Prediction of the Early Effective Treatment Response to Gefitinib Using 18F-FDG MicroPET-CT in Mouse Tumor Xenografts

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Purpose

The antitumor activities of epithelial growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) is significant. Likewise clinical benefits with EGFR-TKI have been demonstrated in various tumor types [1-3]. Unfortunately, responses have been seen in only a subgroup of patients at the cost of potential toxicity for all treated patients [1,4]. There is an urgent need for early identification of the patients who showed benefit from target therapy in order to avoid ineffective treatment.

Conventional anatomic imaging, based on tumor volume measurement, fail to early convey the tumor metabolic effect. As a mature molecular imaging technology, $^{18}$F-FDG PET-CT have been used for measurement of the metabolic response of tumor cells to therapy, including chemotherapy, radiotherapy and target therapy [5-7].

Indeed many clinical trials have shown that $^{18}$F-FDG PET-CT imaging could be a reliable surrogate marker for the detection of early therapeutic responses and clinical benefit [8-10]. Su et al. found a decline in $^{18}$F-FDG uptake in sensitive tumor cell lines within 24 to 48 hours of gefitinib treatment [11]. They also showed rapid decreases of tumor $^{18}$F-FDG uptake in sensitive xenografts within 48 hours of gefitinib treatment.

The purpose of this study was to investigate whether the early changes in $^{18}$F-FDG uptake correlate with the histopathological responses induced by gefitinib in the tumor xenografts and determined whether the changes of $^{18}$F-FDG could be used to early identify those sensitive tumor most likely to benefit from gefitinib therapy in vivo.
Methods and Materials

Xenograft Models

All animal experiments were approved by the PUMA Institutional Animal Research Committee. This study used 16 female Balb/c nude mice 6-8 weeks old and weighing 20-24g. Human epidermoid carcinoma cell lines A431 were grown in DMEM with 10% fetal calf serum, 100 IU/mL of penicillin, and 100 µg/mL of streptomycin. Cells were incubation at 37° in an atmosphere of 5% CO₂. Tumor cell lines A431 were inoculated subcutaneously (2×10⁷ cells per mice) in the right flank of mice.

Experimental Design

Sixteen mice were randomly divided into 2 groups, respectively. Six mice were served as untreated controls, and ten mice received the medicine. The mice in the treated groups was administered intragastrically with 100 mg/( kg·d), while the ones in the control groups with sterile water. When tumors had grown to an approximate size of 150 mm³, the xenografts was used in experiments. ¹⁸F-FDG microPET-CT was performed the day before gefitinib administration (day 0) and at 2 d, 7 d, and 14 d afterward. In the control group, ¹⁸F-FDG microPET-CT images were acquired at the same time points with sterile water administration.

If tumor decreased, it was included in the sensitive group. If tumor increased slowly, which was smaller than the control group, it was included in the insensitive group. Likewise, if the tumor increased more rapidly, which was not significantly different with the control group, it was included in the resistant group (P >0.05). After the experiment, all mice were sacrificed and tumor tissues were rapidly resected.

¹⁸F-FDG microPET-CT Imaging Protocol

The microPET-CT imaging protocol consisted of CT anatomic imaging and PET metabolic imaging. MicroCT scans were performed with an X-ray tube voltage of 80 kV, a current of 500 µA, exposure time 130 ms and rotation steps 120. Mice were injected i.v with 290~320µCi of ¹⁸F-FDG in 200uL saline after 10 min isoflurane (2% in 100% oxygen) anesthesia period, then after 60 minutes, imaging was initiated. During scanning, the mouse was placed prone on the examination bed with isoflurane (2% in 100% oxygen) anesthesia and lasted for 20 minutes. After data acquisition, microPET data were reconstructed with the filtered back-projection reconstruction algorithm.

Quantitative Image Analysis
Images were analyzed with the Inveon Research Workplace. Regions of interest were drawn manually by qualitative assessment covering the entire tumor. The maximal percentage of the injected dose per gram of tissue (%ID/g$_{\text{max}}$) was obtained using ROI drawn around areas of increased tracer accumulation[12]. Tumor volume was generated by summation of voxels within the tomographic planes. The tumor volume response was expressed as $\#\text{Volume}_{\text{day } n} = (\text{Volume}_{\text{day } n} - \text{Volume}_{\text{day } 0}) / \text{Volume}_{\text{day } 0} \times 100$. The %ID/g$_{\text{max}}$ response was also expressed as $\%\text{ID/g}_{\text{max } \text{day } n} = (\%\text{ID/g}_{\text{max } \text{day } n} - \%\text{ID/g}_{\text{max } \text{day } 0}) / \%\text{ID/g}_{\text{max } \text{day } 0} \times 100$, described in previous studies[12].

**Histopathologic Examination**

All the tumors specimens were excised, cut open across the maximum dimension, fixed with 10% formalin, embedded in paraffin, sectioned at 4 mm thick with a microtome, and stained with hematoxylin and eosin (HE) and antibody (Ki-67: M7248, Dako; Glut-1: RB-9052, NeoMarkers), respectively. Pictures that were taken under high magnification fields of view (200×) were analyzed using the professional image analysis software, ImagePro Plus 6.0. The necrotic area, the integrated optical density (IOD) of Glut-1 and the stained cell of Ki-67 in tumor tissue were evaluated[13].

