Morphological Compartmentalization of *CTNNB1* Mutation to Glands and/or Squamous Morules in Endometrial Endometrioid Carcinoma: Practical Implications for Using β-catenin IHC to Guide Localization of DNA Sampling for Mutational Analysis

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Background

In low risk endometrial endometrioid carcinomas (EEC), *CTNNB1* mutation (mut) correlates with decreased recurrence-free survival. β-catenin (β-cat) IHC can be used as a surrogate marker for *CTNNB1*mut. In EEC, squamous morules (SM) express nuclear β-cat IHC (nβ-cat+), yet nβ-cat IHC is negative or very focally positive in glandular components. We hypothesized that detection of *CTNNB1*mut in EEC depends on inclusion of nβ-cat+ tumor cells (including SM) in the area sampled for molecular testing.

Design

With IRB exemption, 10 low grade EECs (FIGO grade 1 or 2), with nβ-cat+ SM were selected from a cohort of EEC cases previously sequenced for *CTNNB1* after tumor microdissection (MD) that was agnostic to inclusion/exclusion of nβ-cat+ cells or SM (agnostic MD). For this study, using β-cat IHC slides as a reference, two areas of each tumor were selectively microdissected (selective MD) (Figure 1):

1) Nuclear β-cat (+) squamous morules (nβ-cat+ SM)
2) Nuclear β-cat (–) glandular (nβ-cat neg gland)

Each of the selective MD foci (n=20) underwent Sanger sequencing for *CTNNB1*. Selective MD results were compared to agnostic MD results. The percent SM cellularity of total tumor area cell volume was estimated.

Results

In 5/10 EEC (Table 1, Cases #1-5), selective MD showed nβ-cat+ SM foci harbored *CTNNB1*mut, while nβ-cat neg gland were *CTNNB1* wild-type (WT) (For example, Figure 1). The agnostic MD had detected a *CTNNB1*mut in only 1 of these cases (Figure 2, A-D). In 3/10 EEC (Cases #6-8), identical *CTNNB1*mut were identified in nβ-cat+ SM as in nβ-cat neg gland; but on re-review, positive nβ-cat staining was identified in the selective MD areas intended to be limited to nβ-cat neg glands. The agnostic MD identified the same *CTNNB1*mut as selective MD in these cases (Figure 2, E-G). A *CTNNB1*mut was not found in the remaining 2 cases (Cases #9-10); in both, SM comprised <1% of the tumor.

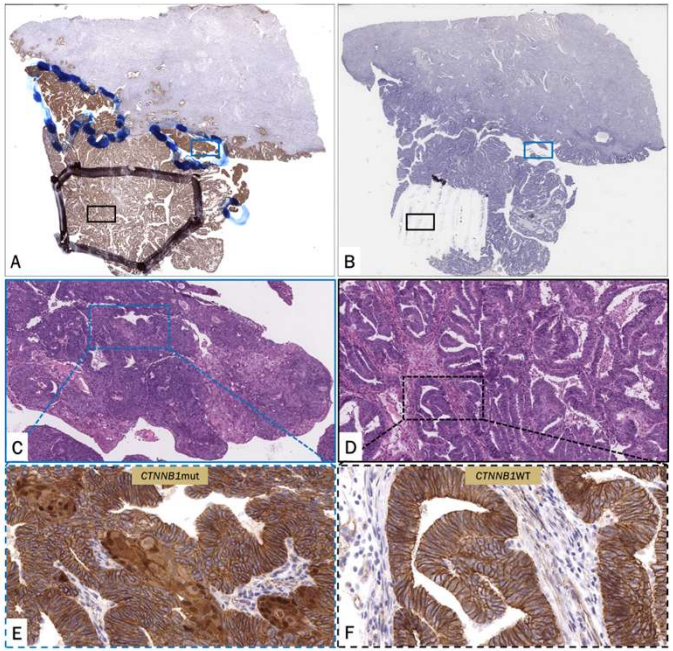

Figure 1. Case #2. A) β-cat IHC with selective MD target areas of nβ-cat+ SM (blue pen) and nβ-cat neg gland (black pen) (whole slide image). B) Post MD hematoxylin-stained (whole slide image). C) Hematoxylin & eosin (HE) squamous morular component (blue box inset from A, 100x). D) HE glandular component (black box inset from A, 100x). E) β-cat IHC in the MD area with SM nuclear positivity (blue box inset from A, 400x). F) β-cat IHC in the MD area with glandular nuclear negativity (black box inset from A, 400x).

