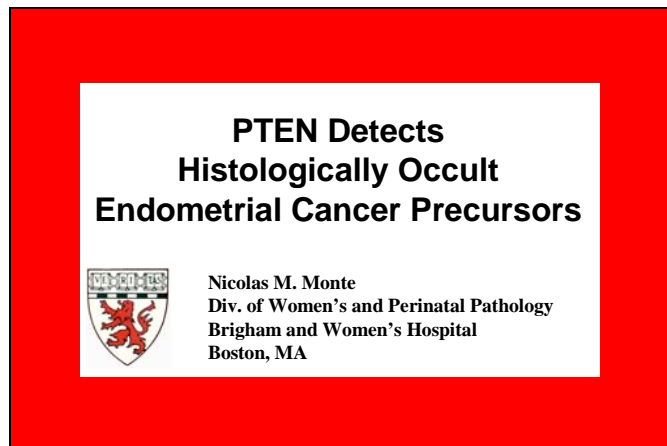


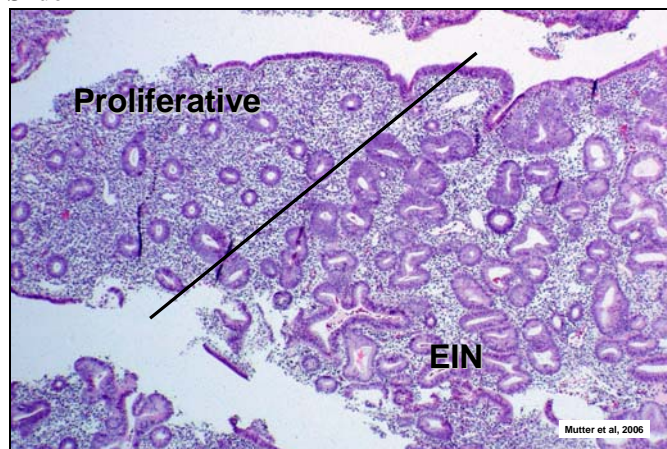
Lecture: PTEN detects histologically occult endometrial cancer precursors
 Speaker: Nicolas Monte

Slide 1

**Introduction**

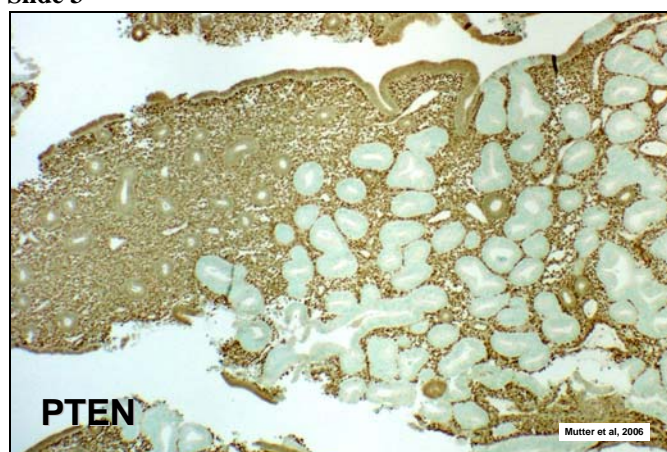
I am Nick Monte, a Research Associate in the laboratory of Dr. George Mutter. This mini-lecture will explain how a biomarker, PTEN, was used to detect occult precursors of endometrial cancer years before they can be diagnosed using current clinical methods.

