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Translational Insights for Pediatric Cancer Biology

RESEARCH ARTICLE

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p53 and *β-Catenin* Expression in Gallbladder Carcinoma: Translational Insights for Pediatric Cancer Biology

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Abstract

Background: The tumor suppressor *p53* and the *Wnt* pathway effector *β-catenin* represent two of the most frequently dysregulated molecular components across human malignancies. Although gallbladder cancer (GBC) predominantly affects adults, the pattern and clinicopathological correlates of *p53* and *β-catenin* dysregulation in GBC may illuminate how these pathways behave in different biological contexts, including developmental and pediatric oncological settings, where analogous pathway alterations drive distinct tumor types. **Objective:** To characterize *p53* and *β-catenin* expression patterns in gallbladder carcinoma by immunohistochemistry, correlate findings with clinicopathological parameters, and discuss in a qualified, hypothesis-generating framework the parallels and divergences between these pathway alterations and those reported in pediatric malignancies. **Methods:** A cross-sectional study analyzed 61 neoplastic and 49 non-neoplastic gallbladder lesions (2017–2021) using standardized immunohistochemistry for *p53* (clone DO-7; Dako/Agilent, 1:100) and *β-catenin* (clone E247; Abcam, 1:200). Expression patterns were correlated with clinicopathological parameters using chi-square and Fisher's exact tests. Findings were discussed in the context of published pediatric cancer literature, with explicit acknowledgment of inter-study methodological heterogeneity. **Results:** *p53* overexpression (defined as >20% nuclear positivity) occurred in 78.2% of GBC cases versus 11.1% of benign lesions, correlating significantly with tumor grade and stage ($p<0.05$). Aberrant *β-catenin* expression (cytoplasmic/nuclear) was observed in 81.8% of malignant cases, with nuclear localization in 36.3%, correlating with poor differentiation. These patterns qualitatively parallel though are not directly quantitatively comparable to those reported in pediatric hepatoblastoma, Wilms' tumor, and pediatric sarcomas. **Conclusion:** *p53* and *β-catenin* dysregulation in GBC exhibits qualitative parallels to patterns reported in pediatric cancers, suggesting shared oncogenic pathway biology. These observations are hypothesis-generating and warrant validation through functional studies in appropriate preclinical models before therapeutic implications can be drawn.

Keywords: *p53*, *β-catenin*, *Wnt* signaling, immunohistochemistry, biomarkers

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Introduction

The molecular landscape of cancer biology has revealed that certain oncogenic pathways particularly those governing cell cycle regulation and developmental signaling are recurrently dysregulated across tumor types, tissue origins, and patient age groups. Among these, the *TP53* tumor suppressor pathway and the canonical *Wnt/β-catenin* signaling axis represent two of the most evolutionarily conserved and clinically consequential molecular programs in human malignancy [1, 2].

Rather than proposing gallbladder carcinoma (GBC) as a direct biological analog of pediatric cancers, this study investigates whether the pattern, frequency, and clinicopathological correlates of *p53* and *β-catenin* dysregulation in a well-characterized adult cohort align with, and can thereby inform, parallel observations in pediatric oncology literature with particular attention to the context-dependent manifestations of these pathway alterations across developmental stages. GBC is explicitly rare in children; the translational value of this study lies not in shared cellular origin but in shared pathway biology.

The embryological connection between the gallbladder and liver ut endoderm provides a modest but biologically meaningful developmental link to hepatoblastoma, the most common pediatric liver malignancy and a tumor characterized by among the highest rates of CTNNB1 (*β-catenin*) mutation of any human cancer (60–90%) [3]. This shared foregut endodermal origin, combined with the well-established role of *Wnt/β-catenin* signaling in biliary and hepatic organogenesis, provides one rationale for examining *β-catenin* pathway behavior across these

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histological contexts [4].

The *Wnt/β-catenin* pathway plays particularly crucial roles in embryonic development and tissue homeostasis, making its dysregulation especially relevant to pediatric cancers arising from developmental abnormalities [4]. The interaction between *p53* and *Wnt* signaling adds further complexity: *p53* has been shown to function as a negative regulator of *β-catenin*-mediated transcription, such that simultaneous disruption of both pathways may be synergistically oncogenic [5].

This translational perspective is particularly relevant in the current era of precision oncology, where molecular characteristics increasingly guide treatment selection independent of tissue of origin [6]. The identification of shared pathway vulnerabilities between adult and pediatric cancers may accelerate hypothesis generation for cross-age therapeutic strategies. Crucially, such hypotheses require validation through functional and preclinical studies before clinical application, and this study is explicitly positioned as an observational, hypothesis-generating contribution.