Case Number	Squamous Morule (%)	Agnostic Microdissection	Selective Microdissection	
		Random tumor	nβ-Cat (+) Squamous Morules	nβ-Cat (-) Glandular (intended)
1	8-10%	Wild-type	c.101G>A;p.G34E	Wild-type
2	5%	Wild-type	c.100G>A, p.G34R	Wild-type
3	5%	Wild-type	c.110C>A; p.S37Y	Wild-type
4	1%	Wild-type	c.110C>T;p.S37S	Wild-type
5	1-3%	c.98C>T; p.S33F	c.98C>T;p.S33F	Wild-type
6	15%	c.121A>G; p.T41A	c.121A>G; p.T41A	c.121A>G; p.T41A
7	15%	c.94G>T; p.D32Y	c.94G>T, p.D32Y	c.94G>T, p.D32Y
8	40%	c.122C>T; p.T41I	c.122C>T; p.T41I	c.122C>T; p.T41I
9	<1%	Wild-type	Wild-type	Wild-type
10	<1%	Wild-type	Wild-type	Wild-type

Table 1. *CTNNB1* mutation status based on agnostic and selective microdissection

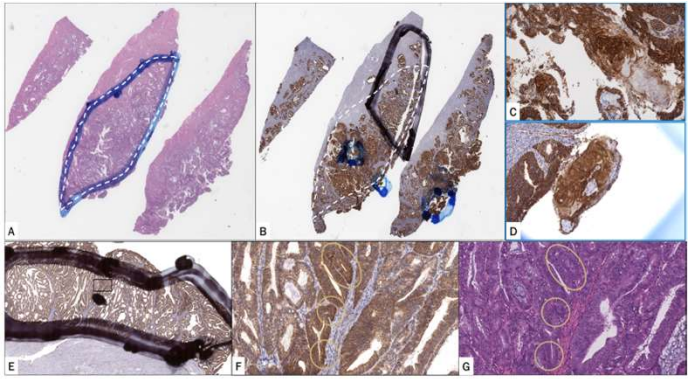

Figure 2. Top row: case #5. A) HE, agnostic MD area of random tumor (blue pen/white dashed line) (whole slide image). B) β-cat IHC with selective MD target areas of nβ-cat+ SM (blue pen), nβ-cat neg gland (black pen), and prior agnostic MD (white dashed line) (whole slide image). C/D) nβ-cat+ SM that were included in agnostic MD (200x). Bottom row: case #6. E) Selective MD area intended to be nβ-cat neg gland (black pen) (20x). F) Intended nβ-cat neg gland selective MD area (black box inset from E), later discovered to include focal non-SM nuclear β-catenin positive glandular cells (yellow circles) (200x). G) HE in area of black box inset from E with β-cat positive glandular cells highlighted by yellow circles (200x).

Conclusion

When present, the specific *CTNNB1*mut in any EEC was identical across all 3 MD areas, supporting that nβ-cat IHC (in glands or in SM) is indicative of *CTNNB1* mutation. In 4 cases with agnostic MD yielding *CTNNB1*WT, the nβ-cat+ SM areas were enriched for *CTNNB1*mut, whereas previously the mutation was below the limit of detection. Studies correlating outcomes in EEC with *CTNNB1* have not systematically addressed inclusion/exclusion of nβ-cat IHC+ foci/SM. It has been unclear whether to include SM in β-cat IHC scoring as a surrogate for *CTNNB1*mut, with suggestion that nβ-cat IHC positivity in SM is normal and thus potentially less significant. Our study shows that the detection of a *CTNNB1*mut depends on tumor area tested, and nβ-cat IHC (including in SM) is a reliable map of *CTNNB1* tumor heterogeneity. As *CTNNB1* mutational status is incorporated into individual EEC prognostication, attention to the area of tumor sequenced is warranted. The results raise the possibility that nβ-cat IHC may be a more informative assay than molecular testing, absent careful microdissection, but further studies are required.