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The galectin-3 serum level is correlated with prognosis of acute on chronic liver failure

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Background

- Insufficient hepatic tissue regeneration is one major reason for poor prognosis in liver failure patients.
- Galectin-3 (Gal-3), one of β- galactoside-binding proteins, its biological function including：
  - cell adhesion regulation
  - Cell chemotaxis and cell activation regulation
  - Cell growth and cell apoptosis regulation
  - Binding to AGEs(advanced glycosylation end products modified proteins), leading to changes in expression levels of some proteins.

Background

- Basic research shows that Gal-3 can promote proliferation of several types of cell, such as cornea cell, renal tubule cell and colonic epithelial cell.
- Animal experiments demonstrate that Gal-3 plays a hepato-protective role in animal models of ischemia / reperfusion injury and non alcoholic fatty liver.
- Clinical studies show that Gal-3 serum levels C of Child-Pugh C alcoholic cirrhosis patients is significantly higher than that of Child-Pugh A alcoholic cirrhosis patients, indicating that Gal-3 levels increased in patients with severe liver injury.
- The role of Gal-3 in liver failure is not clear.

Methods

- Hospitalized patients in liver disease department of the second hospital of nanjing from Jan 2014 to Dec 2014.
  - 76 patients with acute on chronic liver failure (ACLF) caused by HBV
  - 38 patients with severe degree chronic hepatitis B. (TBIL≥171.1ummol/L and PTA＞40%)
  - 26 patients with mild or moderate degree chronic hepatitis B
  - 25 healthy persons as control.

Methods

- Treatment protocols: all the ACLF patients received combined treatment including nutritional support therapy, hepato-protective agent therapy, symptomatic treatment, artificial liver support system if necessary.
- The baseline data, include serum biochemical tests, coagulation function test, serum galectin-3 levels (by ELISA method), symptoms, signs and complications were recorded.
- Follow up: every patients in ACLF group follow up for six months.
- Statistical analysis： Continuous variables were analyzed using independent Student's *t-test.* Mann-Whitney *U test* were used to analyze non normal distribution data. Logistic regression analysis were used to analyze risk factor correlated with prgnosis.

