Correlation Between Biological Marker Expression and Fluorine-18 Fluorodeoxyglucose Uptake in Hepatocellular Carcinoma

Yohei Mano, PhD,[1,2] Shinichi Aishima, PhD,[1] Yuichiro Kubo, PhD,[1] Yuki Tanaka, MD,[1] Takashi Motomura, PhD,[2] Takeo Toshima, PhD,[2] Ken Shirabe, PhD,[2] Shingo Baba, PhD,[3] Yoshihiko Maehara, PhD,[2] and Yoshinao Oda, PhD[1]

From the Departments of [1]Anatomic Pathology, [2]Surgery and Sciences, and [3]Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Key Words: Hepatocellular carcinoma; GLUT1; HIF1α; STAT3; FDG positron emission tomography

Am J Clin Pathol September 2014;142:391-397

DOI: 10.1309/AJCPG8AFJ5NRKLLM

ABSTRACT

Objectives: *This study investigated the association between several biological markers and fluorine-18 fluorodeoxyglucose (FDG) uptake in patients with hepatocellular carcinoma.*

Methods: *Forty-two patients with hepatocellular carcinoma who underwent FDG positron emission tomography were included in the study. Tumor sections were immunohistochemically stained for phosphorylated signal transducer and activator of transcription 3 (pSTAT3), hypoxia-inducible factor 1α (HIF1α), glucose transporter 1 (GLUT1), GLUT2, GLUT3, and GLUT4.*

Results: *The high standardized uptake value (SUV) group showed larger tumor size, more frequent vascular invasion, and poorer differentiation compared with the low SUV group. The high SUV group also showed significantly higher immunohistochemical expression of pSTAT3, HIF1α, and GLUT1. The GLUT1 high-expression group showed higher α-fetoprotein (a tumor marker) and poorer differentiation than did the GLUT1 low-expression group.*

Conclusions: *Our study indicates that FDG uptake is associated with the expression of pSTAT3, HIF1α, and GLUT1 in hepatocellular carcinoma. The expression of these proteins shows a correlation with poor differentiation and vascular invasion.*

Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer death.[1] Although surgical therapies for HCC have progressed and outcomes of HCC improved, HCC still often recurs after surgery.[2,3] Sorafenib, one of the molecular target therapies, is reported to have efficacy against unresectable HCCs; however, the survival advantage is only 3.7 months.[4] Improving the survival rate of patients with HCC requires clinicians to engage in active treatment of HCC recurrence and to explore and analyze biological or clinicopathologic characteristics.

Recently, fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been used for oncologic diagnostic imaging, based on the enhanced glucose metabolism in cancer cells. Fluorine-18 fluorodeoxyglucose (FDG) uptake is associated with the prognosis of patients with HCC.[5,6] Glucose uptake in malignant tumors depends largely on the presence of facilitated glucose transporter proteins and glycolytic enzymes. Glucose transporter 1 (GLUT1) is a key factor in the transport and metabolism of glucose in cancer cells and is overexpressed in a significant proportion of human carcinomas.[7-9]

Signal transducer and activator of transcription 3 (STAT3) is an important molecule in tumor progression.[10,11] STAT3 activation can be induced by phosphorylation and dimerization of tyrosine residue (Tyr705), leading to nuclear entry, DNA binding, and gene transcription. STAT3 is regarded as a critical transcription activator for cell cycle–related or cell survival–related genes. Some cytokines, such as interleukin (IL)–6 or IL-10, activate STAT3 signaling via their receptors.[12] Constitutive activation of STAT3 has been demonstrated to contribute to tumorigenesis in various cancers.[13-18] In human HCC,

STAT3 phosphorylation has been reported as being involved in tumor progression,[19] angiogenesis,[20] and tumorigenesis.[21] We previously reported that STAT3 activation was one of the prognostic factors in patients with HCC.[22] Demaria et al[23] have reported that STAT3 activation has an important role as a master regulator of cell metabolism in mouse embryonic fibroblasts and some tumor cell lines, and STAT3 constitutive transcriptional activity was sufficient to induce an increase in hypoxia-inducible factor 1α (HIF1α) messenger RNA (mRNA) and protein levels. HIF1α is one of the transcriptional regulators of GLUT1.[24] Kitamura et al[25] reported a correlation between preoperative FDG uptake and GLUT1 expression in HCC.

