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Hexokinase-II expression in untreated oral squamous cell carcinoma: comparison with FDG PET imaging

Mei TIAN,*[1,2] Hong ZHANG,*[1] Tetsuya HIGUCHI,*[2] Noboru ORIUCHI,*[2] Yoshiki NAKASONE,*[3] Kuniaki TAKATA,*[4] Nobuaki NAKAJIMA,*[5] Kenji MOGI*[3] and Keigo ENDO*[2]

*[1]*Department of Nuclear Medicine, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China*
*[2]*Department of Nuclear Medicine and Diagnostic Radiology, Gunma University School of Medicine, Maebashi, Gunma, Japan*
*[3]*Department of Oral and Maxillofacial Surgery,* *[4]*Department of Anatomy, and* *[5]*Department of Pathology, Gunma University School of Medicine, Maebashi, Gunma, Japan*

Hexokinase is thought to be one of the key factors of glucose catabolism in the cell. The aim of this study was to investigate the relationship between HK-II expression and ^{18}F-fluoro-2-deoxy-D-glucose (FDG) uptake in human untreated oral squamous cell carcinoma (OSCC). Pre-operatively FDG positron emission tomography (PET) was performed 60 min after FDG injection in all the patients. Maximum standardized uptake value (SUV) was used for evaluation of tumor FDG uptake. Tumor sections were stained immunohistochemically for HK-II. All the tumor sections stained positive for HK-II. Eighteen (95%) tumors in HK-II showed immunostained positive area ≥50%. HK-II findings revealed eleven (58%) tumors with strong intensity, six (32%) with moderate intensity and two with weak intensity (10%). There was no statistically significant correlation between SUV and the expression of HK-II ($p = 0.46$). In conclusion, OSCC showed increased FDG accumulation and overexpression of HK-II. However, we did not find any significant relationship between high FDG uptake and overexpression of HK-II in this patient population, and thus other properties need to be evaluated in order to elucidate key factors responsible for FDG activity in OSCC.

Key words: hexokinase-II, immunohistochemistry, FDG PET, oral squamous cell carcinoma

INTRODUCTION

THE ENHANCED RATE of glucose utilization by tumor cells has been exploited in the positron emission tomography (PET) imaging of tumors using ^{18}F labeled glucose structural analogue fluoro-2-deoxy-D-glucose (FDG), which like glucose is transported into cells by glucose transporters (Glut) and phosphorylated by hexokinase (HK).[1] It is known that HK, especially HK-II, is the initial and rate-limiting enzyme in the glycolytic pathway. HK-II has been suggested as a marker for human malignancies, though it is not consistently found in all malignancies of the same tissue origin.[2–4] However, the underlying mechanisms for FDG uptake in tumors are still a matter of debate. Recently, two studies compared FDG incorporation by pancreatic and breast cancer *in vivo* with the level of expression of Glut-1 and HK-II,[5,6] and found that FDG uptake was not associated with the extent of immunodetectable expression with that of HK-II. Obviously, further study is necessary to correlate increased FDG uptake of tumors with their biological characteristics of HK-II expression, and thereby lead to a better understanding and interpretation of FDG PET imaging. In our previous study, we reported on the relationship between Glut-1 and FDG PET in OSCC.[7] In the present study, we further investigated the expression of HK-II, as well as the association between FDG PET study and the expression of HK-II in untreated OSCC patients.

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For reprint contact: Mei Tian, M.D., M.Sc., Ph.D., Department of Nuclear Medicine and Diagnostic Radiology, Gunma University School of Medicine, 3–39–22 Showa-machi, Maebashi, Gunma 371–8511, JAPAN.

MATERIALS AND METHODS

Nineteen patients (11 male and 8 female; age range, 40–78 years) with OSCC who underwent pre-operative FDG-PET imaging were included in this study. Surgical resection or biopsy confirmed the histological diagnosis. All patients underwent surgical resection or biopsy within 2 weeks following the PET and CT/MRI studies. None of the patients had insulin-dependent diabetes mellitus. Informed consent was obtained from all patients participating in this study.

FDG PET studies were performed using a SET 2400W (Shimadzu Corporation, Kyoto, Japan).[8] Patients fasted at least 4 hours before FDG injection. A whole body image obtained by the simultaneous emission-transmission method with a rotating external source was started at 60 min after the injection of 185–370 MBq of FDG. The imaging protocols of FDG PET were approved by the Institutional Review Board of our institute.

Transaxial and coronal FDG PET images were interpreted by two nuclear physicians in conjunction with the CT or MRI findings until a consensus was reached. Regions of interest (ROIs) method was used to evaluate the FDG uptake and quantitative analysis performed using the standardized uptake value (SUV).