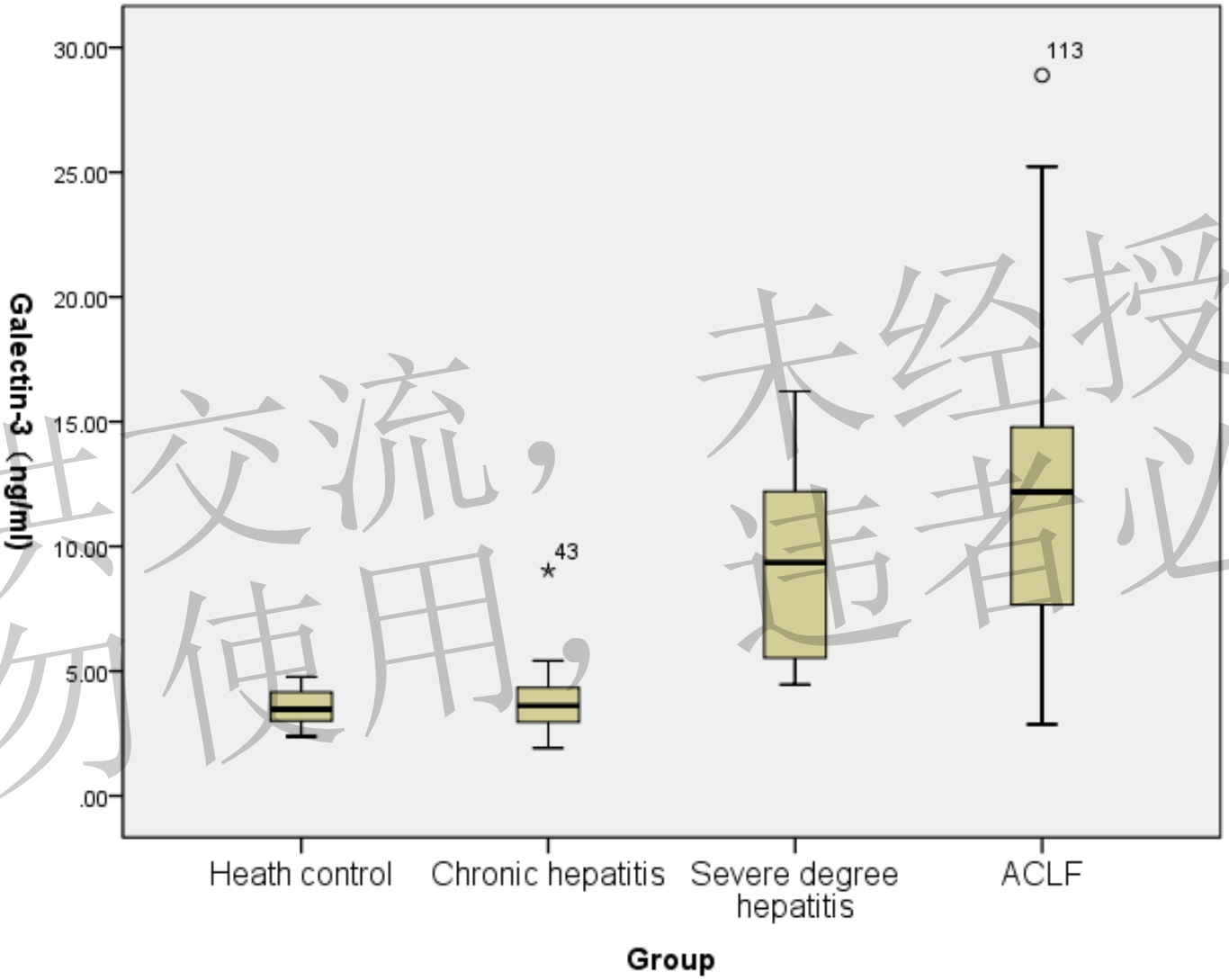

Figure 1. Galectin-3 serum levels at baseline in the four groups

Table 1. Characteristics of patients with different prognosis in the ACLF group

Characteristics	Death(n=40)	Survival(n=36)	t/χ^2/Z value	*P* value
Age(years)	51±13.2	47.9±13.3	t=1.041	0.301
Gender(M:F)	31:9	26:10	χ^2=0.008	0.631
TBIL(umol/L)	387.4±113.8	330.1±107.9	t=2.246	0.028
DBIL(umol/L)	280.9±91.5	252.7±80.5	t=1.423	0.159
ALT(U/L)	127(70.2, 397.7)	164.6(64.4, 381)	Z=—0.27	0.787
AST(U/L)	130.1(102.2, 276.9)	107.5(81.7, 317.1)	Z=—1.176	0.24
ALB(g/L)	31.35(28.5, 33.4)	32.95(30, 35.03)	Z=—2.372	0.018
INR	2.09(1.9, 2.37)	1.87(1.77, 2.07)	Z=—2.549	0.011
K^+(mmol/L)	3.97(3.39, 4.51)	4(3.55, 4.37)	Z=—0.083	0.934
Na^+(mmol/L)	134.75(132.4,137.1)	137.9(135.43, 139.3)	Z=—3.349	0.001
Bun(mmol/L)	6.04 (3.66, 9.38)	4.29(3.1, 6.67)	Z=—2.143	0.032
Cr(umol/L)	70.5(62,82.5)	64.5(53.75,79.5)	Z=—2.222	0.026
AFP（ng/ml）	40.7(5.5, 120.1)	97.4(26.6, 276.2)	Z=—2.217	0.027
CHO(mmol/L)	1.92(1.51, 2.43)	2.09(1.59, 2.58)	Z=—0.924	0.355
TG(mmol/L)	0.33(0.21, 0.7)	0.63(0.46, 1.01)	Z=—2.138	0.033
HDL-C(mmol/L)	0.13(0.11, 0.16)	0.17(0.11, 0.27)	Z=—1.163	0.245
LDL-C(mmol/L)	1.23(0.93, 1.42)	1.38(0.9, 1.64)	Z=—1.199	0.23
FT_3(pg/ml)	2.23(1.67, 2.58)	2.39(1.95, 2.88)	Z=—0.452	0.651
FT_4 (ng/dl)	11.54(9.21, 14.16)	12.24(10.47, 17.29)	Z=—1.278	0.201
TSH(IU/ml)	0.22(0.15, 0.41)	0.68(0.42, 1.13)	Z=—3.356	0.001
Galectin-3(ng/ml)	9.13(5.17,14.26)	13.42(9.87,15.78)	Z=—3.085	0.01
hepatic encephalopathy(n,%)	18 （45）	6 （16.67）	χ^2=7.04	0.008
Hepatorenal syndrome(n,%)	5 （12.5）	4 （11.11）	χ^2=0.035	0.852
GI bleeding(n,%)	8 （20）	2 （5.56）	χ^2=2.311	0.128
Infection(n,%)	32 （80）	15 （41.67）	χ^2=11.799	0.001
Ascites(n,%)	19 （47.5）	1 （2.78）	χ^2=19.544	0.000

