International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print), 2222-503X (Online)
http://www.innspub.net
Vol. 19, No. 3, p. 1-5, 2024

RESEARCH PAPER OPEN ACCESS

Comparative study of biological characteristics between paediatric hepatocellular tumors and adult hepatocellular carcinoma

Kosuke Kudo[*1], Juma Obayashi[1], Koji Fukumoto[3], Hiroaki Kitagawa[1], Shigeyuki Furuta[1], Junki Koike[2]

[1]*Department of Pediatric Surgery, St Marianna University Hospital, 2-16-1 Sugo, Miyamae-ku, Kawasaki, Kanagawa, Japan*

[2]*Department of Pathology, St Marianna University Hospital, 2-16-1 Sugo, Miyamae-ku, Kawasaki, Kanagawa, Japan*

[3]*Department of Pediatric Surgery, Shizuoka Children's Hospital, Urushiyama, Aoi-ku, Shizuoka City, Shizuoka, Japan*

Key words: Hepatoblastoma, Paediatric hepatocellular carcinoma, Adult hepatocellular carcinoma, GPC3

Article Published: 10 December 2024

Abstract

The biological differences between paediatric hepatocytic tumours (pHCT) and adult hepatocellular carcinoma (aHCC) remain unclear. This study aimed to compare the expression of hepatocyte-specific proteins, liver stem cell markers, and sinusoidal endothelial cell proteins in pHCTs and aHCCs using immunohistochemistry. A retrospective analysis identified 31 cases of pHCTs (24 hepatoblastomas and 7 paediatric hepatocellular carcinomas) treated at our hospital and other hospitals, where tumour biopsy or liver resection was performed. Pathological features were compared with 36 cases of aHCC. Tumour markers, including Hep Par-1, Arginase1, Glypican3 (GPC3), AFP, CD56, C-kit, and CD34, were examined. GPC3 was expressed in all pHCTs except for one hepatoblastoma case, while it was detected in only 18/36 aHCC cases, particularly those without chronic liver disease in adjacent tissue. HepPar-1 was expressed in all paediatric and adult tumours except for one pHCT case, and Arginase1 and AFP were universally expressed. CD56 and c-kit were not identified in any tumours, and CD4 expression was absent in sinusoidal microvascular endothelial cells, but CD34 was consistently expressed in all cases. These findings reveal significant biological differences between pHCC and aHCC, highlighting GPC3 as a potential characteristic marker of paediatric hepatocytic tumours.

***Corresponding Author:** Kosuke Kudo ✉ Kosuke.kudo@marianna-u.ac.jp

Introduction

Hepatocellular tumors encompass various types of malignant liver cancer which can arise in both children and adults. In this study, we aimed to compare the biological characteristics of pediatric hepatocellular tumors (hepatoblastoma and pediatric hepatocellular carcinoma) with those of adult hepatocellular carcinoma, and to identify the characteristics of each tumor type.

Pediatric hepatocellular tumors (pHCTs), which include hepatoblastoma (HB) and pediatric hepatocellular carcinoma (pHCC), are among the rarest solid malignancies seen in children. Conversely, adult hepatocellular carcinoma (aHCC) is the main form of liver cancer in adults, and is particularly frequent in individuals with hepatic cirrhosis. Among these hepatic tumors, current understanding of the differences between the biological characteristics of pHCC and aHCC in particular is incomplete, and the elucidation of these differences was therefore the focus of this study.

In this context, we conducted a comparison of the biological characteristics of pHCTs and aHCC by characterizing the protein expression of tumor and sinusoid microvascular endothelial cells that compose the tumor stroma of these tumor types.

Materials and methods

Patients

The study subjects were 31 pediatric patients who underwent tumor biopsy or hepatectomy for pHCTs at St Marianna University Hospital or Shizuoka Children's Hospital during the 42-year period from January 1979 of December 2021, and 36 patients who underwent hepatectomy for aHCC at St Marianna University Hospital in 2021. This

The pathological diagnoses of pHCT and aHCC were based on the International Pediatric Liver Tumor Consensus and the World Health Organization (WHO) classifications, respectively. From among the samples obtained from the study subjects, only samples that contained sufficient tumor tissue and non-tumorous liver tissue to enable diagnosis by a specialist pathologist were selected. These samples were subjected to hematoxylin-eosin (HE) and the immunohistochemical staining.

Immunohistochemical staining was conducted by deparaffinizing 4-μm slices and heating them in citrate buffer solution at 90°C for 40 min, to activate antigens. After cooling at room temperature for 20 min, slices were immersed in 3% H_2O_2-methanol for 5 min to deactivate endogenous peroxidase, and subsequently washed in phosphate buffered saline (PBS).