Pediatric Cancer Landscape and Molecular Parallels

Pediatric Cancer Epidemiology and Unique Characteristics

Pediatric cancers account for less than 1% of all cancer diagnoses globally but represent the leading cause of disease-related mortality in children over one year of age [7]. Unlike adult tumors, which typically accumulate mutations over decades of somatic exposure, pediatric cancers frequently arise from errors in developmental programs and are characterized by fewer somatic point mutations but a higher burden of chromosomal rearrangements, gene fusions, and epigenetic alterations [8]. This developmental origin has important implications for pathway biology: genes that regulate embryogenesis may be more tonically active in developing tissues, increasing both the oncogenic impact of their dysregulation and the potential toxicity of their therapeutic inhibition.

The most common pediatric solid tumors including neuroblastoma, Wilms' tumor, hepatoblastoma, rhabdomyosarcoma, and Ewing sarcoma share certain molecular features despite diverse tissue origins. Among these, *p53* pathway dysfunction and *Wnt/β-catenin* activation are among the most recurrent, appearing across multiple pediatric tumor types in patterns that differ qualitatively from adult cancers in ways that may reflect the developmental biology of their tissues of origin [9].

p53 in Pediatric Malignancies

p53 alterations in pediatric cancers often differ from adult tumors in their mechanism, frequency, and clinical implications. In Li-Fraumeni syndrome, germline *TP53* mutations predispose children to a broad spectrum of malignancies including sarcomas, brain tumors, and adrenocortical carcinoma with penetrance exceeding 90% by age 70 [10]. In sporadic pediatric sarcomas, somatic *p53* alterations occur in approximately 10–20% of cases but consistently associate with particularly adverse prognosis [11]. Pediatric high-grade gliomas harbor TP53 mutations in 30–40% of cases, correlating with treatment resistance [12]. Osteosarcoma shows *p53* pathway alterations in approximately 20–30% of cases, predictive of poor chemotherapy response [13].

An important interpretive note applicable to all IHC-based comparisons in this manuscript: *p53* protein overexpression by IHC is a surrogate for *TP53* missense mutation and does not confirm mutational status or functional consequence. Equally, complete absence of staining (null pattern) may indicate truncating or nonsense mutations resulting in an unstable protein. Both patterns represent *p53* pathway dysfunction but manifest differently by IHC and carry different functional implications. Direct quantitative comparisons between our IHC data and mutation-frequency data from pediatric cancer genomics studies should therefore be interpreted cautiously and are not the primary analytical aim of this work.

β-catenin in Pediatric Cancers

β-catenin pathway dysregulation is particularly prominent in embryonal pediatric cancers, a pattern consistent with the pathway's central role in developmental organogenesis. Hepatoblastoma harbors CTNNB1 mutations in 60–90% of cases across published series, making it one of the most *β-catenin*-driven cancers known [14]. Wilms' tumor shows CTNNB1 mutations in approximately 15% of cases, associated with more aggressive histology [15]. Medulloepithelioma and craniopharyngioma show near-universal *β-catenin* pathway activation, reflecting their origin from developmental progenitor cells [16]. Pediatric low-grade gliomas in certain anatomical locations also show *β-catenin* pathway involvement [17].

The high frequency of *β-catenin* pathway activation in embryonal tumors supports the concept that these malignancies represent, at least in part, hijacked developmental programs rather than sequentially accumulated mutations in mature tissues [3]. This observation is directly relevant to interpreting the *β-catenin* expression data from our GBC cohort in a translational framework: while the mechanisms and frequencies differ, the qualitative association between nuclear *β-catenin* accumulation and malignant behavior is a consistent theme across these diverse tumor types.

Materials and Methods

Study Design

A retrospective cross-sectional observational study was conducted examining gallbladder specimens received in the Department of Pathology, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, between January 2017 and December 2021. The study was approved by the Institutional Ethics Committee. The primary aim was immunohistochemical characterization of *p53* and *β-catenin* expression in gallbladder carcinoma, with findings contextualized through a narrative literature review of pediatric cancer studies reporting analogous data.

Study Population

A total of 61 histologically confirmed neoplastic

gallbladder specimens were included. All cases were reviewed by two qualified pathologists (minimum 10 years' experience in gastrointestinal pathology) and classified according to the WHO Classification of Tumours of the Digestive System. Tumor grade was assigned using standard criteria (well-differentiated/G1, moderately differentiated/G2, poorly differentiated/G3), and pathological stage was determined per AJCC 8th Edition TNM criteria.

The non-neoplastic control cohort comprised 49 cholecystectomy specimens obtained for benign indications (predominantly cholelithiasis with chronic cholecystitis). Control specimens underwent identical histological and immunohistochemical processing.

Immunohistochemical Protocol

Immunohistochemistry was performed on 4-µm sections of formalin-fixed, paraffin-embedded (FFPE) tissue mounted on positively charged slides. All sections were deparaffinized in xylene and rehydrated through graded alcohols to distilled water using standard protocols.