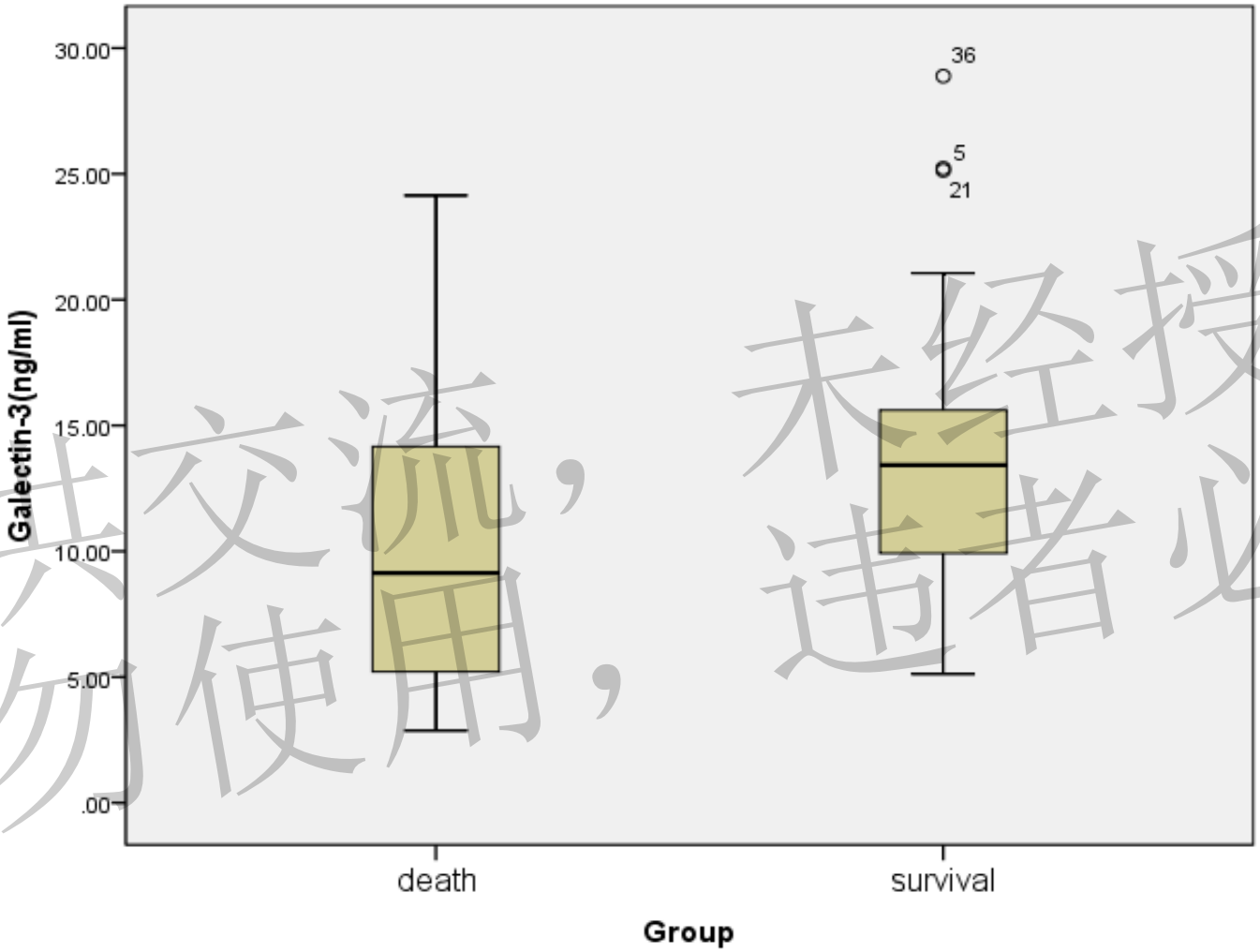

Figure 2. Baseline galectin-3 serum levels of patients with death or survival prognosis in ACLF group.

Table 2. Multivariate Logistic regression analysis of prognostic factors of the ACLF patients

Variables	β	S.E.	Walds	*P* value	OR(95% CI)
TBIL	-2.084	0.630	10.931	0.011	0.124（0.093, 0.367）
INR	-3.520	1.676	4.411	0.036	0.03（0.001, 0.79）
TSH	1.633	0.641	6.492	0.011	5.119（1.458,17.977）
Galectin-3	3.323	1.543	4.639	0.031	27.731（1.349,570.214）
Ascites	-7.223	3.139	5.293	0.021	0.001（0.000, 0.343）

Summarization

- The serum Galectin-3 level of ACLF patients was significantly higher than that of normal persons and chronic hepatitis B patients.
- The serum Galectin-3 level is correlated with prognosis of ACLF, patients with higher Gal-3 levels with better prognosis.
- Upregulation of Galectin-3 acting as protective factor in ACFL.