Slide 2

**Precancerous EIN: Routine Stain**

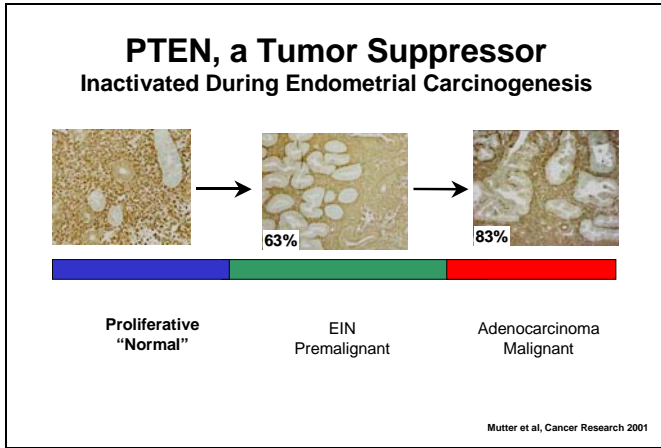
On the right side of this field you can see the crowded glandular architecture and altered cytology that is characteristic of EIN, adjacent to normal proliferative tissue on the left. From previous work we know that these precursor lesions carry a 45-fold increased risk of progression to endometrial cancer. In this case the lesion is large and clearly visible, thus there is no need to use a biomarker for identification. However, with a good marker it would be possible to find small lesions hidden among normal appearing tissue that would otherwise be impossible to detect!

Slide 3

**PTEN Marker**

Using immunohistochemistry, we have found that the tumor suppressor gene PTEN is the sensitive, high-resolution marker that we were looking for. Here the PTEN deficient glands of the EIN lesion present as pale and punched out, a stark contrast to the darkly stained expressing glands on the left side of the field. If you look closely, we are even able to detect several expressing glands that have been overrun directly adjacent to their null counterparts.

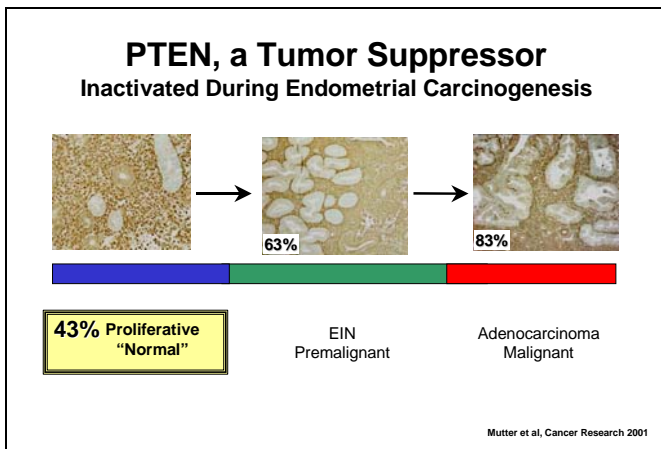
Slide 4



Carcinogenesis

Moving from left to right represents the progression over time from normal, benign tissue through EIN and finally to adenocarcinoma. Through screening, we have found that PTEN is inactivated in more than 3/4 of endometrial cancers, and over half of all EIN's.

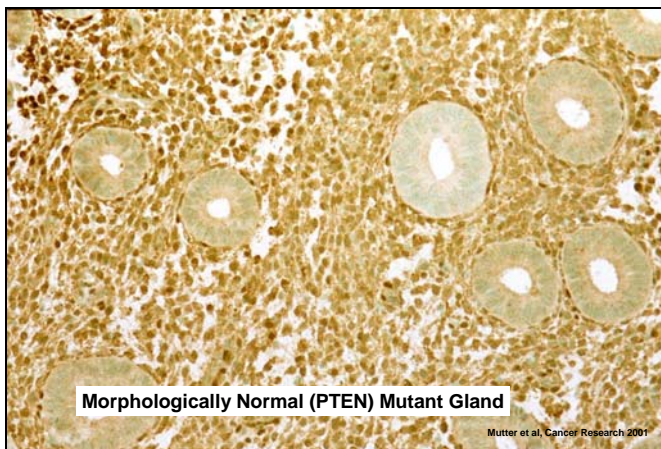
Slide 5



PTEN-null Normal Tissues

What's most interesting is that we find rare, scattered, morphologically normal glands that are PTEN deficient in 43% of pre-menopausal women. Given that cancers arise from normal tissue, we feel that this is an initial step of carcinogenesis.

