ISSN: 2476-2377

Research Article

International Journal of Cancer Research & Therapy

Clinicopathologic Significance of G Protein-Coupled Receptor 81 in Hepatocellular Carcinoma

Hye Won Lee[1], Jee Young Park[1], Ilseon Hwang[1], Byoung Kuk Jang[2], Keun-Gyu Park[3] and Yu Na Kang[1*]

[1]*Department of Pathology*

[2]*Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Korea*

[3]*Department of Internal Medicine, Kyung pook National University School of Medicine, Daegu, Korea*

[*]Corresponding author
Yu Na Kang, Department of Pathology, Keimyung University School of Medicine, 1095 Dalgubeoldae-ro, Dalseogu, Daegu 42601, Korea, E-mail: yunakang@dsmc.or.kr

Submitted: 02 Aug 2017; **Accepted**: 10 Aug 2017; **Published**: 17 Aug 2017

Abstract

Background/ Aims: *Lactate functions as a metabolic key player in cancer in various aspects. G-protein coupled receptor 81 (GPR81), a cell surface lactate receptor, is involved in the metabolism of lactate. However, only a few studies have been conducted on GPR81 expression in cancer, especially hepatocellular carcinoma (HCC). The present study aims to identify the clinical significance of GPR81 expression in HCC and its role as a prognostic factor.*

Methods: *Tissues were obtained from 197 patients who had undergone surgery for HCC. GPR81 expression level was assessed by immunohistochemistry. And the function of GPR81 on HCC cell growth and mobility was explored through cell line studies.*

Results: *GPR81 was overexpressed in the HepG2, Huh7, SNU182 and SK-Hep1 HCC cell lines and HCC tissues compared with that in the THLE-2 normal liver cell lines. Furthermore, high GPR81 expression levels were correlated significantly with disease recurrence. In addition, because of significant differences in cancer proliferation, migration, and invasion depending on the GPR81 expression level in various HCC cell line studies, GPR81 is believed to play a role in promoting aggressive cancer cell behavior.*

Conclusions: *As such, GPR81 expression level was determined to be a useful prognostic factor for predicting HCC progression. The present study is the first to report on GPR81 expression in HCC and its significance. Henceforth, GPR81 is expected to become a highly valuable candidate for future target therapy.*

Keywords: Hepatocellular carcinoma, GPR81 protein, Immunohistochemistry, Prognosis.

Introduction

Recent studies on cancer metabolism have gained attention owing to the increasing knowledge of cancer biology and development of significant therapeutic targets [1]. Malignant cells grow continuously at a rapid speed, resulting in a lack of oxygen and nutrients, such as glucose. Therefore, this requires changes in cellular metabolism. Despite the presence of oxygen in tumor cells, oxidative phosphorylation is inhibited, and the tumor cells become dependent on aerobic glycolysis, in which energy is obtained from fast glycolysis [2]. As a result, a large amount of lactate is generated as the final product [3,4]. Lactate production in cancer has various benefits in terms of survival, and the present study primarily focused on lactate, the final product. First, the lactate itself is one of intrinsic inflammatory mediators that cause chronic inflammation in the tumor microenvironment due to T-cells and macrophages [5]. Second, the increase of extracellular lactate inhibits differentiation of immune cells and plays a role in immune escape [6-8], and various cell line studies have confirmed that cell migration and angiogenesis are induced in a lactate concentration-dependent manner [9-12].

G-protein coupled receptor 81 (GPR81), also known as hydroxyl carboxylic acid receptor 1, a recently discovered lactate receptor, is supposed to involved in the metabolism of lactate [13]. GPR81 was first found in adipose tissue and skeletal muscle, which are major sites of lactate production [14]. An anti-lipolytic effect of lactate through GPR81 activation has been reported, and as a result, it has been studied as a treatment mechanism for dyslipidemia [13-15]. However, experimental studies examining GPR81 in cancer are almost nonexistent. Therefore, the present study conducted an investigation on GPR81 expression in malignant tumors, specifically in hepatocellular carcinoma (HCC).