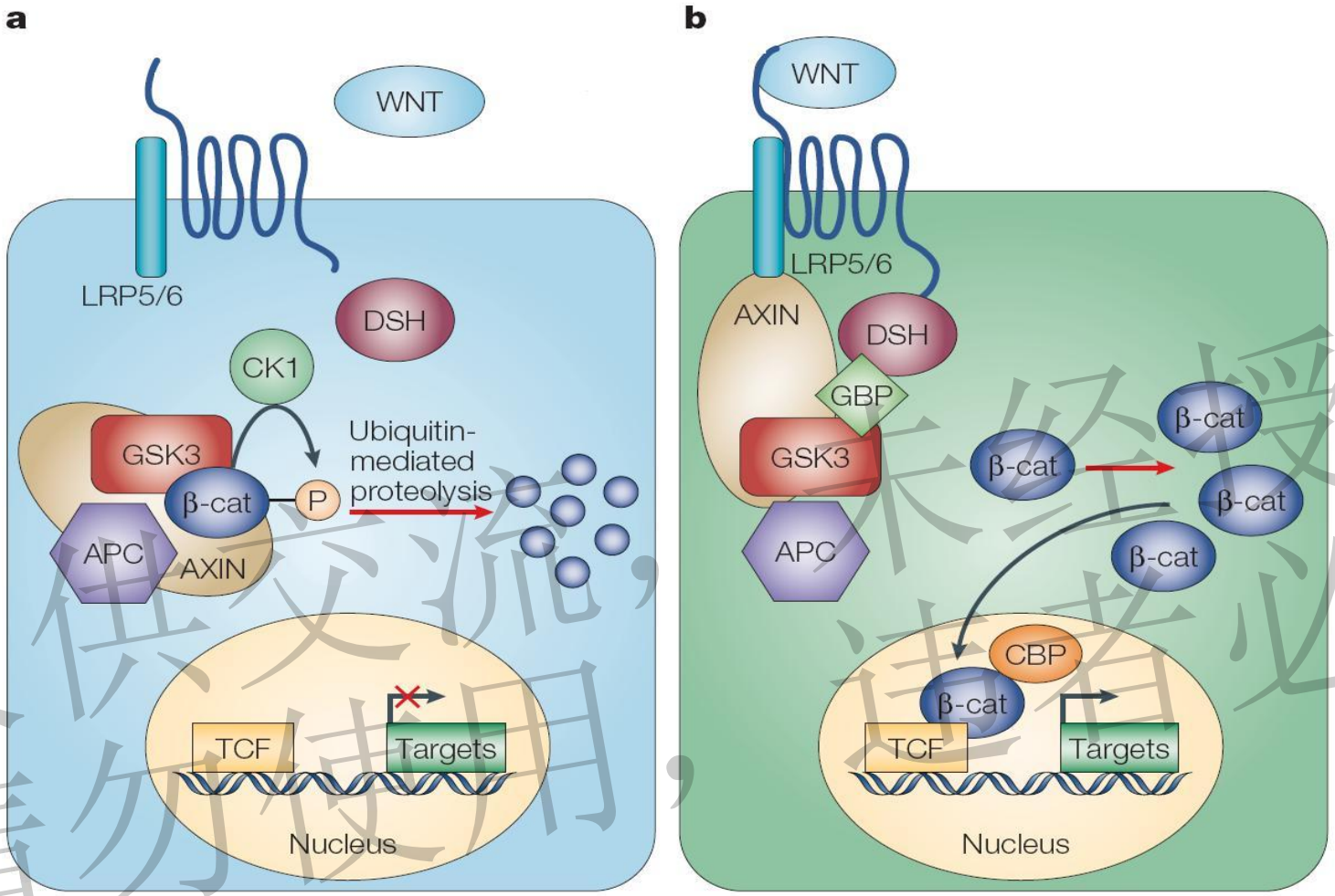

Figure 3. **WNT/β-catenin signalling. (a),** In the absence of active WNT β-catenin is degraded, and prospective target genes are in a repressed state. **(b)** If WNT signalling **is active, β-catenin** degradation is reduced. As β-catenin accumulates, it enters the nucleus, binds to T-cell factor (TCF)- and lymphoid enhancerbinding protein (LEF)-family transcription factors and activates transcription. The components shown are described in more detail in the text; additional pathway components are described on web sites that are linked to the main text. APC, adenomatous polyposis coli; β-cat, β-catenin; CBP, CREB-binding protein; CK, casein kinase; DKK, Dickkopf; DSH, Dishevelled; GBP, GSK3-binding protein; GSK, glycogen synthase kinase; LRP, LDLreceptor-related protein; P, phosphorylation; sFRP, secreted Frizzled-related protein; TCF, T-cell factor.

Human Hepatocytes (HH) culture

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Grouping： Gal-3 SiRNA group, Gal-3 vector group, blank SiRNA or vector group, and blank control group

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To harvest cells at different time points

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To detect the fluorescence expression by fluorescence microscope and Gal-3 protein expression by western blot in HH

