Morphological Compartmentalization of CTNNB1 Mutation to Glands and/or Squamous Morules in Endometrial 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.

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 mut. 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.