Dynamic Contrast-Enhanced Magnetic Resonance Imaging for Early Therapy Evaluation of Combined Anti-EGFR Antibody and Irinotecan in Orthotopic Pancreatic Tumor Xenografts

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Purpose: To evaluate DCE-MRI as an early prognostic tool for effective anti-EGFR therapy with/without concurrent chemotherapy in an orthotopic pancreatic-cancer murine model, and to develop a novel timing-independent DCE-MRI biomarker for early therapy assessment, based on characterization of non-linear tumor response observed during serial imaging.

Methods: Groups 1-4 (n=6/group) of SCID mice bearing orthotopic pancreatic adenocarcinoma (luciferase-positive MIA PaCa-2) were treated with PBS (control), cetuximab (1mg), irinotecan (25mg/kg), or cetuximab plus irinotecan respectively twice weekly for 3 weeks. DCE-MRI was performed on days 0, 1, 2, and 3 after therapy initiation, while anatomical MRI was performed once weekly for 3 weeks. Bioluminescence imaging was performed on days 0 and 21. At day 21, all tumors were collected for CD31 and Ki-67 staining. Groups 5 (n=5) and 6 (n=4) were injected with Tc-99m-cetuximab and Tc-99m-isotype control antibody respectively; SPECT/CT imaging was performed at 6 hours after dosing, and biodistribution studies were performed at 24 hours after dosing. The averaged K^{trans} values in the entire tumor region or within the 0.5-mm thick peripheral tumor region were calculated. The best-fitting 2nd-order polynomial curves for the K^{trans} changes were retrieved, and the quadratic coefficient for each curve was proposed as the novel MR biomarker. K^{trans}, v_e, and tumor-volume measurements made among the groups 1-4 over 3 days (or 21 days for tumor volume) were analyzed using repeated measures analysis of variance (RM ANOVA). Comparisons for a single measurement were done using one-way ANOVA followed by Tukey's HSD (Honestly Significant Differences) test. The pearson correlation coefficient was used to look at the relationships between two variables.

Results: *In vivo* SPECT/CT images visualized the significantly higher tumor uptake of Tc^{99m}-cetuximab than of Tc^{99m}-isotype control antibody, which was confirmed by biodistribution study; the tumor uptake of Tc^{99m}-cetuximab was 19.0±0.6 %ID/g, which was significantly higher than that of Tc^{99m}-isotype control antibody (6.5±1.2 %ID/g) ($p<0.001$), whereas the blood concentration of Tc^{99m}-cetuximab (10.4±0.5 %ID/g) was not different from that of Tc^{99m}-isotype control antibody (10.2±2.8 %ID/g) ($p=0.949$). Figure 1 shows representative dynamic contrast-enhanced MR images of a mouse at (A) 1 minute before and (B) 2 minutes after gadoteridol injection using the same intensity scale with (C,D) K^{trans} and (E,F) v_e maps of the (C,E) entire tumor regions or (D,F) 0.5-mm thick peripheral tumor regions. The boundary of the tumor region is indicated with a red dotted circle in Figs. 1A and 1B. The change in K^{trans} values of groups 1-4 for 3 days after therapy initiation were 83±33%, 63±28%, 17±16%, and 12±18% respectively in the entire tumor region without statistical difference among any of the groups. However, when analyzed in peripheral tumor region, change in K^{trans} values were 102±16%, 42±21%, 15±6%, and -19±5% respectively (Fig. 2). The significant suppression of K^{trans} increase was detected after irinotecan ($p=0.008$) or combination therapy ($p<0.001$). The change in v_e values of groups 1-4 for 3 days after therapy initiation were 67±42%, 28±9%, 8±16%, and -12±10% respectively in the entire tumor region, while those in the peripheral tumor region were 77±43%, 26±12%, 13±11%, and -20±7% respectively, but no significant difference was detected among groups in either region. Figure 3 shows tumor-volume change of groups 1-4 during 21 days after therapy initiation; the mean tumor volumes of all four groups increased about 20% during the first 3 days without statistical difference ($p>0.050$). However, for the entire 3 weeks, the tumor-volume increase was significantly suppressed by either monotherapy or combined therapy ($p<0.050$). The K^{trans} changes observed for 3 days in the peripheral region were significantly correlated with tumor-volume changes ($p<0.001$) and bioluminescence-signal changes ($p=0.050$). The microvessel densities (CD31 stained) of groups 1-4 were 1.60±0.25%, 0.64±0.13%, 0.53±0.09%, and 0.27±0.05% respectively, while the proliferating cell densities (Ki-67 stained) of groups 1-4 were 85.3±1.8%, 70.3±5.4%, 70.8±4.4%, and 48.3±7.1% respectively. The K^{trans} changes for 3 days in peripheral region were significantly correlated with microvessel densities ($p=0.002$) and proliferating-cell densities ($p=0.001$). Of interest, the mean K^{trans} changes of all 4 groups in peripheral tumor region followed second-order polynomial curves, validated with high R^2 values (≥0.73). **The quadratic coefficient of each curve was proposed as a novel DCE-MR based biomarker**; the mean value of novel biomarker of groups 1-4 were 0.050±0.026, -0.015±0.028, -0.071±0.021, and -0.082±0.013 respectively, and those of groups 3 and 4 were significantly lower than that of control ($p=0.006$ and 0.003 respectively). The values of the novel biomarker were significantly correlated with tumor-volume changes ($p<0.001$), bioluminescence-signal changes ($p=0.019$), microvessel densities ($p=0.002$), and proliferating-cell densities ($p=0.001$).