Antigen retrieval was performed by heat-induced epitope retrieval (HIER) in sodium citrate buffer (10 mM, pH 6.0) using a calibrated pressure cooker for 20 minutes at 120°C, followed by cooling at room temperature for 30 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 minutes. Non-specific background was blocked with 5% normal goat serum in PBS for 20 minutes at room temperature. Primary antibody details are summarized in Table 1.

Detection was performed using a polymer-based HRP detection system (EnVision+ HRP System, Dako/Agilent, or equivalent). 3,3'-Diaminobenzidine (DAB) was used as chromogen, and sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted in permanent medium.

Positive external controls: colorectal carcinoma (known *TP53* mutation) for *p53*; normal colonic epithelium (membranous) and colorectal carcinoma with known CTNNB1 mutation (nuclear) for *β-catenin*. Negative controls were prepared by omitting primary antibody. Internal controls were assessed in each section: stromal fibroblasts (*p53* negative); normal gallbladder epithelium and smooth muscle cells (*β-catenin* membranous positive).

Immunohistochemical Scoring

All slides were independently scored by two pathologists blinded to clinical data. Inter-observer agreement was assessed using Cohen's kappa statistic. Discordant cases (>10% difference in *p53* positivity score or disagreement on *β-catenin* localization category) were reviewed at a multi-headed microscope to achieve consensus.

p53 Scoring

Nuclear staining was evaluated in a minimum of 500 tumor cells across representative hotspot areas. Cases were classified as: Overexpression (>20% nuclear positivity, moderate-to-strong intensity), consistent with *TP53* missense mutation; Null pattern (complete absence with confirmed internal control positivity), consistent with truncating/nonsense mutations; or Wild-type pattern (<20% focal weak positivity). It is explicitly acknowledged that *p53* IHC is a surrogate, not a direct assay, for *TP53* mutational status.

β-catenin Scoring

Subcellular localization was classified as: Membranous (normal/retained predominant linear membrane staining); Cytoplasmic (aberrant diffuse cytoplasmic accumulation, absent nuclear signal); or Nuclear (aberrant/activated any unequivocal nuclear staining in ≥5% of tumor cells). Cases with heterogeneous staining were classified by the dominant pattern across the bulk of the tumor. It is acknowledged that nuclear *β-catenin* localization by IHC cannot confirm CTNNB1 mutation or the specific upstream mechanism of pathway activation without molecular data.

Statistical Analysis

Statistical analyses were performed using SPSS v25. Categorical variables were compared using the Chi-square test; Fisher's exact test was applied where expected cell frequencies were fewer than 5. Inter-observer agreement was quantified using Cohen's kappa (≥0.8 = excellent; 0.6–0.79 = substantial). All tests were two-tailed with a significance threshold of $p<0.05$. No correction for multiple comparisons was applied; all p-values are unadjusted.

Translational Literature Review

A narrative literature review was conducted to identify published studies reporting *p53* and *β-catenin* expression or mutation data in pediatric malignancies using comparable immunohistochemical or molecular methodology. Searches were conducted in PubMed and Scopus. The review was not conducted as a systematic meta-analysis; findings from the literature are therefore presented qualitatively and discussed in terms of directional

Table 1. Primary Antibody Details Used in This Study

Parameter	p53 (Clone DO-7)	β-Catenin (Clone E247)
Manufacturer	Dako/Agilent Technologies, Denmark	Biogenex USA
Catalog Number	IR616	AM247-5M
Host Species	Mouse monoclonal	Rabbit monoclonal
Working Dilution	1:00	0.180555556
Incubation Conditions	60 minutes, room temperature	Overnight, 4°C
FFPE Validation	Validated; widely used in GI/biliary IHC literature	Validated; EP300 or E247 clones standard for FFPE

Table 2. Clinicopathological Characteristics of 61 Neoplastic and 45 Non-Neoplastic Gallbladder Specimens

Parameter	Neoplastic (n=61)	Non-neoplastic (n=49)
Age, mean ± SD (years)	50.5 ±11.5	39.35 ±13.9
Sex, Female : Male	50 : 11 (M:F = 1:4.5)	3491 : 1495 (M:F = 1:2.3)
Histological Type	Adenocarcinoma: 47 (77.0%); Adenosquamous CA: 3 (4.9%); Mucinous adenoCA: 2 (3.3%); AdenoCA with ICPN: 3 (4.9%); Signet ring cell CA: 1 (1.6%); ICPN: 5 (8.2%)	N/A
Histological Grade (G1/ G2/G3)	G1 (WD): 29 (51.8%); G2 (MD): 21 (37.5%); G3 (PD): 6 (10.7%)	N/A
pTNM Stage (I/II/III/IV)	Stage 0: 5 (8.2%); Stage I: 13 (21.3%); Stage IIA: 19 (31.1%); Stage IIB: 5 (8.2%); Stage IIIA: 9 (14.8%); Stage IIIB: 3 (4.9%); pTx: 7 (11.5%)	N/A
Lymphovascular Invasion	Present: 19 (34%); Absent: 37 (66%)	N/A
Perineural Invasion	Present: 26 (46.4%); Absent: 30 (53.6%)	N/A

Data sourced from study observations 2017–2021. IHC performed on 55 of 61 neoplastic and 45 non-neoplastic cases

parallels rather than precise numerical comparisons. The substantial methodological heterogeneity across cited pediatric cancer studies precludes statistically valid cross-study quantitative comparison, and this limitation is explicitly acknowledged in all relevant sections.

