

Signal Transducer and Activator of Transcription 3 for the Differentiation of Hepatocellular Carcinoma from Cirrhosis

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Abstract

Background: Overexpression and constitutive activation of signal transducer and activator of transcription (STAT) 3 have been suggested in the tumorigenesis of many human cancers, including multiple carcinomas, melanoma, and lymphoma. The diagnosis of hepatocellular carcinoma (HCC) in lobectomy specimens is usually straightforward, but distinguishing cirrhosis from well-differentiated HCC can be challenging in core biopsies. Our aims were to investigate the expression level of STAT3 and phosphorylated STAT3 (pSTAT3) in HCC and cirrhosis, and the application of STAT3 in the differential diagnosis of HCC and cirrhosis.

Methods: Sixty cases were divided into three groups: patients with HCC only (Group 1), HCC and cirrhosis (Group 2), and cirrhosis only (Group 3). Formalin-fixed and paraffin-embedded tissue sections were stained immunohistochemically for STAT3, pSTAT3, and CD163. The values obtained from the tissue sections of each group were compared in statistical analysis.

Results: STAT3 showed a high level in HCC and was a significant marker for differentiating HCC from cirrhosis ($P < 0.0001$). The odds ratio between HCC and cirrhosis increased 34.4 times when the intensity of STAT3 increased by 1 level. Spearman's correlation and Chi-square tests also demonstrated that expression level of STAT3 did not correlate with age, gender, or the presence of a cirrhotic background.

Conclusions: STAT3 staining differs significantly in HCC and cirrhosis. The findings reinforce the role of STAT3 in the tumorigenesis of HCC and provide a useful marker to differentiate HCC from cirrhosis in challenging liver biopsies.

Key words: Cirrhosis; Differential Diagnosis; Hepatocellular Carcinoma; Signal Transducer and Activator of Transcription 3

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer in men and seventh most prevalent in women. HCC has become the second leading cause of cancer mortality worldwide, and most patients have poor prognosis related to late diagnosis.^[1] Although surgery is the best choice of treatment and gene-based treatments are being developed,^[2] only a few patients can be cured. Thus, surveillance of high-risk patients may reduce mortality. High-risk patients usually include those with cirrhosis caused by hepatitis B or C, or alcoholic or nonalcoholic steatohepatitis.^[3] The prevalence of cirrhosis in patients with HCC is 80–90% worldwide.^[4] Usually, the diagnostic tools of HCC include the serum tumor marker α -fetoprotein (AFP),^[5] radiographic imaging, and liver

biopsy, and liver biopsy is superior to the other tests, with a 96% sensitivity and 95% specificity.^[6] However, the diagnosis of HCC sometimes can be difficult. Although lobectomy specimens may be straightforward to make a diagnosis, the distinction of well-differentiated HCC from cirrhosis-based hyperplastic nodules in needle biopsies is not easy.^[7] According to the different structure

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and cytological characteristics, HCC can be divided into undifferentiated, poorly differentiated, moderately differentiated, and well differentiated. There are difficulties in histological identification, such as cirrhosis with hepatocellular adenoma and well-differentiated HCC.^[7] Early HCC is seen as a carcinoma *in situ*, displaying high proliferation and differentiation, and its differential diagnosis from dysplastic nodules mainly depends on a series of morphological characteristics, which are often hard to identify, especially in biopsy specimens.