Primary antibodies were then applied. Histological staining was conducted using a Histoanalyzer 48A automated histostaining system (Nichirei), with the primary antibodies reacted for 60 min, the secondary antibodies for 30 min, and 3,3-diaminobenzidine (DAB) for 1 min. The primary antibodies and their dilutions were as follows: anti-GPC3 monoclonal antibody (mouse, clone 1G12, Nichirei, 1:1 dilution); anti-human hepatocyte antibody (Hep Par-1 mouse, clone OCH1E5, DAKO Cytomation [Glostrup Denmark], 1:1000 dilution); anti-Arginase-1 monoclonal antibody (rabbit, clone EP261, Nichirei, 1:1 dilution); Novocastra TM liquid mouse monoclonal antibody CD56 (NCAM) (human, clone CD564, Leica BIOSYSTEMS [product code NCL-L-CD56-504], 1:200 dilution); polyclonal rabbit anti-human CD117 c-kit (rabbit, Dako code A4502, 1:200 dilution); CD4 monoclonal antibody (mouse, clone 4B12, Nichirei, 1:2 dilution); and CD34 monoclonal antibody (mouse, clone NU-4A1, Nichirei, 1:200 dilution).

For statistical analysis, Fisher's exact test was used with a significance level of <0.05.

Results and discussion

Table 1 presents the tumor histological types seen in the patients. Among the 31 patients with pHCT, 24 were HB and 7 were pHCC. The histological subtypes of the 24 cases of HB included 12 cases of the fetal type, 7 of the embryonal type, and 5 of the combined

fetal and embryonal type. The pHCC grade was highly differentiated in 3 cases, moderately differentiated in 1, and poorly differentiated in 3. The grade of the 36 cases of aHCC was highly differentiated in 15 cases, moderately differentiated in 4, and poorly differentiated in 17.

Table 2 presents the GPC3 expression status of tumor cells in each tumor type. Overall, with the exception of 1 case of HB, GPC3 was expressed in all the cases of pHCT. GPC3 expression was seen in 18/36 cases of aHCC; in all of these cases, chronic liver disease was not apparent in non-tumorous hepatic tissue ($p < .05$). HepPar-1 expression was evident in all of the pediatric and adult hepatocellular tumors, excluding 1 case of pHCC, while Arginase 1 and AFP were expressed in all cases. CD56 and c-kit expression were not identified in any of the tumors (Fig. 1). CD4 expression was not apparent in sinusoid microvascular endothelial cells within any of the tumors, but CD34 was expressed in all cases.

Table 1. Overview of patients with hepatocellular tumors

Case	Diagnosis	Number of case	Age	HBV	HCV
1	Adult hepatocellular carcinoma	36	71.86 Â± 8.2	2	4
2	Hepatoblastoma	24	9.95 Â± 2.35	0	0
3	Paediatric hepatocellular carcinoma	7	3.89 Â± 2.79	0	1

Table 2. Results of hepatocellular tumor immunostaining

Diagnosis	Her Par-1	Alginase1	GPC3	AFP	CD34
Adult hepatocellular carcinoma	100% (36/36)	100% (36/36)	50% (18/36)	100% (36/36)	86% (31/36)
Hepatoblastoma	100% (7/7)	100% (7/7)	85% (6/7)	100% (7/7)	100% (7/7)
Paediatric hepatocellular carcinoma	92% (22/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)

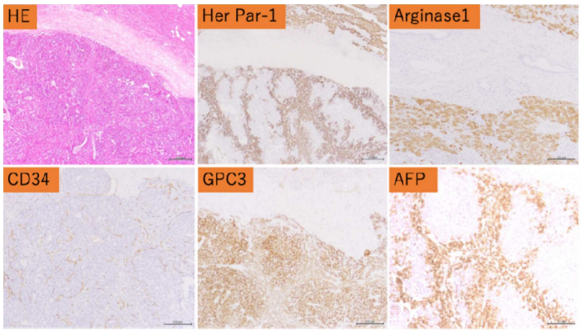

Fig. 1. Example immunostaining results of a hepatoblastoma

This tumor exhibits the morphology of fetal-type hepatoblastoma on HE staining. HepPar-1 and Arginase1 are both positive, staining to approximately the same extent as non-tumorous hepatic cells. The stromal microvascular endothelial cells are positive for CD34, while tumor cells are diffusely positive for GPC3, and some tumor cells are positive for AFP.