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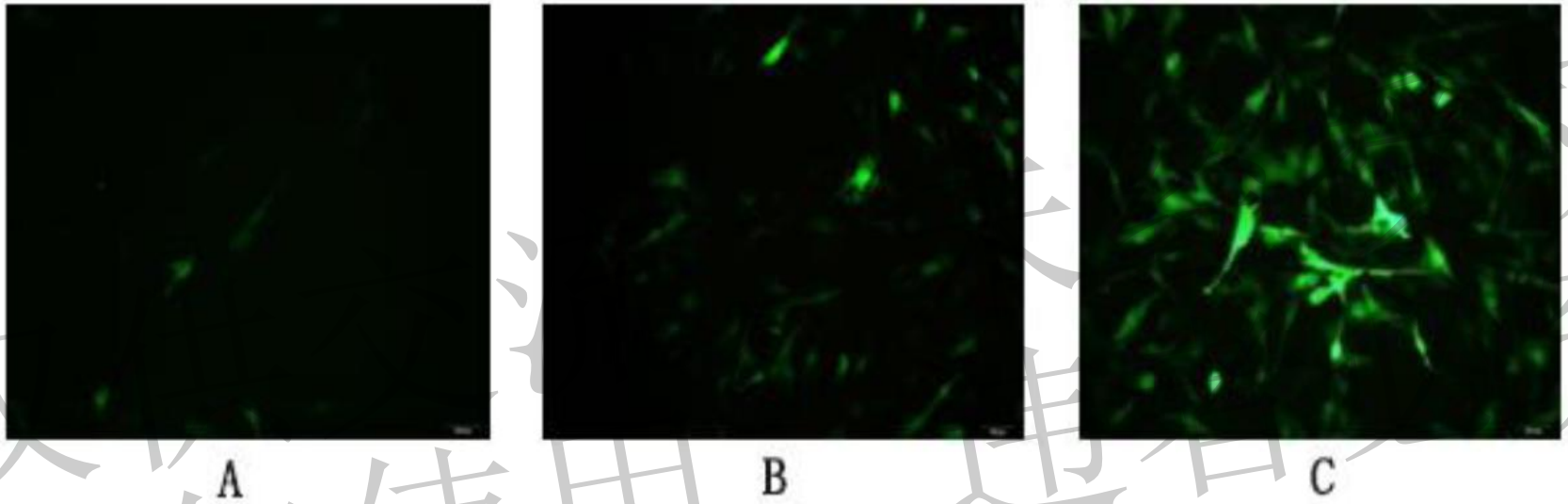
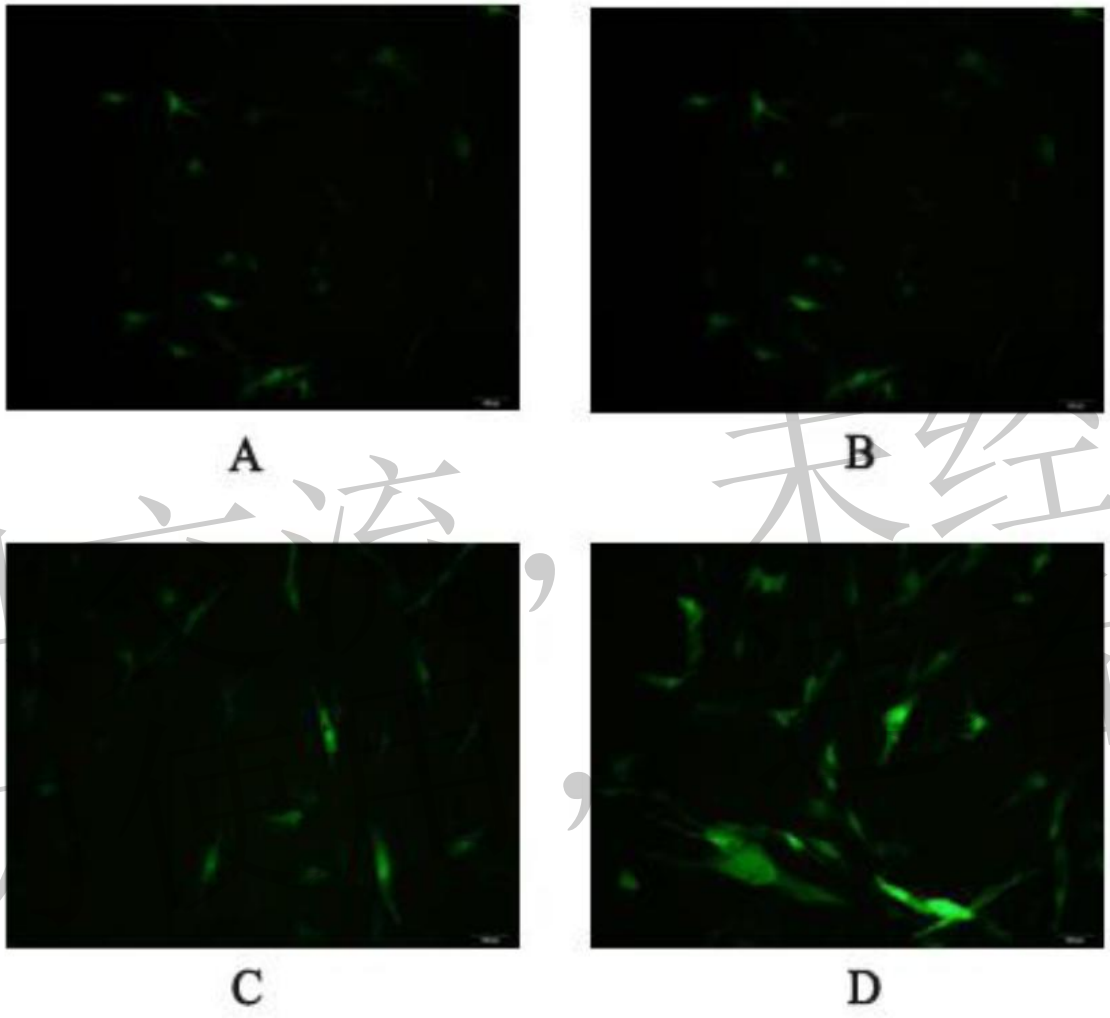
The proliferation ability of HH was detected by CCK-8 method. The protein levels of axin, total β-catenin, active β-catenin, total GSK-3β, and active GSK-3β were detected by western blot method.