HCC is the great majority of primary liver cancer in adults and is a leading cause of death from cancer worldwide [16]. Up to now, there have been many studies on diagnostic genes, prognostic factors, and genes for target therapy. However, even in hepatectomy, which

are expected to show the best effects, the five-year survival rate and recurrence rate were 30-50% and 70-85%, respectively [17]. And the only chemotherapeutic agent that is currently used and effective against HCC is Sorafenib, a multikinase inhibitor [18]. Therefore, there is an urgent need for the development of a new target that can increase the treatment response when personalized medicine is applied in parallel with localized treatment for HCC. Lactate is a metabolic key player, furthermore, tumor cell itself tries to regulate lactate level, because a continued increase of lactate suppresses continued tumor cell growth [19,20]. Therefore, studies regarding lactate and lactate-regulating genes, GPR81 can be considered very meaningful works in the fight against cancer. There have been previous studies that have confirmed that there is very low GPR81 expression in normal hepatocytes although this is a mouse model for lipolysis [21]. The present study began with the assumption that if lactate increases in HCC of human tissue, GPR81 expression would also increase.

Materials and Methods

Patients and tissue specimens

A total of 197 cases were obtained by retrieval of the pathology reports of patients who underwent surgery for HCC at the Keimyung University Dongsan Hospital from January 2000 to December 2010. The clinicopathological parameters of the patients were re-evaluated by a review of the patients' medical records and microscope slides of hematoxylin and eosin stain. The tumor-node-metastasis (TNM) stage was evaluated according to the seventh edition of the American Joint Committee on Cancer staging system. Patients dying of causes other than HCC and follow-up loss were excluded from this study. This study was officially approved by the Institutional review board. All study participants provided informed consent.

Tissue Microarrays (TMA) and Immunohistochemistry

GPR81 expression level was assessed by immunohistochemistry. All primary HCC samples were formalin fixed and paraffin embedded. The paraffin blocks containing representative tumor lesions were selected after review of the corresponding hematoxylin and eosin stain slides. One to three representative lesions from each case were marked on the source blocks and cored with a 3.0-mm diameter cylindrical device manually. And then, the each core was re-embedded into the recipient blocks. The TMA paraffin blocks were cut into to 4-μm thick slices, which were attached to slides. They were then soaked in xylene for 5 minutes three times for paraffin removal, passed through a moisturizing process, and washed with distilled water. Microwave oven antigen retrieval from tissue sections processed using 10mm sodium citrate buffer (pH 6.0). Immunohistochemistry for the GPR81 antigen was performed using an autostainer (LV360-2D) and UltraVision LP Kit (TL-060-HD) from Lab Vision Corporation (Fremont, CA, USA), according to the manufacturer's protocol. The rabbit polyclonal GPR81 antibody (1:100, NLS2095, Novus, Littleton, CO, USA) was applied for primary antibody. The slides were counterstained with hematoxylin. After the autostainer process, the slides were dehydrated through 100% alcohol, cleared and mounted with permanent mounting media. Tissue from pancreatic adenocarcinoma and pancreatic islet cell known to have high GPR81 expression were used as external positive control. The slides incubated without primary antibody were used as negative control. Positive and negative controls stained appropriately.

Evaluation of Immunohistochemical Staining

GPR81 was predominantly immunostained as cytoplasmic pattern, and it considered positive. GPR81 was stained in most of tumor cells, but intensities of GPR81 staining were diverse in the each case. GPR81 expression was assessed according to staining intensity, and was scored from 1 to 3 as follows: 1, weak staining; 2, moderate staining; and 3, strong staining. Representative examples of immunostaining are shown in **Fig. 1**. All sections were evaluated blinded to clinicopathological features or clinical outcome. In the adjacent normal tissues, GPR81 was sparsely expressed in the cytoplasm of vascular and lymphatic endothelial cells, bile ductular cells and histiocytes.

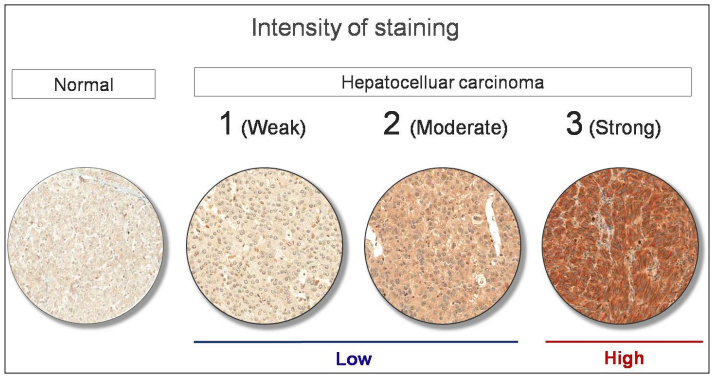

Fig.1. Immunohistochemical analysis of GPR81 expression in hepatocellular carcinoma tissues. The GPR81 stains were divided into low (scores 1 and 2) and high (score 3) expression groups for statistical analysis, according to intensity of staining.