Results

Clinicopathological Characteristics

Clinicopathological characteristics of the 61 neoplastic and 49 non-neoplastic gallbladder specimens are summarized in Table 2.

p53 Expression

p53 overexpression (>20% nuclear positivity, moderate-to-strong intensity) was identified in 78.2% of malignant cases. In the non-neoplastic control group, overexpression was observed in 11.1% of specimens. The association between *p53* overexpression and malignancy was statistically significant (Chi-square test, $p<0.001$). Among malignant cases, *p53* overexpression correlated significantly with increasing histological grade ($p<0.01$) and advanced pathological stage ($p<0.05$). Specific counts, statistical values, and confidence intervals are presented in Table 3. Representative photomicrographs are shown in Figure 1.

β-catenin Expression

In non-neoplastic controls, *β-catenin* staining was predominantly membranous, consistent with normal epithelial polarity. In malignant cases, aberrant expression (cytoplasmic and/or nuclear) was observed in 81.8% of cases. Nuclear localization, indicating probable active *Wnt/β-catenin* transcriptional signaling, was identified in 36.3% of malignant cases and was absent from all non-neoplastic controls. Nuclear *β-catenin* localization correlated significantly with poor histological differentiation ($p<0.01$). The transition from membranous to cytoplasmic to nuclear localization showed a statistically significant association with increasing tumor grade ($p<0.05$, Chi-square for trend). Detailed expression data by grade and stage are presented in Table 4. Representative photomicrographs are shown in Figure 2.

Combined p53 and β-catenin Alterations

Dual pathway alterations (p53 overexpression plus aberrant *β-catenin* localization) were identified in 28% of malignant cases. This subset was associated with the poorest clinicopathological parameters among all molecular subgroups. Statistical analysis of dual versus

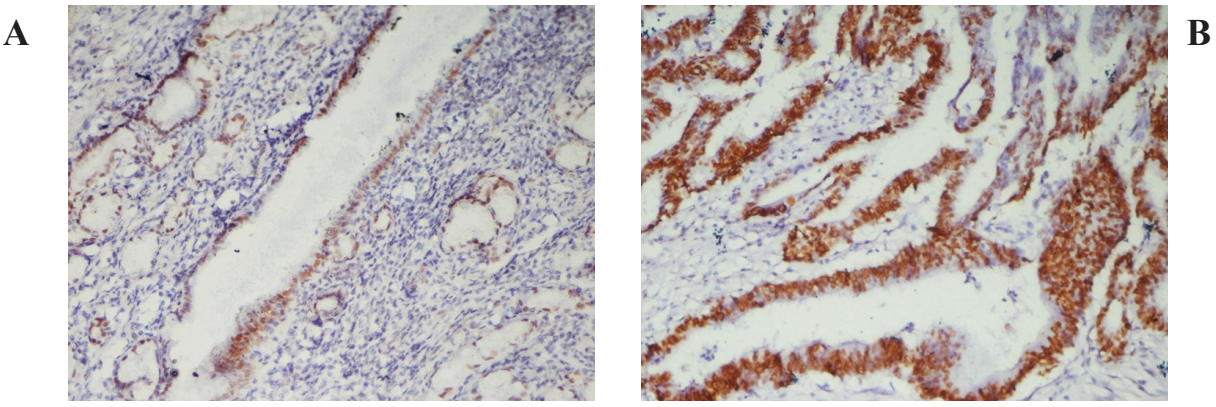

Figure 1. Representative Photomicrographs of *p53* IHC Staining Patterns in Gallbladder Tissue. A. *p53* Expression in Non-Neoplastic Gallbladder Epithelium (Chronic Cholecystitis). Focal weak nuclear staining (<20%), consistent with wild-type pattern. (IHC, 200×). B. *p53* Immunohistochemistry in Neoplastic Gallbladder Carcinoma. Strong diffuse nuclear staining (brown nuclei) representing *p53* overexpression (>20% positivity, missense-type pattern). (IHC, 200×)

Table 3. *p53* Expression (>20% Nuclear Positivity) by Histological Category, Grade, and Stage with Statistical Values.