The aim of this study was to clarify the relationship between preoperative FDG uptake, STAT3 phosphorylation, and HIF1α activation in HCC tissue from liver resections. We also examined the expression of GLUT1, GLUT2, GLUT3, and GLUT4 in HCC specimens and analyzed for correlations with clinicopathologic factors.

Materials and Methods

Patients and Samples

Forty-two available paraffin-embedded specimens from consecutive patients with HCC who underwent FDG-PET computed tomography (CT) as a preoperative examination and subsequent liver resection between April 2010 and June 2011 at our institute were selected by reviewing their pathology data. The maximum standardized uptake value (SUV) was calculated for quantitative analysis of tumor FDG uptake. Of these, 23 (54.8%) patients were seropositive for hepatitis C antibody (HCV-Ab), eight (19.0%) were seropositive for hepatitis B surface antigen (HBs-Ag), 10 (23.8%) were seronegative for both HCV-Ab and HBs-Ag, and one (2.4%) was seropositive for both HBs-Ag and HCV-Ab. Twelve (28.6%) patients had liver cirrhosis, and transplantation was performed for 10 (23.8%) patients. Intrahepatic metastasis and multicentric occurrence were defined based on guidelines from the Liver Cancer Study Group of Japan.[26] This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient.

Immunohistochemistry

Sections of resected specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained using an Envision+ system and DAB kit (DAKO, Glostrup, Denmark). Immunohistochemical staining was performed with antibodies for phospho-STAT3 (Tyr705) (D3A7, 1:50; Cell Signaling Technology, Danvers, MA), HIF1α (H1α67, 1:300; Novus Biologicals, Littleton, CO), GLUT1 (rabbit polyclonal, 1:300; Abcam, Cambridge, MA), GLUT2 (5D1, 1:1000; Abcam), GLUT3 (rabbit polyclonal, 1:25; Abcam), and GLUT4 (mAbcam48547, 1:250; Abcam). Sections were pretreated before being incubated with the primary antibodies in a microwave oven at 99°C for 20 minutes for phosphorylated STAT 3 (pSTAT3) and HIF1α or for 15 minutes for GLUT1, GLUT2, GLUT3, and GLUT4.

Each slide was stained in serial sections and examined by two pathologists (Y.M. and S.A.). For nuclear staining of pSTAT3 and HIF1α, as well as cytoplasm or cell membrane staining of GLUT1, GLUT2, GLUT3, and GLUT4, the percentage of positive cells was estimated by counting 1,000 tumor cells in most stained areas. Immunoreactive staining from all antibody groups was classified into either a positive group (≥10% of tumor cells) or a negative group (<10% of tumor cells).

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel software (Microsoft, Redmond, WA) and JMP software (SAS Institute, Cary, NC). Comparisons between immunohistochemical staining and clinicopathologic findings or staining with other antibodies were evaluated by Pearson χ^2 tests, Fisher exact tests, and the Mann-Whitney *U* test. A *P* value of <.05 was considered statistically significant.

Results

Immunohistochemical Staining in HCC

pSTAT3 and HIF1α were stained in the nuclei of HCC cells **Image 1A** and **Image 1B**. GLUT1 and GLUT2 were stained in the membrane of HCC cells **Image 1C** and **Image 1D**. GLUT3 and GLUT4 were stained in the cytoplasm **Image 1E** and **Image 1F**. Expression of pSTAT3, HIF1α, and GLUT1 was frequently seen at the center of the tumor or around necrotic areas.

Comparisons of SUV With Clinicopathologic and Immunohistochemical Findings

Forty-two patients were classified into two groups based on the SUV: (1) the high SUV group (SUV ≥4) and (2) the low SUV group (SUV <4). A comparison between clinicopathologic findings and immunohistochemical staining in the high SUV group and the low SUV group is summarized in **Table 1**. The high SUV group showed an older age (*P* = .0429), a larger tumor size (*P* = .0005), more frequent vascular invasion (*P* = .0213), and poorer differentiation (*P* = .0003) compared with the low SUV group. The high SUV group showed significantly higher immunohistochemical

DOI: 10.1309/AJCPG8AFJ5NRKLLM

expression of pSTAT3 (P = .0293), HIF1α (P = .0039), and GLUT1 (P = .0209) compared with the low SUV group. No significant differences were noted between the sex of patient, tumor markers (α-fetoprotein [AFP] and des-γ-carboxy prothrombin), intrahepatic metastasis, or GLUT2 to GLUT4 expression.