Cell Lines and Western Blotting

Human HCC cell lines (HepG2, Huh7, PLC/PRF/5, SNU182, SNU398 and SK-Hep1) and immortalized normal hepatocyte cell line (THLE-2) were acquired from Kyung pook National University School of Medicine (Daegu, Korea). And cells were cultured basically according to the American Type Culture Collection (ATCC) protocols under strictly controlled conditions. Briefly, HCC cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Lonza, Cambridge, UK). For THLE-2 cell culture, precoated plate (mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin dissolved in BEBM medium) was prepared. And BEGM Bullet kit (CC3170, Lonza, Cambridge, UK) with 5 ng/mL EGF and 70 ng/mL phosphoethanolamine, 10% FBS was used. The cultures were maintained at 37 °C with a gas mixture of 5% CO_2 and 95% air. GPR81 expression was identified in cell lines using Western blot analysis. It was performed as previously describe and same antibody was applied as immunohistochemistry [22].

Cell Transfection with siRNA

The siRNAs On-Target plus Smart Pool reagent for GPR81 siRNA was purchased from Dharmacon Inc. (Lafayette, CO, USA). Transfection was performed using 100 nmol/L siRNA and 7.5μL Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Cell proliferation

To verify and support the immunohistochemistry, additional cell line studies were performed. THLE-2, SK-Hep1, SNU182 and HepG2 were targeted for experiments. At the time of seventy two hours after transfection using siRNA for GPR81, photographs were taken

and the transfected and untreated cell groups were counted under a microscope manually.

Migration assay

A wound healing assay was performed to detect HCC cell migration. Control and siRNA targeting GPR81 transfected SNU182 cells were seeded into 12-well tissue culture plates at a density of 2×10^5 cells per well. A sterile pipette tip was used to scratch the cell monolayers at the center of the well. The resulting gap distance is equal to the outer diameter of the end of the tip. After scratching, the well was washed with medium to remove the detached cells and replenished with fresh medium. The cells were maintained in a CO2 incubator, and observed after 24 hours. The photographs were taken by using a microscope at initial and end points.

Invasion Assay

Cell invasion was measured by using the Transwell migration apparatus (Costar, Cambridge, MA, USA). Briefly, cultured control and transfected cells were loaded 1×10^5 cells per well into the top of a 24-well invasion chamber assay plate with serum free media. The 10% FBS was added to the bottom chamber as an attractant. The chambers were assembled and maintained in an incubator for 24 hours. The cells on the upper surface were removed with gentle swabbing, and then the migrated cells on the bottom surface of the chamber were fixed with 20% methanol for 20 minutes and stained with 0.5% crystal violet for 15 minutes. The stained cells were observed under a microscope at × 200 magnification.

Statistical Analysis

For statistical analyses, the immunohistochemical staining scores were grouped into low (scores 1 to 2) and high (score 3) expression. Cross tabulations with $\chi 2$-statistic and Fisher's exact test were constructed in order to evaluate the association between GPR81 expression and clinicopathological parameters; age, gender, tumor number, histologic grade (Edmondson-Steiner's), TNM stage, disease recurrence and distant metastases. For survival analysis, two end points were considered, i.e., disease relapse (defined as either a local recurrence or metastasis) and expire with disease. Kaplan-Meier curves were used to estimate the distributions of disease-free survival (DFS) and overall survival (OS), and the differences in survival between the groups were compared using the log-rank test. DFS was measured as the duration from surgical resection to clinical evidence of disease relapse, or the last follow-up in patients with no evidence of recurrence or metastasis. The period of OS was measured from the date of surgical resection to the date of death or the last follow-up. The median duration of follow-up was 1361 days (range 8 to 3519). With TNM staging, stage I was considered as early, and stages II to III as advanced. All statistical analyses were carried out using the SPSS version 19.0 software package (SPSS-IBM Inc, Chicago, IL, USA), and $p < 0.05$ was considered statistically significant.