As a member of the signal transducer and activator of transcription (STAT) family, STAT3 has received much attention during the past decade^[8] It is involved in many processes such as immune responses, inflammation, and tumorigenesis.^[9-11] The overexpression and constitutive activation of STAT3 have been suggested in many human cancers, and intervention in its pathway is also used in the treatment of cancer.^[12-15] Accumulating evidence implies that STAT3 is involved in HCC development, directly or indirectly.^[16-18] Thus, exploring the possible biological effects of STAT3 in HCC and cirrhosis will be an important issue. In HCC, STAT3 is supposed to be a critical oncogenic transcription factor, and it may enhance hepatic fibrosis by regulating transforming growth factor β expression.^[19] STAT3 can promote cell proliferation and prevent apoptosis by regulating expression of numerous apoptosis-related proteins, including Bcl-2, Mcl-1, survivin, and cyclin D1,^[13] and the carcinogenesis could be promoted by STAT3 though AAG8 (aging-associated gene 8 protein, encoded by the SIGMAR1 gene) both *in vitro* and *in vivo*.^[20] STAT3 also could bond with the promoter of vascular endothelial growth factor, promoting the angiogenesis and lymphangiogenesis of HCC and adjacent tissues.^[9,21]

In this study, we examined by immunohistochemistry the expression level of STAT3, phosphorylated STAT3 (pSTAT3), and CD163 in tissues from HCC and cirrhosis patients. In contrast to pSTAT3 and CD163, our results indicate that staining for STAT3 differs significantly in HCC and cirrhosis. The findings reinforce the role of STAT3 in the tumorigenesis of HCC and provide a useful marker to differentiate HCC from cirrhosis in challenging liver biopsies.

METHODS

Ethical approval

This study was performed with informed consent from the patients and approvals from the Peking Union Medical College Hospital and Blood Diseases Hospital.

Surgical specimens

Sixty liver samples were from Peking Union Medical College Hospital and Blood Diseases Hospital, between 2013 and 2015. Diagnostic criteria of liver cancer/cirrhosis were as described previously.^[22] None of the patients received preoperative chemotherapy or radiotherapy. The specimens were routinely fixed in formalin and embedded in paraffin, and the histopathological diagnosis based on multiple blocks

was confirmed by two independent pathologists without prior knowledge of each patient's clinical information.

Immunohistochemical staining

Immunohistochemical staining assay was performed in the Department of Pathology in Peking Union Medical College Hospital. Four-micrometer paraffin-embedded tissue sections were deparaffinized with xylene rinse and rehydrated in a graded ethanol series. Antigen retrieval was enhanced by autoclaving slides in sodium citrate buffer (pH 6.0) for 30 min, and subsequent cooling at 25°C for 30 min. Endogenous peroxidase activity was quenched by 10 min of incubation in 3% hydrogen peroxide in methanol. After washing three times with phosphate-buffered saline, the slides were incubated with primary antibodies at 4°C overnight: polyclonal antibody to STAT3 (1:50 dilution, sc-7179; Santa Cruz Biotechnology, USA); monoclonal antibody to pSTAT3 (Tyr 705) (1:500 dilution, 9145; Cell Signaling Technology, USA); and monoclonal antibody to CD163 (1:50 dilution, NB110-59935; Novus Biologicals, USA). Antigen-antibody complexes were detected by the avidin-biotin-peroxidase method with 3,3'-diaminobenzidine tetrahydrochloride. Finally, the slides were counterstained with hematoxylin and examined by light microscopy. All the immunostaining was performed on the same tissue block for each case.

Evaluation of signal transducer and activator of transcription 3, phosphorylated signal transducer and activator of transcription 3 and CD163 immunohistochemical staining

The results for staining were assigned a combined score based on the proportion and intensity of staining. The proportion represented the estimated fraction of cells positive for nuclear/cytoplasmic staining (Score 0, no positive cells; Score 1, positive cells $\leq 25\%$; Score 2, positive cells 26–50%; Score 3, positive cells 51–75%; and Score 4, positive cells $\geq 76\%$). The intensity represented the estimated average nuclear/cytoplasmic staining intensity of positive cells. The staining intensity was scored as negative (0), weak (1), moderate (2), and strong (3). The combined score was expressed as the sum of the proportion and intensity scores and then divided into four major scores: Score 0, summed Grades 0–1; Score 1, summed Grades 2–3; Score 2, summed Grades 4–5; and Score 3, summed Grades 6–7. The rare cases with discordant scores were re-evaluated and scored on the basis of consensual opinion.

Statistical analysis

All calculations were performed using SAS version 9.2 statistical software (SAS Inc., Cary, North Carolina, USA). Data are expressed as means \pm standard deviation (SD). To compare values obtained from these groups, statistical analyses were performed using analysis of variance (ANOVA) or Chi-square test, the relationships between STAT3 and other variables were analyzed using Spearman's rank correlation, and logistic regression analysis was used to estimate the role of STAT3 in differentiating HCC from cirrhosis. The values of $P < 0.05$ were considered statistically significant.

RESULTS

Signal transducer and activator of transcription 3 showed a high level in hepatocellular carcinoma

The sixty cases were divided into three groups: patients with HCC only (Group 1), HCC and cirrhosis (Group 2), and cirrhosis only (Group 3). The characteristics of gender and age in each group are shown in Table 1. The ANOVA and Chi-square test were performed and indicated that there was no significant difference in age ($P = 0.78$) and gender ($P = 0.25$) among the three groups. Hematoxylin-eosin and immunohistochemical staining showed a clear boundary between the tumor and normal tissues [Figure 1a and 1c]. Cells next to the tumor were squeezed into a shuttle shape, and tumor cells had a high degree of malignancy [Figure 1b]. All the liver cancer tissues showed abnormal karyograms with fat vacuoles. Immunohistochemical staining also showed that STAT3 was significantly activated [Figure 1c and 1d]. However, cirrhosis tissue showed normal karyograms, and mainly Scores 0 and 1 were detected. In Group 1, the positive rate of STAT3 was 94% (30/32 cases), and Scores 2 and 3 accounted for 72% of the 30 cases. In Group 2, we performed immunohistochemical staining of cancer and cirrhotic tissue. In HCC tissues, all cases were STAT3 positive, whereas in cirrhotic tissues, only two cases were positive for STAT3 (Score 1). In the cirrhotic tissues in Group 3, STAT3 was positive in 47% (9/19 cases), including 37% staining weakly positive and 10% staining moderately positive [Figure 1e].

Table 1: Clinical parameters for 60 samples from patients with hepatocellular carcinoma or cirrhosis

Parameters	Group 1 (n = 32)	Group 2 (n = 9)	Group 3 (n = 19)	Statistics	P
Age (years)	55 ± 13	55 ± 9	58 ± 12	0.25*	0.7776
Gender (male), n	20	8	11	2.77†	0.2507

*F value; † χ^2 value.

We also detected another two signaling molecules (pSTAT3 and CD163) in the two tissues. pSTAT3 is phosphorylated at Tyr705 and forms dimers that can be translocated into the nucleus, where it combines with DNA and regulates target gene transcription.^[10] Therefore, expression of pSTAT3 may be higher in tumor tissues. CD163 staining may be positive in cirrhosis. It is a macrophage lineage-specific hemoglobin-haptoglobin scavenger receptor and a specific marker of activated monocytes/macrophages. It is expressed exclusively on macrophages (Kupffer cells) in liver diseases, such as hepatitis and cirrhosis.^[23] However, unlike STAT3, the results from pSTAT3 and CD163 studies did not show specificity for HCC or cirrhosis.

Signal transducer and activator of transcription 3 could be a significant marker for differentiating hepatocellular carcinoma from cirrhosis

To clarify the difference in STAT3 expression between the two groups, we performed logistic regression analysis from the 28 cirrhosis and 47 liver cancer tissue slices, showing that STAT3 was a significant marker for differentiating HCC from cirrhosis ($P < 0.0001$). The odds ratio between HCC and cirrhosis increased 34.4 times when the intensity of STAT3 increased by 1 level [Table 2]. This showed that STAT3 may could serve as an effective marker to differentiate liver cancer from cirrhosis.

To establish whether age, gender, or the presence of a cirrhotic background affected the score in HCC tissue, Spearman's correlation and Chi-square test were performed and demonstrated that expression level of STAT3 did not correlate with these factors [Table 3].

