Review

DOI: 10.5582/ddt.2016.01052

Advances of diagnostic and mechanistic studies of γ-glutamyl transpeptidase in hepatocellular carcinoma

Jufeng Xia[1], Peipei Song[2], Zhipeng Sun[3], Tatsuo Sawakami[1], Mingku Jia[4], Zhigang Wang[4,*]

[1] *Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;*
[2] *Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa-shi, Chiba, Japan;*
[3] *Oncology Surgery Department, Peking University Ninth School of Clinical Medicine, Beijing, China;*
[4] *Hepato-Biliary-Pancreatic Surgery Division, The Second Affiliated Hospital of Jilin University, Changchun, China.*

Summary **Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second major cause of cancerous deaths in the world, accounting for 80-90% of all cases of liver cancer with an assessed global incidence of 782,000 new cases and approximate 746,000 deaths in 2012. Preoperative laboratory data (des-γ carboxyprothrombin (DCP), α-fetoprotein (AFP), Indocyanine green retention 15 min (ICG-R15), and γ-glutamyl transferase (GGT)) should be completely assessed before deciding a treatment and predicting prognosis in order to improve the prognosis for patients with HCC. A few recent studies have suggested GGT as an independent prognostic indicator in cases with HCC. And the data of our and other research teams revealed that combination of GGT and ICG-R15 or other factors may improve the efficiency of GGT as a prognostic predictor. In addition of clinical studies, a few mechanistic studies had been performed and GGT was suggested to promote tumor progression and poor prognosis through inducing DNA damage and genome instability, releasing reactive oxygen species to activating invasion-related signaling pathway, blocking chemotherapy, regulating microRNAs, and managing CpG island methylation. Although there were a few mechanistic studies, further and accurate researches were still in need.**

Keywords: γ-Glutamyl transferase (GGT), indocyanine green retention 15 min (ICG-R15), prognosis, risk factor, hepatocellular carcinoma (HCC)

1. Introduction

Hepatocellular carcinoma (HCC) is the second chief culprit of cancer deaths worldwide. HCC caused a global incidence of 782,000 new sufferers and almost 746,000 deaths in 2012 (*1*). At present, hepatic resection is considered as the first treatment option for early stage HCC. Although improved diagnostic methods, surgical techniques, and perioperative period management have lead to better results (*2-5*), the striking rate of recurrence after hepatectomy is still a barrier that deteriorated patient prognosis, with a cumulative rate of 50-60% at 3 years and 60-80% at 5 years (*6,7*). As a result, there is an urgent need for surgeons to find out how to predict prognosis and take interventional measures as early as possible.

Up to now, certain risk factors of the prognosis of HCC have been studied, and some factors such as microvascular invasion (MVI), poor differentiation, and tumor size have been validated as important risk factors impairing prognosis after hepatectomy (*8*). Lately, a great number of studies on various subgroups of cases, such as patients with hepatitis B virus (HBV)-related HCC, hepatitis C virus (HCV)-related HCC, noncirrhotic HCC, non-alcoholic fatty liver disease-related HCC, or multinodular tumors, have investigated risk factors which predict prognosis of sufferers with HCC (*9,10*). And for these years, a series of biochemistry factors, such as α-fetoprotein (AFP), des-γ-carboxyprothrombin (DCP), indocyanine green retention 15 min (ICG-R15),

Released online in J-STAGE as advance publication August 18, 2016.

**Address correspondence to:*
Dr. Zhigang Wang, Hepato-Biliary-Pancreatic Surgery Division, The Second Affiliated Hospital of Jilin University, No 218, Ziqiang Road, Changchun 130041, China.
E-mail: flybirdgang@163.com

Table 1. Factors related to prognosis for patients with HCC

Examinations	Indicators	Applications
Laboratory data	AFP	Tumor marker in liver cancer
	DCP	Tumor marker in HCC
	GGT	Diagnostic marker for liver disease
	ICG-R15	Biomarker for liver reserve function
	GP73	Diagnostic marker for liver disease
	ALT	Biomarker for inflammation and liver injury
	HBsAg	Biomarker for HBV infection
	Platelet count	Diagnostic marker for liver disease
	COMP	Biomarker for liver fibrosis and early HCC
	AGE	Biomarker for cancer growth, and metastasis
Imaging data	Tumor size	
	Tumor number	
	Vascular invasion	
Pathological data	Tumor differentiation	
	Microvascular invasion	
	Intrahepatic metastasis	

AFP: α-fetoprotein; DCP: Des-γ-carboxyprothrombin; GGT: γ-glutamyl transferase; ICG-R15: Indocyanine green retention 15 min; GP73: Golgi protein 73; ALT: Alanine aminotransferase; HBsAg: Surface antigen of the hepatitis B virus; COMP: Cartilage oligomeric matrix protein; AGE: Advanced glycation end products.

and γ-glutamyl transpeptidase (GGT), had been studied and utilized as risk predictor for tumor progression and prognosis. Among them, GGT attracted more attention for its advantage of predicting the postoperatively prognosis.