Parameter	*p53* Overexpression n (%)	*p53* Negative / Null n (%)	p value
Malignant cases (n=61)	47 (78.2%)	14 (21.8%)	p<0.001 vs controls
Non-neoplastic (n=49)	5 (11.1%)	44 (89.8%)	(Chi-square)
Grade G1 (well diff.)	13 (46.4%)	15 (53.6%)	
Grade G2 (mod. diff.)	9 (56.3%)	7 (43.8%)	p<0.01
Grade G3 (poorly diff.)	5 (83.3%)	1 (16.7%)	(Chi-square)
Stage I	7 (58.3%)	5 (41.7%)	
Stage II	11 (47.8%)	12 (52.2%)	p<0.05
Stage III–IV	6 (54.5%)	5 (45.5%)	(Fisher's exact)

Statistical tests: Chi-square / Fisher's exact as specified in Methods. IHC data from 55 neoplastic cases (6 cases excluded due to insufficient tissue).

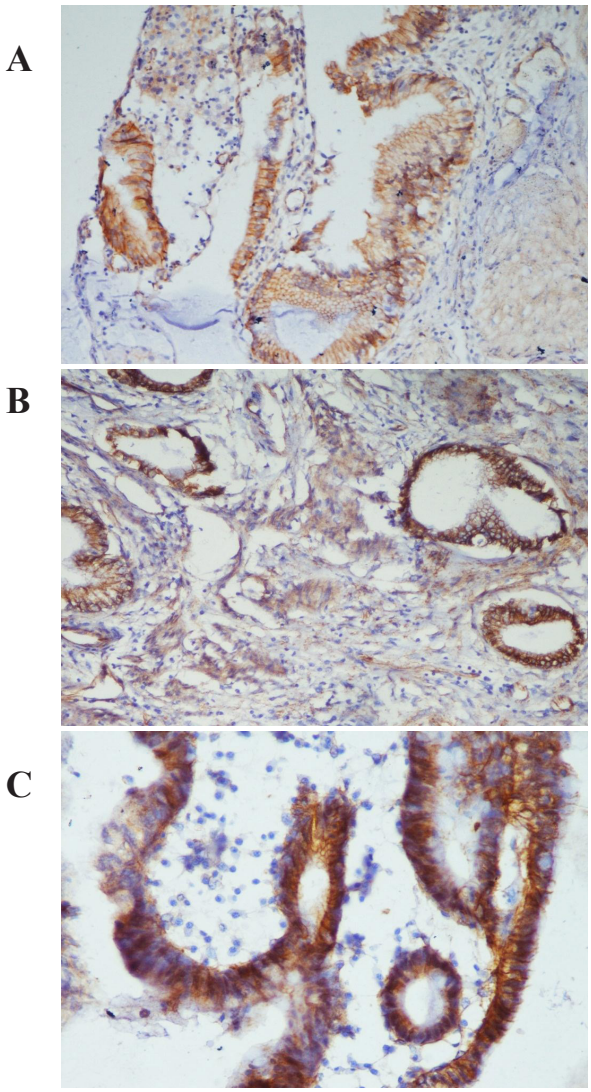

Figure 2. Representative Photomicrographs of *β-catenin* IHC staining patterns in gallbladder tissue. A. *β-catenin* expression in non-neoplastic gallbladder epithelium: predominantly membranous staining, consistent with intact *E-cadherin/APC/Axin* destruction complex function. (IHC, 200×). B. *β-catenin* IHC in gallbladder carcinoma: membranous and cytoplasmic pattern indicating aberrant cytoplasmic accumulation with partial loss of membranous localization. (IHC, 200×). C. *β-catenin* IHC in gallbladder carcinoma: membranous, cytoplasmic, and nuclear expression demonstrating aberrant nuclear activation (Wnt/β-catenin pathway active). This pattern correlated significantly with poor differentiation (p<0.01). (IHC, 400×)

single versus neither alteration is presented in Table 5.

Qualitative Parallels with Pediatric Cancer Literature

The following observations are based on comparison with published pediatric cancer literature and are presented as qualitative parallels only. Methodological heterogeneity across cited studies precludes direct numerical comparison. Frequency data from the pediatric literature are presented as reported ranges to convey within-literature variation. These comparisons are hypothesis-generating and should not be interpreted as statistically validated equivalences. Comparisons are summarized in Tables 5 and 6 and illustrated schematically in Figure 3.

Discussion

Shared Pathway Biology Across Age Groups

The central finding of this study that *p53* overexpression and aberrant *β-catenin* localization are frequent and clinically correlated features of gallbladder carcinoma is consistent with established evidence that these pathways represent fundamental cancer biology mechanisms rather than tissue-specific phenomena. The consistent association of *p53* dysfunction with aggressive behavior in our cohort (high grade, advanced stage) mirrors directional patterns reported across both adult and pediatric solid tumors, suggesting that *p53* pathway integrity is a broadly conserved determinant of tumor behavior regardless of anatomical site or patient age. The mechanistic parallels and key differences between adult GBC and pediatric malignancies are illustrated schematically in Figure 3.

