closer to adenocarcinoma. In this model, unopposed estrogens are a key promoting factor, and progression towards cancer is accompanied by increased cytologic atypia and increased gland complexity. There are two fundamental problems with this traditional endometrial hyperplasia model. First is its structure as a continuum without discrete biologic or morphologic thresholds subdividing groups. Thus, there have been a number of attempts to variably sub-divide the universe of endometrial hyperplasias into differing numbers of subgroups. In the absence of identified biologic thresholds that correspond to diagnostic criteria, it becomes difficult to really understand how many discrete categories should be defined. The most commonly discussed threshold, that between atypical and nonatypical hyperplasias, is poorly reproducible and fails to cleanly segregate low and high risk lesions. The second problem with the traditional hyperplasia model is the incorrect assumption that the entire endometrial field gradually evolves in a continuum of slow change.

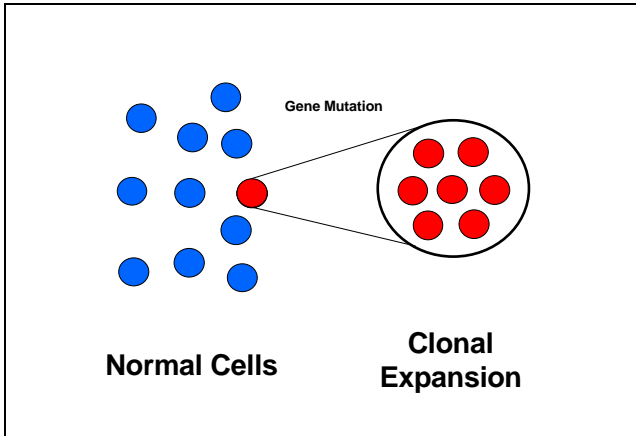
We now know that the effects of estrogens, which may cause an altered glandular growth, in fact may be continuous and progressive over time. In contrast to this, a premalignant lesion emerges from the endometrial field with a very different histologic appearance. It is quite discontinuous from the presentation of the background endometrium which may or may not have not been affected by unopposed estrogens.

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**Multistep Carcinogenesis: Genetics View**

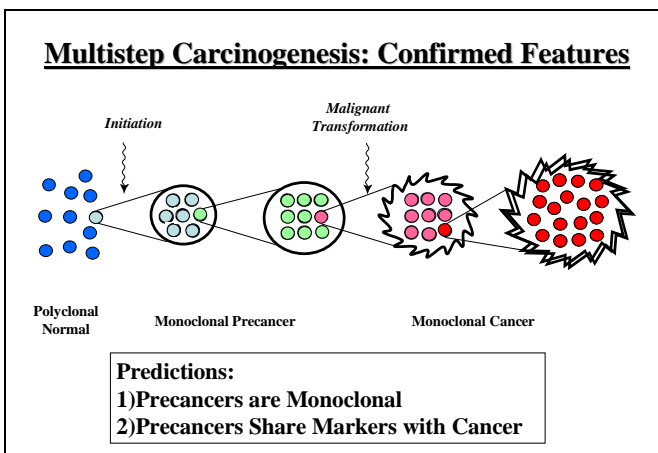
Our understanding of epithelial carcinogenesis has changed rather dramatically in the past decade. Endometrial adenocarcinoma, like other epithelial tumor types, arises through accumulation of multiple genetic mutations within individual cells. Not all of the genes associated with these steps are known for endometrial cancer. Those which have been implicated in the endometrioid pathway include PTEN, beta-catenin, K-ras, and DNA mismatch repair genes like MSH1. In an individual patient, the combination and sequence of acquisition of multiple genetic changes may be unique.

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**Clonal expansion of mutated cells**

Many thousands of mutated cells must be present within a tissue before they will be evident under a light microscope as abnormally shaped glands. This very simple idea, that genetic mutation is associated with the appearance of new lesions, requires that the affected cells undergo clonal expansion after mutation. This results in physically proximate clusters of cells, all of which share the same genetic mutation, and demonstrate an altered appearance.