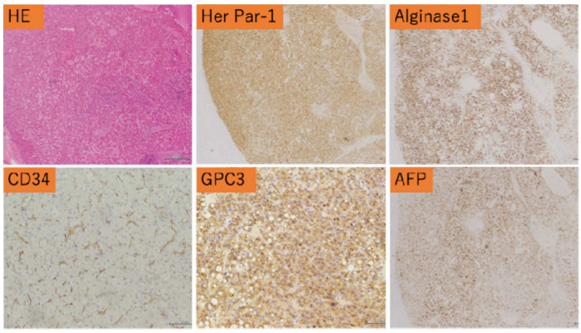

Fig. 2. Example immunostaining results of a pediatric hepatocellular carcinoma

This tumor exhibits the morphology of highly differentiated hepatocellular carcinoma (HE staining). The tumor cells are diffusely positive for HepPar-1, Arginase1, and GPC3. The stromal microvascular endothelial cells are positive for CD34, while some tumor cells are positive for AFP.

Overall, our results showed that GPC3 was expressed with high frequency in pediatric hepatocellular tumors (Fig. 2). In cases of adult hepatocellular carcinoma, GCP expression was observed in 18 cases that developed from non-cirrhotic liver, including 6 in which it occurred in patients with normal livers and no underlying chronic liver disease; however, it was not evident in the other 18 cases (Fig. 3). In light of the fact that GPC3 is expressed in liver cells in the normal fetal liver (Iglesias *et al.*, 2008), the

expression of GPC3 in pHCTs suggests that these tumors may be derived from fetal liver cells.

Overall, our results showed that the frequency of GPC3 expression in aHCC was approximately 50%, and that its expression frequency differed greatly between cases of aHCC that had developed from normal non-cirrhotic livers and those that had developed from cirrhotic livers. This indicates that the mechanism of tumorigenesis may differ between these two groups. The frequency of GPC3 expression in aHCC has varied greatly between different studies. In reports from Western countries (Zynger *et al.*, 2008) the frequency of GPC3 expression tends to be higher, with GPC3 widely being regarded as a tumor marker for aHCC.

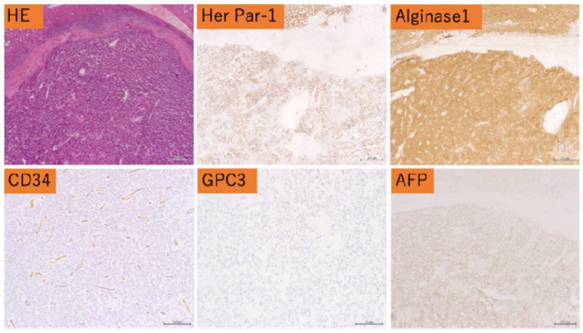

Fig. 3. Example immunostaining results of an adult hepatocellular carcinoma
This tumor exhibits the morphology of highly differentiated hepatocellular carcinoma on HE staining. HepPar-1 and Arginase1 are both positive, staining to approximately the same extent as non-tumorous hepatic cells. Stromal microvascular endothelial cells are positive for CD34. All tumor cells are negative for GPC3, while some are positive for AFP

Conversely, in Asia, most cases of HCC stem from viral hepatitis (Nakatsura *et al.*, 2003; Kinoshita *et al.*, 2015), which may be why GPC3 expression is lower. In Asia, hepatocellular carcinoma predominantly develops after hepatitis B or C infection, which may affect GPC3 expression. In Western countries, alcoholic liver disease and non-alcoholic fatty liver disease are the primary causes of hepatocellular carcinoma, which may partly explain the geographic variation in expression. This geographic variation may further reflect molecular-level differences in the pathogenesis and progression of hepatocellular carcinoma, and could potentially be of significance for the choice of treatment.

GPC3 is a cell-surface heparan sulfate proteoglycan involved in cell growth and differentiation. In particular, it plays an important role in fetal development, and is known to be expressed in high frequency in tumor cells (Cottereau *et al.*, 2013). GPC3 is also known as the gene responsible for Simpson-Golabi-Behmel syndrome, a disorder carrying a high risk of pediatric malignant tumors. The GPC3 gene plays a role as a tumor suppressor gene, and its loss and abnormal function are believed to be involved in carcinogenesis and cancer progression. Conversely, GPC3 is expressed at high levels in many tumor tissues, and it has been suggested that it may be implicated in abnormal cell growth and tumor formation. High GPC3 expression may further promote the growth of tumor cells and be involved in malignant transformation. GPC3 has therefore attracted attention as a target for immunotherapy in tumor tissues. A recent study (Tsuchiya *et al.*, 2018) reported that progress is being made in immunotherapy targeting GPC3, and that this treatment method may be particularly promising for GPC3-positive hepatocellular carcinoma. Clinical trials of therapies utilizing GPC3-specific T-cell receptors (TCRs) and chimeric antigen receptor T-cell (CAR-T-cell) therapies targeting GPC3 are currently underway. These treatment methods specifically aim to attack tumor cells with high expression GPC3 levels, and are regarded as promising therapeutic approaches for pHCCs and specific aHCCs.