DISCUSSION

We retrieved sixty cases from our surgical pathology files. Formalin-fixed, paraffin-embedded tissue sections were

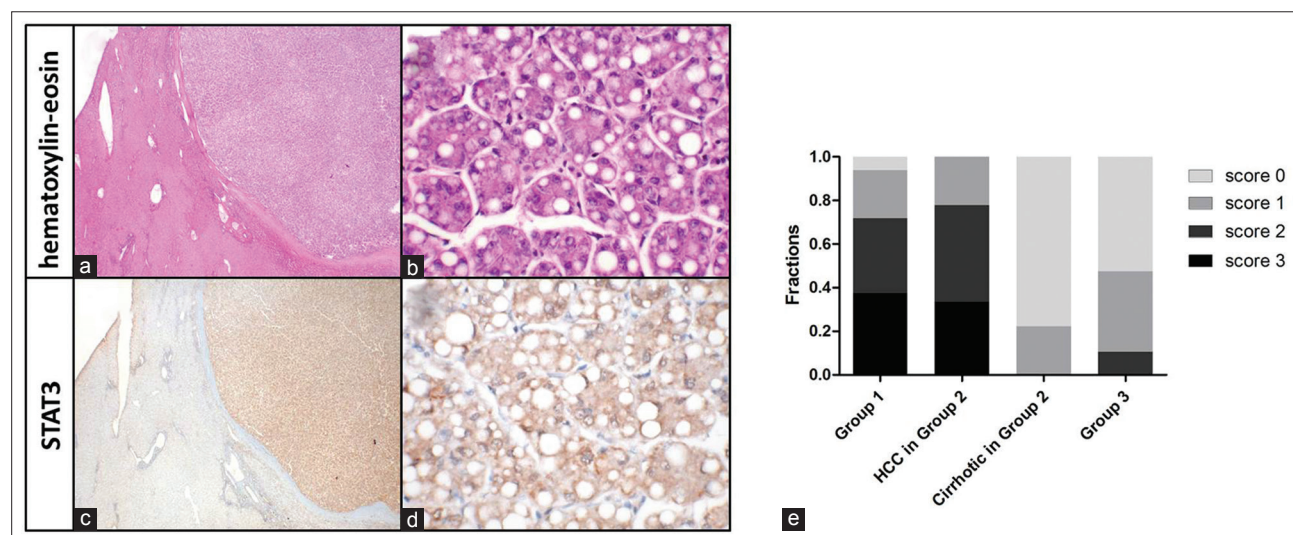


Figure 1: The staining and score fractions of signal transducer and activator of transcription 3. (a-d) H and E, ([a and b], original magnification, $\times 400$) and immunohistochemical analysis of signal transducer and activator of transcription 3 ([c and d], original magnification, $\times 400$) in hepatocellular carcinoma tissue. (e) Fractions and distributions of the scores in different groups. STAT: Signal transducer and activator of transcription.

Table 2: Relationship between STAT3 immunoreactivity and clinical parameters in hepatocellular carcinoma and cirrhotic tissues

Pathological type	Score				P	OR	95% Wald confidence limits
	0	1	2	3			
HCC, n	2	9	15	15	<0.0001	34.4	9.44; 125.14
Cirrhosis, n	17	9	2	0			

STAT: Signal transducer and activator of transcription; HCC: Hepatocellular carcinoma; OR: Odds ratio.

Table 3: Gender and age distribution of hepatocellular carcinoma patients, n

Items	Score				Statistics	P
	0	1	2	3		
Gender (male)	2	7	9	10	1.800*	0.78
Age (years)						
≤50	1	4	5	5	-0.015†	0.93
51-70	1	3	7	10		
>71	0	2	3	0		
Cirrhotic background (yes)	0	2	4	3	0.850*	1.00

* χ^2 values; †Spearman r_s value.

stained immunohistochemically for STAT3, pSTAT3, and CD163, and STAT3 was positive in most HCC tissues, and the scores of the STAT3 level in HCC tissues were significantly higher than in cirrhosis tissues. Logistic regression indicated that STAT3 could be a significant marker for differentiating HCC from cirrhosis.

Most HCC is caused by progressive liver fibrosis and cirrhosis, and liver cancer may be a multistep, incremental process starting from cirrhotic nodules and precancerous lesions progressing to malignant transformation. The main factors that influence the prognosis of HCC include tumor grade, liver function, and general health situation^[24] and tumor staging also is an independent predictor of survival. For early HCC patients, surgery, local treatment, and liver transplantation may improve the cure rate. Thus, it is necessary to establish an early diagnostic method, and monitoring and early detection of patients with cirrhosis to identify tumor is the best treatment. Although biopsy is still the gold standard, sometimes, it is restricted. For example, blood clotting disorder caused by advanced cirrhosis or too little sample obtained from ultrasound-guided, fine-needle aspiration often hinders laparoscopic liver biopsy. During pathological diagnosis, immunohistochemistry can play an important auxiliary role. Some alternative immunological markers, such as HepPar1, glypican-3, CD34, and AFP, are helpful in differential diagnosis.^[6,7,25-27]

Much evidence suggests that STAT3 plays a significant role in the development of liver cancer.^[18,28,29] Constitutive activation of STAT3 exists in human liver cells and tissues.^[30] Continued activation of STAT3 in cancer cells leads to permanent changes in the genes that control cellular

processes and can promote tumorigenesis, angiogenesis, and metastasis by up-regulating cyclin D, c-myc, BCL-x1, BCL-2, vascular endothelial growth factor, and basic fibroblast growth factor.^[21,30] STAT3 also plays an important role in liver inflammation and fibrosis. STAT3 protects liver cells from damage, and cytokines such as interleukin (IL)-6 and IL-22 ameliorate fatty liver and promote liver regeneration by activating STAT3 in liver cells.^[31,32] Our results indicated that the positive rate of STAT3 was higher in HCC (>95%) than in cirrhotic tissues, and the cases of Score 2 and Score 3 in HCC tissues account for 73%, which accounted only for 7% in cirrhosis tissues [Table 2]. Therefore, expression level of STAT3 can be used as a specific index for the identification of liver cancer and cirrhosis.

Sensitivity is important when judging detection methods. Recent technological advance has significantly increased the detection rate of abdominal ultrasound, spiral computed tomography (CT), and magnetic resonance imaging (MRI), but they still have limitations. The accuracy of ultrasound is limited by the ability of the operator. The sensitivity of CT and MRI for HCC is related to tumor size and is high for tumors ≥ 2 cm and low for tumors < 1 cm. CT is not sensitive enough for detecting tumor progression in cirrhosis patients. MRI provides additional functional approaches for diagnosis and grading,^[33] but it has low efficiency in differentiating malignant from nonmalignant liver diseases. A variety of biochemical indicators, including AFP, have low diagnostic sensitivity for HCC.

Although the tissue sections of HCC were sufficient and usually detected by immunohistochemistry in clinical practice, immunohistochemistry for cirrhosis was not applied frequently. Hence, we only achieved 28 cirrhosis sections. Considering the error of the sample size between HCC and cirrhosis, we detected 41 HCC tissue sections for statistical analysis. The sample size could be enlarged for further study. In addition, our results indicated that the application of STAT3 in the differential diagnosis of HCC and cirrhosis in clinical was feasible, and we need to go deeper in the mechanism of the role of STAT3 in future.