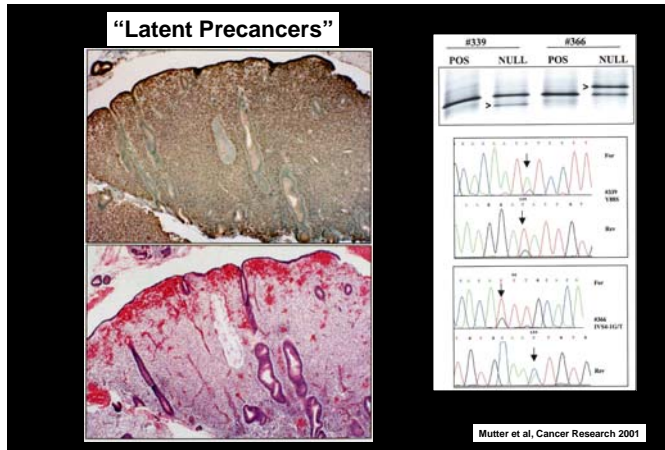
Slide 6



PTEN Stain

These PTEN deficient glands have no change in architecture or cytology from normal tissue, though they have acquired genetic alterations distinct from neighboring PTEN expressing glands. Loss of PTEN is due to acquired somatic mutations and not downregulation of the protein or an inherited germline effect.

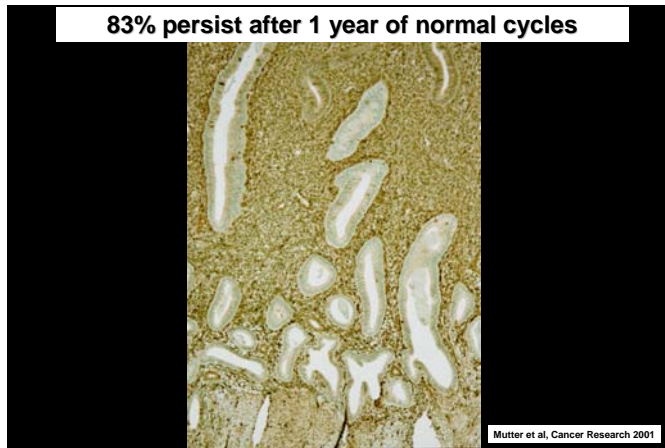
Slide 7



Latent Precancers are Mutated

We proved this by microdissecting both PTEN null and expressing glands followed by DNA analysis and as you can see in these figures, in the PTEN deficient glands we found sequence confirmed mutations while the expressing glands contain wild type DNA. We refer to these lesions as “latent precancers” because they have mutations commonly found in cancer but have not yet developed any recognizable histologic phenotype by routine stains. However, simply showing that mutations exist does not mean that these glands are carcinogenic. These mutated glands would have no significance if they were simply shed at each menstrual cycle.

Slide 8



Latent Precancers Persist for Years

But, when we resampled women one year later we found that null glands persisted in 83% of cases. This could be because the mutant glands are represented in the reserve population of cells in the basal layer that regenerate the functionalis each month. The important thing to note is that these glands do indeed persist for years. It's now appropriate to ask, for those normal appearing glands that do persist, are they capable of malignant transformation to cancer?

Slide 9

PTEN Immunohistochemistry Screen

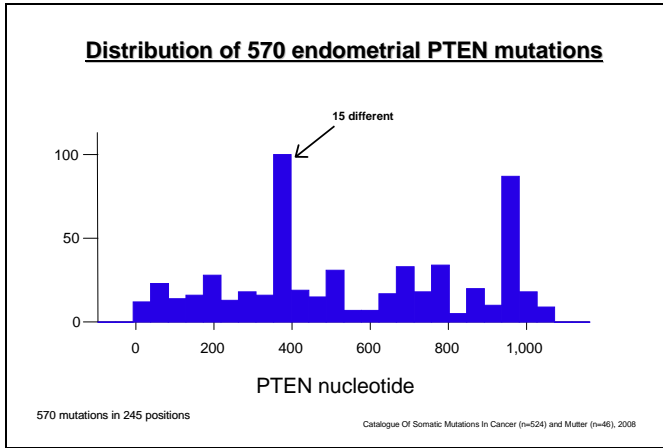
Time → Benign → Neoplastic (EIN or Cancer) → N=58

