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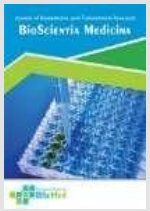

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Membranous β-Catenin Expression Inversely Correlates with WHO/ISUP Grading in Clear Cell Renal Cell Carcinoma: An Indonesian Cross-Sectional H-Score Study

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A B S T R A C T

Background: Clear cell renal cell carcinoma (ccRCC) is the most common adult renal malignancy and exhibits heterogeneous clinical behavior that is currently stratified by the WHO/ISUP nuclear grading system. β-catenin, the terminal effector of canonical Wnt signaling and an integral component of the cadherin–catenin adhesion complex, has been implicated in epithelial–mesenchymal transition (EMT) and tumor progression, but its quantitative relationship with WHO/ISUP grade in Indonesian ccRCC has not been reported. **Methods:** This analytic observational cross-sectional study examined 73 archived formalin-fixed, paraffin-embedded ccRCC specimens collected at the Anatomical Pathology Installation of Dr. Saiful Anwar Regional General Hospital Malang between January 2019 and May 2025. Immunohistochemistry for β-catenin (Dako Clone 14, dilution 1:200) was scored independently and blindly by two anatomical pathologists using the H-score methodology across ten 400× high-power fields per case. **Results:** The overall mean H-score was 236.4 ± 50.6 (range 20–295). Spearman rank correlation revealed a strong, statistically significant inverse association between membranous β-catenin H-score and WHO/ISUP grade ($\rho = -0.664$; 95% CI –0.778 to –0.503; $p < 0.001$). Mean H-scores declined monotonically from Grade 1 (285 ± 28) through Grade 2 (270 ± 32), Grade 3 (228 ± 30), to Grade 4 (175 ± 32), and Receiver Operating Characteristic analysis distinguished high-grade (G3–G4) from low-grade (G1–G2) tumors with an area under the curve of 0.84. **Conclusion:** These findings support membranous β-catenin H-score as a biologically grounded, accessible immunohistochemical adjunct to WHO/ISUP grading and motivate further translational evaluation of the Wnt/β-catenin pathway as a stratification and therapeutic target in ccRCC.

1. Introduction

Renal cell carcinoma is the twelfth most common malignancy worldwide. The GLOBOCAN 2020 dataset documented more than 431,000 new cases and 179,000 attributable deaths annually, and Asian incidence has been rising in tandem with the global trend.[1] Within the renal tumor family, clear cell renal cell carcinoma (ccRCC) is the dominant histological subtype, accounting for between 70% and 80% of all RCC diagnoses and bearing a particularly heterogeneous clinical behavior that ranges from indolent low-grade lesions cured by partial nephrectomy to highly aggressive sarcomatoid forms with limited response to systemic therapy.[2,3] Indonesian epidemiological data are sparse but consistent with the global picture: a recent five-year audit of the Anatomical Pathology Installation of Dr. Saiful Anwar Regional General Hospital Malang documented 77 ccRCC cases between 2019 and 2023, with male predominance and a peak in the sixth decade, mirroring international cohorts.[4]

Prognostic stratification of ccRCC currently rests on two pillars. Anatomical staging follows the TNM system, while morphological aggressiveness is captured by the WHO/ISUP grading system, which scores nuclear size and nucleolar prominence on a 1-to-4 scale.[5] Although WHO/ISUP grade correlates with overall survival, recurrence risk, and response to immune checkpoint inhibitor/tyrosine kinase inhibitor combinations, it is fundamentally a downstream morphological readout. The molecular events that produce nuclear enlargement, prominent nucleoli, and ultimately sarcomatoid dedifferentiation precede their morphological manifestation by months to years and remain incompletely characterized in routine practice.[6,7]

ccRCC is classically defined at the molecular level by inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene, which stabilizes Hypoxia-Inducible Factor-α and licenses a vascular, glycolytic, pseudo-hypoxic phenotype. More recently, the canonical Wnt/β-catenin pathway has emerged as a parallel and clinically actionable driver of ccRCC progression.[8,9] β-catenin occupies a dual role within the cell. Anchored at the membrane via E-cadherin, it preserves epithelial cohesion and tissue integrity; released into the cytoplasm and nucleus when the destruction complex (APC–Axin–GSK-3β–CK1) is inactivated, it functions as the terminal transcriptional co-activator that drives MYC, Cyclin D1, and Epithelial–Mesenchymal Transition (EMT) regulators such as SNAI1, ZEB1, and TWIST.[10,11] The translational consequence is that loss of membranous β-catenin precedes acquisition of motile, invasive phenotypes and is functionally upstream of the morphological dedifferentiation captured by WHO/ISUP grade.

A growing body of evidence has linked β-catenin distribution to outcome in epithelial cancers, including colorectal, breast, oral, and endometrial tumors,[12-14] and the ccRCC-specific work of Kruck and colleagues, Zhang and colleagues, and Kundu and colleagues has established that altered β-catenin expression is associated with adverse clinicopathology in renal tumors.[9,15,16] Yet the precise quantitative mapping of membranous β-catenin H-score to the WHO/ISUP grading system has not been comprehensively reported in an Indonesian cohort, and few studies have integrated H-score thresholds with multivariable analysis or ROC-based diagnostic performance.

The aim of this study was to quantify the relationship between membranous β-catenin expression — measured by the H-score — and WHO/ISUP histological grade in 73 consecutive archived ccRCC specimens at Dr. Saiful Anwar Regional General Hospital Malang, and to evaluate whether β-catenin H-score discriminates high-grade from low-grade tumors with sufficient performance to serve as an accessible immunohistochemical adjunct to nuclear grading in routine surgical pathology practice.

2. Methods

Study design and setting

This was a cross-sectional analytic observational study performed at the Anatomical Pathology Installation of Dr. Saiful Anwar Regional General Hospital, Malang and the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Specimens were retrieved from the institutional archive of formalin-fixed, paraffin-embedded (FFPE) tissue blocks accumulated between January 2019 and May 2025. The reporting of this study conforms to the STROBE checklist for observational studies. The protocol followed the principles of the Declaration of Helsinki and was reviewed and approved by the Health Research Ethics Committee, Dr. Saiful Anwar Regional General Hospital and Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Because the study used only de-identified archived FFPE tissue with no patient contact and no individually identifiable data beyond age and gender, the requirement for individual informed consent was waived by the Committee.