A：5MOI；B：10MOI；C：20MOI

Figure 4. Fluorescence expression of different MOI gradient in SiRNA group



A：50MOI；B：100MOI；C：150MOI；D：200 MOI

Figure 5. Fluorescence expression of different MOI gradient in vector group

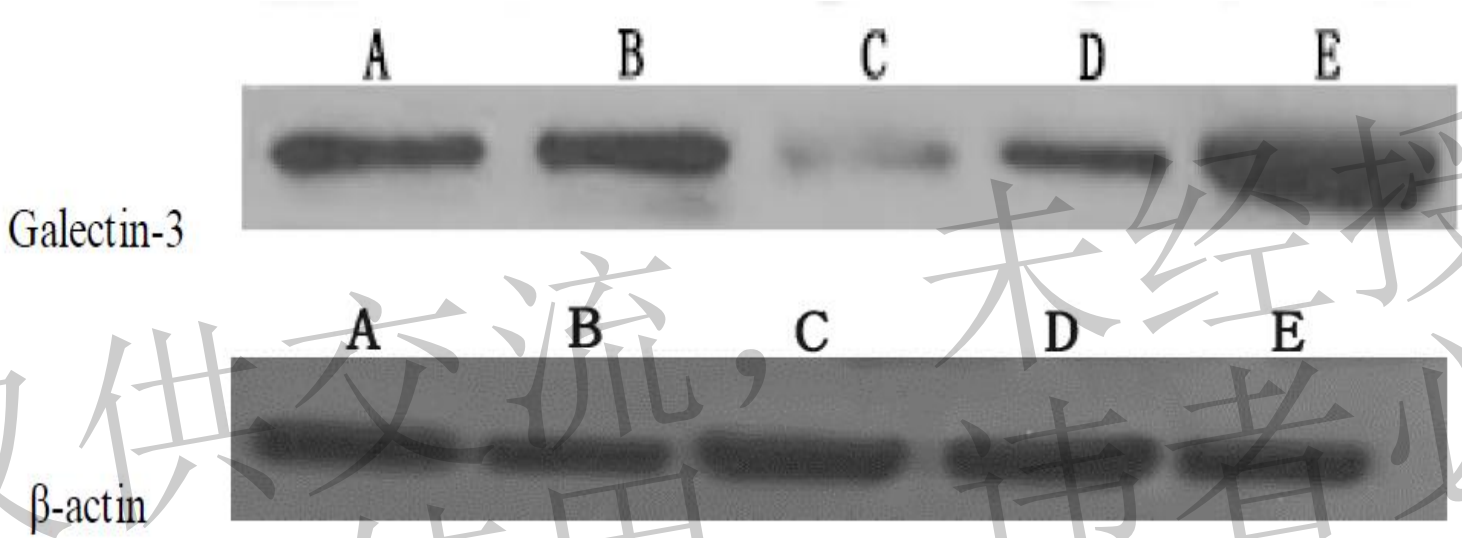


Figure 6. Galectin-3 expression detected by western blot of the five group
Note: A. Blank control; B. Blank SiRNA; C. Gal-3 SiRNA; D. blank vector; E. Gal-3 vector.