Type	PTEN		n	%
	Benign	Neoplastic		
???	null → null	→ null	24	41%
Independent	wt → wt	→ null	11	19%
Independent	null → null	→ wt	5	9%
Not Informative	wt → wt	→ wt	18	31%

Experimental Design

In the following are experiments we have studied the characteristics of these mutant glands, over time, in individual women. In particular, what we are interested in are clones that might progress to cancer. We identified 58 women with a diagnosis of EIN or Cancer who also had antecedent benign biopsies and performed PTEN screens at two or more time points. Of the screened cases 1/3 were not informative, in another 1/3 we were unable to show the persistence of null clones - indicating that the tissues were different. however, in 41% of the screened cases we witnessed PTEN null glands in both the neoplastic biopsy and prior benign biopsies. The big question in this segment of cases is whether these glands are the result of clonal conservation or the product of independent PTEN inactivation events. This cannot be answered by immunohistochemistry so we instead must look to genetic analysis. What we need is a clone specific marker.

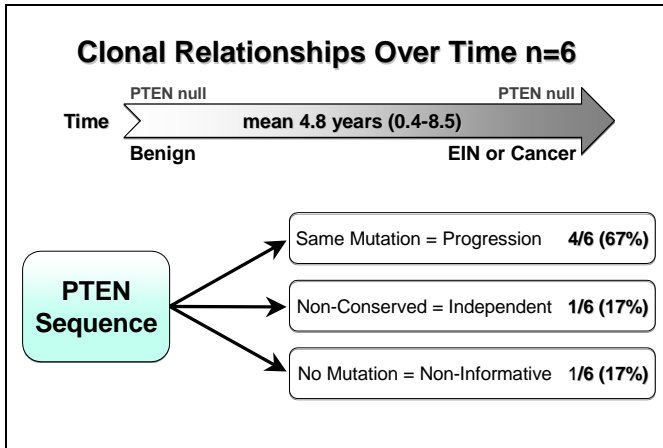
Slide 10



PTEN Mutations are Clone Markers

Here you see the PTEN gene and its pattern of mutations in 570 endometrial cancers, notice that they are spread around randomly across 1000 bases. Unlike some genes that tend to accumulate mutations at very specific loci, PTEN mutations are instead distributed across the entire length of the gene. Even in areas of this graphic where there appear to be hot spots, in fact these peaks are comprised of many different mutations. Therefore, if we have two different, independent mutations the likelihood of them being identical is less than 1%. Unlike screening by immunohistochemistry, individual PTEN mutations are unique clonal markers.

Slide 11



Clonal Relationships Over Time

We have begun to sequence the DNA of null glands isolated by laser microdissection in both benign and subsequent neoplastic specimens, separated by a mean of approximately 5 years. Here I can summarize the 6 cases we have analyzed at the level of PTEN sequence. Of the data we have, 17% were not informative, another 17% did not have conserved mutations, however 2/3 of cases have identical mutations at multiple time points, indicating that the same clone has persisted from benign, histologically normal appearing tissue through neoplastic transformation to EIN or cancer. I would now like to show you some of the cases we examined:

Slide 12

Case 05-174: Conservation of PTEN Clone

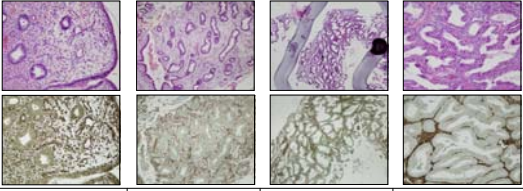
Diagnosis	Proliferative	Proliferative	Proliferative	EIN
Time(Yrs)	0	0	5.2	5.2
Protein	normal, present	null, absent	normal, present	null, absent
Mutation	wild type	c.433delT	wild type	c.433delT

Conservation of Clone, Example 1

Moving from left to right across the slide represents the passage of time. Both null and expressing glands are shown for each sample with corresponding information below each paired set of images. Here we see the conservation of a PTEN clone that arose in normal appearing proliferative tissue and has persisted over a period of 5.2 years, progressing to EIN.

