Clinicopathologic significance of slug expression in human intrahepatic cholangiocarcinoma

Ke-Jun Zhang, Bing-Yuan Zhang, Kun-Peng Zhang, Li-Min Tang, Shi-Song Liu, Dong-Ming Zhu, Dian-Liang Zhang

AIM: To explore the expression and function of slug, a transcriptional repressor, in human intrahepatic cholangiocarcinoma (IHCC) and identify its role in IHCC progression.

METHODS: Expression of slug was detected in 36 cases of IHCC and 12 cases of normal intrahepatic bile ducts and liver parenchyma by immunohistochemistry. The patients were divided into low slug expression group (<20% of carcinoma cells stained) and high slug expression group (≥20% of carcinoma cells stained). Slug expression was correlated with clinicopathological parameters of IHCC patients. The patients were defined as short-term survivors if their survival time was <12 mo and as long-term survivors if their survival time was ≥12 mo.

RESULTS: Slug was not expressed in normal liver epithelium samples, lowly expressed in 15 tissue samples (10-, 5+) and highly expressed in 21 tissue samples (16++, 5+++). The survival rate of patients with a low slug expression was 33.3% (n = 5) and 66.7% (n = 10), respectively. The survival rate of patients with a high slug expression was 61.9% (n = 13) and 38.1% (n = 8), respectively (P = 0.02). Lymph node metastasis was found in 4 (26.7%) out of the 15 patients with a low slug expression and in 14 (66.7%) out of the 21 patients with a high slug expression, respectively. The incidence rate of lymph node metastasis increased with the increasing slug expression level (P = 0.003), and higher in patients with a high slug expression than in those with a low slug expression. Slug expression did not significantly correlate with the tumor size and stage or histologic grade, or with the gender and age of patients.

CONCLUSION: Slug expression is a novel prognostic marker for IHCC with lymph node metastasis.

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Key words: Intrahepatic cholangiocarcinoma; Transcriptional repressor Slug; Clinicopathologic significance

Peer reviewer: Dr. Theodora Choli-Papadopoulou, Associate Professor, Aristotle University of Thessaloniki, School of Chemistry, Department of Biochemistry, Thessaloniki, 55124, Greece

Zhang KJ, Zhang BY, Zhang KP, Tang LM, Liu SS, Zhu DM, Zhang DL. Clinicopathologic significance of slug expression in human intrahepatic cholangiocarcinoma. World J Gastroenterol. 2010; 16(20): 2554-2557 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i20/2554.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i20.2554

INTRODUCTION

Intrahepatic cholangiocarcinoma (IHCC) is the second...
most common liver cancer in adults, accounting for approximately 10% of all primary liver cancers. Complete surgical resection is the only procedure for IHCC. The prognosis of IHCC is very poor because of its advanced stage when diagnosed and early metastasis. The prognostic factors for IHCC include curative resection, hepatitis B surface antigen (HBsAg), as well as tumor, node and metastasis (TNM) stage, tumor size, lymph node metastasis, perineural invasion, portal vein invasion, and intrahepatic metastasis.

Slug, a member of the snail family of transcription factors, plays a crucial role in regulation of epithelial-mesenchymal transition by suppressing several epithelial markers and adhesion molecules, including E-cadherin. It has been shown that slug is overexpressed in breast, esophageal squamous cells, colorectal, gastric carcinomas and lung adenocarcinoma, accompanying increased lymph node metastasis. In this study, we investigated whether slug expression is correlated to the invasion and lymph node metastasis of IHCC and whether it is a significant prognostic factor after surgical resection.

MATERIALS AND METHODS

Patients and tissue samples
From October 1998 to December 2008, 36 IHCC patients (21 men and 15 women) at the age of 31-76 years (mean: 60 ± 9 years) underwent liver resection at the Second Department of General Surgery in Affiliated Hospital of Qingdao University (China). All the patients were histologically confirmed to have IHCC. Thirty-six patients underwent hepatectomy with lymph node dissection in 30, and common bile duct resection with biliary reconstruction in 18. Lymph node metastasis, lymphovascular invasion, and distant metastasis were detected in 18, 12, and 8 patients, respectively. Clinicopathologic parameters were recorded and compared.

Histologic examination
Resected specimens were cut along the largest diameter, fixed with formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin (HE). Tumor diameter, bile duct spread type, vascular and lymphatic duct permeation of cancer cells, perineural invasion of cancer cells, and lymph node metastasis were observed and recorded.