Slide 7

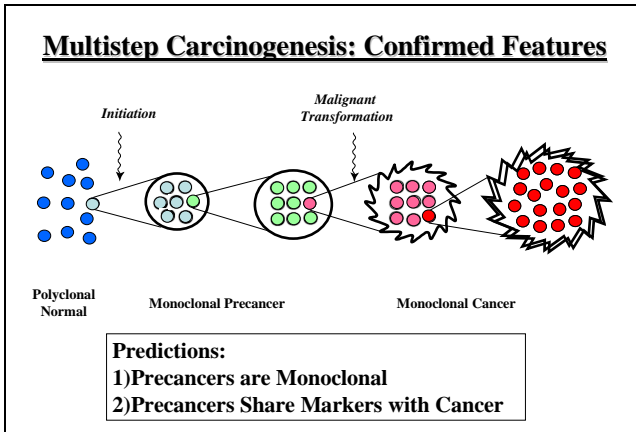
**Multistep Carcinogenesis: Integrated View**

Let us now consider the histologic implications of a multi-step model of carcinogenesis. In the beginning, normal parent tissues all have an identical genotype. The appearance of a mutation, an initiation which begins carcinogenesis, is the impetus for the first clonal expansion. This generates a clone of adjacent cells which may have a distinctive appearance identifiable as a premalignant lesion. It is possible, in fact likely, that multiple mutations accumulate during the premalignant phase of tumor development. At this point, the lesion within the endometrial compartment is a benign premalignant neoplasm, one which is prone to malignant conversion.

When a minimum level of cumulative genetic damage has developed, malignant transformation occurs. Here, a mutated clone becomes aggressive and develops the capability to metastasize. These are adenocarcinomas. In fact, this process of nested or sequential clonal evolution continues even once a lesion is already malignant. Note that progression events are defined by an expansion of each new clone from a single cell in the preceding clone. This is the basis for the notion that the entire endometrial field does not evolve as a group of cells towards cancer. Rather, what occurs is that individual clones expand, each having a different histologic appearance than the tissue from which it arose. The presentation of new mutations is punctuated by emergence of completely new and differing appearing groups of endometrial glands.

This model has now been confirmed through a series of molecular studies undertaken in the endometrium, as well as other epithelial tumor types which have precursor lesions. There are two significant features of a precursor lesion in this

Slide 7 (continued)



particular molecular scenario. First, premalignant lesions, or precancerous lesions, are monoclonal. This contrasts to the source normal tissues from which they arise. Second, the premalignant lesions have genetic alterations which are carried forward to the resultant cancers. This idea of lineage continuity between the physical cells of a precursor lesion and the resultant carcinoma is the ultimate biologic link between these two processes. Other, equally important features of premalignant lesions are defined clinically, such as increased cancer risk.

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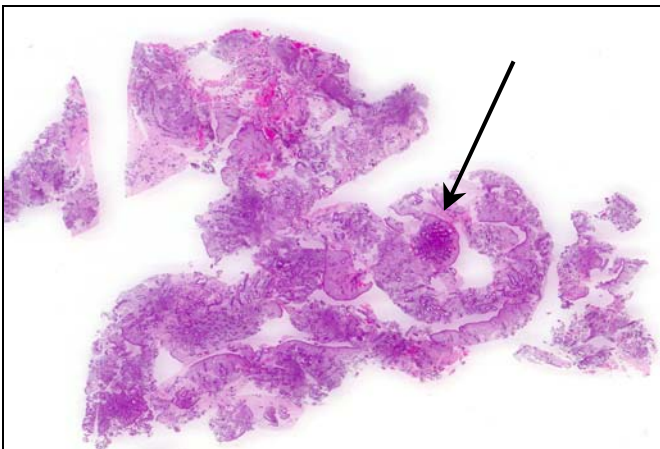
Monoclonal Origin

- Point origin and expansile growth
 Select Relevant Fields
- Lesion-Normal contrast
 Compare Internally

Histologic Significance of Monoclonal Growth

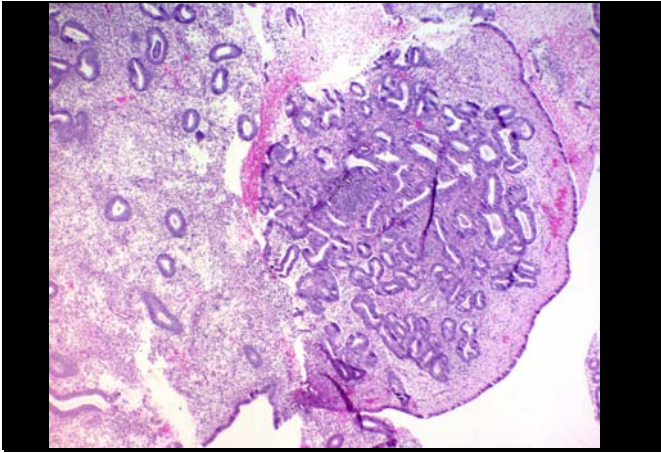
This experimentally confirmed monoclonal origin of premalignant endometrial lesions has several direct consequences for practicing pathologists. The first is that premalignant lesions originate at a single cell and grow outwards. This means that the lesions begin as localizing processes, only over time spreading to involve the entire endometrial compartment. Secondly, if one observes a lesion before it has occupied the entire endometrial compartment, it is possible to compare lesional glands to background normal glands in the same specimen.