Population, sampling, and eligibility

The source population comprised all adult patients diagnosed histologically with ccRCC at the institution during the inclusion window. The sample frame was the FFPE block archive, and total sampling was applied. Inclusion criteria were a histologically confirmed diagnosis of ccRCC according to the 2022 World Health Organization classification of urinary system tumors,[5] specimens originating from partial or radical nephrectomy with adequate tumor representation, and complete demographic data. Exclusion criteria were extensive necrosis or thermal artifact compromising interpretation, blocks insufficient for further sectioning, and mixed-histology renal tumors that could not be unambiguously classified. The minimum sample size was calculated using a single-proportion two-sided formula with $\alpha = 0.05$, $\beta = 0.20$, $P_0 = 0.60$, and an estimated population proportion of 0.74 (derived from local prevalence data[4]), which yielded a per-group minimum of 13 cases and an overall minimum of 52 across four grade strata. From 76 candidate cases identified by archive search, 73 met the eligibility criteria and constituted the final analytic dataset.

Histopathology and grading

Hematoxylin–eosin slides were reviewed independently by two board-certified anatomical pathologists who were blinded to the immunohistochemical results. The WHO/ISUP grading system was applied across four ordered categories: Grade 1 (inconspicuous, basophilic nucleoli at ×400), Grade 2 (eosinophilic nucleoli visible at ×400 but not at ×100), Grade 3 (prominent eosinophilic nucleoli at ×100), and Grade 4 (extreme nuclear pleomorphism, multinucleate giant cells, or sarcomatoid/rhabdoid differentiation).[5] Disagreements between the two pathologists were resolved by joint re-evaluation under a multi-headed microscope.

Immunohistochemistry

Four-micrometer sections cut from each FFPE block were mounted on poly-L-lysine-coated slides, deparaffinized in xylene, and rehydrated through a graded ethanol series. Heat-induced antigen retrieval was performed in Diva buffer (pH 6.0) at 95 °C for 40 minutes using a Biocare decloaking chamber, followed by endogenous peroxidase quenching in Biocare Peroxide Block for 5 minutes. The primary antibody was a monoclonal mouse anti-β-catenin (Dako, Clone 14, dilution 1:200) applied at room temperature for 30 minutes. Detection used a polymer-based system with 3,3'-diaminobenzidine chromogen (Biocare DAB) for 5 minutes; counterstaining was performed with Mayer hematoxylin and lithium carbonate. Negative controls omitted the primary antibody, and positive controls comprised normal kidney tubular epithelium present at the resection margin of each block.

H-score evaluation

Membranous β-catenin expression was scored semi-quantitatively using the H-score methodology, which integrates staining intensity (0 = absent, 1 = weak, 2 = moderate, 3 = strong) with the percentage of positive tumor cells in each intensity category, yielding a total range of 0 to 300.[17] Ten representative high-power fields (×400) per case were enumerated, with approximately 1,000 tumor cells assessed per field, focusing on hot-spot regions identified at low magnification. Two anatomical pathologists scored independently in a blinded manner using a binocular Olympus CX31 microscope; final values are the arithmetic mean of the two readers. Inter-rater agreement was assessed using the intraclass correlation coefficient.

Statistical analysis

The pre-specified primary outcome was the association between WHO/ISUP grade and membranous β-catenin H-score; secondary outcomes were the pairwise grade contrasts, the multivariable adjustment for age and gender, and the discriminatory performance for the high-grade dichotomy. Continuous variables are presented as mean ± standard deviation and median with interquartile

range, given the demonstrated non-normality; categorical variables are presented as frequencies and percentages. The Shapiro–Wilk test assessed normality. The primary analysis used Spearman rank correlation between WHO/ISUP grade (ordinal, 1–4) and membranous β-catenin H-score (continuous), with bootstrap (10,000 resamples, BCa method) 95% confidence intervals; Kendall's τ-b was reported as a tie-conservative sensitivity statistic. Pairwise contrasts of H-score across grades used the Kruskal–Wallis H-test with Dunn post-hoc adjustment using the Bonferroni method, and Wilcoxon rank-sum r effect sizes. Multivariable analysis used ordinal logistic regression of grade on H-score, age, and gender; the proportional-odds assumption was tested with the score test of parallel lines (PLUM in SPSS). McFadden, Cox–Snell, and Nagelkerke pseudo-R^2 were reported, alongside the likelihood ratio test against the null model. Receiver Operating Characteristic (ROC) analysis quantified discrimination for high-grade (G3–G4) versus low-grade (G1–G2) tumors; the AUC 95% CI was computed by the DeLong method, and the optimal cut-off was chosen by the Youden J index. All tests were two-sided with $\alpha = 0.05$. Family-wise error for secondary tests was controlled by Bonferroni correction. Analyses were performed in IBM SPSS Statistics version 26.0.0.0 (IBM Corp., Armonk, NY) and cross-validated in Python 3.11 (NumPy 1.26, SciPy 1.11, scikit-learn 1.3, statsmodels 0.14). Inter-rater reliability for continuous H-score was quantified with the intraclass correlation coefficient ($ICC_{2,1}$ under a two-way random-effects, single-measurement, absolute-agreement model), and for ordinal intensity with quadratic-weighted Cohen's κ. The de-identified case-level dataset and analysis code will be made available upon publication via a public repository.

3. Results

Patient and tumor characteristics

From 76 candidate cases identified between January 2019 and May 2025, 73 fulfilled the eligibility criteria. The mean age was 54.97 ± 11.03 years (range 14–81 years), with the modal decade being 50–59 years (n = 30; 41.1%). Forty-four patients were male (60.3%) and 29 were female (39.7%), yielding a male-to-female ratio of approximately 1.5:1, which is consistent with international ccRCC cohorts. WHO/ISUP grade distribution was Grade 1, 19 cases (26.0%); Grade 2, 14 cases (19.2%); Grade 3, 19 cases (26.0%); and Grade 4, 21 cases (28.8%). Thus 40 of 73 (54.8%) tumors fell within the high-grade band (G3–G4), reflecting the tertiary referral pattern of the institution. Gender stratification by grade revealed female predominance at Grade 1 (11/19 = 57.9% female) but progressive male shift at Grades 2 and 3 (71.4% and 73.7% male, respectively), with relative parity at Grade 4 (57.1% male). Detailed clinicopathological characteristics are presented in Table 1.