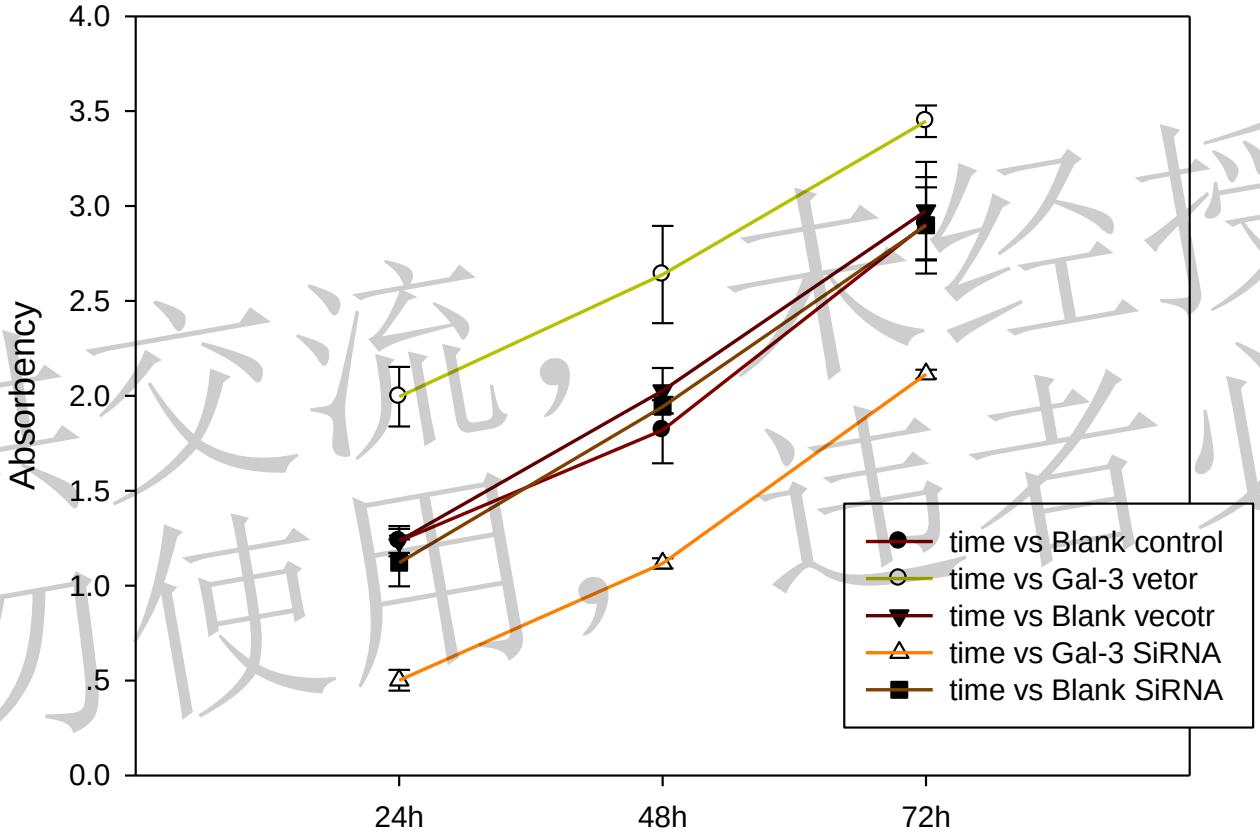


Figure 7. Cell proliferation ability detected by CCK8 method at different time points of the five group

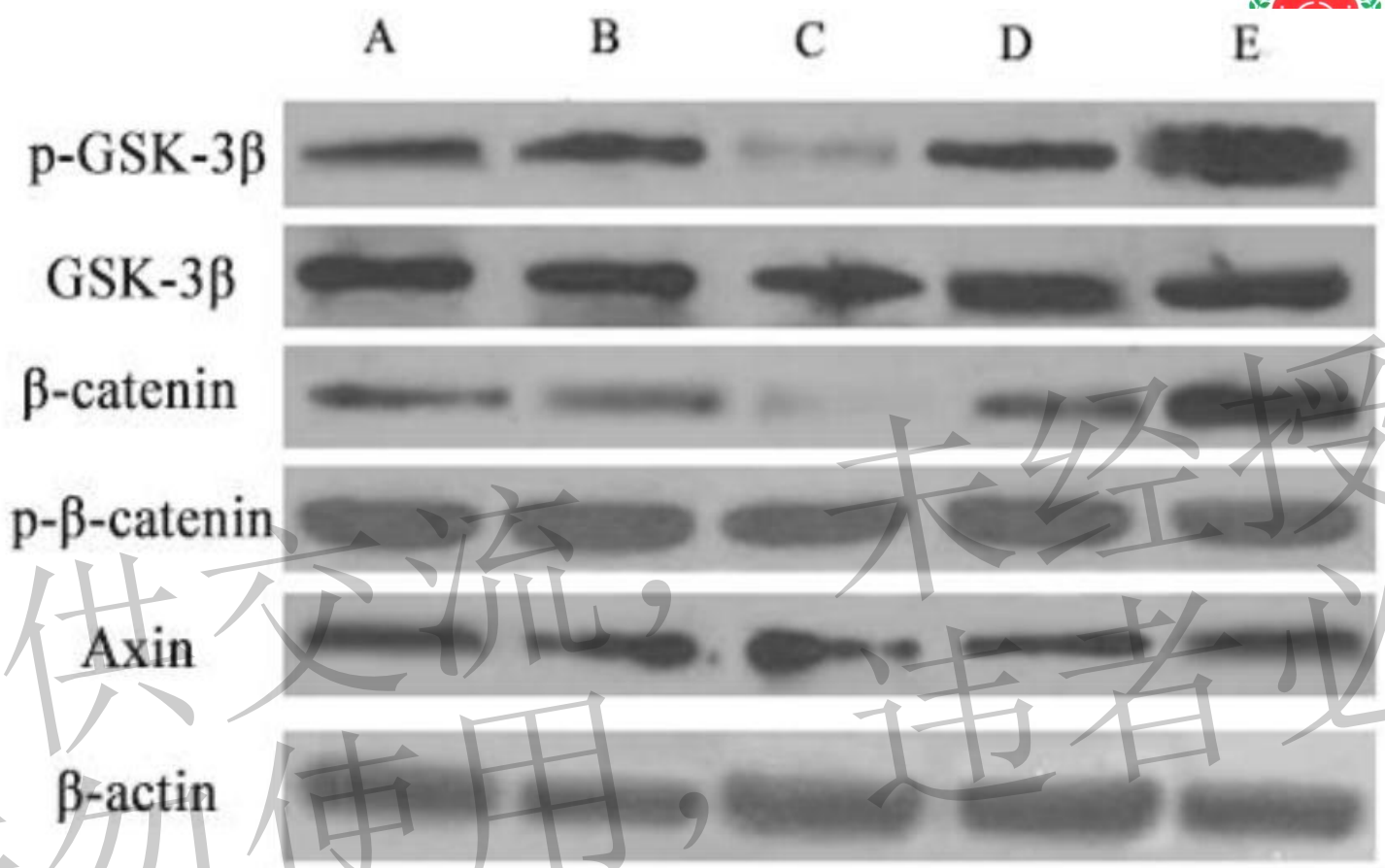


Figure 8. Wnt/β-catenin pathway protein expression detected by western blot method of the five group

Note: A. Blank control; B. Blank SiRNA; C. Gal-3 SiRNA; D. blank vector; E. Gal-3 vector.