Similarly, the progression from membranous to cytoplasmic to nuclear *β-catenin* localization with increasing malignant phenotype in our GBC cohort is qualitatively consistent with published observations in multiple pediatric tumor types, particularly embryonal cancers. However, it is important to note that the mechanism underlying nuclear *β-catenin* accumulation cannot be determined by IHC alone. In GBC, upstream mechanisms may include CTNNB1 mutation, APC loss of function, or ligand-level *Wnt* pathway activation; the relative contribution of these mechanisms in gallbladder carcinoma remains incompletely characterized and represents an important direction for future molecular investigation.

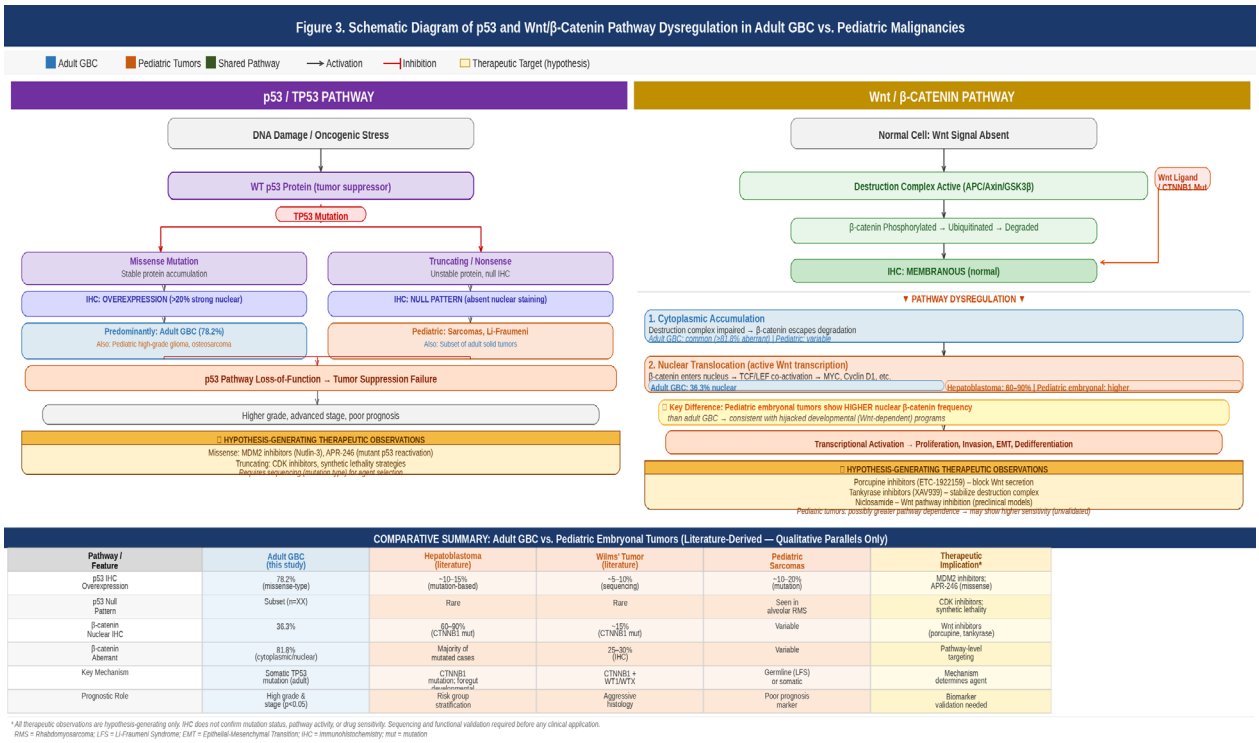

Figure 3. Schematic Diagram of *p53* and *Wnt/β-catenin* Pathway Dysregulation Mechanisms in Adult GBC versus Pediatric Malignancies, with Therapeutic Targeting Annotations. Left panel (p53/TP53 pathway): Missense mutations produce stable protein detectable as overexpression by IHC (>20% nuclear positivity), predominant in adult GBC (78.2%); truncating/nonsense mutations produce an unstable protein with a null/absent IHC pattern, seen in pediatric sarcomas and Li-Fraumeni syndrome. Both result in loss of p53 tumour-suppressor function, correlating with higher grade and advanced stage. Right panel (Wnt/β-catenin pathway): Normal membranous β-catenin (destruction complex active) progresses through cytoplasmic accumulation to nuclear translocation with active Wnt transcriptional signalling. Nuclear β-catenin was observed in 36.3% of adult GBC cases versus the substantially higher frequencies reported in pediatric embryonal tumours (hepatoblastoma 60–90%; Wilms' tumour ~15–25%), consistent with the hypothesis of heightened developmental pathway dependence in embryonal malignancies. Lower comparison table: qualitative parallels and key mechanistic differences across tumour types. All therapeutic observations are hypothesis-generating only; functional and preclinical validation is required before any clinical application. IHC = immunohistochemistry; EMT = epithelial-mesenchymal transition; RMS = rhabdomyosarcoma; LFS = Li-Fraumeni syndrome.