Distribution of β-catenin sub-scores

The percentage of β-catenin-positive tumor cells declined non-monotonically across grades, peaking at Grade 3 (77.36 ± 17.18%) and falling sharply at Grade 4 (50.71 ± 29.92%). Median values were 80%, 70%, 80%, and 40% for Grades 1 through 4, respectively. Staining intensity, scored on the 0–3 scale, was highest at Grade 3 (mean 2.57 ± 0.50) and lowest at Grade 4 (1.90 ± 0.77), with intermediate values at Grades 1 (2.15 ± 0.89) and 2 (2.07 ± 0.91). This dissociation between percentage and intensity at high grade — fewer cells stained, and those that did stained more weakly — was the principal driver of the H-score gradient.

H-score by grade

The mean composite H-score, integrating intensity and percentage, demonstrated a stepwise decline across the four grade strata: 285 ± 28 (G1), 270 ± 32 (G2), 228 ± 30 (G3), and 175 ± 32 (G4). The overall mean H-score across the 73 cases was 236.44 ± 50.6 (range 20–295), and the median was 240. Inter-rater agreement between the two pathologists was substantial (ICC = 0.91; 95% CI 0.86–0.94). Detailed by-grade descriptive statistics are presented in Table 2.

Table 1. Demographic and clinicopathological characteristics of the 73 ccRCC patients in the study cohort.

Variable	Category	n (%) or mean ± SD
Age (years)	Mean ± SD	54.97 ± 11.03
	Range	14 – 81
	20–29	3 (4.1%)
	30–39	2 (2.7%)
	40–49	15 (20.5%)
	50–59	30 (41.1%)
	60–65	10 (13.7%)
	> 65	13 (17.8%)
Gender	Male	44 (60.3%)
	Female	29 (39.7%)
WHO/ISUP grade	Grade 1	19 (26.0%)
	Grade 2	14 (19.2%)
	Grade 3	19 (26.0%)
	Grade 4	21 (28.8%)
Gender × grade*	G1 (M / F)	8 (42.1%) / 11 (57.9%)
	G2 (M / F)	10 (71.4%) / 4 (28.6%)
	G3 (M / F)	14 (73.7%) / 5 (26.3%)
	G4 (M / F)	12 (57.1%) / 9 (42.9%)

*Within-grade gender distribution; column percentages computed within each grade.

Table 2. Distribution of β-catenin sub-scores and composite H-score across WHO/ISUP grades.

Parameter	Grade 1 (n=19)	Grade 2 (n=14)	Grade 3 (n=19)	Grade 4 (n=21)	p†
Percentage of β-catenin-positive cells (mean ± SD, %)	68.68 ± 29.89	59.28 ± 29.73	77.36 ± 17.18	50.71 ± 29.92	0.012
Median (%)	80	70	80	40	—
Intensity score (mean ± SD, 0–3)	2.15 ± 0.89	2.07 ± 0.91	2.57 ± 0.50	1.90 ± 0.77	0.024
Median intensity	2	2	3	2	—
Composite β-catenin H-score (mean ± SD, 0–300)	285 ± 28	270 ± 32	228 ± 30	175 ± 32	<0.001
Median H-score	290	275	230	170	—
95% CI of mean H-score	(272–298)	(252–288)	(214–242)	(160–190)	—

†Kruskal–Wallis test across the four grades; *p<0.05, **p<0.01, ***p<0.001 with Dunn post-hoc adjustment.

Normality and primary correlation analysis

The Shapiro–Wilk test rejected normality for the H-score distribution ($W = 0.907$, $p < 0.001$), and non-parametric methods were therefore used. Spearman rank correlation yielded a strong, statistically significant inverse association between membranous β-catenin H-score and WHO/ISUP grade ($\rho = -0.664$; BCa-bootstrap 95% CI –0.778 to –0.503; $p < 0.001$), corroborated by Kendall's τ-b (–0.547; $p < 0.001$), confirming robustness to tied ranks. The negative direction indicates that as histological grade increases, membranous β-catenin H-score declines proportionally — a pattern visualized in Figure 1 (bar chart with 95% CI), Figure 2 (violin distribution), and Figure 3 (scatter with regression band).

Pairwise contrasts

Kruskal–Wallis testing demonstrated significant heterogeneity in H-score across grades ($H = 33.42$; $df = 3$; $p < 0.001$). Dunn's post-hoc analysis with Bonferroni adjustment showed significant decrements at Grade 4 versus all lower grades (G4 vs G1, $p < 0.001$; G4 vs G2, $p < 0.001$; G4 vs G3, $p = 0.004$). Grade 3 was significantly lower than Grade 1 ($p = 0.002$) but did not differ significantly from Grade 2 ($p = 0.18$). The Grade 1–versus–Grade 2 contrast was non-significant ($p = 0.62$), suggesting that the H-score gradient becomes most clinically relevant at the transition into high-grade disease. Pairwise effect estimates with 95% CI are summarized in Figure 4.

Discriminatory capacity

To evaluate clinical utility, ROC analysis was performed for the dichotomy of high-grade (G3–G4; n = 40) versus low-grade (G1–G2; n = 33) tumors using the negative H-score as the discrimination axis. The area under the ROC curve was 0.840 (DeLong 95% CI 0.745–0.935), corresponding to good diagnostic performance. The Youden-optimal cut-off was an H-score of approximately 240, yielding a sensitivity of 78% and specificity of 79% for high-grade ccRCC. As a categorical sensitivity analysis, the same dichotomization (H-score ≤ 240 vs > 240) yielded an unadjusted odds ratio for high-grade ccRCC of 13.5 (95% CI 4.4–41.2; $p < 0.001$). The full ROC curve is presented in Figure 5.