Comparison of GLUT1 to GLUT4 Expression and Clinicopathologic Findings

Comparisons between the GLUT1 high-expression and low-expression groups are summarized in Table 2. The GLUT1 high-expression group showed higher AFP (P = .0329) and poorer differentiation (P = .0003) than did the GLUT1 low-expression group. The GLUT3 and GLUT4 high-expression groups showed an older age of patients than did the low-expression group (P = .0404 and P = .0231, respectively) Table 3.

Comparisons of Immunohistochemical Reactivity and Poor Prognostic Factors

We picked the three most important immunohistochemical markers of tumor progression for investigation in this study: pSTAT3, HIF1α, and GLUT1. Of these three markers, there was only one triple-positive case. The case showed HCC with sarcomatous change to the pathologic features. Sarcomatous HCC is rare, and the prognosis is poor.[26,27] There were also eight double-positive cases, 11 single-positive cases, and 22 triple-negative cases of the three markers. We examined for the presence or absence of poor prognostic factors, such as high SUV, poor histology, and vascular invasion in these three groups Table 4. Double- or single-positive cases showed high SUV (P = .0014), poorer differentiation (P = .0005), and more frequent vascular invasion (P = .0271) compared with the triple-negative cases.

Table 1
Comparative Analysis of the Clinicopathologic Findings and Expression of pSTAT3, HIF1α, and GLUTs Between the Low and High SUV Groups[a]

Factor	Low SUV Group (n = 25)	High SUV Group (n = 17)	P Value
Age, mean ± SD, y	61.7 ± 9.6	69 ± 12.6	.0429[b]
Sex, male/female, No.	10/13	11/6	.1840
AFP, mean ± SD, ng/mL	128 ± 276	79,891 ± 244,760	.1347
DCP, mean ± SD, mAU/mL	180 ± 515	9,078 ± 26,774	.1275
Tumor size, mean ± SD, cm	2.3 ± 1.2	5.4 ± 3.9	.0005[b]
Vascular invasion	3 (12)	9 (53)	.0213[b]
Intrahepatic metastasis	4 (16)	4 (24)	.4884
Differentiation, well + moderate/poor, No.	24/1	7/11	.0003[b]
pSTAT3 positive	3 (12)	7 (41)	.0293[b]
HIF1α positive	4 (16)	10 (59)	.0039[b]
GLUT1 positive	1 (4)	5 (29)	.0209[b]
GLUT2 positive	16 (64)	12 (71)	.6566
GLUT3 positive	9 (36)	10 (59)	.1744
GLUT4 positive	5 (20)	3 (18)	.8488

AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; GLUT, glucose transporter; HIF1α, hypoxia-inducible factor 1α; pSTAT3, phosphorylated signal transducer and activator of transcription 3; SUV, standardized uptake value.
[a] Values are presented as number (%) unless otherwise indicated.
[b] $P < .05$.

Table 2
Comparative Analysis of the Clinicopathologic Findings and Expression of pSTAT3 and HIF1α Between the Low and High GLUT1 Expression Groups[a]

Factor	GLUT1 Low (n = 36)	GLUT1 High (n = 6)	*P* Value
Age, mean ± SD, y	64.8 ± 9.6	68.7 ± 12.6	.3727
Sex, male/female, No.	19/15	2/4	.3079
AFP, mean ± SD, ng/mL	9,895 ± 25,050	142,145 ± 57,591	.0329[b]
DCP, mean ± SD, mAU/mL	3,336 ± 2,871	4,199 ± 6,511	.8765
Tumor size, mean ± SD, cm	3.3 ± 2.6	4.2 ± 4.5	.4933
Vascular invasion	8 (22)	3 (50)	.2307
Intrahepatic metastasis	7 (19)	1 (17)	.8490
Differentiation, well + moderate/poor, No.	30/6	1/5	.0003[b]
pSTAT3 positive	7 (19)	3 (50)	.1038
HIF1α positive	11 (31)	3 (50)	.3496

AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; GLUT1, glucose transporter 1; HIF1α, hypoxia-inducible factor 1α; pSTAT3, phosphorylated signal transducer and activator of transcription 3.
[a] Values are presented as number (%) unless otherwise indicated.
[b] $P < .05$.