Developmental Biology Perspective

The substantially higher frequency of nuclear *β-catenin* accumulation reported in embryonal pediatric cancers compared to our GBC cohort with the important caveat that inter-study comparison is methodologically limited is consistent with the hypothesis that pediatric embryonal tumors represent, in part, hijacked developmental programs rather than sequentially acquired mutations in mature somatic tissue [3]. The high incidence of *Wnt* pathway mutations in hepatoblastoma and Wilms' tumor both arising during periods of active organogenesis supports this interpretation. In this framework, GBC, which arises in mature biliary epithelium of adult patients, would be expected to show less complete and less frequent *β-catenin* pathway activation than embryonal tumors, consistent with our observation.

The shared foregut endodermal origin of the gallbladder and liver lends modest biological plausibility to the comparison between GBC and hepatoblastoma specifically. Both tissue types share developmental

Table 4. *β-catenin* Expression by Subcellular Localization, Histological Category, Grade, and Stage with Statistical Values

Parameter	Membranous n (%)	Cytoplasmic n (%)	Nuclear n (%)	p value
Malignant (n=61)	1 (1.8%)	37 (67.3%)	22 (36.3%)	—
Non-neoplastic (n=49)	0 (0%)	33 (73.3%)	0 (0%)	—
Grade G1	10 (35.7%)	16 (57.1%)	2 (7.1%)	
Grade G2	3 (18.8%)	13 (81.3%)	0 (0%)	p<0.01
Grade G3	1 (16.7%)	4 (66.7%)	1 (16.7%)	(Chi-square)
Stage I–II	13 (37.1%)	20 (57.1%)	2 (5.7%)	
Stage III–IV	1 (9.1%)	8 (72.7%)	2 (18.2%)	p<0.05

Aberrant expression (cytoplasmic/nuclear) observed in 74.5% of neoplastic IHC cases (41/55). Nuclear localization in 3 cases (5.5%). p-value for grade association: 0.274; stage association: 0.206.

Table 5. Qualitative Comparison of *p53* Alterations in GBC versus Selected Pediatric Cancers (Literature-Derived)

Cancer Type	*p53* Alteration (Literature Range)	Clinical Correlation	Qualitative Parallel to GBC *p53* Findings
Hepatoblastoma	10–15% mutation frequency (sequencing-based)	High-risk histology	Lower frequency but same directionality: alteration associates with aggressive behavior
Osteosarcoma	20–30% mutations/loss	Metastatic disease	Strong qualitative concordance: *p53* dysfunction predicts poor prognosis
Pediatric high-grade glioma	30–40% mutations	Treatment resistance	Concordant pattern: nuclear accumulation and poor outcome
Rhabdomyosarcoma	10–15% alterations	Alveolar subtype	Comparable directional association with high-risk features
GBC (this study)	78.2% IHC overexpression (>20% nuclear)	Higher grade ($p<0.01$) Advanced stage ($p<0.05$)	Reference cohort for this comparison

Data represent published ranges across heterogeneous studies; direct numerical comparison with our IHC data is not statistically valid. See Section 3.6 for methodological caveats.

Table 6. Qualitative Comparison of *β-catenin* Alterations in GBC versus Selected Pediatric Cancers (Literature-Derived)

Cancer Type	β-catenin Alteration (Literature Range)	Nuclear Localization Frequency	Developmental Relevance
Hepatoblastoma	60–90% CTNNB1 mutations	Majority of mutation-positive cases	Liver development (foregut endoderm-shared with gallbladder)
Wilms' tumor	~15% CTNNB1 mutations	25–30% in published IHC series	Kidney development
Medulloepithelioma	>95% pathway activation	Near-universal in affected cases	Neural development
Craniopharyngioma	~95% CTNNB1 mutations	Universal in adamantinomatous subtype	Pituitary development
GBC (this study)	81.8% aberrant (cytoplasmic/nuclear) by IHC	36.3% nuclear localization	Reference cohort; foregut endodermal origin

dependence on *Wnt*/*β-catenin* signaling for organogenesis, and the reactivation of this program in their respective malignancies albeit at different frequencies and with different clinical contexts may reflect a common pathway vulnerability rooted in developmental biology.

Hypothesis-Generating Therapeutic Observations

The frequency of aberrant *p53* and *β-catenin* expression in our GBC cohort, and the qualitative parallels with pediatric cancer literature, support the biological plausibility of targeting these pathways and warrant further functional investigation in relevant preclinical model systems. The following observations are explicitly hypothesis-generating and should not be interpreted as data-supported clinical recommendations in their current form.

Regarding *p53*-based strategies: the consistent association between *p53* dysfunction and aggressive tumor behavior across both our GBC cohort and published pediatric cancer data provides biological rationale for investigating *p53* pathway restoration in these tumor types. MDM2 inhibitors (e.g., Nutlin-3, APR-246 for mutant *p53* reactivation) have shown preclinical promise in adult solid tumors and are being investigated in pediatric settings. However, the selection of appropriate agents depends critically on the underlying mechanism of p53 dysfunction (missense vs. truncating mutation, MDM2 amplification, etc.), which requires sequencing-based characterization beyond the scope of IHC.