Conclusion: This study supports the clinical use of DCE-MRI to evaluate an anti-EGFR therapy combined with chemotherapy for pancreatic adenocarcinoma, identifies a novel timing-independent DCEMRI based biomarker, and proposes further application of this therapeutic surveillance strategy in human subjects to ultimately achieve more favorable patient-specific clinical outcomes.

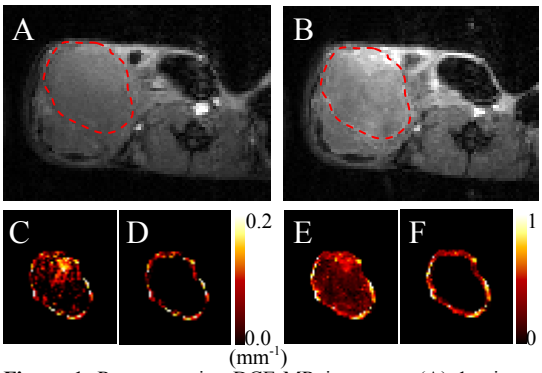

Figure 1. Representative DCE-MR images at (A) 1 minute before and (B) 2 minutes after gadoteridol injection with (C,D) K^{trans} and (E,F) v_e maps of the (C,E) entire tumor regions or (D,F) 0.5-mm thick peripheral tumor regions.

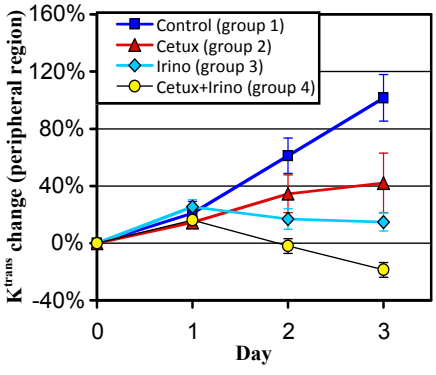

Figure 2. K^{trans} changes of groups 1-4 during 3 days post therapy in the 0.5-mm thick peripheral tumor region.

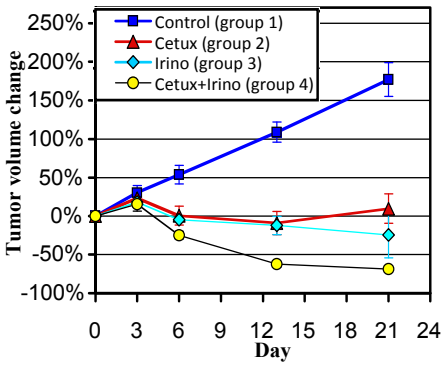

Figure 3. Tumor volume changes of groups 1-4 during 21 days after therapy initiation.