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**Localizing Lesion: Low Magnification**

Here we have a low power view of an endometrial pipelle biopsy. You can see that one fragment has more densely clustered glands than the others. This is the expected appearance of such a clonal process. Selection of a fragment likely to contain a premalignant lesion occurs under very low power magnification.

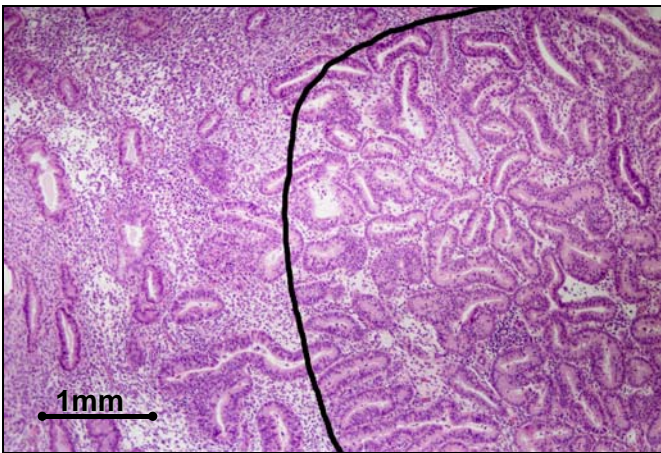
Slide 10



Localizing Lesion: Detail

Here you see, on the right, a premalignant EIN lesion that has a different architecture and a subtly different cytology than the background endometrium from which it has emerged on the left.

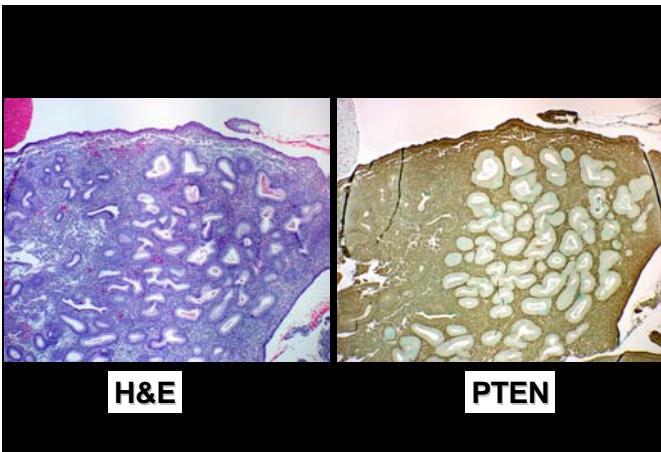
Slide 11



Perimeter of Localizing Lesion

The perimeter of discrete early lesions can be identified. We can now consider whether lesion size might be a relevant variable either for diagnosis or prognosis. As premalignant lesions expand, on the periphery glands are somewhat rarefied in a centripetal manner, extending into adjacent stroma.

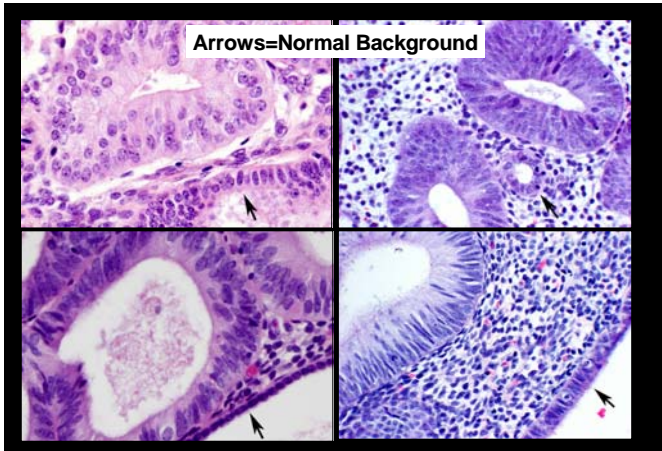
Slide 12



PTEN Marker defines a lesion

Another way to visualize the clonal nature of premalignant lesions is to use molecular markers. In this case, you see on the right, an immunohistochemical stain for the PTEN tumor suppressor protein. The protein is present in endometrial stroma and in normal endometrial glands, indicated by an intense brown stain. In contrast, there is a tight cluster of endometrial glands missing PTEN protein. These share a mutation of the PTEN gene, and are offset from the background by architecture and cytology.