GGT is an enzyme that transfers γ-glutamyl functional groups (*11*). It exists in the cell membranes of many tissues and involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, certain L-amino acids, and peptides, leaving the cysteine product to preserve intracellular homeostasis of oxidative stress. There are increasing amount of researches suggesting that GGT may play an important role of predicting prognosis for patients with HCC.

2. GGT as a predictive biomarker in clinical investigations

In Table 1, laboratory data, imaging data, and pathological data have identified some indicators as risk factors of prognosis for sufferers with HCC (*12-15*). Microvascular invasion (MVI), tumor size, and tumor number indicated in imaging data are considered as risk factors for prognosis. And imaging studies have been given weight before deciding a treatment and predicting the prognosis for patients with HCC. However, as some research have revealed, a tumor may recur in approximately 60.0% of sufferers with a single tumor smaller than 2.0 cm (*16*). As a result, more methods to predict prognosis risk factors are urgently needed besides imaging data. Pathological data cannot validate pathologic changes prior to operation. In contrast, laboratory testing of AFP, DCP, ICG-R15, and GGT can be performed before surgery. Therefore, these indicators should be considered as a way to select a treatment and predict survival and recurrence for patients with HCC.

Patients with positive laboratory data for AFP, DCP, ICG-R15, and GGT have a higher risk of poor prognosis (*17-19*). These sufferers should be administrated with more effective treatments including anatomical hepatectomy, liver transplantation, preoperative and postoperative transcatheter arterial chemoembolization (TACE), and timely follow-up. Laboratory data for DCP and AFP are correlated to malignant conditions such as MVI and metastasis. ICG-R15 is suggested to be correlated to liver function (*17,20*). Lately, GGT has been validated as an independent prognostic risk indicator for patients with HCC (*18,21*).

GGT is a critical enzyme which catalyzes the hydrolysis of glutathione and the transfer of γ-glutamyl residues, and GGT has been widely utilized as a biomarker for some tumors, such as lung cancer and ovarian cancer. GGT was researched and employed as a liver function indicator in the 1960s to 1970s (*22*). An increasing level of GGT can be detected in patients with hepatitis, steatosis, cirrhosis, or HCC at various stage (*23,24*). Up to now, a great number of clinical studies have reported a high level of abnormal GGT in sufferers with primary or secondary HCC. According to a study by Tsutsumi *et al*., detection of mRNA expression of GGT could be a useful method for diagnosis of HCC at the early stage because GGT mRNA may change from type-I to type-II during the progress of HCC (*25*). But GGT is found to be abnormal in most cases with different liver diseases, and a large number of various diseases and conditions (such as pancreatitis, obesity, and alcohol abuse) can also lead to high expression levels of GGT (*25-27*). Therefore, GGT was not regarded as a useful tumor indicator for detecting HCC for a long time. GGT was utilized as a diagnostic tumor biomarker for liver disease with a high sensitivity of 83-100%, but it only has a low specificity of 32% (*10*). Therefore, long since GGT was not utilized as an

Table 2. Investigation of GGT as a prognostic factor based on different subgroups of patients with HCC

Authors	Patients	Treatment	Results
Song *et al*. (*36*), 2015	384 cases	Hepatectomy	GGT > 50 U/L is significantly associated with poor RFS
Wang *et al*. (*37*), 2014	288 cases	Hepatectomy	GGT > 55 U/L is significantly associated with poor RFS
Zhao *et al*. (*34*), 2013	266 cases with multi-nodular HCC	Hepatectomy	GGT > 130 U/L was a preoperative predictor for microvascular invasion
Chen *et al*. (*38*), 2014	154 cases	TACE	GGT > 85 U/L is significantly associated with poor RFS
Hung *et al*. (*39*), 2013	150 cases	TACE and chemotherapy	GGT > 100 U/L is significantly associated with poor RFS
Nishigawa *et al*. (*40*), 2013	74 cases with HBV-related HCC	Entecavir	GGT > 50 U/L is found to be significant prognostic factors linked to RFS
Nishigawa *et al*. (*41*), 2014	368 cases with solitary HCC	Radiofrequency ablation	GGT > 80 U/L is significantly associated with poor RFS