Results

Clinicopathological Variables

The patients'characteristics, incidence of disease progression and status of the patients are illustrated in **Table 1**. The study subjects comprised 163 (82.8%) men and 34 (17.3%) women with aged 29-76 years (mean 55 years). Ninety six patients (48.7%) had stage I disease; 62 patients (31.5%), stage II and 39 patients (19.8%), stage III, respectively. The histologic grades of tumor were as follows: 2 (1.0%) grade 1, 38 (19.3%) grade 2, 109 (55.3%) grade 3 and 48 (24.4%) grade 4. There were 72 patients without evidence of disease relapse and 125 patients (63.5%) had recurrent disease. A total of 43 patients (21.8%) died of the disease during the follow-up period. Low expression of GPR81 revealed lower histologic grade. These were statistically significant results ($p < 0.05$). Increased risk for recurrence was significantly related to high GPR81 expression ($p < 0.05$). On the other hand, GPR81 had no significant association with age, gender, presence of cirrhosis and possibility of disease-related death. High GPR81 expression tended to increase number of tumor and higher TNM stage, but it did not acquired statistical significance.

Table 1: Association between GPR81 Expression and Clinicopathological Parameter in Hepatocellular Carcinoma

Parameter	GPR81		p-value
	Low N (%)	High N (%)	
Age (years)			0.886
≤ 55	38 (19.3)	59 (29.9)	
> 55	36 (18.3)	64 (32.5)	
Gender			0.457
Male	60 (30.5)	103 (52.3)	
Female	14 (7.1)	20 (10.2)	
Cirrhosis			0.089
Absent	42 (21.3)	53 (26.9)	
Present	32 (16.2)	70 (35.5)	
Tumor number			0.067
Single	65 (33.0)	112 (56.9)	
Multiple	9 (4.6)	11 (5.6)	
Histologic grade			0.024*
Grade 1-2	22 (11.2)	18 (9.1)	
Grade 3-4	52 (26.4)	105 (53.3)	
TNM stage			0.09
I	37 (18.8)	59 (29.9)	
II-III	37 (18.8)	64 (32.5)	
Recurrence			0.036*
Absent	50 (25.4)	22 (11.2)	
Present	24 (12.2)	101 (51.3)	
Status of the patients			0.665
Alive	63 (32.0)	91 (46.2)	
Disease-related death	11 (5.6)	32 (16.2)	
Total	74 (37.6)	123 (62.4)	

N: number; TNM: tumor-node metastasis. p-values of $\chi 2$-tests are indicated; *: statistically significant ($p < 0.05$).

Survival Analyses

Kaplan-Meier curves revealed that the high expression of GPR81 was associated with poor disease-free survival and worse overall survival; however, the difference did not reach statistical significance ($p > 0.05$) (**Fig. 2**). The estimated five-year survival rate of low expression group (73.0%) is comparatively different from that of high expression group (53.7%).

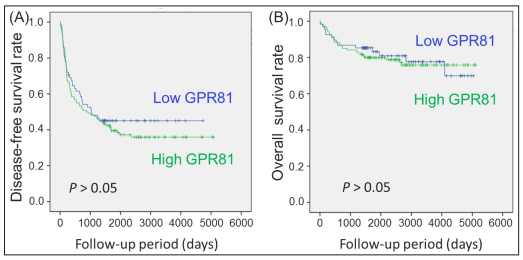

Fig.2. Disease-free survival (a) and overall survival (b) according to GPR81 expression groups in patients with hepatocellular carcinoma. High expression of GPR81 was associated with poor disease-free survival and worse overall survival; however, the difference did not reach statistical significance ($p > 0.05$).

Western Blot Analysis of GPR81 Expression

Initially GPR81 expression was identified in normal hepatocytes, THLE-2, and HepG2, Huh7, PLC/PRF/5, SNU182, SNU398 and SK-Hep1 HCC cell lines using Western blotting. GPR81 was highly expressed in HepG2, Huh7, SNU182 and SK-Hep1 cells, whereas PLC/PRF/5 and SNU398 revealed sparse expression. Normal hepatocytes, THLE-2 had only low levels of GPR81 expression (**Fig. 3**). Particularly HepG2, SNU182 and SK-Hep1 were selected for further functional analyses.

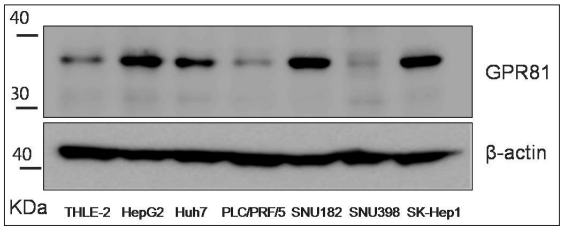

Fig.3. Western blot analysis of GPR81 expression level in various hepatocellular carcinoma cell lines. GPR81 expression was highly expressed in the hepatocellular carcinoma cell lines, such as HepG2, Huh7, SNU182 and SK-Hep1. But only normal hepatocytes, THLE-2 showed low GPR81 expression.