Slide 13

Case K-9672: Conservation of PTEN Clone



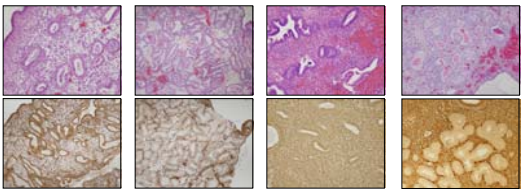
Diagnosis	Proliferative	Proliferative	Secretory	Cancer
Time(Yrs)	0	0	0.3	7.0
Protein	normal, present	null, absent	null, absent	null, absent
Mutation	wild type	IVS1-8 del 9, c.800 del A	IVS1-8 del 9, c.800 del A	IVS1-8 del 9, c.800 del A

Conservation of Clone, Example 2

Here is another example that has very similar findings. In this patient we see the conservation of two PTEN mutations, both mutations are present in two independent samplings of normal tissue 7 years before clinically diagnosed cancer.

Slide 14

Case 05-176: Conservation of PTEN Clone with additional mutation



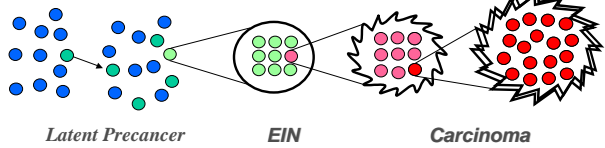
Diagnosis	Secretory	Secretory	Polyp	EIN in Polyp
Time(Yrs)	0	0	8.4	8.4
Protein	normal, present	null, absent	normal, present	null, absent
Mutation	wild type	956 del ACTT	wild type	956 del ACTT; 925 G>A; IVS 3 +58T>A

Progressive Mutation

You can imagine that in addition to histological progression there can be genetic progression as well. Here we see 3 mutations at the time of her EIN, 8 years earlier at her initial sampling only 1 mutation is present, but it is the same that is carried forward.

Slide 15

Preclinical Disease, "Latent Precancers"



Latent Precancer

EIN

Carcinoma

Latent Precancers:

- Are detectable with markers
- Are common
- Persist for years
- May Progress to cancer

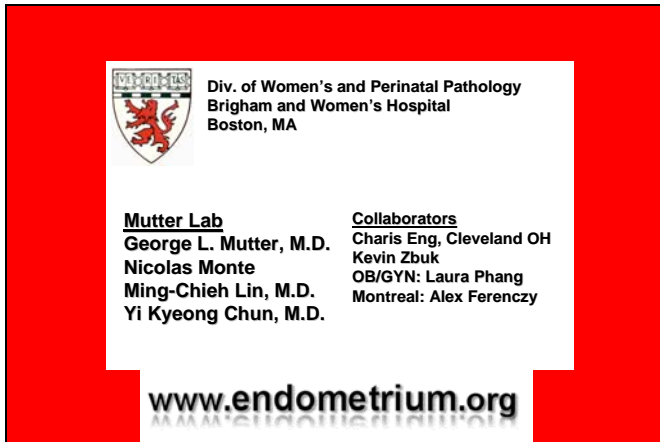
Questions for the future

- Clone Turnover Dynamics
- Targets for Prevention

Model Confirmed, With Questions

As you have just seen, there are detectable cells in normal tissue that progress to later cancers. We believe that some of these latent precancers are probably killed off by other anti-cancer mechanisms, but it is obvious that that they do have the ability to persist for long periods of time. If these lesions are indeed the first step of carcinogenesis, other interventions that cause ablation of these cells may have the capacity to prevent future disease.

Slide 16



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www.endometrium.org

Conclusion

This work was presented by myself, Nick Monte, at the 2008 Annual Meeting of the United States and Canadian Academy of Pathology.

It was performed in the Women's and Perinatal Pathology Division of the Department of Pathology at Brigham and Women's Hospital in conjunction with the team shown here.

Thank you for joining us for this Mini-Lecture at www.endometrium.org.