DOI: 10.1309/AJCPG8AFJ5NRKLLM

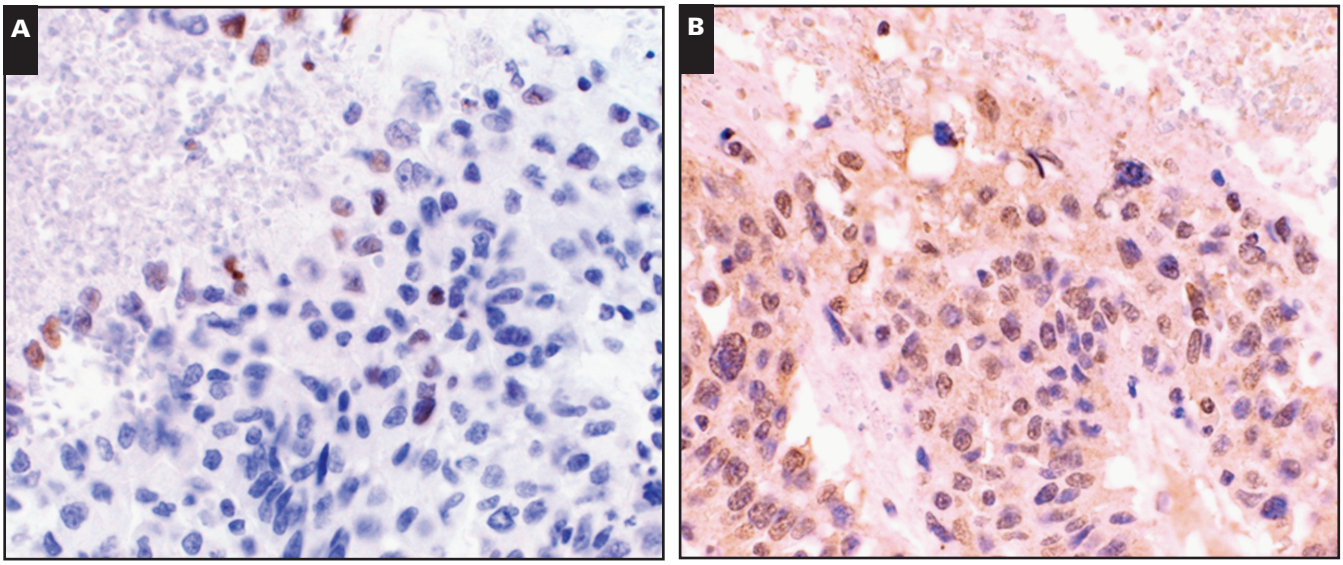

Image 1 Immunohistochemical staining in hepatocellular carcinoma (HCC) cells (×200). **A**, Phosphorylated signal transducer and activator of transcription 3 was stained in the nuclei of HCC cells. **B**, Hypoxia-inducible factor 1α was stained in the nuclei of HCC cells.

Discussion

Most cancer cells metabolize glucose by aerobic glycolysis. This is known as "the Warburg effect." Inducible HIF1α transcription factor lies at the crossroads between anaerobic and aerobic glycolysis.[28] Some growth factors and oncogenes can also increase HIF1α activity via enhanced protein translation mediated by the PI3K-induced mammalian target of rapamycin pathway.[29]

STAT3 plays a central metabolic role on many levels in cancer. STAT3 has been reported as one of the molecules modulating HIF1α expression and activation,[30,31] and it was recently reported that STAT3 played an important role in the regulation of mitochondrial activity.[23] STAT3 inhibition may contribute to a novel therapeutic strategy to target cancer.