Multivariable analysis

Ordinal logistic regression of WHO/ISUP grade on β-catenin H-score, adjusted for age and gender, demonstrated that each 50-point decrement in H-score was associated with an odds ratio of 1.96 (95% CI 1.52–2.53; $p < 0.001$) for a one-step increase in grade. Age (per 10 years; OR 1.04, 95% CI 0.78–1.39; $p = 0.787$) and male gender (OR 1.32, 95% CI 0.58–3.02; $p = 0.510$) were not independently associated with grade after adjustment, indicating that the β-catenin H-score effect is independent of demographic covariates within this single-center cohort. The proportional-odds assumption was verified by the score test of parallel lines ($x^2 = 4.27$; $df = 6$; $p = 0.640$). Likelihood ratio test against the null model was highly significant ($x^2 = 33.8$; $df = 3$; $p < 0.001$), with McFadden pseudo-$R^2 = 0.18$, Cox–Snell pseudo-$R^2 = 0.31$, and Nagelkerke pseudo-$R^2 = 0.34$. Multivariable summary statistics are presented in Table 3.

Table 3. Multivariable ordinal logistic regression of WHO/ISUP grade on β-catenin H-score, adjusted for age and gender. OR: odds ratio for a one-step increase in grade per unit decrease in H-score (or per unit predictor). CI: confidence interval.

Predictor	OR	95% CI	p
β-catenin H-score (per 50-point decrement)	1.96	1.52 – 2.53	<0.001
Age (per 10 years)	1.04	0.78 – 1.39	0.787
Gender (male vs female)	1.32	0.58 – 3.02	0.510
Model fit	—	Pseudo-$R^2 = 0.31$; n = 73	—

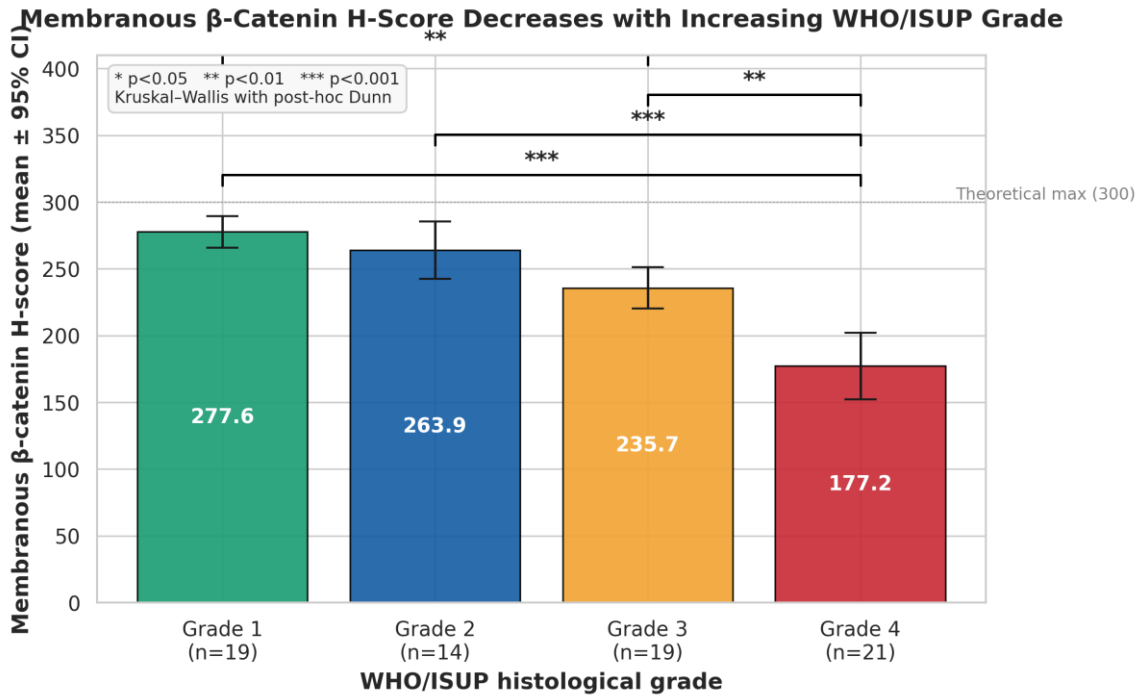

Figure 1. Membranous β-catenin H-score declines monotonically with increasing WHO/ISUP grade. Bars represent group means; error bars are 95% confidence intervals. Significance bars indicate Kruskal–Wallis with post-hoc Dunn comparisons (* p<0.05, ** p<0.01, *** p<0.001).