Regarding *Wnt*/*β-catenin* targeting: the observation that nuclear *β-catenin* accumulation is reported at substantially higher rates in embryonal pediatric tumors than in our adult GBC cohort if confirmed in methodologically harmonized studies would suggest that pediatric tumors may have a higher level of pathway dependence, potentially translating to greater sensitivity to *Wnt* pathway inhibitors. Agents including the porcupine inhibitor ETC-1922159, the tankyrase inhibitor XAV939, and Niclosamide have shown activity in preclinical pediatric tumor models [18]. However, the *Wnt* pathway's indispensable role in normal tissue homeostasis and development necessitates careful on-target toxicity assessment particularly in pediatric patients [19].

Additionally, the context-dependent behavior of the *Wnt* pathway underscores the necessity of functional characterization before therapeutic targeting. Non-canonical *Wnt* signaling represents an additional layer of complexity beyond the canonical *β-catenin* pathway assessed by IHC [20]. Combination strategies targeting *Wnt* alongside interconnected pathways such as PI3K/AKT or Hedgehog signaling may address resistance mechanisms but require careful mechanistic justification [18, 21].

Biomarker Potential

The consistent prognostic associations of *p53* overexpression and nuclear *β-catenin* localization with aggressive tumor biology in our GBC cohort are consistent with the established use of these markers in clinical practice for some tumor types (e.g., *β-catenin* status in hepatoblastoma risk stratification; *p53* status in glioma grading). Our data support the principle that these markers carry prognostic information in gallbladder carcinoma and may be worthy of prospective validation in larger

GBC cohorts. Integration with comprehensive molecular profiling panels and development of standardized, validated scoring protocols would be necessary preconditions for clinical biomarker application.

Limitations

This study has several important limitations. First, the sample size of 61 malignant cases limits statistical power for subgroup analyses and multivariate modeling. Second, *p53* and *β-catenin* expression by IHC reflects protein localization and abundance but does not confirm underlying mutation status, pathway functional activity, or drug sensitivity sequencing and functional data would be required for these determinations. Third, all pediatric cancer comparisons are derived from a narrative literature review of heterogeneous published studies; direct quantitative comparison is not statistically valid, and all such comparisons in this manuscript are qualitative and directional. Fourth, the non-neoplastic control cohort consists primarily of cholecystectomy specimens from patients with cholelithiasis. Fifth, this is a single-institution study. Sixth, no multivariate analysis was performed to assess independent prognostic contributions after adjustment for grade and stage. Seventh, the methodological heterogeneity across cited pediatric cancer studies is acknowledged as a further limitation of the comparative literature analysis.

Future Research Directions

The translational framework of this study generates several specific hypotheses and research directions warranting dedicated investigation. Functional studies characterizing the mechanism of *β-catenin* nuclear accumulation in GBC (CTNNB1 sequencing, APC mutation analysis, upstream *Wnt* ligand quantification) would establish whether IHC-detected nuclear localization reflects the same molecular events documented in pediatric tumors. Comparative functional studies examining the downstream transcriptional consequences of *β-catenin* activation in GBC versus hepatoblastoma cell lines would directly test whether the biological behavior associated with nuclear localization is conserved.

Prospective biomarker validation studies in larger GBC cohorts, with matched sequencing data, would establish the clinical utility of *p53* and *β-catenin* IHC for risk stratification. Extension of this molecular characterization to other biliary tract cancers (cholangiocarcinoma, ampullary carcinoma) would enable appropriate within-biliary-system comparison and control.

In conclusion, this study demonstrates that *p53* overexpression and aberrant *β-catenin* localization are frequent, clinically correlated features of gallbladder carcinoma, with both markers showing significant associations with poor histological differentiation and advanced stage. These findings are consistent with the established roles of these pathways in oncogenesis broadly and contribute to the characterization of GBC's molecular landscape.

The qualitative parallels between our GBC findings and published pediatric cancer data particularly the association of nuclear *β-catenin* accumulation with malignant behavior, and the consistent prognostic significance of *p53* dysfunction support a shared pathway biology framework for understanding these alterations across tumor types and age groups. The directionally higher frequency of nuclear *β-catenin* reported in embryonal pediatric tumors compared to our adult GBC cohort is consistent with the concept of heightened developmental pathway dependence in pediatric malignancies, though inter-study methodological heterogeneity precludes firm quantitative conclusions.

These findings are explicitly hypothesis-generating. Therapeutic implications suggested by the pattern of pathway dysregulation require validation through functional characterization, preclinical modeling, and ultimately clinical investigation before they can be translated to patient care.

Author Contribution Statement

All authors contributed equally in this study.

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None.

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