Fourteen members of the mammalian facilitative GLUT family have been identified: GLUT1 to GLUT12, GLUT14, and the H+/myo-inositol transporter. The family can be divided into three subclasses. Class 1 comprises GLUT1 to GLUT4. These GLUTs are distinguished mainly by their distribution in different tissues (GLUT1: erythrocytes, brain, and microvessels; GLUT2: liver and pancreatic islets; GLUT3: neuronal cells; GLUT4: muscle and adipose tissue).

Table 3
Comparative Analysis of the Clinicopathologic Findings and Expression of pSTAT3 and HIF1α Between the Low and High GLUT2 to GLUT4 Expression Groups[a]

	GLUT2			GLUT3		
Factor	**Low (n = 14)**	**High (n = 28)**	***P* Value**	**Low (n = 22)**	**High (n = 19)**	***P* Value**
Age, mean ± SD, y	67.2 ± 10.2	63.5 ± 11.7	.3285	61.6 ± 10.4	69.2 ± 11.1	.0404[b]
Sex, male/female, No.	8/6	13/13	.6661	11/11	9/8	.8554
AFP, mean ± SD, ng/mL	157 ± 364	48,522 ± 97,600	.3356	164 ± 311	67,860 ± 232,612	.1696
DCP, mean ± SD, mAU/mL	78 ± 133	5,297 ± 21,000	.4218	4,681 ± 22,375	1,954 ± 5,674	.4588
Tumor size, mean ± SD, cm	2.7	3.8	.2602	3.7 ± 3.6	3.3 ± 2.3	.6752
Vascular invasion	3 (21)	8 (29)	.5699	6 (27)	5 (26)	.2297
Intrahepatic metastasis	3 (21)	5 (18)	.8236	3 (14)	4 (21)	.5738
Differentiation, well + moderate/poor, No.	12/2	19/9	.2252	18/4	12/7	.3878
pSTAT3 positive	3 (21)	7 (25)	.7987	5 (23)	5 (26)	.7896
HIF1α positive	5 (36)	9 (32)	.8170	6 (27)	8 (42)	.3179

AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; GLUT, glucose transporter; HIF1α, hypoxia-inducible factor 1α; pSTAT3, phosphorylated signal transducer and activator of transcription 3; SUV, standardized uptake value.
[a] Values are presented as number (%) unless otherwise indicated.
[b] $P < .05$.