HepPar-1 and Arginase 1 were found to be expressed at equivalent levels in both pediatric and adult hepatocellular tumors; however, the hepatic stem cell markers CD56 and c-kit were not expressed in any tumors, while the involvement of hepatic stem cells in tumorigenesis remains unknown. CD4, a marker of hepatic sinusoidal endothelial cell differentiation, was not observed in any case, either pediatric or adult, whereas CD34, a marker of undifferentiated mesenchymal cells, was present in both pediatric and adult HCCs. This showed that, in both children and adults, the microvessels that form the tumor stroma of

hepatocellular tumors do not comprise endothelial cells differentiated into sinusoidal endothelial cells.

This study was limited by the small sample size, and its results may therefore only be generalized with caution. Further in-depth analysis of factors affecting GPC3 expression is also required. Further studies of a larger number of patients will be necessary in future, as will the elucidation of the genes and molecular mechanisms related to GPC3 expression. It will also be important to investigate the differences in GPC3 expression between Asian and Western countries, and to assess their clinical significance.

Conclusion

In this study, we identified differences in the biological characteristics of pHCC and aHCC, with results suggesting that GPC3 expression may be a characteristic marker of paediatric tumors. This highlights the necessity of developing different therapeutic approaches to hepatocellular tumors in children and adults. Further studies to develop new treatment methods based on these characteristics are anticipated.

References

Cottereau E, Mortemousque I, Moizard M, Bürglen L, Lacombe D, Gilbert-Dussardier B, Sigaudy S, Boute O, David A, Faivre L, Amiel J, Robertson R, Ramos FV, Bieth E, Odent S, Demeer B, Mathieu M, Gaillard D, Van Maldergem L, Baujat G, Maystadt I, Héron D, Verloes A, Philip N, Cormier-Daire V, Frouté MF, Pinson L, Blanchet P, Sarda P, Willems M, Jacquinet A, Ratbi I, van den Ende J, Lackmy-Port Lis M, Goldenberg A, Bonneau D, Rossignol S, Toutain A. 2013. Phenotypic spectrum of Simpson-Golabi-Behmel syndrome in a series of 42 cases with a mutation in GPC3 and review of the literature. American Journal of Medical Genetics Part C: Seminars in Medical Genetics **163C,** 92–105. DOI: 10.1002/ajmg.c.31360

Iglesias BV, Centeno G, Pascuccelli H, Ward F, Peters MG, Filmus J, Puricelli L, de Kier Joffé EB. 2008. Expression pattern of glypican-3 (GPC3) during human embryonic and fetal development. Histology and Histopathology **23**, 1333–1340.

Kinoshita Y, Tanaka S, Souzaki R, Miyoshi K, Kohashi K, Oda Y, Nakatsura T, Taguchi T. 2015. Glypican 3 expression in pediatric malignant solid tumors. European Journal of Pediatric Surgery **25**, 138–144.
DOI: 10.1055/s-0034-1393961.

Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, Hosaka S, Beppu T, Ishiko T, Kamohara H, Ashihara H, Katagiri T, Furukawa Y, Fujiyama S, Ogawa M, Nakamura Y, Nishimura Y. 2003. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochemical and Biophysical Research Communications **306**, 16–25. DOI: 10.1016/S0006-291X(03)00908-2.

Tsuchiya N, Hosono A, Yoshikawa T, Shoda K, Nosaka K, Shimomura M, Hara J, Nitani C, Manabe A, Yoshihara H, Hosoya Y, Kaneda H, Kinoshita Y, Kohashi K, Yoshimura K, Fujinami N, Saito K, Mizuno S, Nakatsura T. 2018. Phase I study of glypican-3-derived peptide vaccine therapy for patients with refractory pediatric solid tumors. OncoImmunology **7**(1), e1377872.
DOI: 10.1080/2162402X.2017.1377872.

Zynger DL, Gupta A, Luan C, Chou PM, Yang GY, Yang XJ. 2008. Expression of glypican 3 in hepatoblastoma: An immunohistochemical study of 65 cases. Human Pathology **39**(2), 224–230.
DOI: 10.1016/j.humpath.2007.06.006.