effective risk factor for the detection of liver disease. Nevertheless, GGT has important clinical significance as a predictive biomarker of prognosis. This finding was reported by researches based on different subgroups of cases published over the past five years. In the light of a study by Sheen *et al*., patients with HCC with type-II GGT mRNA had poorer prognosis, such as worse results, earlier recurrence, and higher death rates (*28*). A few studies of cases with HCC receiving hepatectomy have suggested a relationship between increasing levels of GGT and decreasing level of survival rate for patients with HBV-related HCC, Child-Pugh A liver function, or multi-nodular tumors (*29*). Moreover, a few studies have showed the predictive value of GGT in cases with unresectable HCC who received TACE or chemotherapy (*30-35*). In a clinical study of our research team, patients operating characteristic curves of 384 cases with single primary HCC who received hepatectomy were charted to validate the topgallant cutoff value of GGT was 50 U/L for recurrence-free survival (RFS) and 100 U/L for survival. After above analysis, GGT > 50 U/L was considered as a preoperative independent predictor impairing 1-, 3-, and 5-years RFS; GGT >100 U/L was considered as a independent predictor impairing 1-, 3-, and 5-years survival before operation. These results further validate the function of GGT as a preoperatively independent predictor correlated with tumor recurrence and overall survival in cases with HCC.

In Table 2, sufferers with high levels of GGT were apt to commit early recurrence and lower overall survival rate, including sufferers with multi-nodular HCC, HBV-related HCC, and those who received TACE, radiofrequency ablation, or entecavir. Hepatectomy, ultrasonography, CT and MR imaging and a timely follow-up are advised for these patients

Table 3. GGT combined with other risk factors

Authors	Patients	Factors
Song *et al*. (*36*), 2015	384 cases	GGT + ICG-R15
Norman *et al*. (*42*), 2015	187 cases	GGT + COMP
Cho *et al*. (*43*), 2014	337 cases	GGT + MPV
Kan *et al*. (*44*), 2014	90 cases	GGT + AGE
Hou *et al*. (*45*), 2013	79 cases	GGT + AFP + GP73

GGT: γ-glutamyl transferase; ICG-R15: Indocyanine green retention 15 min; COMP: Cartilage oligomeric matrix protein; MPV: Mean platelet volume; AGE: Advanced glycation end products; AFP: α-fetoprotein; GP73: Golgi protein 73.

(*29,36-40*). The combination of GGT and other indicators, such as tumor size, tumor number, MVI, or laboratory data for AFP and DCP, should be paid more attention when deciding a treatment and predicting the curative results for patients with HCC (*41*).

As shown in Table 3, recently, besides AFP and DCP there were more laboratory indicators which were thought highly of prognostic prediction and were combined with GGT to predict the recurrence. Norman *et al*. reported that combination of cartilage oligomeric matrix protein (COMP) > 15 U/L and GGT > 50 U/L was associated with cirrhosis and poor prognosis for patients with HCC (*42*). In 2014, Cho *et al*. published their research results that combination of mean platelet volume (MPV) and GGT was considered as malignant indicator (*43*). And in the same year, research result from Kan *et al*. suggested that advanced glycation end products combined with GGT would be indicators for non-B or non-C HCC (*44*). In 2013, Hou *et al*. reported that the combination of α-fetoprotein (AFP), Golgi protein 73 (GP73), and GGT might serve as a potential predictive method for HCC (*45*). In a clinical study of our team, GGT and ICG-R15 were focused on as

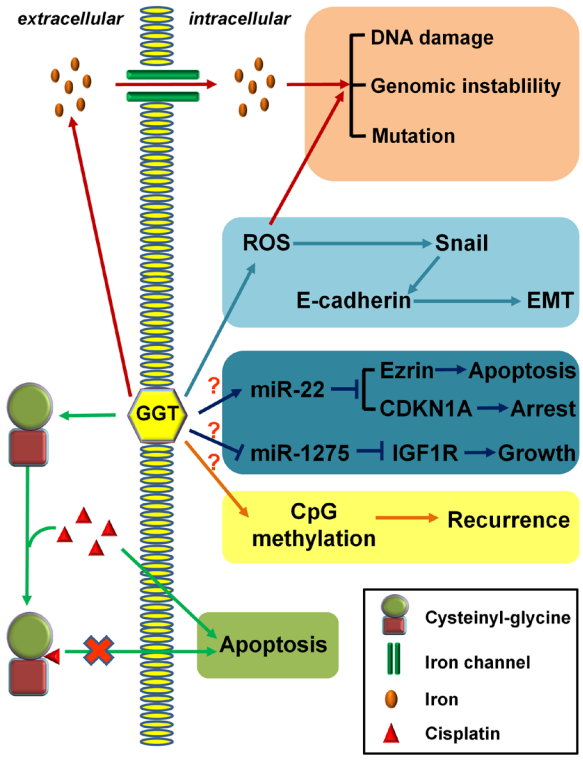

Figure 1. The molecular mechanisms of GGT in HCC.

