

Multiparametric Imaging for Therapy Response to Cytotoxic and Cytostatic Agents in Xenograft Mice

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Introduction

Chemotherapeutic (cytotoxic) agents form the basis of most anti-cancer treatment protocols for advanced malignancies. In the past decade, novel signal transduction inhibitors (CTIs, cytostatic) have revolutionized treatment strategies by incorporating targeted therapies that in some cases supplant cytotoxic agents. Unlike cytotoxic drugs, STIs are design to selectively inhibit a particular oncologic pathway that is implicated in oncogenesis. Colorectal cancer (CRC) represents a major health burden and is the second-leading cause of cancer-related death in the US. Among the many new pathways being exploited for cancer treatment, is the insulin-like growth factor receptor (IGF1R) signaling pathway, which appears to be a robust target in CRC (e.g., over-expressed in more than 50% of CRC patients). Small-molecule tyrosine kinase inhibitors (TKI) of the IGF1R have an ability to suppress uncontrolled CRC cell proliferation. Unlike cytotoxic chemotherapeutic agents, most novel STIs exhibit modest tumor regression, therefore, there is significant need for establishing functional pharmacodynamic endpoints for inhibition of a particular oncopathway. In the present study we established functional imaging-based end-points (DW-MRI, MRS and FDG-PET) to detect the therapeutic responses to an IGF1R/IR TKI (cytostatic) and compared them to those of a classic chemotherapeutic agent, irinotecan (cytotoxic) in CRC mouse models.

Methods

Female nude xenograft mice, inoculated with human CRC cell lines (sensitive and resistant to IGF1R inhibition), underwent treatment with an IGF1R TKI (40 mg/kg) by daily oral gavage. IGF1R resistant tumors were treated with irinotecan (given as an ip injection twice a week) to increase the therapeutic efficacy. Diffusion-weighted MRI, 2-deoxy-2-¹⁸F-fluoro-D-glucose positron emission tomography (FDG-PET) and ¹⁸F-choline (D4-¹⁸FCH) PET were performed at the baseline (prior to treatment), day 2-3 and day 25-28 of treatment. By the end of the study, tumors were harvested, extracted and underwent *ex vivo* high-resolution MRS for expanded metabolic profiling. ADC, glucose and choline uptake as well as absolute metabolite concentrations are reported for DW-MRI, FDG/ FCH-PET and MRS, respectively. Each group contained at least 4 animals, with 8 tumors (one per flank) in each data set (unless reported otherwise); an unpaired t-test was applied to determine statistical significance (with $p < 0.05$ values).

Results

DW-MRI: No significant changes in ADC values between the baseline and day 3/ day 25 of treatment were observed in IGF1R TKI treated CRC mice, independent of their sensitivity to IGF1R inhibition (for IGF1R sensitive tumor, $ADC = 1.31 \pm 0.19$ at baseline vs. 1.19 ± 0.25 at day 25, n.s., $n = 16$). The only treatment group which exhibited increased ADC values, were mice treated with the irinotecan combination (Figure 1, $p = 0.02$). **Metabolic PET and MRS:** Decreased glucose uptake ($p = 0.05$) by FDG-PET was apparent in IGF1R TKI treated sensitive xenografts as early as 3 days of treatment when no changes in tumor size were yet detected. A significant decrease in tumor size (MRI, $p = 0.001$, $n = 16$), choline uptake and membrane choline-containing phospholipids ($p = 0.02$ and 0.03 , respectively), as well as a complete inhibition of glucose uptake ($p = 0.0001$) and lactate production ($p = 0.001$) was seen after 28 days of IGF1R TKI treatment in sensitive xenografts (Figure 2). No changes in FDG-PET, MRS, or tumor progression were observed in IGF1R-resistant xenografts. In the irinotecan treated group, an initial slight increase in glucose uptake (day 3, $p = 0.07$) was observed followed by a slight decrease in glucose uptake by PET (day 28, $p = 0.05$). There was a significant decrease in adenosine ($p = 0.01$) and nucleotide ($p = 0.02$) pools in irinotecan-treated tumors by MRS. A slight decrease in phosphocholine (PCho, $p = 0.05$) and a significant increase in glycerophosphocholine (GPC, $p = 0.02$) with decreased [PCho/GPC] ratios correlated well with a moderate decrease in tumor growth by irinotecan.

Conclusions

In the presented study we established specific pharmacodynamic end-points for therapeutic responses to a novel cytostatic STI (decreased glucose uptake, decreased lactate production, decreased choline uptake and metabolism by PET and MRS) in contrast to cytotoxic effects of a chemotherapeutic agent (increased ADC by DW-MRI and increased phospholipid and nucleotide breakdown by MRS). Thus, metabolic and functional multiparametric imaging can be used clinically to assess characteristic metabolic inhibition (cytostatic) versus cell death induction (cytotoxic) in CRC patients receiving these agents.

Figure 1: ADC mapping/ Irinotecan

Figure 2: FDG-PET, MRS/ sensitive (LS1034) and resistant (LS174T) tumors