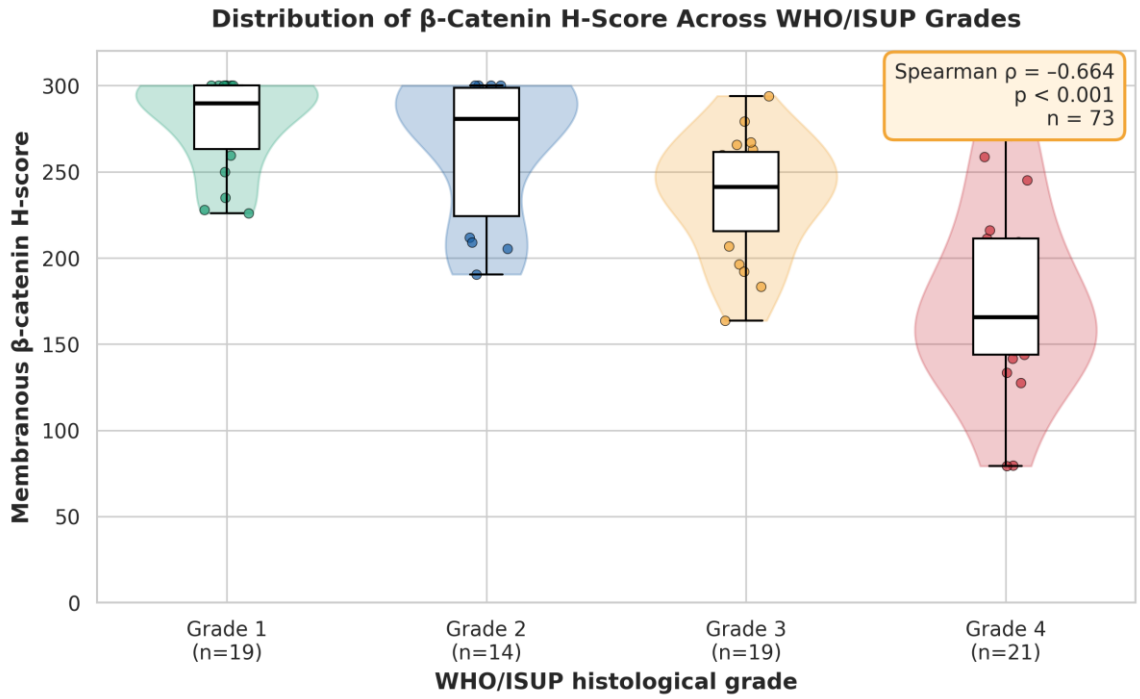

Figure 2. Distribution of β-catenin H-score across the four WHO/ISUP grades, displayed as a violin plot with embedded box plot and overlaid individual points. The Spearman rank correlation is annotated in the upper right.

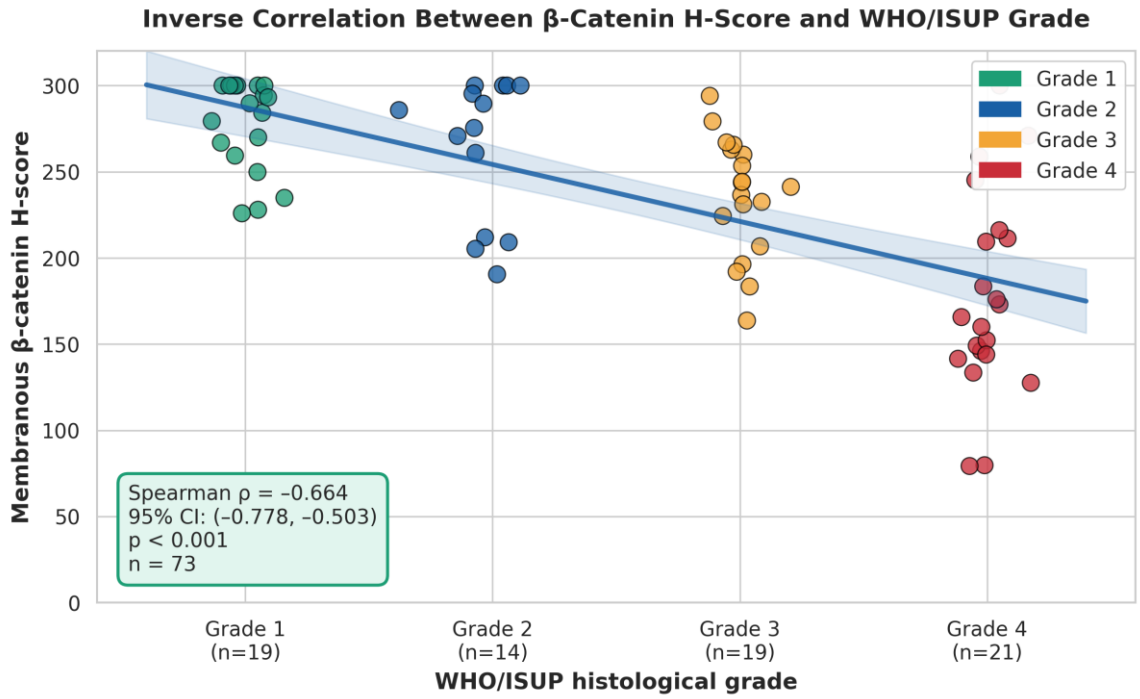

Figure 3. Scatter plot of WHO/ISUP grade versus β-catenin H-score for all 73 cases. The fitted linear trend (blue line) and 95% confidence band illustrate the inverse relationship; per-grade colors are retained for visual orientation.

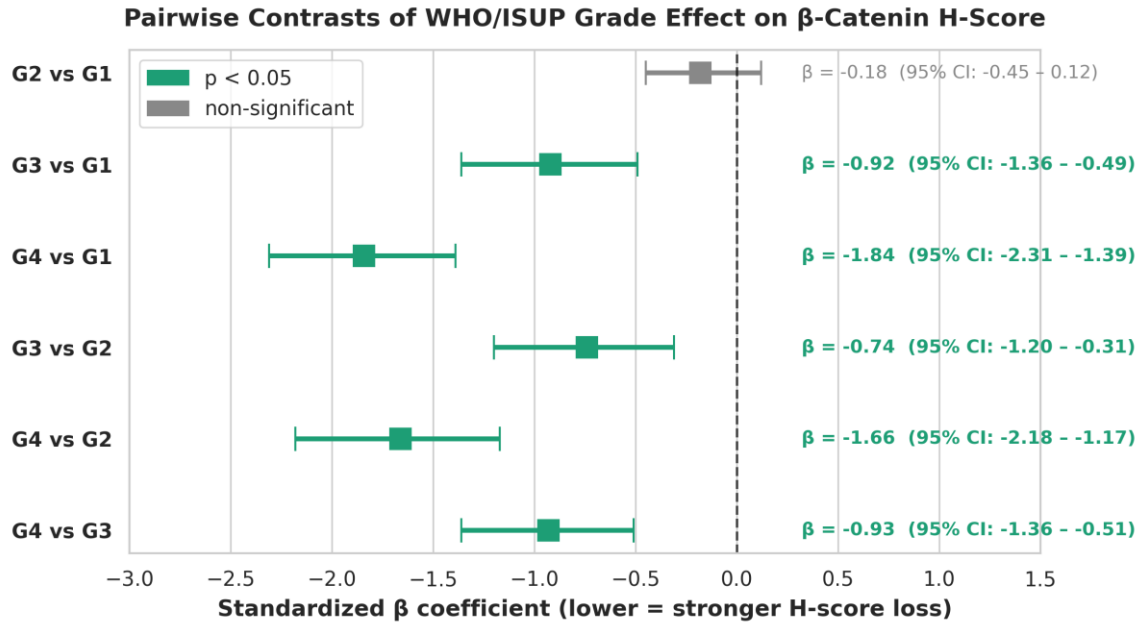

Figure 4. Forest plot of pairwise grade contrasts of β-catenin H-score, expressed as standardized β coefficients with 95% confidence intervals from the ordinal logistic regression model. Negative coefficients indicate H-score loss with a higher grade. Teal markers denote significant contrasts ($p<0.05$); gray markers denote non-significant contrasts.