Tumor Cell Proliferation Study

To investigate the relationship between GPR81 expression and tumor cell proliferation, the present study assessed whether the depletion of GPR81 could suppress the propagation of cancer cells. After 72 hours, the GPR81 siRNA-treated SK-Hep1, SNU182, HepG2 cells did not grow well. Whereas a marked increase was seen in control during this same period of time and behaved as such. These differences were statistically significant ($p < 0.05$). Inhibition of GPR81 expression had no significant effect on THLE-2 cell proliferation (Fig.4).

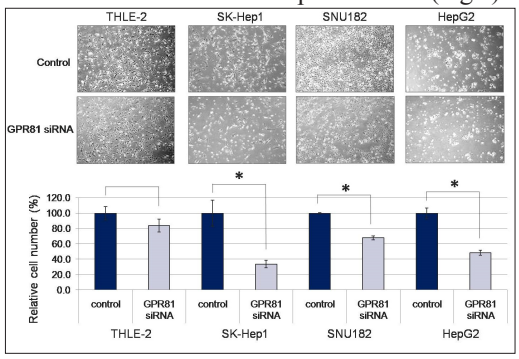

Fig.4. GPR81 expression related to hepatocellular carcinoma cell proliferation. After the transfection of GPR81 siRNA, GPR81 expression showed the significant decrease in tumor cell growth compared with control. But inhibition of GPR81 expression on THLE-2 cell line was not significant. *: statistically significant ($p < 0.05$).

Wound Healing Assay and Invasion Assay

The wound healing and invasion assays were conducted to evaluate the effects of GPR81 expression on HCC cell migration and invasion. In wound healing assays, as shown in **Fig. 5**, the migration rate of siRNA treated SNU182 cells was significantly reduced compared with control. Consistent with the wound healing assay results, reducing GPR81 expression also substantially inhibited cell invasion through a membrane in the invasion assay (**Fig. 6**). The same results applied in both SNU182 and SK-Hep1 cells.

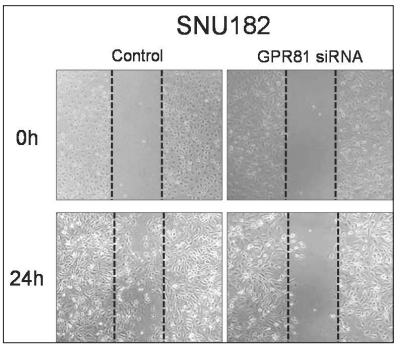

Fig.5. Migration-scratch assay of GPR81 siRNA on hepatocellular carcinoma cell line, SNU182. It revealed that GPR81 knockdown by GPR81 siRNA markedly inhibited cell migration potential.

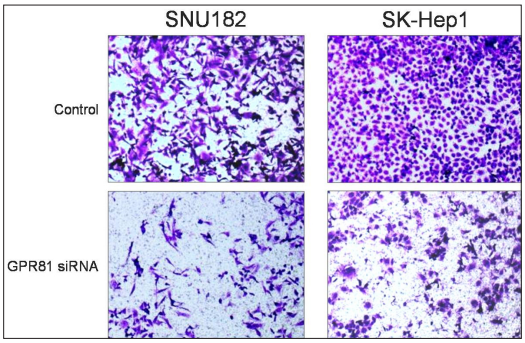

Fig.6. Invasion assay of GPR81 siRNA on hepatocellular carcinoma cell lines, SNU182 and SK-Hep1. The GPR81 siRNA revealed a significant suppression of cell invasion on all of SNU182 and SK-Hep1 cells.