Our logistic analysis showed that the odds ratio between HCC and cirrhosis increased 34.4 times when the intensity of STAT3 increased by 1 level. Spearman's correlation and Chi-square tests demonstrated that the expression level of STAT3 did not correlate with age, gender, or the presence of a cirrhotic background. In future research, we will enlarge the sample size to determine its specificity and sensitivity more accurately. The findings could reinforce the role of STAT3 in the tumorigenesis of HCC and provide a useful marker to differentiate HCC from cirrhosis in challenging liver biopsies. Besides its differential diagnostic function, inhibition of STAT3 activation in patients with HCC could be used as a therapeutic target. Cytokines and small molecules that activate liver cell STAT3 can be used for the treatment of acute liver injury, fatty liver, and alcoholic hepatitis. Therefore, STAT3 warrants further research.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012;379:1245-55. doi: 10.1016/S0140-6736(11)61347-0.
2. Zhou XN, Li GM, Xu YC, Zhao TJ, Wu JX. Knockdown of decoy receptor 3 impairs growth and invasiveness of hepatocellular carcinoma cell line of HepG2. *Chin Med J* 2016;129:2623-9. doi: 10.4103/0366-6999.192775.
3. Miller ZA, Lee KS. Screening for hepatocellular carcinoma in high-risk populations. *Clin Imaging* 2016;40:311-4. doi: 10.1016/j.clinimag.2015.11.010.
4. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: Incidence and risk factors. *Gastroenterology* 2004;127 5 Suppl 1:S35-50. doi: 10.1053/j.gastro.2004.09.014.
5. Charrière B, Maulat C, Suc B, Muscari F. Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma. *World J Hepatol* 2016;8:881-90. doi: 10.4254/wjh.v8.i21.881.
6. Bialecki ES, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB (Oxford)* 2005;7:26-34. doi: 10.1080/13651820410024049.
7. Shafizadeh N, Kakar S. Diagnosis of well-differentiated hepatocellular lesions: Role of immunohistochemistry and other ancillary techniques. *Adv Anat Pathol* 2011;18:438-45. doi: 10.1097/PAP.0b013e318234abb4.
8. Santoni M, Massari F, Del Re M, Ciccarese C, Piva F, Principato G, *et al.* Investigational therapies targeting signal transducer and activator of transcription 3 for the treatment of cancer. *Expert Opin Investig Drugs* 2015;24:809-24. doi: 10.1517/13543784.2015.1020370.
9. Siveen KS, Nguyen AH, Lee JH, Li F, Singh SS, Kumar AP, *et al.* Negative regulation of signal transducer and activator of transcription-3 signalling cascade by lupeol inhibits growth and induces apoptosis in hepatocellular carcinoma cells. *Br J Cancer* 2014;111:1327-37. doi: 10.1038/bjc.2014.422.
10. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, *et al.* Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat Med* 2004;10:48-54. doi: 10.1038/nm976.
11. Li XP, Yang XY, Biskup E, Zhou J, Li HL, Wu YF, *et al.* Co-expression of CXCL8 and HIF-1 α is associated with metastasis and poor prognosis in hepatocellular carcinoma. *Oncotarget* 2015;6:22880-9. doi: 10.18632/oncotarget.4412.
12. Huynh J, Etemadi N, Hollande F, Ernst M, Buchert M. The JAK/STAT3 axis: A comprehensive drug target for solid malignancies. *Semin Cancer Biol* 2017;45:13-22. doi: 10.1016/j.semcancer.2017.06.001.
13. Santoni M, Conti A, Piva F, Massari F, Ciccarese C, Burattini L, *et al.* Role of STAT3 pathway in genitourinary tumors. *Future Sci OA* 2015;1:FSO15. doi: 10.4155/fso.15.13.
14. Momtaz S, Niaz K, Maqbool F, Abdollahi M, Rastrelli L, Nabavi SM. STAT3 targeting by polyphenols: Novel therapeutic strategy for melanoma. *Biofactors* 2017;43:347-70. doi: 10.1002/biof.1345.