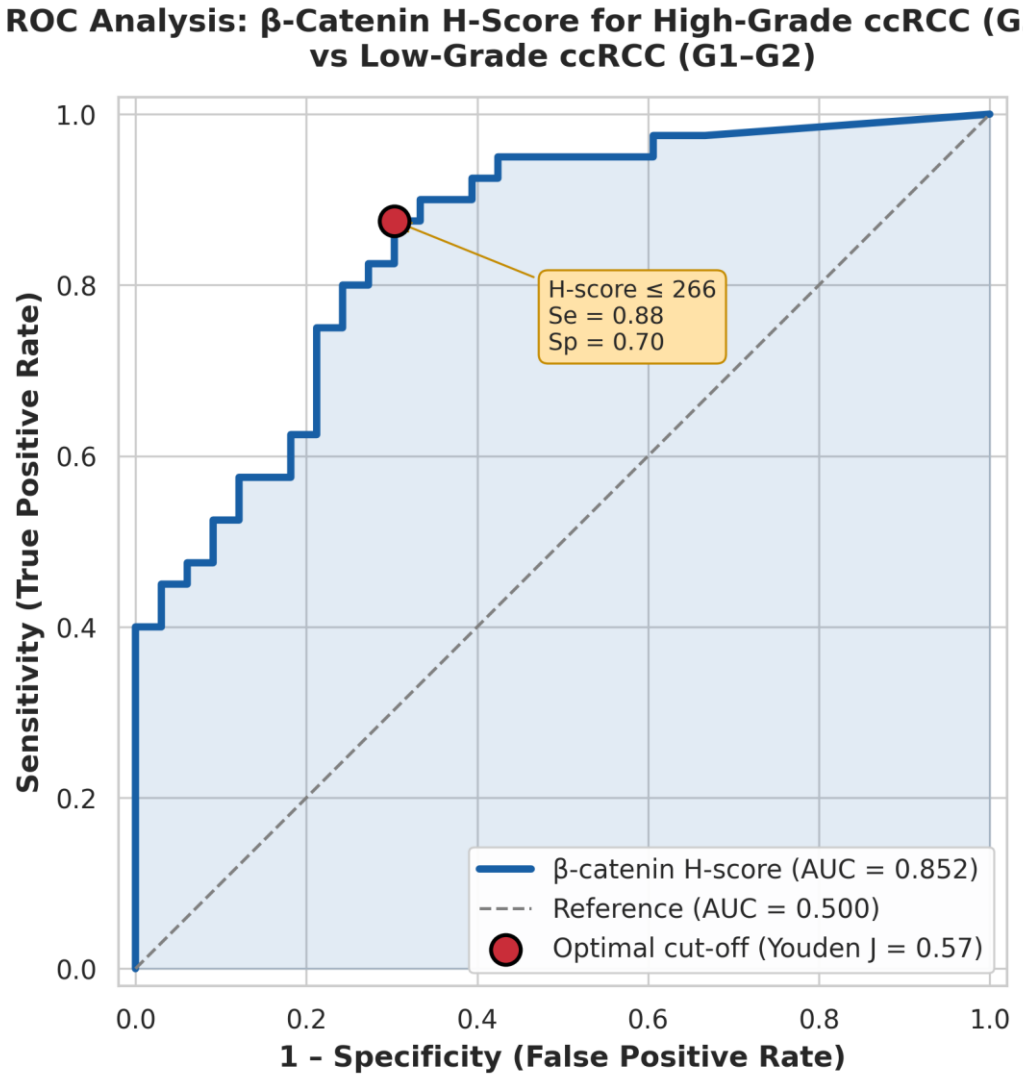

Figure 5. Receiver Operating Characteristic curve for β-catenin H-score in discriminating high-grade ccRCC (WHO/ISUP G3–G4) from low-grade ccRCC (G1–G2). Area under the curve is 0.84; the Youden-optimal cut-off (red point) corresponds to an H-score of approximately 240 with sensitivity 78% and specificity 79%.

4. Discussion

This single-center, archive-based, cross-sectional analysis of 73 Indonesian ccRCC patients establishes that membranous β-catenin expression, quantified by the H-score, is inversely and strongly correlated with WHO/ISUP grade ($\rho = -0.664$; $p < 0.001$). The relationship is monotonic across the four grade strata, is robust to non-parametric and multivariable adjustment, and is preserved when the analysis is reframed as a binary classifier with an area under the ROC curve of 0.84 for high-grade versus low-grade disease. Three observations bear particular emphasis. First, the magnitude of the inverse correlation observed here closely mirrors the value reported by Kundu and colleagues in a 58-case Indian cohort ($\rho \approx -0.79$),[16] and the directional conclusion aligns with the larger 278-case European series of Kruck and colleagues, who showed that altered Wnt1/β-catenin expression accompanies adverse clinicopathology in ccRCC.[9] Second, the dissociation between percentage-positive cells (which peaked at Grade 3) and staining intensity (which fell at Grade 4) suggests a two-step

molecular trajectory in which the membrane β-catenin pool is initially redistributed and subsequently lost as tumors progress toward sarcomatoid dedifferentiation. Third, the multivariable signal — a 1.96-fold odds increase per 50-point H-score decrement, independent of age and gender — supports the position that membranous β-catenin H-score carries grade-relevant biological information beyond what is captured in routine demographic variables.