DOI: 10.1309/AJCPG8AFJ5NRKLLM

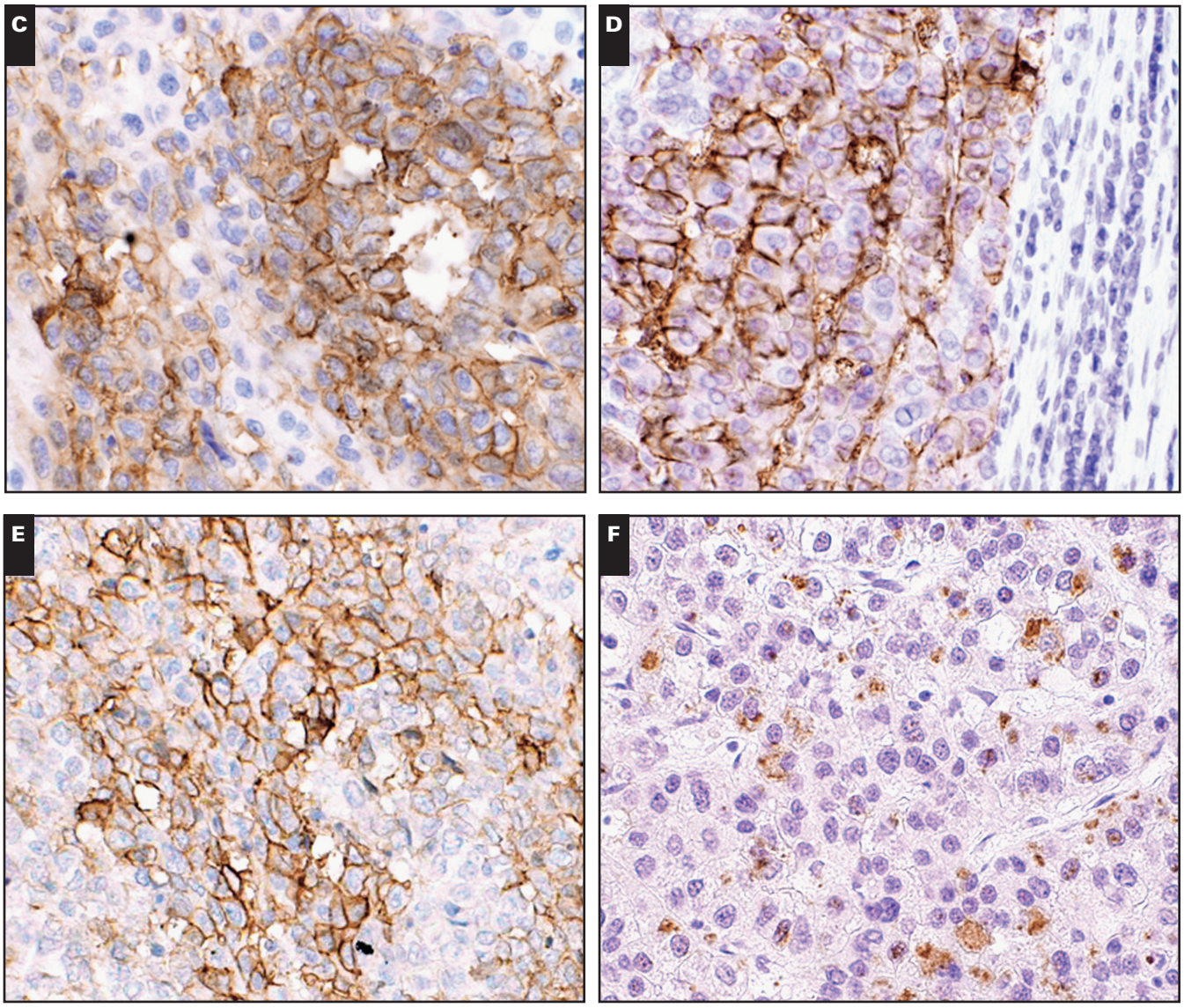

Image 1 (cont) **C**, Glucose transporter 1 (GLUT1) was stained in the membrane of HCC cells. **D**, GLUT2 was stained in the membrane of HCC cells. **E**, GLUT3 was stained in the membrane. **F**, GLUT4 was stained in the cytoplasm.

GLUT4		
Low (n = 34)	**High (n = 8)**	***P* Value**
62.9 ± 11.2	73.6 ± 6.9	.0231[b]
21/13	2/6	.0601
38,884 ± 177,171	22 ± 41	.5178
4,247 ± 19,116	564 ± 1,506	.4892
3.7 ± 3.1	2.5 ± 1.3	.3357
9 (26)	4 (50)	.4510
7 (21)	1 (13)	.5769
26/8	5/3	.3255
7 (21)	3 (38)	.3123
10 (29)	4 (50)	.2264

In this study, we examined the four types of GLUT in class 1. We observed that HCCs with high SUV showed poorer differentiation, larger tumor size, and frequent vascular invasion. The results indicate that HCCs with high SUV have a greater malignant potential. Immunohistochemically, these tumor cells showed immunoreactivity for pSTAT3, HIF1α, and GLUT1. The activation or overexpression of these molecules is correlated with a poor prognosis.[22,25,32] In the present study, statistical relationships between pSTAT3 activation, HIF1α activation, and GLUT1 expression were not observed. Previous studies have reported GLUT1 expression being regulated by blood glucose levels and p53 expression.[33,34] Manalo et al[35] demonstrated that HIF1α activated transcription of numerous genes. STAT3, HIF1α, and GLUT1 may affect glucose

DOI: 10.1309/AJCPG8AFJ5NRKLLM

Table 4
Comparison of Immunohistochemical Reactivity and Poor Prognostic Factors

Factor	Triple Negative (n = 22), No. (%)	Single Positive (n = 11), No. (%)	Double Positive (n = 8), No. (%)	*P* Value
High SUV	3 (14)	7 (64)	6 (75)	.0014
Poor differentiation	0	6 (55)	4 (50)	.0005
Vascular invasion	2 (9)	5 (45)	4 (50)	.0271

SUV, standardized uptake value.

uptake in different ways. Fillies et al[36] reported no significant correlation between HIF1α activation and GLUT1 expression in squamous cell carcinoma. In the present study, the cases with high expression of these proteins showed high SUV, poor differentiation, and a high frequency of vascular invasion (Table 4). To clarify our observations of STAT3, HIF1α, and the GLUT1 signaling pathway, it will be necessary to conduct larger series investigations or to perform in vitro studies.

