EVALUATION OF SALMONELLA-BASED SUICIDE GENE TRANSFER IN A RODENT TUMOR MODEL USING IN VIVO 19F MR SPECTROSCOPY.

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Objective

Early recognition of the success of an anti-cancer therapy will help to select or optimize a therapy on an individual basis. In that context, the use of fluorine containing drugs, such as 5-FC and 5-FU, that are being applied in some of these therapies may allow for a non-invasive detection using ^{19}F MRS. The lack of correlation between 5-FU intra-tumor activity and 5-FU plasma levels furthermore necessitates such a non-invasive screening (6). The present study aimed to correlate the *in vivo* ^{19}F-MRS-observable conversion of 5-FC to 5-FU with the anti-tumor effects of the attenuated *Salmonella* CD-vector system. The intra-tumoral colonization of the TAPET-CD and hypoxic levels were also determined to explain differences observed in anti-tumoral activity.

Materials and Methods

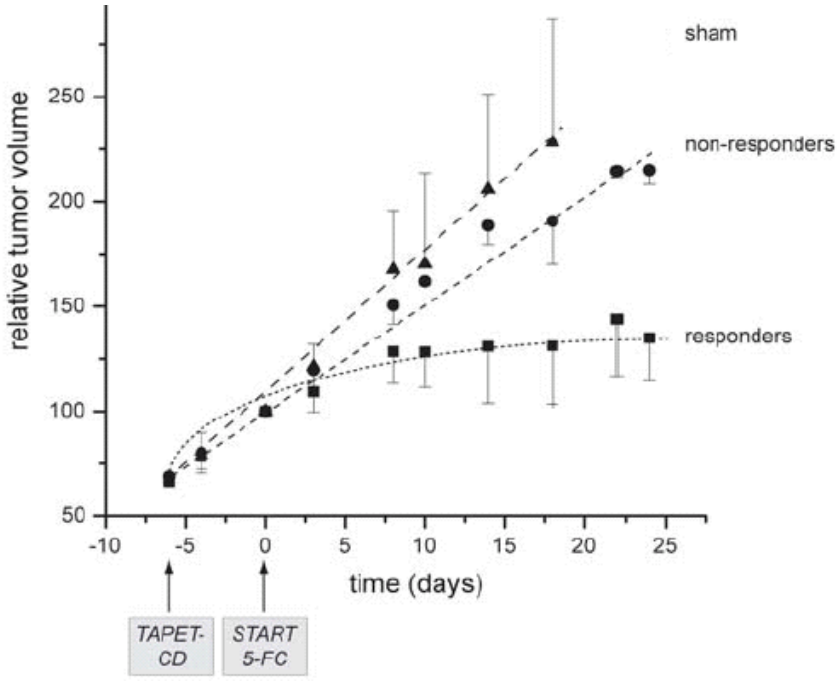
Attenuated *Salmonella typhimurium* VNP20047 recombinant for cytosine deaminase (TAPET-CD, VION Pharmaceuticals, Inc., New Haven, CT) were monitored for 5-FC to 5-FU conversion in subcutaneous xenografted (nu/nu mouse) human colorectal carcinoma (HCT116).

To determine *in vivo* the intra-tumoral conversion activity of the recombinant bacteria ^{19}F-MRS measurements were done in a 4.7 Tesla BIOSPEC horizontal magnet (Bruker), equipped with a double-tuned (^{19}F-^{1}H) surface coil of 1 cm diameter, positioned underneath the tumor. Animals were anaesthetized with Sodium Pentobarbital (Nembutal® 0.1ml/100 g body weight) for stable positioning. Serial ^{19}F spectra (TR= 0.75s, NA= 1024, no decoupling) were acquired every 15 minutes, while the animal environment in the magnet bore was kept at 36°C.

The tumors were injected with $5x10^7$ bacteria when the initial tumor volumes reached >300 mm^3. In one group (n=12) 5-FC was administered systematically each day starting from 7 days post TAPET-CD administration. A control group (n=4) received saline instead of 5-FC according the same schedule. The tumors were measured in three orthogonal directions using a Vernier caliper and tumor volumes were calculated with the formula LxHxWxπ/6. At the end of the follow-up period, animals were killed and tumors excised. Tumor homogenates were prepared to quantify bacterial colonization and the presence of CD. Perchloric acid extracts were made to perform in vitro ^{19}F MRS.The experiments were approved by the Ethical Committee for Animal Experiments of the KULeuven, in agreement with national guidelines.

Results and Discussion

In vivo ^{19}F-MRS was used repeatedly to evaluate the conversion of 5-FC to 5-FU (a measure for the intra-tumoral bacterial CDase activity) in each animal. Animals in which a 5-FU signal could be detected during the follow-up period were marked as 'responders' (9/12), the others as 'non-responders (3/12). Comparison of the average growth curves of both groups with the saline-treated group indicated a difference in response to the therapy for the 'responders' *versus* control and non-responders (Fig. 1). The in vitro ^{19}F MRS data confirmed the in vivo results. Remarkably, no difference in TAPET-CD tumor colonization was observed and Western blots on tumor homogenates revealed that CDase protein was detectable in all tumors. This indicates that the presence of metabolically active bacteria alone in the tumor do not necessarily guarantee anti-tumor effects and that invasive measurements for evaluation not necessarily correlate with (expected) treatment outcome. Since no obvious histological differences were apparent amongst the tumors, our observations probably indicate that variations in prodrug diffusion to the bacterial colonization sites (hypoxic/necrotic regions) are responsible for the differential effects. Most interestingly, it stresses the importance of a non-invasive real-time imaging technique such as ^{19}F MRS to allow a prediction of the success of this TAPET-CD/5-FC therapy. Further evaluation is in progress.



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