Immunohistochemistry
Mouse monoclonal anti-slug antibodies diluted at 1:100 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and secondary antibodies to rabbit anti-mouse IgG (Bost, Wuhan, China) were used for immunostaining. Formalin-fixed and paraffin-embedded tissue blocks were cut into 4-μm thick sections. After deparaffinization and blocking of endogenous peroxidase with 0.3% hydrogen peroxide in methanol for 30 min, the sections were immersed in 10 mmol/L citrate buffer (pH 6.0), dried in a microwave oven for 25 min, and washed with 50 mmol/L Tris-buffered saline (TBS; pH 7.6). After preincubation with normal goat serum (DAKO, Hamburg, Germany), the sections were incubated with appropriate primary antibodies for 1 h at room temperature, washed with TBS, incubated with secondary antibodies (diluted at 1: 50) for 30 min, and washed again with TBS. Peroxidase reaction was visualized using 0.05% diaminobenzidine tetrahydrochloride (DAB) containing 0.01% hydrogen peroxide. The sections were counterstained with hematoxylin. IHC results were assessed with a scoring system as previously described.

Immunohistochemical staining was expressed as percentage of positively stained carcinoma cells. Slug expression in cytoplasm of cells was evaluated. The percentage of positively stained carcinoma cells was graded as: ≤ 5% of carcinoma cells stained, ++: ≥ 5% - < 20% of carcinoma cells stained, +++: ≥ 20% - < 50% of carcinoma cells stained, and ++++: ≥ 50% of carcinoma cells stained. Additionally, the examined cases were divided into low slug expression group and high slug expression group for statistical analysis.

Statistical analysis
All statistical analyses were carried out using the SPSS 11.0 software (SPSS, Chicago, USA). Differences between slug expression in tumor and its adjacent nontumorous tissues were analyzed using paired Student’s t test. Spearman bivariate correlation was used to determine positive or negative correlations between slug protein expression and clinicopathologic parameter of IHCC patients. Relation between slug protein expression and clinicopathologic parameters of IHCC patients was analyzed using the Pearson χ² test. P < 0.05 was considered statistically significant.

RESULTS

Histologic observation
Slug was detected in 26 of 36 tumor tissue samples (72.2%). Weak staining (+), moderate staining (++), and strong staining (++++) were found in 5, 16, and 5 tumor tissue samples, respectively. Slug was weakly expressed in 15 (- and +) tumor tissue samples and highly expressed in 26 (++ and ++++) tumor tissue samples, respectively. The positive slug staining (≥ 5% of carcinoma cells stained) was observed in cytoplasm of carcinoma cells. Slug was not expressed in normal intrahepatic bile ducts or liver parenchyma of 12 normal epithelial tissue samples.

Relation between slug expression and clinicopathologic features
The relation between slug expression and clinicopathologic parameters of IHCC patients is summarized in Table 1. The percentages of distant metastasis, positive nodal metastasis, and positive lymphatic invasion were significantly higher in patients with a high slug expression than in those with a low slug expression, as were the percentages of recurrence in hematogenous and lymph nodes.

The expression rate of slug was 66.7% and 33.3% in patients with a long survival time (> 12 mo) and a short
In this study, the expression of slug protein was determined in 38.1% and 61.9% of long-term survivors and short-term survivors, respectively ($P = 0.04$, Table 1).

### Table 1: Relation between slug expression and clinicopathologic parameters

|                     | Slug   | $P$ value |
|---------------------|--------|-----------|
|                     | Low expression ($n = 15$) | High expression ($n = 21$) |
| Gender              |        |           |
| Male ($n = 21$)     | 9      | 12        |
| Female ($n = 15$)   | 6      | 9         | > 0.050 |
| Age (yr)            |        |           |
| < 60 ($n = 17$)     | 8      | 9         | < 0.001 |
| ≥ 60 ($n = 19$)     | 7      | 12        | > 0.050 |
| Stage               |        |           |
| I - II ($n = 21$)   | 6      | 15        | < 0.005 |
| III - IV ($n = 15$) | 9      | 6         | > 0.050 |
| Size                |        |           |
| TI-2 ($n = 21$)     | 8      | 13        | < 0.005 |
| T3-4 ($n = 15$)     | 7      | 8         | > 0.050 |
| Histologic grading  |        |           |
| G1 ($n = 19$)       | 10     | 9         | < 0.005 |
| G2 + G3 ($n = 17$)  | 5      | 12        | > 0.050 |
| Lymph node metastasis |    |           |
| Positive ($n = 18$) | 4      | 14        | < 0.005 |
| Negative ($n = 18$) | 11     | 7         | < 0.005 |
| Lymphovascular invasion | |           |
| Positive ($n = 12$) | 3      | 9         | < 0.005 |
| Negative ($n = 24$) | 12     | 12        | < 0.005 |
| Distant metastasis  |        |           |
| Positive ($n = 8$)  | 1      | 7         | < 0.001 |
| Negative ($n = 28$) | 14     | 14        | < 0.001 |
| Hematogenous recurrence | |           |
| Positive ($n = 12$) | 3      | 9         | < 0.010 |
| Negative ($n = 24$) | 12     | 12        | < 0.010 |
| Lymph node recurrence |      |           |
| Positive ($n = 8$)  | 2      | 6         | < 0.005 |
| Negative ($n = 29$) | 13     | 16        | < 0.005 |
| Survival time (yr)  |        |           |
| < 1                 | 5      | 13        | < 0.005 |
| ≥ 1                 | 10     | 8         | > 0.050 |

survival time (< 12 mo), respectively. A high slug expression was seen in 38.1% and 61.9% of long-term survivors and short-term survivors, respectively ($P = 0.04$, Table 1).