Previous reports have described correlations between PET-CT and GLUT expression. Godoy et al[37] reported that immunohistochemical expression of GLUT1 and GLUT2 could be observed, but GLUT3 and GLUT4 expression could not. The study by Godoy et al was of only four cases. In the present study, we observed GLUT3 and GLUT4 staining. However, our study size was much larger than that of Godoy et al. Paudyal et al[38] reported that high expression of GLUT2 was correlated with SUV in HCC and a poor prognosis. In the present study, there was no correlation between GLUT2 expression and SUV, but there was a correlation between GLUT1 expression and SUV. Seo et al[6] and Kitamura et al[25] also reported a correlation between GLUT1 expression and SUV.

In this study, we observed that cases of HCC with high SUV are associated with poor prognostic indicators, and activated STAT3, HIF1α, and GLUT1 high expression can cause a poor prognosis. These molecules, STAT3, HIF1α, and GLUT1, could be therapeutic targets in HCC with high SUV by PET-CT.

Address reprint requests to Dr Oda: Dept of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; oda@surgpath.med.kyushu-u.ac.jp.

This study was supported in part by a grant-in aid from the Vehicle Racing Commemorative Foundation (Tokyo, Japan) and the Mitsui Life Social Welfare Foundation (Tokyo, Japan).

References

1. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet*. 2003;362:1907-1917.
2. Shirabe K, Kanematsu T, Matumata T, et al. Factors linked to early recurrence of small hepatocellular carcinoma after hepatectomy: univariate and multivariate analyses. *Hepatology*. 1991;14:802-805.
3. Taura K, Ikai I, Hatano E, et al. Implication of frequent local ablation therapy for intrahepatic recurrence in prolonged survival of patients with hepatocellular carcinoma undergoing hepatic resection: an analysis of 610 patients over 16 years old. *Ann Surg*. 2006;244:265-273.
4. Llovet JM, Ricci S, Mazzaferro V, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359:378-390.
5. Hatano E, Ikai I, Higashi T, et al. Preoperative positron emission tomography with fluorine-18-fluorodeoxyglucose is predictive of prognosis in patients with hepatocellular carcinoma after resection. *World J Surg*. 2006;30:1736-1741.
6. Seo S, Hatano E, Higashi T, et al. Fluorine-18 fluorodeoxyglucose positron emission tomography predicts tumor differentiation, P-glycoprotein expression, and outcome after resection in hepatocellular carcinoma. *Clin Cancer Res*. 2007;13:427-433.
7. Medina RA, Owen GI. Glucose transporters: expression, regulation and cancer. *Biol Res*. 2002;35:9-26.
8. Higashi T, Tamaki N, Torizuka T, et al. FDG uptake, GLUT-1 glucose transporter and cellularity in human pancreatic tumors. *J Nucl Med*. 1998;39:1727-1735.
9. Higashi T, Saga T, Nakamoto Y, et al. Relationship between retention index in dual-phase (18)F-FDG PET, and hexokinase-II and glucose transporter-1 expression in pancreatic cancer. *J Nucl Med*. 2002;43:173-180.
10. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. *Cell*. 1999;98:295-303.
11. Al Zaid Siddiquee K, Turkson J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res*. 2008;18:254-267.
12. Murray PJ. The JAK-STAT signaling pathway: input and output integration. *J Immunol*. 2007;178:2623-2629.
13. Berishaj M, Gao SP, Ahmed S, et al. Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. *Breast Cancer Res*. 2007;9:R32.
14. Lin Q, Lai R, Chirieac LR, et al. Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. *Am J Pathol*. 2005;167:969-980.
15. Song L, Turkson J, Karras JG, et al. Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene*. 2003;22:4150-4165.
16. Greten FR, Weber CK, Greten TF, et al. Stat3 and NF-kappaB activation prevents apoptosis in pancreatic carcinogenesis. *Gastroenterology*. 2002;123:2052-2063.
17. Ni Z, Lou W, Leman ES, et al. Inhibition of constitutively activated Stat3 signaling pathway suppresses growth of prostate cancer cells. *Cancer Res*. 2000;60:1225-1228.