**Statistical Analysis**

Results are presented as mean value ± SD. The tumor responses in each group were statistically evaluated by ANOVA. Statistical analyses were performed to correlate the amount of necrosis and the Ki-67 and Glut-1 expression in the specimen to the change in %ID/g$_{\text{max } \text{day } 14}$. $P$ values less than 0.05 were considered statistically significant.
Results

Changes of Tumor Volume measured by MicroCT

According to the A431 tumor volume at 14 days of therapy, six was sensitive, four was insensitive and none was resistant to gefitinib. Before therapy, there was no significant difference in A431 tumor volume among the 3 groups. The tumor volume in the 3 groups increased gradually, but no significant differences were seen among the groups on day 2 ($P = 0.167$). On day 7, size was significantly lower in the sensitive group than in the insensitive and control groups ($P_{\text{insensitive}} = 0.034$, $P_{\text{control}} = 0.000$). Volume was significantly different among the 3 groups on day 14 (Fig. 1).

Changes of $^{18}$F-FDG uptake measured by MicroPET

The A431 tumor $^{18}$F-FDG uptake did not significantly differ among the three groups before therapy. However, on day 2, the %ID/$g_{\text{max}}$ decreased obviously in the sensitive group. The %ID/$g_{\text{max}}$ decreased slightly in the insensitive group. The average changes of A431 tumor $^{18}$F-FDG uptake were (-30.92 ± 6.66)%, (-5.68 ± 6.95)% and (7.72 ± 3.85)% in the sensitive group, the insensitive group and the control group, respectively ($P = 0.000$, for all). There was significant difference in the changes of A431 tumor $^{18}$F-FDG uptake among those 3 groups at any time point. The %ID/$g_{\text{max}}$ in the control group gradually increased but, until day 7, decreased slightly. The sample transaxial images of xenografts were shown in Fig. 2.

Analysis of Histopathologic Findings

All the degree of tumor tissues necrosis was evaluated. In A431 tumor, on day 14, necrosis was obvious in three groups. The tumor wall became thinner, and the necrotic areas larger, in the sensitive group than in the insensitive and control groups. The necrotic fraction was (90.57%±4.77%), (79.90%±7.76%) and (69.04%±7.80%) in the sensitive, insensitive, and control groups, respectively. Statistically significant necrotic fractions were seen in the sensitive and insensitive groups ($P = 0.045$), in the sensitive and control groups ($P = 0.000$), or in the insensitive and control groups ($P = 0.042$) on day 14.

The immunohistochemical analyses revealed a significant reduction of the Glut-1 and Ki-67 in the sensitive group after 14 days treatment. The Glut-1 and Ki-67 decreased slightly in the insensitive group (Fig. 3).
Fig. 1: Changes in A431 tumor volume at various time points. No significant differences were seen among the groups on days 0, 2 (P >0.05). Significant differences were seen among the groups on days 7, 14.

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Fig. 2: MicroPET-CT imagings in A431 tumor before and after gefinitib treatment. (A) The sensitive tumor FDG uptake decreased obviously on day 2, and decreased slightly on days 7, 14. The tumor volume gradually decreased in the sensitive group. (B) The insensitive tumor FDG uptake gradually decreased on days 2, 7, 14. The tumor volume gradually increased in the insensitive group. (C) The tumor FDG uptake in control group gradually increased, but, until day 7, decreased slightly. The tumor volume increased in the control group.

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Fig. 3: Immunohistochemical study of the effects of gefitinib on tumor tissue. Sections (4 µm thick) of formalin-fixed and paraffin-embedded tumors from mice (A-E) after 14 days gefitinib treatment were stained with antibodies against Glut-1 (A-C), and Ki-67 (D and E). The Glut-1 and Ki-67 expressions were more inhibited in sensitive tumor than the insensitive and control groups after 14 days gefitinib treatment.

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Conclusion

On day 2 after gefitinib administration, the sensitive group exhibited an up to 30% decrease of metabolic activity and the insensitive group showed a less 10% decrease in $^{18}$F-FDG uptake ($P = 0.000$). Immunohistochemistry showed that gefitinib strongly inhibited Glut-1 and Ki-67 expressions in tumor tissue from sensitive xenografts. That is, $^{18}$F-FDG uptake change can effectively predict the pathological response, and more early prompted the effectiveness of gefitinib targeted therapy. The early changes of tumor $^{18}$F-FDG uptake are closely related with sensitivity to target therapy.

Our findings suggest that early response assessment by PET-CT could help to identify sensitive tumors on the basis of a significant decrease in glucose metabolism by tumors. $^{18}$F-FDG PET-CT may be useful for the in vivo detection for EGFR-TKI treatment. The technique is sensitive and noninvasive, and can guide oncologists in choosing patients who will benefit from gefitinib treatment. However, further clinical studies are needed to estimate the optimal cutoff value of $^{18}$F-FDG uptake decline after 2 days of gefitinib administration for the target therapy.
References


Personal Information

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