### DISCUSSION

Slug (Snail), a snail-related zinc-finger transcription factor, is critical for the normal development of neural crest-derived cells and loss-of-function. It has been shown that slug mutations contribute to piebaldism and Waardenburg syndrome type 2 in a dose-dependent manner and slug is aberrantly expressed in a number of tumors, thus contributing to tumorigenesis\[13,16\]. It has been reported that overexpression of slug plays a role in progression of many malignancies such as breast, colon, and gastric cancers\[13,14,16\]. Slug up-regulation correlates with deeper invasion, lymph node metastases, and stage of esophageal squamous cell carcinoma\[14\]. It was reported that slug up-regulation is significantly related with distant metastases and advanced pTNM stage of gastric carcinoma\[16\]. In this study, however, we were unable to find a significant association between slug up-regulation and lymph node metastases of IHCC.

In this study, the expression of slug protein was detected by immunohistochemistry in 26 (72.2%) out of 36 IHCC tissue samples, indicating that slug is expressed in IHCC. However, slug was not expressed in normal intrahepatic bile ducts or liver parenchyma.

The poor prognosis of IHCC patients with a high slug expression seemed to be related to the high incidence of lymph node metastases. Slug was closely correlated with lymph node invasion, lymphovascular invasion, and distant metastasis ($P < 0.005$). The patients with lymphatic invasion and distant metastasis had a high slug expression. Furthermore, the higher recurrence of lymph node metastasis in patients with a high slug expression also indicated that slug might play an important role in lymph node metastasis of IHCC. In various other cancers, such as breast cancer\[14\], and esophageal squamous cell carcinoma\[14\], slug is positively correlated with lymph node metastasis, also indicating that slug may play an important role in invasion of sinusoidal space and portal tract. Nevertheless, the precise mechanism underlying lymphatic duct invasion and lymph node metastasis has yet to be clarified. Another mechanism underlying the affinity of cancer cells for lymphatic tissue may also present. Therefore, it is necessary to determine the precise mechanism underlying lymph node metastasis of IHCC.

In this study, high slug expression was significantly related to a short survival time of IHCC patients and was an independent factor for poor prognosis of IHCC patients. The outcome after surgery was significantly poorer in patients with a high slug expression than in those with a low slug expression, suggesting that slug may be involved in the progression of IHCC and serve as a marker for predicting the malignancy of IHCC after surgery.

It has been shown that slug is expressed in several malignancies, such as breast and gastric cancer\[13,14,16\]. Slug expression is independently correlated with tumor invasiveness in breast cancer\[13,15\]. It has also been shown that slug is present in all infiltrating ductal carcinomas with lymph node metastases\[14\] and slug expression is inversely correlated with the differentiation of breast cancer\[13\]. In our experiment, no correlation was found between slug expression and tumor differentiation, which may be attributed to the limited number of cases.

In conclusion, slug is expressed in IHCC and is an independent indicator of poor prognosis. Slug can be sued as a marker for predicting the outcome of patients with IHCC after surgical resection. IHCC patients with positive slug expression should be followed up carefully.

### COMMENTS

#### Background

The incidence of intrahepatic cholangiocarcinoma (IHCC) has been increasing in recent years. However, its cause remains largely unknown. Prognosis of IHCC is very poor because of early metastasis. Several recent studies have shown that slug is overexpressed in many cancers accompanying lymph node metastasis, which is related to shorter survival time of IHCC patients.

#### Research frontiers

In this study, slug was overexpressed, which is related to increased lymph node metastasis and shorter survival time of IHCC patients.

#### Innovations and breakthroughs

Recent reports have highlighted the importance of slug in carcinogenesis. Slug
is overexpressed in many cancers including IHCC, which is related to increased lymph node metastasis and shorter survival time of IHCC patients.

**Applications**

This study may foster a future strategy against IHCC by elucidating whether slug expression is correlated to invasion and lymph node metastasis of IHCC, and whether slug expression may be a significant prognostic factor after surgical resection.

**Terminology**

Slug, a member of the snail family of transcription factors, plays a crucial role in regulation of epithelial-mesenchymal transition by suppressing several epithelial markers and adhesion molecules, including E-cadherin.