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**Cytologic Change in Emergent Clones**

These premalignant clones contain cytologic changes. Each of the four panels shows a premalignant EIN lesion, in different patients. All have background normal endometrial glands, indicated by an arrow. The neoplastic premalignant glands, those without arrows in each panel, have a very different cytology when compared to the matched normals from the individual patient. The absolute cytologic appearance of premalignant endometrial lesions however, varies tremendously between patients. In every case, there is a change in cytology from each patient's normal to premalignant glands. EIN gland epithelial cells have nuclei that vary in size and extent of polarization. Similarly, cytoplasmic features of affected premalignant glands may also vary. In the lower right example you see more abundant luminal cytoplasm than in the others. Not all EIN lesions contain glands with classical cytologic atypia. Classic atypia is usually described as loss of nuclear polarity and acquisition of prominent nucleoli, features seen in the top two examples here. EIN lesions without classical atypia will always demonstrate a cytologic divergence from that patient's normal background endometrial glands, seen in the two lower panels.

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Endometrial Intraepithelial Neoplasia (EIN)

- A premalignant clonal neoplasm
- Precursor of endometrioid endometrial carcinoma
- Cancer risk is elevated 45 fold
- Is not subdivided into Grades
- Is different than diffuse hormonal effects (benign endometrial hyperplasia)
- New criteria do not correspond to old WHO hyperplasia categories

Endometrial Intraepithelial Neoplasia (EIN)

To summarise, premalignant lesions, which are called endometrial intraepithelial neoplasia, are clonal neoplasms. They are precursors of endometrioid endometrial adenocarcinomas. Patients with EIN lesions have an approximately 45-fold increased cancer risk. Currently, there is no basis to subdivide EIN lesions into grades or sub-types, these don't have clinical relevance. All of them are simply known as EIN. The diffuse hormonal effects of unopposed estrogen are quite different and distinctive compared to EIN. In a later lecture, I will be telling you more about this class of estrogen effects which we now refer to as benign endometrial hyperplasia, really a sequence of changes rather than a single entity. EIN diagnostic criteria include features which have never been part of the old World Health Organization hyperplasia diagnostic schema. For this reason, EIN lesions do not correspond rigidly to a particular subclass in the old hyperplasia system. Evaluation of each lesion using new criteria is required, rather than simple extrapolation from the older terminology and criteria.


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EIN Nomenclature	Topography	Functional Category	Treatment
Benign endometrial hyperplasia (Unopposed Estrogen effect)	Diffuse	Estrogen Effect	Hormonal therapy
EIN Endometrial Intraepithelial Neoplasia	Focal progressing to diffuse	Precancer	Hormonal or surgical
Carcinoma	Focal progressing to diffuse	Cancer	Surgical stage-based

EIN Diagnostic Schema

Here we see a diagnostic schema including this premalignant cancer entity of EIN. There are effectively three entities that need to be described in defining this class of premalignant lesions. EIN is a monoclonal focally originating premalignant lesion that is most commonly treated today by hysterectomy. There is clinical interest in defining efficacy of hormonal or progestin therapies, something that must be resolved by future studies. EIN lesions differ from the diffuse field effects caused by estrogens, a process secondary to an underlying hormonal abnormality. Lastly, EINs may progress to adenocarcinoma, something they have to be distinguished from. Management of endometrial adenocarcinoma requires an accurate clinical assessment of the extent or distribution of disease. This is most commonly accomplished by pathologic evaluation of a hysterectomy specimen.

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INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
 Pathology and Genetics of
 Tumours of Female Genital Organs
 Lyon 16 - 20 March 2002

EIN Diagnostic Criteria (all must be met)

EIN Criterion	Comments
Architecture	Area of Glands>Stroma (VPS<55%)
Cytology	Cytology differs between architecturally crowded focus and background.
Size >1 mm	Maximum linear dimension exceeds 1mm.
Exclude mimics	Benign conditions with overlapping criteria: Basalis, secretory, polyps, repair, etc..
Exclude Cancer	Carcinoma if mazelike glands, solid areas, or significant cribriforming

WHO "Bluebook" 2003

EIN Diagnostic Criteria: Summary

In the next mini lecture, we will be going through specific diagnostic criteria which will allow you to identify premalignant EIN lesions in routine patient material. There are both architectural and cytologic criteria. In fact all five criteria listed here must be met in order to make an EIN diagnosis. Only lesions with a threshold size of 1mm or more in a single fragment have been correlated with increased cancer likelihood. Smaller lesions should not be diagnosed as EIN. The most difficult part of diagnosis is excluding the many mimics which have some or many of the first three diagnostic features. Accurate diagnosis of EIN therefore requires familiarity with a very broad range of entities other than EIN itself.

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Introduction to Premalignant Endometrial Disease

THE END

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Conclusion

This ends our discussion of premalignant endometrial disease. This is George Mutter signing off from endometrium.org in Boston.