predictors of prognosis in patients with single primary HCC who received hepatectomy (*36*). GGT > 100 U/L was considered as a preoperative independent predictor correlated with survival, and GGT > 50 U/L + ICG-R15 > 10% were considered as preoperative independent predictor correlated with tumor recurrence. Sufferers with GGT > 50 U/L + ICG-R15 > 10% commonly had a worse 1-, 3-, and 5-years RFS, and this was also true in cases with a tumor < 5 cm in size. These results indicate that combination of high levels of GGT and ICG-R15 should be paid more attention as a preoperative indicator correlated with prognosis for patients with single primary HCC receiving hepatectomy.

3. GGT as a functional macromolecule in mechanistic studies

The reason why GGT is significantly correlated to high level of recurrence and low level of survival has not yet to be illustrated. As shown in Figure 1, there are five possible mechanisms: *i*) GGT may be correlated to poor prognosis *via* leading to DNA damage and subsequent oncogenesis; *ii*) GGT may be correlated to the degree of malignant outcomings, such as MVI, metastasis, and epithelial-mesenchymal transition (EMT) through promoting certain signaling pathways; *iii*) GGT may be correlated to worse chemotherapeutic results by blocking the permeation of chemotherapy medicine into tumor cell; *iv*) GGT may be correlated to recurrence by regulating certain nucleic acid molecule to promote tumor growth and survival; *v*) GGT may be correlated to drug resistance and recurrence of HCC through leading to CpG island methylation in certain regains in genome.

An increasing number of researches have clarified mechanisms of GGT over the recent years. In a study, it was suggested that GGT lead to DNA damage, genomic instability, and oncogenesis-related mutations by promoting the uptake of iron (*46*), and the role of iron playing in carcinogenesis was already reported by Weinberg *et al*. (*47*). This mechanism is suggested to cause the death of normal liver cells or the destroying of normal liver function. The pro-oxidant function of GGT has been revealed and the subsequent product reactive oxygen species (ROS) may activate some intra- and extracellular molecular signaling pathways (*48*). Lately, ROS were suggested to induce EMT through the Snail/E-cadherin signaling pathway (*49*) and to promote inflammation and invasion by the NF-κB signaling pathway (*50,51*). Another research of U937 lymphoma cells discovered that GGT may play a role in anti-apoptosis (*52*). A research team has revealed that cysteinyl-glycine, which is catalyzed by GGT, is able to combine with cisplatin to form complexes which are not easily transported through the cell membrane (*53,54*).

In a study, it was revealed that increasing level of GGT was significantly related with changes of expression level of miR-22 and miR-1275 (*55*). In other researches, the abnormal expression levels of these two microRNAs were testified to promote anti-apoptosis and tumor growth (*56-58*). A recent study suggested that the up-regulated level of GGT was associated with the CpG island methylation in certain regains in genome, and CpG island methylation had already been reported to be correlated to tumor progression in various cancers (*59*). These mechanisms are considered to explain the progression and poor prognosis of HCC. Although the significance of molecular mechanisms of GGT to worse liver function, progression, and prognosis of HCC is indicated, these molecular mechanisms should be illustrated in further studies. From these studies it is not difficult for us to find that GGT may play a significant role in tumor progression and poor prognosis through various signaling pathways and mechanisms, thus GGT may become a novel target for tumor treatment in addition of as a predictor.

4. Conclusions

In conclusion, preoperative laboratory data (DCP, AFP, ICG-R15, and GGT) should be completely assessed before deciding a treatment and predicting prognosis in order to improve the prognosis for patients with HCC. A few recent studies have suggested GGT as an independent prognostic indicator in cases with HCC. In study of our research team, it was suggested that the preoperative role of GGT > 50 U/L and ICG-R15

> 10% as independent prognostic indicator for tumor recurrence in cases with single primary HCC who received hepatectomy. Patients with up-regulated levels of GGT and ICG-R15 had a worse 1-, 3-, and 5-years RFS. Therefore, combination of high levels of GGT and ICG-R15 could be useful for assessing prognosis postoperatively. In addition, some novel combination methods also may improve the prediction of prognosis of patients with HCC. Although a few molecular mechanisms of GGT were reported and these findings shed light on future functional study of GGT, the further and accurate mechanistic analysis was still in need.

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(Received July 16, 2016; Revised August 2, 2016; Accepted August 7, 2016)