**Peer review**

The results of this study support the use of novel biomarkers in diagnosis and treatment of IHCC. The histological identification of slug expression was carefully done and the conclusion is evidence-based.

**REFERENCES**

1. Hammill CW, Wong LL. Intrahepatic cholangiocarcinoma: a malignancy of increasing importance. J Am Coll Surg 2008; 207: 594-605
2. Khan SA, Miras A, Pelling M, Taylor-Robinson SD. Cholangiocarcinoma and its management. Gut 2007; 56: 1755-1756
3. Ben-Menachem T. Risk factors for cholangiocarcinoma. Eur J Gastroenterol Hepatol 2007; 19: 615-617
4. Lee WJ, Park JW. [Review: analysis of survival rate and prognostic factors of intrahepatic cholangiocarcinoma: 318 cases in single institute] Korean J Hepatol 2007; 13: 125-128
5. Nakagohri T, Kinosita T, Konishi M, Takahashi S, Gotofuda N. [Surgical treatment for intrahepatic cholangiocarcinoma] Nippon Rinsho 2006; 64 Suppl 1: 469-475
6. Tao LY, He XD, Qu Q, Cai L, Liu W, Zhou L, Zhang SM. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a case-control study in China. Liver Int 2009; Epub head of print
7. Bartlett DL. Intrahepatic cholangiocarcinoma: a worthy challenge. Cancer J 2009; 15: 255-256
8. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 2002; 3: 155-166
9. Barrallo-Gimeno A, Nieto MA. Evolutionary history of the Snail/Scratch superfamily. Trends Genet 2009; 25: 248-252
10. Jethwa P, Naqvi M, Hardy RG, Hotchin NA, Roberts S, Spychal R, Tselepis C. Overexpression of Slug is associated with malignant progression of esophageal adenocarcinoma. World J Gastroenterol 2008; 14: 1044-1052
11. Hajra KM, Chen DY, Fearon ER. The SLUG zinc-finger protein represses E-cadherin in breast cancer. Cancer Res 2002; 62: 1613-1618
12. Bolos V, Peinado H, Pérez-Moreno MA, Fraga MF, Esteller M, Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. J Cell Sci 2003; 116: 499-511
13. Côme C, Magnino F, Bibeau F, De Santa Barbara P, Becker KF, Theillet C, Savagner P. Snail and slug play distinct roles during breast carcinoma progression. Clin Cancer Res 2006; 12: 5395-5402
14. Uchikado Y, Natsugoe S, Okamura H, Setoyama T, Matsumoto M, Ishigami S, Aikou T. Slug Expression in the E-cadherin preserved tumors is related to prognosis in patients with esophageal squamous cell carcinoma. Clin Cancer Res 2005; 11: 1174-1180
15. Shioiri M, Shida T, Koda K, Oda K, Seike K, Nishimura M, Takano S, Miyazaki M. Slug expression is an independent prognostic parameter for poor survival in colorectal carcinoma patients. Br J Cancer 2006; 94: 1816-1822
16. Castro Alves C, Rosivatz E, Schott C, Hollweck R, Becker I, Sarbia M, Carneiro F, Becker KF. Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and Snail in the down-regulation of E-cadherin. J Pathol 2007; 211: 507-515
17. Shih JY, Tsai MP, Chang TH, Chang YL, Yuan A, Yu CJ, Lin SB, Liou GY, Lee ML, Chen JJ, Hong TM, Yang SC, Su JL, Lee YC, Yang PC. Transcription repressor slug promotes carcinoma invasion and predicts outcome of patients with lung adenocarcinoma. Clin Cancer Res 2005; 11: 8070-8078
18. Tamada S, Shibahara H, Higashi M, Goto M, Batra SK, Imai K, Yonezawa S. MUC4 is a novel prognostic factor of extrahepatic bile duct carcinoma. Clin Cancer Res 2006; 12: 4257-4264
19. Pérez-Mancera PA, González-Herrero I, Pérez-Caro M, Gutiérrez-Cianca N, Flores T, Gutiérrez-Adán A, Pintado B, Sánchez-Martín M, Sánchez-García I. SLUG in cancer development. Oncogene 2005; 24: 3073-3082
20. Martin TA, Goyal A, Watkins G, Jiang WG. Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. Ann Surg Oncol 2005; 12: 488-496
21. Kurrey NK, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol Oncol 2005; 97: 155-165
22. Gupta PB, Kupferwasser C, Brunet JP, Ramaswamy S, Kuo WL, Gray JW, Naber SP, Weinberg RA. The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. Nat Genet 2005; 37: 1047-1054

S-Editor Wang JL  L-Editor Wang XL  E-Editor Ma WH