Paraffin sections were processed for anti-HK-II immunostaining and routine hematoxylin staining. The immunohistochemical staining was carried out using the same protocol as in our previous study.[7] In brief, sections were incubated for anti-HK-II (1:1000 dilution by PBS, PH 7.4, Chemicon International Inc., Temecula, CA) antibody as a primary antibody for 90 min at room temperature.

Immunohistochemical analysis for Glut-1 and HK-II was independently performed by two well-experienced pathologists who were unaware of the PET results. The intensity of the staining was graded as negative, weak, moderate or strong, and the percentage of positive stained cells was categorized according to a semiquantitative scale as 0%, 1–10%, 10–50% and 50–100%. The overall staining result was scored from 0–4 according to the intensity and positive rate of the staining.[7]

Table 1 Immunostaining results

Characteristic	No. of patients
HK-II	
% positive area	
<10%	0
10%–50%	1
>50%	18
Instensity	
Weak	2
Moderate	6
Strong	11
Score	
0	0
1	0
2	2
3	6
4	11

RESULTS

Immunostaining intensity and the proportion of stained cells were summarized in Table 1. HK-II expression was positive in all OSCC specimens and eighteen (95%) tumors with positive stained area ≥50%. Of them, eleven (58%) tumors showed strong intensity, six (32%) moderate intensity and two weak intensity (10%). Correlations between moderate and well differentiated OSCC regarding to immunostaining were evaluated according to three scales of % positive area, intensity and staining score for HK-II expression shown in Table 2. High values were found in each of the three scales for HK-II staining. Table 3 summarized the findings of immunohistochemical analysis using anti-Glut-1 and anti-HK-II. It was noted that there was a strong positive correlation between the positive area of Glut-1 and HK-II in the higher T grade (T3 + T4) group ($r = 0.99$, $p = 0.0001$). The results of the statistical comparison between tumor SUV and the immunostaining results were shown in Table 4. All the tumors were visualized on FDG-PET images except for those of Patient 6 and Patient 16 with SUVs of less than 2.0. The tumor SUV ranged from 1.36 to 14.10 with an average FDG SUV of 5.53 ± 3.48 for all tumors. As shown in Table 2, the average SUV was 5.77 ± 2.93 for the moderately differentiated tumors and 5.36 ± 3.96 for the well differentiated tumors but this difference was not statistically significant ($p = 0.61$). Regarding the T grade, average FDG uptake of SUV in higher T grades (T3 + T4)

Table 2 Expression of HK-II in OSCC: relationships to tumor differentiation and TNM stage

	All	Tumor differentiation			TNM stage		
		Moderate	Well	p	T1 + T2	T3 + T4	p
No.	19	8	11		14	5	
HK-II, % positive area	92.11 ± 14.37	90.00 ± 20.70	93.64 ± 8.09	0.30	90.00 ± 16.17	98.00 ± 4.47	0.15
HK-II intensity	2.46 ± 0.70	2.38 ± 0.74	2.55 ± 0.69	0.31	2.43 ± 0.76	2.60 ± 0.55	0.33
HK-II, staining score	3.47 ± 0.70	3.38 ± 0.74	3.55 ± 0.69	0.31	3.43 ± 0.76	3.60 ± 0.55	0.33
Tumor SUV	5.53 ± 3.48	5.77 ± 2.93	5.36 ± 3.96	0.61	4.60 ± 3.52	8.13 ± 1.65	0.04

Table 3 Relationship between staining results of Glut-1 and HK-II in OSCC: relationships to tumor differentiation and TNM stage

	Moderate	Well	T1 + T2	T3 + T4	Total
Glut-1 vs. HK-II					
% positive area	0.39 (0.36)	0.44 (0.19)	0.39 (0.17)	0.99 (0.0001)	0.39 (0.98)
intensity	0.18 (0.68)	0.15 (0.67)	0.08 (0.79)	0.61 (0.31)	0.19 (0.43)
staining score	0.18 (0.68)	0.15 (0.67)	0.08 (0.79)	0.61 (0.31)	0.19 (0.43)

Values are r (correlation coefficient), with the probability value (p) within parentheses

Table 4 Correlation between FDG SUV and staining results of HK-II in OSCC: relationships to tumor differentiation and TNM stage

	Moderate	Well	T1 + T2	T3 + T4	Total
HK-II vs. SUV					
% positive area	0.40 (0.34)	–0.23 (0.50)	–0.003 (0.99)	–0.41 (0.54)	0.10 (0.68)
intensity	0.34 (0.43)	0.11 (0.75)	0.13 (0.67)	0.38 (0.57)	0.18 (0.46)
staining score	0.34 (0.43)	0.11 (0.75)	0.13 (0.67)	0.38 (0.57)	0.18 (0.46)

Values are r (correlation coefficient), with the probability value (p) within parenteses

was significantly higher than that in lower T grades (T1 + T2) (8.13 ± 1.65 vs. 4.60 ± 3.52, p = 0.04). In Table 4, there was no statistically significant correlation between SUV and HK-II (p = 0.46).

DISCUSSION

FDG uptake, known as a marker of glucose metabolism, has been used to visualize, stage, and monitor progression of human cancers with PET. However, the molecular mechanisms of increased FDG uptake in tumor cells as compared to normal cells are still not fully understood and are the subject of intense research efforts. Several fundamental studies have focused on the expression of glucose transporters and HK activity to define the role of these two classes of genes in the regulation of FDG uptake.[9] Recently, the number of known glucose transporters has been expanded considerably and thirteen sugar transporters, Glut-1 to Glut-12 and HMIT; gene name SLC2A, have been recognized.[10] Furthermore, it has been found that, the number of distinct gene products, together with the presence of several different transporters in certain tissues and cells, indicates that glucose delivery into cells is a process of considerable complexity. In addition, the phosphorylation of glucose is catalysed by HK, of which there are four (HK-I to IV) in mammalian tissues. HK converts the incoming glucose to glucose-6-phosphate, the initial phosphorylated intermediate of the glycolytic pathway and an important precursor for further glucose utilization in normal and cancer tissues. Compared with normal tissues, a number of mechanisms are associated with changes in HK activity seen in tumors, such as HK gene dosage, increased transcription, modulation of HK promoter activity, and increased mitochondrial binding of HK." At the genetic level the tumor cell adapts metabolically by first increasing the gene copy number of HK-II.[12] Tumor cells grow more rapidly than normal cells, resulting in an increased expression of glycolytic enzymes, including HK, which has been detected in resected tumors from patients with lung, gastrointestinal and breast cancer,[11] and increased HK activity, together with increased glucose transport by tumor cells, has been exploited in cancer imaging using FDG PET.[5,6,13] The cytoplasmic staining finding of HK-II in our study confirmed the above mentioned studies and suggested that enzyme is bound to mitochondria. We also found "nuclear staining" in several cases in this study. This is a kind of mitochondrial staining, located close to the nucleus, which can be falsely scored as nuclear staining when using light microscopy.[13]

Ross CD et al.[14] suggested that the high rate of glucose utilization (indicated by hexokinase activity) found in more poorly differentiated tumors has a higher component of aerobic oxidative metabolism and a relatively lower contribution from anaerobic metabolism than do the rates found in more differentiated tumors. However, in our immunohistochemical study, in which even a higher level of expression of HK-II was found, we have not observed such relationships between well and moderate differentiation tumors. This may be due to the heterogeneity of expression in individual cells and cell populations.

The results of this study initially confirmed that HK-II was predomiantly expressed in all examined patients with OSCC. The positive area of HK-II ranged from 40% to 100% in patients with OSCC, and the tumors could be divided into the three higher scores of 2, 3 and 4 according to both positive area and intensity. This split reflects the metabolic heterogeneity of tumors, with the majority containing high HK-II.

In our study, there was no statistically significant

correlation found with HK-II expression and FDG SUV. Similarly, in a previous study, FDG uptake correlated better with FDG phosphorylating activity of mitochondrial preparations rather than the level of expression of the Glut-1 or HK-I and II genes in cultured cells.[15] Furthermore, the amount of Glut-1 associated with plasma membrane preparations, which should be the only fraction of active protein, also showed higher and lower levels in the different cell lines, indicating that these cell lines adequately represent higher and lower level plasma membrane Glut-1-containing cells.[15] In other words, higher levels of glucose transporter protein do not guarantee increased FDG uptake by cancer cells, with metabolic trapping via phosphorylation of FDG appearing more likely as the rate-determining step in FDG metabolism procedure. However, further studies also should be performed to confirm the key factors of FDG phosphorylation. In addition, we found a significantly strong positive correlation between Glut-1 and HK-II in the higher T grade (T3 + T4) group ($r = 0.99$, $p = 0.0001$), but not in the lower T grade. This may reflect the fact that the activities of Glut-1 and HK-II are different between higher and lower T grades.

In conclusion, OSCC showed increased FDG accumulation and overexpression of HK-II. However, we did not find any significant relationship between high FDG uptake and overexpression of HK-II in this patient population, and thus other properties need to be evaluated in order to elucidate key factors responsible for FDG activity in OSCC.

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