Loss of CD73 promotes mutant β-catenin oncogenic activity in endometrial cancer

Rebecca M. Hirsch[1,2,5], Sunthoshini Premsankar[1], Hannah N. Lee[1], Lilly Chiou[1,5], Katherine C. Kurnit[3], Pierre D. McCrea[4], Russell R. Broaddus[1,5], Cyrus Vaziri[1,5], and Jessica L. Bowser[1,5]

[1]Department of Pathology and Laboratory Medicine, The University of North Carolina School of Medicine; [2]Curriculum in Cell Biology and Physiology , UNC School of Medicine; [3]Department of Obstetrics and Gynecology, Section of Gynecologic Oncology, University of Chicago; [4]Department of Genetics, University of Texas MD Anderson Cancer Center, USA; [5]Lineberger Comprehensive Cancer Center, UNC Chapel Hill

Background and Hypothesis

- Patients with low grade, early-stage endometrial cancer (EC) are generally cured by surgery. However, 15-20% recur and have poor clinical outcomes.
- Exon 3 *CTNNB1* (β-catenin) mutations can disrupt β-catenin degradation. They are enriched in low grade EC and associate with poor clinical outcomes. However, only half of patients with *CTNNB1* mutations recur.
- **Question: does CD73 loss in EC explain recurrence in β-catenin mutant tumors?**
- CD73 is a cell surface enzyme that generates extracellular adenosine (Figure 1A).
- We have shown that CD73 loss associates with poor survival in EC and that extracellular adenosine rescues epithelial integrity and moves wild-type β-catenin to the membrane (Figure 1B).
- **Hypothesis: CD73 sequesters mutant β-catenin to the membrane to prevent its oncogenic transcriptional activity and disease recurrence.**

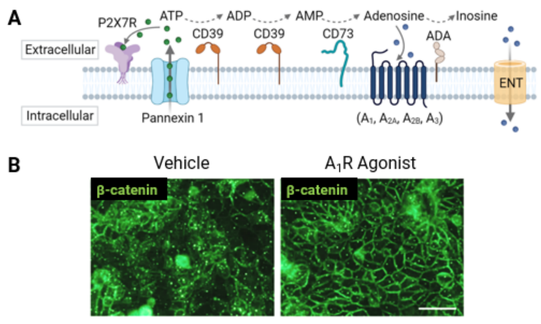
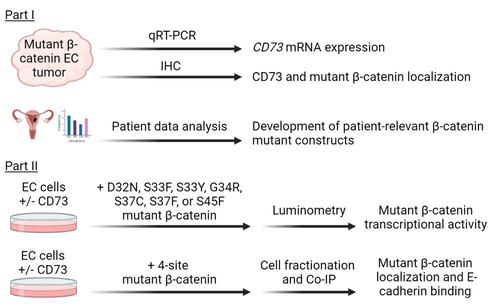


Figure 1: (A) Schematic of CD73 and the purinergic pathway. (B) IF staining of β-catenin in EC cells treated with either vehicle or an adenosine receptor 1 (A1R) agonist.

Methods



Results, Part I

Figure 2: CD73 loss associates with EC recurrence and nuclear localization of mutant β-catenin

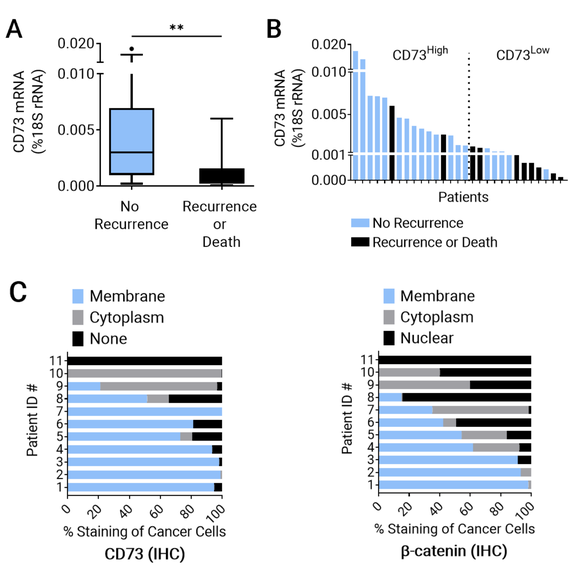


Figure 2: (A), (B) CD73 mRNA expression in mutant β-catenin endometrial tumors. **P < 0.05; Mann-Whitney test. (B) Tumor IHC staining for CD73 and β-catenin in mutant β-catenin endometrial tumors. Correlation between nuclear β-catenin and membrane/absent CD73 = 0.79; P = 0.005, Spearman correlation. (B-C) Individual bars represent different patient tumors.

Figure 3: Development of myc-tagged constructs of patient-relevant β-catenin mutants for mechanistic studies

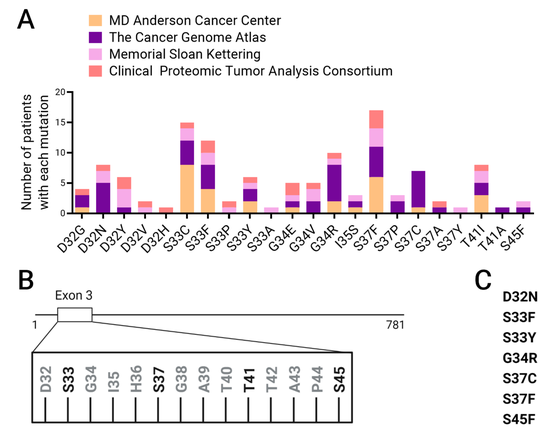


Figure 3: (A) Missense mutations collected from 4 cohorts of EC patients. (B) Exon 3 region of β-catenin. Phosphorylation sites are in black. (C) Individual missense mutation constructs obtained for mechanistic studies.