Discussion

Lactate, a final product of glucose metabolism, is commonly maintained at high level in the fast-growing cancer cell environment [2]. In addition, it is known to affect the growth and maintenance of cancer cells in various aspects as mentioned previously [19]. Among several factors associated with lactate, GPR81 is a recently discovered lactate receptor that has been studied as one of the treatment mechanisms for dyslipidemia as it exhibits anti-lipolytic effects [14,15]. Furthermore, there have been reports that GPR81 plays a role in organ damage or ischemic brain injury caused by inflammation [23]. However, only few studies have been conducted on GPR81 expression in cancer. Roland et al. have suggested that GPR81 is associated with survival in pancreatic adenocarcinoma [24]. High expression of GPR81 was observed in most of the excised pancreatic cancer tissues confirmed on immunohistochemistry. In vitro studies as well as animal experiments have revealed that high GPR81 expression is associated

with tumor maintenance, growth, and metastasis. Moreover, it was proven that GPR81 is required for the expression of proteins involved in lactate metabolism, such as monocarboxylate transporter (MCT), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), and CD147. The most recent study on breast cancer also showed that GPR81 plays a critical role in the survival of tumors cells [25]. Two previous studies reported that high expression of GPR81 was associated with aggressive behavior of cancer [24,25]. Similarly, the HCC analysis results in present study are consistent with the results of previous studies. As the result of evaluating the relationship between GPR81 expression and its clinicopathological values in HCC, significant correlation was observed between GPR81 expression and disease recurrence. Also one of the well-known prognostic factors, histologic grade was related to GPR81 expression. High GPR81 expression tended to recur more and increase the possibility of disease related death on survival analysis. Various cell line studies showed that GPR81 contributes to proliferation, migration and invasion, which supports immunohistochemistry results. Taken together, these results indicate that GPR81 plays a role in regulating the cell proliferation, migration and invasion of HCC cells.

Based on these results, GPR81 is believed to play a role in promoting aggressive behavior of HCC. Moreover, this is likely to be associated with lactate. The mechanism of interaction between lactate and GPR81 has not yet been determined in terms of lactate metabolism and lactate transport. Therefore, the effect of GPR81 on lactate metabolism, i.e., its association with related genes verified only in the previous pancreatic cancer study may need to be confirmed also in the HCC study as additional research [24]. Cancer cells, themselves attempt to thoroughly maintain the lactate level because the increase of lactate disables consistent growth [20]. Hence, studies on GPR81-related mechanism may provide significant basis for the development of target therapies for HCC, which is resistant to chemotherapy. GPR81 could be a highly effective candidate for future target therapy.

Furthermore, GPR81 is associated with Peroxisome proliferator-activated receptor-γ (PPARγ), which is involved in the reduction of adipogenesis, and PGC-1α, which is involved in mitochondrial fatty acid oxidation within adipose tissue or white adipose tissue browning, among various mechanisms causing cancer cachexia, a loss of fat [26-28]. Most of all, GPR81 has a direct inhibitory effect on lipolysis. Thus, GPR81 negatively affects survival and quality of life, and it is expected to alleviate on cancer cachexia, which occurs in 50% of HCC cases [29].

It is well known that chronic hepatitis is a major risk factor of HCC, and long chronic hepatitis is ultimately converted to malignant transformation. It is also known that inflammasome, which is recently gaining spotlight in the field of neoplasm, consists of a nucleotide-binding domain, leucine-rich family, pyrin domain-containing 3 (NLRP3), an apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC) adaptor and caspase-1. The inflammasome plays a role in maturation and secretion of the proinflammatory cytokines, and causes inflammation [29]. However, there has been a recent report related to lactate that GPR81, is required for anti-inflammatory effects, i.e., the inhibition of a proinflammatory response and mediates them through inflammasomes [21]. Such associations with inflammasomes suggest that GPR81 plays a certain role in a series of process in which chronic hepatitis is converted to carcinoma. Hence, studies on the association between GPR81 and inflammasomes and the underlying mechanism may provide a clue for conversion of chronic hepatitis to HCC.

GPR81 is believed to play a role in promoting aggressive cancer cell behavior based on these study results. As such, GPR81 expression level is determined to be a useful prognostic factor for HCC progression. The present study is the first to report on GPR81 expression in HCC and its significance. These results may provide a novel prognostic marker in human HCC and contribute to elucidate the process of HCC development and progression. A further understanding of the mechanisms underlying the role of GPR81 in hepatocellular carcinogenesis will likely help in the identification of novel approaches for diagnosis and therapy of HCC.

Acknowledgements

This work was supported by grants (HI16C1501) from the Korea Health technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea.