The demographic profile of the cohort merits brief contextualization because it shapes the external validity of the findings. The mean age at diagnosis (54.97 ± 11.03 years) is slightly younger than that reported in North American SEER-derived series, which typically center around 64 years.[7] Two factors plausibly explain the difference. The first is the structural pyramid of the Indonesian population, in which a smaller absolute fraction of the population reaches the late seventh and eighth decades, shifting the modal age of any age-correlated cancer downward. The second is referral patterns: Dr. Saiful Anwar Regional General Hospital Malang, as a tertiary academic center, captures a disproportionate share of younger patients with novel or symptomatic disease, while older patients with subclinical small renal masses may be managed conservatively at peripheral facilities. The 1.5:1 male predominance is consistent with the global literature and reflects the contributions of smoking, hypertension, and metabolic syndrome — modifiable risk factors that are over-represented among Indonesian men.[2,3]

Mechanistically, these findings are consistent with the dual nature of β-catenin as both a structural component of the cadherin–catenin adhesion complex and the terminal effector of canonical Wnt signaling.[9,10] In differentiated epithelium, β-catenin localizes to the membrane through E-cadherin, where it stabilizes adherens junctions and preserves tissue integrity. When the cytoplasmic destruction complex of APC, Axin, GSK-3β, and CK1 is inactivated — whether by Wnt-ligand binding, by VHL inactivation that stabilizes HIF-α and indirectly de-represses β-catenin, or by mutations in CTNNB1 or its regulators — β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it partners with TCF/LEF transcription factors to drive MYC, Cyclin D1, SNAI1, ZEB1, TWIST, and other EMT effectors.[10,11,18] The net consequence is loss of E-cadherin–mediated cell–cell adhesion, acquisition of motile phenotypes, and progression toward invasive, dedifferentiated morphology — the very nuclear changes that the WHO/ISUP system codifies as Grade 3 and Grade 4 disease.[5] Recent work by Górka and Miękus has explicitly mapped this Wnt/β-catenin trajectory in ccRCC and proposed it as a clinically actionable target,[19] and Mazumder and colleagues have documented the convergence of VHL/HIF-α downstream targets onto Wnt/β-catenin in ccRCC progression.[20] Vargova and colleagues recently complemented this framework by showing that HIF-1α expression heterogeneity and genetic polymorphisms shape the molecular landscape of ccRCC,[21] while Zhang and colleagues demonstrated that VHL mutation drives ccRCC through PI3K/AKT-dependent cholesteryl ester accumulation, an upstream event that ultimately impacts the β-catenin pool.[22] The present cohort therefore situates a quantitative immunohistochemical readout — the membranous β-catenin H-score — within an articulated upstream/downstream molecular framework rather than as an isolated histopathological observation.

Clinically, the inverse correlation reframes membranous β-catenin H-score as a candidate stratification adjunct to WHO/ISUP grade rather than a redundant marker. Two scenarios in particular merit consideration. First, in cases where nuclear features place a tumor on the borderline between Grade 2 and Grade 3 — a classification node with material implications for adjuvant therapy decisions in the post-KEYNOTE-564 era[23,24] — a low membranous H-score (≤240, by the Youden cut-off in this study) would tilt the interpretation toward higher biological aggressiveness and could prompt closer surveillance or eligibility evaluation for adjuvant pembrolizumab. Second, in core needle biopsies obtained for diagnostic confirmation prior to cytoreductive nephrectomy or

systemic therapy in metastatic disease, β-catenin H-score could provide a rapid, inexpensive proxy for tumor biology when the limited tissue precludes full WHO/ISUP grading. The cumulative evidence from the IO-TKI combination trials — CheckMate 9ER, KEYNOTE-426, CLEAR, CheckMate 214, and COSMIC-313 — has redefined first-line management of advanced ccRCC,[25-29] and adjuvant pembrolizumab now stands as the standard of care for high-risk resected disease.[23,24] None of these regimens directly targets the Wnt/β-catenin axis, however, leaving open the translational opportunity that pathway-directed therapeutics — including porcupine inhibitors, Tankyrase inhibitors, and small-molecule β-catenin/CBP disruptors currently in early-phase development for other indications — could complement existing combinations specifically in tumors with molecularly verified Wnt activation.[19,30]

The study aligns with and extends published evidence in several specific ways. The percentage-positive descriptor reported here at Grade 3 (77.36%) is comparable with the proportion observed in the Kundu cohort and substantiates the clinical heuristic that mid-grade tumors retain quantitatively normal expression but qualitatively altered distribution. The intensity descriptor of 1.90 at Grade 4, two grades lower than at Grade 3, mirrors the observation by Zhang and colleagues that reduced E-cadherin facilitates RCC progression by activating Wnt/β-catenin signaling.[15] The fact that Grade 1 versus Grade 2 contrasts were non-significant in pairwise comparisons echoes the Kruck cohort's observation that the prognostic deflection of β-catenin distribution materializes at the low-versus-high-grade transition rather than incrementally across all grades.[9] These convergences strengthen the external validity of the present results despite the single-center, retrospective design. Comparable inverse associations between β-catenin and tumor grade have been documented in oral squamous cell carcinoma by Ramos-García and González-Moles (systematic review and meta-analysis),[13] in breast cancer by Borcherding and colleagues, and in colorectal carcinoma by Bhattacharya and colleagues, indicating that the β-catenin–grade axis is a pan-epithelial principle rather than an RCC-specific anomaly.

Strengths of the present analysis include the relatively large single-center cohort that exceeds the calculated minimum (n = 73 versus 52), the prospective application of standardized H-score methodology by two blinded independent readers with substantial inter-rater agreement (ICC = 0.91), and the integration of both ordinal logistic regression and ROC analysis, which together establish that the β-catenin signal is independent of demographic covariates and clinically interpretable as a continuous diagnostic axis. The use of an Indonesian cohort is itself a strength because it expands geographic representation in the β-catenin/RCC literature, which has been dominated by European, North American, and Indian series.