15. Geiger JL, Grandis JR, Bauman JE. The STAT3 pathway as a therapeutic target in head and neck cancer: Barriers and innovations. *Oral Oncol* 2016;56:84-92. doi: 10.1016/j.oraloncology.2015.11.022.
16. Xie J, Zhang Y, Zhang Q, Han Y, Yin J, Pu R, *et al.* Interaction of signal transducer and activator of transcription 3 polymorphisms with hepatitis B virus mutations in hepatocellular carcinoma. *Hepatology* 2013;57:2369-77. doi: 10.1002/hep.26303.
17. Yang YM, Lee CG, Koo JH, Kim TH, Lee JM, An J, *et al.* Ga12 overexpressed in hepatocellular carcinoma reduces microRNA-122 expression via HNF4a inactivation, which causes c-Met induction. *Oncotarget* 2015;6:19055-69. doi: 10.18632/oncotarget.3957.
18. Wu J, Zhang J, Shen B, Yin K, Xu J, Gao W, *et al.* Long noncoding RNA lncTCF7, induced by IL-6/STAT3 transactivation, promotes hepatocellular carcinoma aggressiveness through epithelial-mesenchymal transition. *J Exp Clin Cancer Res* 2015;34:116. doi: 10.1186/s13046-015-0229-3.
19. Yang C, Zheng SD, Wu HJ, Chen SJ. Regulatory mechanisms of the molecular pathways in fibrosis induced by MicroRNAs. *Chin Med J* 2016;129:2365-72. doi: 10.4103/0366-6999.190677.
20. Sun B, Kawahara M, Ehata S, Nagamune T. AAG8 promotes carcinogenesis by activating STAT3. *Cell Signal* 2014;26:1863-9. doi: 10.1016/j.cellsig.2014.04.001.
21. Haura EB, Turkson J, Jove R. Mechanisms of disease: Insights into the emerging role of signal transducers and activators of transcription in cancer. *Nat Clin Pract Oncol* 2005;2:315-24. doi: 10.1038/nconp0195.
22. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008;134:1752-63. doi: 10.1053/j.gastro.2008.02.090.
23. Kong LQ, Zhu XD, Xu HX, Zhang JB, Lu L, Wang WQ, *et al.* The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma. *PLoS One* 2013;8:e59771. doi: 10.1371/journal.pone.0059771.
24. Nathan H, Schulick RD, Choti MA, Pawlik TM. Predictors of survival after resection of early hepatocellular carcinoma. *Ann Surg* 2009;249:799-805. doi: 10.1097/SLA.0b013e3181a38eb5.
25. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morengi E, *et al.* Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology* 2007;45:725-34. doi: 10.1002/hep.21531.
26. Libbrecht L, Severi T, Cassiman D, Vander Borgh S, Pirenne J, Nevens F, *et al.* Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006;30:1405-11. doi: 10.1097/01.pas.0000213323.97294.9a.
27. Zhou F, Shang W, Yu X, Tian J. Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment. *Med Res Rev* 2017. [Epub ahead of print]. doi: 10.1002/med.21455.
28. Gao B. Cytokines, STATs and liver disease. *Cell Mol Immunol* 2005;2:92-100.
29. Quétier I, Brezillon N, Duriez M, Massinet H, Giang E, Ahodantin J, *et al.* Hepatitis B virus HBx protein impairs liver regeneration through enhanced expression of IL-6 in transgenic mice. *J Hepatol* 2013;59:285-91. doi: 10.1016/j.jhep.2013.03.021.
30. Calò V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N, *et al.* STAT proteins: From normal control of cellular events to tumorigenesis. *J Cell Physiol* 2003;197:157-68. doi: 10.1002/jcp.10364.
31. Ki SH, Park O, Zheng M, Morales-Ibanez O, Kolls JK, Batailler R, *et al.* Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: Role of signal transducer and activator of transcription 3. *Hepatology* 2010;52:1291-300. doi: 10.1002/hep.23837.
32. Horiguchi N, Ishac EJ, Gao B. Liver regeneration is suppressed in alcoholic cirrhosis: Correlation with decreased STAT3 activation. *Alcohol* 2007;41:271-80. doi: 10.1016/j.alcohol.2007.04.008.
33. Zou LQ, Chen J, Pan L, Jiang JZ, Xing W. Comparison of magnetic resonance elastography and diffusion-weighted imaging for staging hepatic fibrosis. *Chin Med J* 2015;128:620-5. doi: 10.4103/0366-6999.151659.