Results, Part II

Figure 4: Loss of CD73 increases TCF/LEF activity for different human site-specific β-catenin mutants

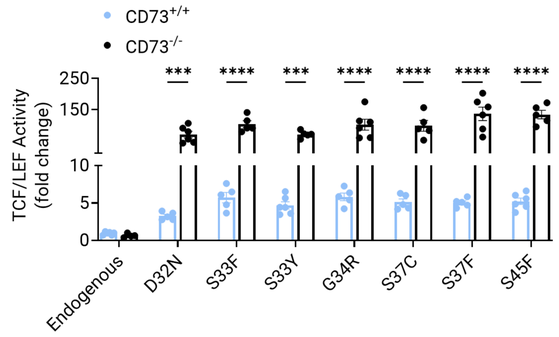


Figure 4: TCF/LEF activity measured by luciferase activity. Values are fold change vs endogenous. Data are mean ± SEM. **** P < 0.0001 vs. Endogenous; *** P < 0.0005, ****P < 0.0001; 2-way ANOVA and Sidak's post test.

Figure 5: Re-expression of CD73 in CD73-deficient cells decreases TCF/LEF activity for human site-specific β-catenin mutants

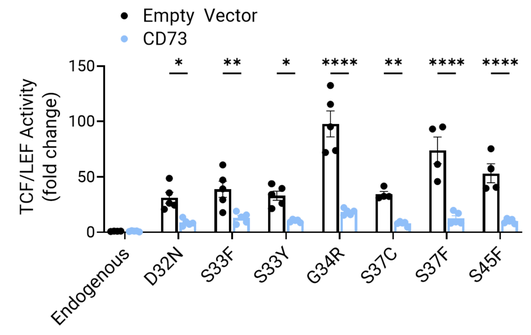


Figure 5: TCF/LEF activity measured by luciferase activity. Values are fold change vs endogenous. Data are mean ± SEM. *P < 0.05, ** P < 0.01, *** P < 0.0005; 2-way ANOVA with Sidak's post test.

Figure 6: Proof-of-concept data that CD73 sequesters mutant β-catenin to the membrane

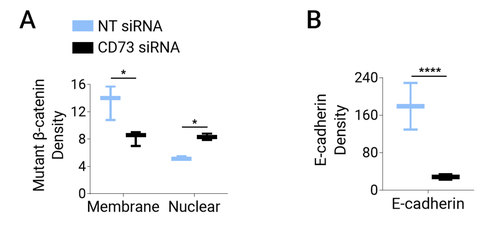


Figure 6: (A) Cellular fractionation with 4-site mutant β-catenin. (B) Co-IP of 4-site mutant β-catenin with E-cadherin. *P < 0.05, ****P , 0.0001, 2-sided T test.

Conclusions and Future Directions

Conclusions

- CD73 predicts recurrence and β-catenin localization in patients with mutant β-catenin tumors (Part I).
- We have characterized human patient-specific β-catenin mutants (D32N, S33F, S33Y, G34R, S37C, S37F, and S45F). These mutants induce TCF/LEF transcriptional activity (Part II).
- CD73 loss increases TCF/LEF transcriptional activity for all individual β-catenin mutants (Part II).
- CD73 loss increases nuclear mutant β-catenin and decreases membranous β-catenin/E-cadherin interactions (Part II).
- CD73 likely protects against EC recurrence by sequestering mutant β-catenin to the membrane with E-cadherin (Figure 7).

Future Directions

- Perform RNA-seq to identify transcriptional targets of specific mutants in the presence and absence of CD73
- Perform cellular fractionations to determine if CD73 can sequester various forms of mutant β-catenin to the membrane

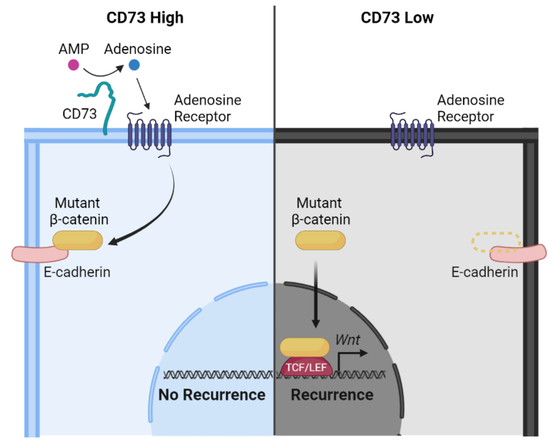


Figure 7: Schematic showing how CD73-derived adenosine restrains mutant β-catenin in EC (left, blue) and how loss of CD73 in mutant β-catenin tumors can drive recurrence (right, black).

Acknowledgements

Funding: Program in Translational Medicine T32 GM122741, UNC Lineberger Comprehensive Cancer Center, NIH/NCI SPORE in Uterine Cancer.

Figures made with BioRender.