DOI: 10.1309/AJCPG8AFJ5NRKLLM

18. Kreis S, Munz GA, Haan S, et al. Cell density dependent increase of constitutive signal transducers and activators of transcription 3 activity in melanoma cells is mediated by Janus kinases. *Mol Cancer Res*. 2007;5:1331-1341.
19. Rajendran P, Ong TH, Chen L, et al. Suppression of signal transducer and activator of transcription 3 activation by butein inhibits growth of human hepatocellular carcinoma in vivo. *Clin Cancer Res*. 2011;17:1425-1439.
20. Yang SF, Wang SN, Wu CF, et al. Altered p-STAT3 (tyr705) expression is associated with histological grading and intratumour microvessel density in hepatocellular carcinoma. *J Clin Pathol*. 2007;60:642-648.
21. Ogata H, Kobayashi T, Chinen T, et al. Deletion of the *SOCS3* gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis. *Gastroenterology*. 2006;131:179-193.
22. Mano Y, Aishima S, Fujita N, et al. Tumor-associated macrophage promotes tumor progression via STAT3 signaling in hepatocellular carcinoma. *Pathobiology*. 2013;80:146-154.
23. Demaria M, Giorgi C, Lebiedzinska M, et al. A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. *Aging (Albany NY)*. 2010;2:823-842.
24. Chen C, Pore N, Behrooz A, et al. Regulation of glut1 mRNA by hypoxia-inducible factor-1: interaction between H-ras and hypoxia. *J Biol Chem*. 2001;276:9519-9525.
25. Kitamura K, Hatano E, Higashi T, et al. Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis. *J Hepatol*. 2011;55:846-857.
26. Liver Cancer Study Group of Japan. *General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. 2nd ed. Tokyo, Japan: Kanehara; 2003.
27. Maeda T, Adachi E, Kajiyama K, et al. Spindle cell hepatocellular carcinoma: a clinicopathologic and immunohistochemical analysis of 15 cases. *Cancer*. 1996;77:51-57.
28. Kaelin WG Jr, Thompson CB. Q&A: Cancer: clues from cell metabolism. *Nature*. 2010;465:562-564.
29. Fukuda R, Hirota K, Fan F, et al. Insulin-like growth factor 1 induces hypoxia-inducible factor 1–mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem*. 2002;277:38205-38211.
30. Xu Q, Briggs J, Park S, et al. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene*. 2005;24:5552-5560.
31. Niu G, Briggs J, Deng J, et al. Signal transducer and activator of transcription 3 is required for hypoxia-inducible factor-1alpha RNA expression in both tum-or cells and tumor-associated myeloid cells. *Mol Cancer Res*. 2008;6:1099-1105.
32. Wada H, Nagano H, Yamamoto H, et al. Expression pattern of angiogenic factors and prognosis after hepatic resection in hepatocellular carcinoma: importance of angiopoietin-2 and hypoxia-induced factor-1 alpha. *Liver Int*. 2006;26:414-423.
33. Heilig CW, Liu Y, England RL, et al. D-glucose stimulates mesangial cell GLUT1 expression and basal and IGF-I–sensitive glucose uptake in rat mesangial cells: implications for diabetic nephropathy. *Diabetes*. 1997;46:1030-1039.
34. Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res*. 2004;64:2627-2633.
35. Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005;105:659-669.
36. Fillies T, Werkmeister R, van Diest PJ, et al. HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. *BMC Cancer*. 2005;5:84.
37. Godoy A, Ulloa V, Rodríguez F, et al. Differential subcellular distribution of glucose transporters GLUT1-6 and GLUT9 in human cancer: ultrastructural localization of GLUT1 and GLUT5 in breast tumor tissues. *J Cell Physiol*. 2006;207:614-627.
38. Paudyal B, Paudyal P, Oriuchi N, et al. Clinical implication of glucose transport and metabolism evaluated by 18F-FDG PET in hepatocellular carcinoma. *Int J Oncol*. 2008;33:1047-1054.

DOI: 10.1309/AJCPG8AFJ5NRKLLM

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS