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REVIEW

Application of Positron Emission Tomography Molecular Probes in Hepatocellular Carcinoma Biological Imaging[△]

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Biological behavior is a hot issue in hepatocellular carcinoma (HCC) study. Positron emission tomography (PET), a biological imaging technique, has been widely applied in many types of tumors. It is capable of noninvasive detection of biological behavior. Different radiotracers provide different information of HCC, including glucose/lipid metabolism, DNA synthesis, and apoptosis. In addition, radiotracer uptake relates to biological and clinical prognostic markers. In this article we review the application of several existing and novel radiotracers in PET in HCC study.

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THE clinical application of positron emission tomography (PET) in hepatocellular carcinoma (HCC) is expanding, covering staging and grading, treatment monitoring, and estimation of long-term prognosis. Functional imaging with PET has been reported to offer advantages over anatomic imaging modalities. PET using [¹⁸F] 2-deoxy-2-fluoro-D-glucose (FDG) reveals the difference between benign and malignant liver tumors.¹ Many recent reports indicated that ¹⁸F-FDG and other radiotracers imaging could help distinguishing primary HCC from metastatic HCC.²⁻⁵

Like many other types of carcinoma, HCC shows gene or protein alterations which are associated with the ma-

lignant phenotype. Compared with normal tissue, HCC tissue exhibits angiogenesis, reporter expression, increased glucose metabolism, protein synthesis, increased cell proliferation, and apoptosis. All of these processes could be detected with PET imaging. This article reviews useful and novel radiotracers for imaging the biological characteristics of HCC.

Relationship between FDG uptake and clinical markers of molecular characteristic

The glucose analogue FDG is a surrogate marker for glucose metabolism and the most common radiotracer for PET. It has long been known that increased glycolysis is a feature of malignant tumors compared with normal tissue.⁶ Yun et al² found higher FDG uptake correlated well with more rapid cell growth. Several clinical studies have also proved that the increased FDG uptake was correlated with tumor differentiation.⁷⁻⁹ The detection rate of ¹⁸F-FDG PET was found higher in poorly differentiated HCC lesions than

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in moderately or well differentiated HCC lesions, whereas choline and acetate demonstrated the opposite results.^{4,5} Besides, higher FDG uptake was a predictor for larger tumor size, shorter survival, and higher rates of recurrence.¹⁰⁻¹³ It also had a strong correlation with elevated alpha-fetoprotein level in detecting HCC no larger than 5 cm in size.¹⁴ It has recently been reported that HCC prognosis may be predicted with ¹⁸F-FDG PET.¹⁵ In a series of clinical trials, FDG uptake was also found to be a good predictor of survival time, tumor aggressiveness, and microvascular tumor invasion.¹⁶⁻¹⁸ In addition, the molecular mechanism of FDG accumulation within cells has been investigated *in vitro* and *in vivo*. In recent studies, glucose transporters, glycolytic enzyme, and FDG uptake have demonstrated a close relationship.¹⁹⁻²¹ Ahn et al²² reported that cells with overexpression of hexokinase-II (HK-II) showed higher (1.5- to 2-fold) cell survival and lower resistance (2- to 8-fold) to the anticancer agent cisplatin compared with the nontransfected cell line, and FDG uptake was significantly higher after transfection, confirming that FDG uptake could serve as a surrogate marker of prognosis in HCC. In rat model studies, the intratumoral distribution of FDG correlated significantly with the expression of Glut-1 ($P<0.001$), Glut-3 ($P<0.001$), and HK-II ($P<0.01$).^{19,23} However, a recent study found only weak correlation between FDG uptake and Glut-1 expression.²⁴ In a research involving 31 patients, significant correlation was found between F-FDG uptake and overall expression of Glut-2 ($P=0.002$) and HK-II ($P=0.04$).²⁰ The prognosis of the patients with standardized uptake value (SUV) ≥ 2 was significantly worse than that of the patients with SUV < 2 ($P=0.005$), and similar result was observed in the comparison between patients with positive Glut-2 and those with negative Glut-2 ($P=0.03$), suggesting that SUV could be considered as an important prognostic factor.

Other potential metabolism radiotracers for HCC imaging

¹¹C-acetate was originally used in imaging cardiac oxidative metabolism. It is rapidly converted into acetyl coenzyme A (CoA) after cellular uptake and dominantly enters the tricarboxylic acid cycle for CO₂ generation. Acetate has now been proposed as an alternative radiotracer for detecting tumors not revealed by FDG PET,^{4,25} because human HCCs or cancer cells with low glycolysis show remarkable ¹¹C-acetate uptake.² Although the metabolic pathway of ¹¹C-acetate in tumor cells is not well understood, some studies showed that increased acetate uptake reflected the enhanced lipid synthesis and higher level of fatty acid synthase.^{26,27} In addition, some key enzymes

such as acetyl-CoA synthetase (ACAS) 1 and ACAS2 appear to be important in acetate uptake and acetate-dependent lipid synthesis.^{2,28} Salem et al²⁹ also found that high acetate uptake was associated with *de novo* lipogenesis, and the main metabolites in this process were phosphatidylcholine and triacylglycerol. The overall sensitivity of acetate in detecting HCC lesions may be better than that of FDG.^{4,30} The primary HCC lesions with low FDG uptake demonstrate increased acetate uptake.³⁰ However, acetate was not sensitive for detection of poorly differentiated HCC lesions,⁴ so the pattern of FDG uptake and that of acetate appear to complement each other in HCC imaging.² Two different groups of researchers evaluated the sensitivity of both acetate and FDG in metastatic HCC lesions, finding the sensitivity of dual-tracer imaging in detecting metastatic HCC to be 98% and 85.7% respectively.^{3,31} However, the widespread use of ¹¹C-acetate is limited by the short half-life of ¹¹C (20 minutes). Fluoroacetate (FAC), an analog of acetate, is metabolized to fluoroacetyl-CoA and then fluorocitrate, which cannot be further metabolized to CO₂ and water,³² thus trapped in the cell in proportion to oxidative metabolism. ¹⁸F-FAC has been evaluated as a PET agent for imaging prostate cancer and glioblastoma,^{33,34} but there is no report yet about FAC imaging for HCC.

Choline is incorporated into cells through phosphorylcholine synthesis and integrated into the cell membrane phospholipids.³⁵ The use of ¹¹C-choline PET has been reported for the detection of tumors in the brain and prostate, even for tumor recurrence.³⁶⁻³⁸ Yamamoto et al⁵ and Salem et al³⁹ both found that ¹¹C-choline PET had a higher overall detection rate for HCC lesions than FDG or acetate, meanwhile the level of choline kinase was significant higher in HCC than that in surrounding hepatic tissue ($P=0.001$).

Methionine is transferred into cells by various amino acid transporters and was incorporated into proteins. Zhao et al⁴⁰ found ¹¹C-methionine uptake significantly lower in the granuloma than in the liver tumor, whereas ¹⁸F-FDG and ³H-fluorothymidine (FLT) were not able to differentiate granulomas from tumors, suggesting that ¹¹C-methionine has the potential to accurately distinguish malignant tumors from benign lesions, particularly granulomatous lesions.

The indicator of cellular proliferation is DNA synthesis, which can be measured using radiolabelled thymidine or its analogues. A thymidine analogue, 3'-deoxy-3'-¹⁸F-FLT, has been introduced for imaging tumoral proliferation. It was trapped within the cytosol after being monophosphorylated by thymidine kinase-1, a principle enzyme in the salvage pathway of DNA synthesis.⁴¹ Presently, ¹⁸F-FLT has been found to be useful for noninvasive assessment of

the proliferation rate of several types of cancer, such as colorectal, oesophageal, breast, and laryngeal cancers.⁴²⁻⁴⁵ Especially in lung cancer, the FLT uptake correlated significantly with proliferative activity as indicated by the Ki-67 index.⁴⁶⁻⁴⁹ This kind of radiotracer specially reflecting proliferative activity may be suitable for evaluation of therapy response, yet there has been few report about FLT imaging in liver cancer. Eckel et al⁵⁰ evaluated FLT uptake in HCC and surrounding liver tissue by calculating mean and maximum SUV. The result showed higher FLT uptake in HCC than in the surrounding liver tissue, and a significant positive relationship between the proliferation marker MIB-1 and the mean SUV ($P=0.02$). Besides, higher FLT uptake seems to be associated with shorter survival time.

Novel and potential probes applied in HCC biological studies

Tumor hypoxia is an important biological characteristic and exhibits increased resistance to most types of cancer therapy.^{51,52} Noninvasive assessment of the tumor hypoxia could help identifying tumor heterogeneity for biologically based forcal ablative therapies and indicating prognosis for therapy outcome.^{53,54} A variety of different hypoxia markers have been labeled with radionuclides to image tumor hypoxia in PET. Misonidazo (MISO) and iodoazomycin galactopyranoside (IAZG) are clinically useful tracers for tumor hypoxia, with their respective advantages and disadvantages. Riedl et al⁵⁵ compared their uptake in the same rats with liver tumors and peritoneal metastasis in a dynamic PET imaging study. Because of a more rapid initial renal elimination, the tumor/normal tissue ratios at 3 hour for ¹⁸F-FMISO ranged from 1.2 to 2.3, higher than the corresponding ratios for ¹²⁴I-IAZG (1.05-1.35) in almost all tumors. Consequencely, the ¹⁸F-MISO images were of better diagnostic quality than the ¹²⁴I-IAZG images in the hepatoma model. 2-(2-nitro-1[H]-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5) is another potential hypoxia marker. In a study on ¹⁸F-EF5 imaging in a rat model of liver tumor, tumor/muscle ratios ranged from 0.82 to 1.73 120 minutes after injection and 1.47 to 2.95 180 minutes after injection.⁵⁶ Tumors became easily visible by 60 minutes after injection, supporting the use of EF5 as another promising agent for tumor hypoxia imaging. All the tracers discussed above are potential complements to ¹⁸F-FDG in detection of HCC lesions.

Angiogenesis is also one of the biological characteristics of HCC. It is a process depending on the balance between promoting and inhibiting factors, involving growth factors and reporters, adhesion molecule, enzyme, etc. Recently the application of PET in angiogenesis imaging at

functional and molecular levels has been reported.⁵⁷⁻⁵⁹ The key molecular imaging makers in angiogenesis include integrins, vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR), matrix metalloproteinases, and Hypoxia/HIF1. Intergrins, a family of cell adhesion molecules, are composed of different α - and β - subunits. Intergrin $\alpha_v\beta_3$, which plays an important role in regulating angiogenesis, is expressed in a lot of tumors such as glioblastoma, ovarian cancer, and prostate cancer,⁵⁷⁻⁵⁹ but not found in HCC so far. VEGF/VEGFR imaging in colorectal cancer liver metastases has been reported.⁶⁰ However, there was no correlation between ¹¹¹In-bevacizumab uptake and VEGFR-A expression. PET using H₂¹⁵O and ⁶⁸Ga-1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid-albumin also presents a quantitative measure of tumor blood perfusion or volume, thereby providing functional relevant data of angiogenesis.⁶¹ Those radiotracers offer a novel approach for non-invasive monitoring of tumor angiogenesis, and for evaluation of the efficacy of anti-angiogenic therapies.

¹¹C-SA5845 and ¹¹C-SA4503 are novel tracers for tumor. Kawamura et al⁶² found that ¹¹C-SA5845 and ¹¹C-SA4503 were accumulated in AH109A cell and tissues. Meanwhile in PET studies in rabbits, the uptake of ¹¹C-SA5845 was found relatively higher than that of ¹¹C-SA4503 in the VX-2 carcinoma, because the density of sigma2 receptors in the VX-2 tissue was much higher compared with that of sigma1 receptors. Based on those findings, ¹¹C-SA5845 and ¹¹C-SA4503 may be applied in PET imaging of sigma receptor-rich tumors including liver tumor.

Apoptosis is another important biological process of tumor. NST-732 and Annexin-V are useful apoptosis probes *in vitro* or *in vivo*.^{63,64} Both of them could accumulate within cells in the early stage of apoptosis *via* crossing the membrane into the cell.⁶³ Although there is no report about apoptosis imaging of HCC, these apoptosis markers are potential for clinical PET imaging of tumor apoptosis.

CONCLUSION

Unlike tissue sampling studies, PET imaging studies allow analysis of the entire tumor at molecular and cellular levels. This imaging modality is considered not only for staging and prognosis, but also for assessment of biological behavior of tumors (cell proliferation, apoptosis, angiogenesis, invasion, and metastasis). Researchers have so far found many biological markers related with biological characteristics of cancer, and applied existing and novel

tracers in PET imaging in HCC studies. We should pay more attention to the relationship between radiotracer uptake value and biological markers expression, and make more specialized PET tracers available for understanding the tumor biological behavior *in vivo*. It is possible that in the near future, biological imaging with PET tracers would be routinely applied in clinical cancer trials and personalized molecular therapy.

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