References

1. Vander Heiden MG (2011) Targeting cancer metabolism: a therapeutic window opens. Nature reviews Drug discovery 10: 671-684.
2. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nature reviews Cancer 4: 891-899.
3. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324: 1029-1033.
4. Warburg O (1956) On the origin of cancer cells. Science 123: 309-314.
5. Yabu M, Shime H, Hara H, Saito T, Matsumoto M, et al. (2011) IL-23-dependent and -independent enhancement pathways of IL-17A production by lactic acid. International immunology 23: 29-41.
6. Dietl K, Renner K, Dettmer K, Timischl B, Eberhart K, Dorn C, et al. (2011) Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. Journal of immunology 184: 1200-1209.
7. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, et al. (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 109: 3812-3819.
8. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, et al. (2006) Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. Blood 107: 2013-2021.
9. Baumann F, Leukel P, Doerfelt A, Beier CP, Dettmer K, et al. (2009) Lactate promotes glioma migration by TGF-beta2-dependent regulation of matrix metalloproteinase-2. Neuro-oncology 11: 368-380.
10. Beckert S, Farrahi F, Aslam RS, Scheuenstuhl H, Konigsrainer A, et al. (2006) Lactate stimulates endothelial cell migration. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 14: 321-324.
11. Stern R (2008) Hyaluronidases in cancer biology. Seminars in cancer biology 18: 275-280.
12. Vegran F, Boidot R, Michiels C, Sonveaux P, Feron O (2011) Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kappaB/IL-8 pathway that drives tumor angiogenesis. Cancer research 71: 2550-2560.
13. Ahmed K (2011) Biological roles and therapeutic potential of hydroxy-carboxylic Acid receptors. Frontiers in endocrinology 2: 51.

14. Ahmed K, Tunaru S, Tang C, Muller M, Gille A, et al. (2010) An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. Cell metabolism 11: 311-319.
15. Cai TQ, Ren N, Jin L, Cheng K, Kash S, et al. (2008) Role of GPR81 in lactate-mediated reduction of adipose lipolysis. Biochemical and biophysical research communications 377: 987-991.
16. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, et al. (2015) Global cancer statistics, 2012. CA: a cancer journal for clinicians 65: 87-108.
17. Lu WP, Dong JH (2014) Hepatectomy for hepatocellular carcinoma in the era of liver transplantation. World journal of gastroenterology : WJG 20: 9237-9244.
18. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. (2008) Sorafenib in advanced hepatocellular carcinoma. The New England journal of medicine 359: 378-390.
19. Hirschhaeuser F, Sattler UG, Mueller-Klieser W (2011) Lactate: a metabolic key player in cancer. Cancer research 71: 6921-6925.
20. Doherty JR, Cleveland JL (2013) Targeting lactate metabolism for cancer therapeutics. The Journal of clinical investigation 123: 3685-3692.
21. Hoque R, Farooq A, Ghani A, Gorelick F, Mehal WZ (2014) Lactate reduces liver and pancreatic injury in Toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity. Gastroenterology 146: 1763-1774.
22. Lee HW, Choe M (2012) Expression of EZH2 in renal cell carcinoma as a novel prognostic marker. Pathology international 62: 735-741.
23. Shen Z, Jiang L, Yuan Y, Deng T, Zheng YR, et al. (2015) Inhibition of G protein-coupled receptor 81 (GPR81) protects against ischemic brain injury. CNS neuroscience & therapeutics 21: 271-279.
24. Roland CL, Arumugam T, Deng D, Liu SH, Philip B, et al. (2014) Cell surface lactate receptor GPR81 is crucial for cancer cell survival. Cancer research 74: 5301-5310.
25. Staubert C, Broom OJ, Nordstrom A (2015) Hydroxycarboxylic acid receptors are essential for breast cancer cells to control their lipid/fatty acid metabolism. Oncotarget 6: 19706-19720.
26. Jeninga EH, Bugge A, Nielsen R, Kersten S, Hamers N, et al. (2009) Peroxisome proliferator-activated receptor gamma regulates expression of the anti-lipolytic G-protein-coupled receptor 81 (GPR81/Gpr81). The Journal of biological chemistry 284: 26385-26393.
27. Sun VC, Sarna L (2008) Symptom management in hepatocellular carcinoma. Clinical journal of oncology nursing 12: 759-766.
28. Tisdale MJ (2009) Mechanisms of cancer cachexia. Physiological reviews 89: 381-410.
29. Wei Q, Mu K, Li T, Zhang Y, Yang Z, et al. (2014) Deregulation of the NLRP3 inflammasome in hepatic parenchymal cells during liver cancer progression. Laboratory investigation; a journal of technical methods and pathology 94: 52-62.