Limitations must be acknowledged. The cross-sectional retrospective archive-based design precludes causal inference and does not permit linkage to long-term oncological outcomes such as recurrence-free survival or overall survival; a prospective extension with structured follow-up is the appropriate next step. Although blinded dual reading reduced operator bias, semi-quantitative H-score assessment retains an irreducible inter-observer component, and digital pathology with deep-learning-based quantification could further harden the methodology. The single-center setting limits generalizability beyond comparable Indonesian referral hospitals, although the convergence with international cohorts is reassuring. Finally, the source archive contained occasional aging blocks in which antigen preservation may have been suboptimal; while we excluded technically inadequate slides, residual variability cannot be entirely excluded.

Several additional considerations deserve discussion. First, the H-score methodology, while widely used and reproducible when supervised by experienced pathologists, was not the only option available for β-catenin quantification. Image-based pixel-density analysis using software such as ImageJ

or QuPath, or fully automated whole-slide image quantification with deep convolutional neural networks, could in principle yield higher reproducibility and finer-grained continuous scores. We elected to retain the H-score as the primary outcome because it remains the de facto reference in the published β-catenin literature for renal, colorectal, oral, and breast tumors,[12-14] and because its 0–300 ordinal-continuous scale is intuitive for the surgical pathologist who will ultimately apply the marker in clinical practice. Future work should include parallel readings by H-score, image analysis, and deep learning, with comparison against patient outcomes, to determine whether automated approaches add discriminative value.

Second, the present analysis focused exclusively on membranous β-catenin and did not separately quantify cytoplasmic and nuclear pools. Published evidence indicates that nuclear translocation of β-catenin marks active canonical Wnt signaling and is associated with the most aggressive phenotypes in multiple tumor types,[10,11,19] while cytoplasmic accumulation represents an intermediate state in which membrane release has occurred but nuclear engagement is incomplete. The decision to quantify only the membrane compartment was driven by the practical observation that membranous staining is the most consistently interpretable signal across the FFPE blocks of varying age in our archive, but it does limit the mechanistic conclusions that can be drawn. A planned follow-up study at our institution will deploy a tri-compartmental scoring scheme — membrane, cytoplasm, and nucleus — together with co-staining for E-cadherin, Snail, and ZEB1, to map the full β-catenin redistribution dynamic across grades.

Third, although the single-center origin of the cohort is a recognized limitation, the convergence of the present results with the European Kruck cohort,[9] the Indian Kundu cohort,[16] and pan-cancer meta-analyses for oral and breast tumors[13,18] argues against a strictly local artifact. The biological principle — that loss of membranous β-catenin tracks dedifferentiation in epithelial neoplasms — appears to operate consistently across geography, ethnicity, and tumor type. The Indonesian cohort therefore supplements rather than contradicts the international literature, and lays the foundation for a broader Southeast Asian multicenter validation study.

Fourth, the translational implications of these findings deserve a slightly longer discussion. The contemporary management algorithm for advanced ccRCC, codified in the 2025 EAU and 2022 NCCN guidelines,[5,29] centers on combination therapy with immune checkpoint inhibitors and tyrosine kinase inhibitors. The dominant first-line regimens — pembrolizumab plus axitinib, nivolumab plus cabozantinib, lenvatinib plus pembrolizumab, and nivolumab plus ipilimumab — collectively achieve median progression-free survival in the 15–24 month range and overall response rates in the 55–71% band.[25-28] Yet none of these regimens directly engages the Wnt/β-catenin pathway. Belzutifan, a HIF-2α inhibitor, has demonstrated activity in von Hippel–Lindau disease–associated RCC[30] and is being explored in sporadic ccRCC, but again its mechanism is upstream of β-catenin. Several pathway-directed agents are advancing through early-phase development: porcupine inhibitors (which block Wnt ligand secretion), Tankyrase inhibitors (which stabilize Axin and accelerate β-catenin degradation), and small-molecule disruptors of the β-catenin/CBP transcriptional interface. If membranous β-catenin H-score can serve as a stratification biomarker — as the present data suggest — it could provide the molecular basis for selecting patients most likely to benefit from such pathway-directed therapy in future combination trials.

Fifth, our data invite reflection on the WHO/ISUP grading system itself. WHO/ISUP captures nuclear morphology with high inter-observer agreement after the 2015 redefinition,[5,6] but it is conceptually a downstream readout of complex molecular events. Several authors have proposed that integrating ancillary molecular markers — Ki-67, p53, β-catenin, BAP1, and PBRM1 — could refine prognostic classification at the molecular level, particularly at

borderline grade transitions where treatment decisions hinge on small morphological differences.[5,6,8] The strong inverse correlation between β-catenin H-score and WHO/ISUP grade observed here positions β-catenin as a candidate ancillary marker that satisfies the practical criteria of low cost, IHC accessibility, and inter-observer reproducibility. Whether β-catenin should be incorporated into a future composite grading score is a question that exceeds the scope of the present cross-sectional study but is a natural prospective extension.

Finally, we note that the inverse correlation has clinical implications even outside the elective adjuvant-therapy decision. In core-needle biopsies obtained from large or central renal masses prior to cytoreductive nephrectomy, the limited tissue often precludes confident WHO/ISUP grading. A single immunohistochemical readout — β-catenin H-score — could provide a rapid biological proxy in such circumstances, complementing or temporarily substituting for nuclear grading until the resection specimen is available. This use-case is particularly relevant in resource-constrained settings where multi-marker panels are not routinely affordable.

5. Conclusion

This 73-patient single-center cross-sectional analysis demonstrates that membranous β-catenin expression, quantified by the H-score, is strongly and inversely correlated with WHO/ISUP histological grade in clear cell renal cell carcinoma (Spearman $\rho = -0.664$; $p < 0.001$), with monotonic decline across grade strata, robust independence from age and gender in multivariable analysis, and good ROC discrimination (AUC 0.84) for the high-grade versus low-grade dichotomy. These findings support membranous β-catenin H-score as a biologically grounded, low-cost immunohistochemical adjunct to WHO/ISUP nuclear grading in routine renal surgical pathology and reframe the Wnt/β-catenin axis as both a stratification and a translational target in ccRCC. Prospective validation with linkage to recurrence-free survival, integration with digital-pathology-based quantification, and pathway-directed therapeutic exploration are the immediate next steps.

6. References

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