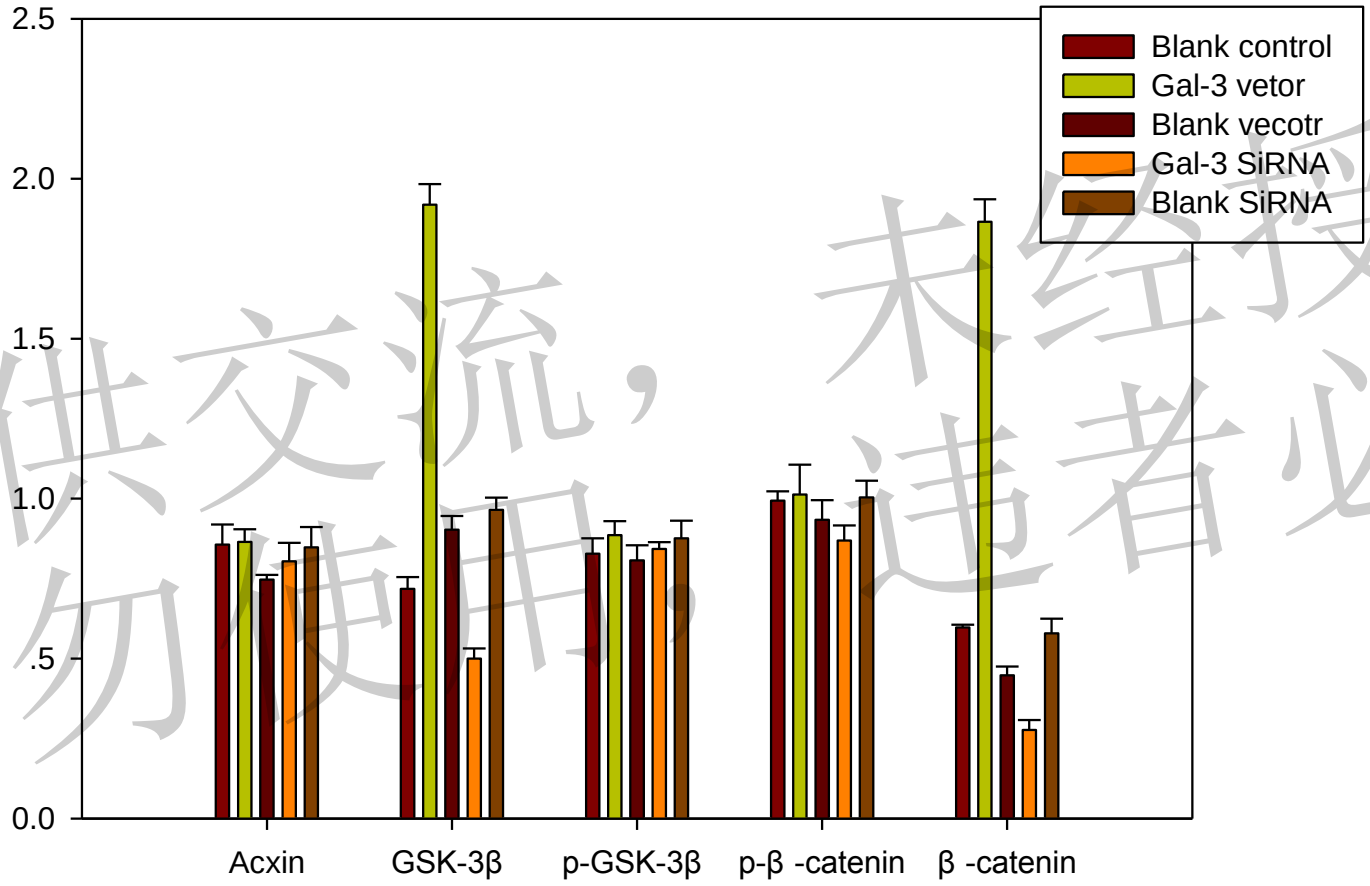


Figure 9. Effects of overexpression or down-regulation of Gal-3 on Wnt/ beta -catenin pathway in HH cells

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- The Galectin-3 serum level in was significantly increased in ACLF patients, and the level of elevation were related to prognosis, which may be the protective factor.
- Overexpression or downregulation of Galectin-3 can promote or inhabit HH cells proliferation, and activation of Wnt/ beta -catetin pathway may be the mechanism.
- Galectin-3 may affect the prognosis of ACLF by promoting the regeneration of liver tissue.