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Biology Contribution

Reverse-Contrast Imaging and Targeted Radiation Therapy of Advanced Pancreatic Cancer Models

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Summary

The recent development of genetically engineered mouse models of pancreatic cancer constitutes a significant advance for experimental therapy development against this deadly disease. However, neither these models nor orthotopic tumors can be easily irradiated, owing to limitations in x-ray detection

Purpose: To evaluate the feasibility of delivering experimental radiation therapy to tumors in the mouse pancreas. Imaging and treatment were performed using combined CT (computed tomography)/orthovoltage treatment with a rotating gantry.

Methods and Materials: After intraperitoneal administration of radiopaque iodinated contrast, abdominal organ delineation was performed by x-ray CT. With this technique we delineated the pancreas and both orthotopic xenografts and genetically engineered disease. Computed tomographic imaging was validated by comparison with magnetic resonance imaging. Therapeutic radiation was delivered via a 1-cm diameter field. Selective x-ray radiation therapy of the noninvasively defined orthotopic mass was confirmed using γH2AX staining. Mice could tolerate a dose of 15 Gy when the field was centered on the pancreas tail, and treatment was delivered as a continuous 360° arc. This strategy was then

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of abdominal organs. This hinders development of more effective protocols to treat pancreatic cancer by irradiation, a major modality for clinical management, or to elucidate resistance mechanisms. We demonstrate a noninvasive imaging technique, which is then used to deliver large radiation doses to tumors in the mouse pancreas. This opens the way for studies of radiation therapy and chemoradiation in the most appropriate disease models available.

used for radiation therapy planning for selective delivery of therapeutic x-ray radiation therapy to orthotopic tumors.

Results: Tumor growth delay after 15 Gy was monitored, using CT and ultrasound to determine the tumor volume at various times after treatment. Our strategy enables the use of clinical radiation oncology approaches to treat experimental tumors in the pancreas of small animals for the first time. We demonstrate that delivery of 15 Gy from a rotating gantry minimizes background healthy tissue damage and significantly retards tumor growth.

Conclusions: This advance permits evaluation of radiation planning and dosing parameters. Accurate noninvasive longitudinal imaging and monitoring of tumor progression and therapeutic response in preclinical models is now possible and can be expected to more effectively evaluate pancreatic cancer disease and therapeutic response.

Introduction

Research to better understand and combat cancer has benefitted from animal models that more closely resemble human cancer. The use of subcutaneous xenografts has largely been replaced by orthotopic tumors and genetically engineered immune competent mice, which spontaneously develop tumors in relevant organs. These models offer various advantages, including a tumor microenvironment that better represents that of human tumors, possession of gene expression profiles that may match patient samples, and a clinically relevant primary site from which the disease may metastasize (1-5). These models are particularly valued for testing treatment strategies, because cancer researchers may have been misled about the likely value of experimental therapies by relying on the unique biology of subcutaneous tumors (6).

This development poses a problem for experimental radiation therapy in that it is difficult to deliver meaningful doses to an orthotopic tumor without serious damage to adjacent normal structures. Recent technical achievements have led to the development of small animal combined radiation therapy/computed tomography (CT) units (7, 8). This brings 2 novel advances to experimental radiation therapy: (*1*) they allow delivery of radiation dose to tissue volumes identified by CT; and (*2*) by enabling delivery of the treatment dose from multiple angles, or in a continuous rotational arc, they spare normal tissue and thus allow larger doses to be delivered to the tumor. These 2 characteristics more closely recapitulate the clinical paradigm of irradiation planning and delivery. However, dose delivery to pancreatic tumor models is further complicated because of the lack of CT contrast of the organ. Anatomic localization and delineation of the pancreas in mice is very challenging because the organ is not a defined solid secondary retroperitoneal organ (as it is in humans) but rather a thin membrane spread throughout the upper abdomen. To date, the only radiation therapy in experimental abdominal tumors has been attempted using the xenobiotic luciferase reporter, as performed by Tuli et al (9, 10).

Here we explore an alternative approach that does not require a reporter. By injecting a large volume of dilute CT contrast agent intraperitoneally (IP), we achieve both physical separation of the abdominal structures and CT visualization, because tissue is outlined by x-ray opaque medium. The technique enables us to rapidly define the pancreas and its enclosed tumor. The use of small volumes of IP contrast agent has previously been described (11), allowing the detection of liver metastases and ovarian and Wilms cancer (12-14), and large-volume injection of saline has been used in the context of ultrasound imaging (15). To the best of our knowledge this is the first report to apply a contrast agent IP to precisely deliver experimental radiation therapy and monitor pancreatic disease. We have called this technique "reverse contrast CT," and we have developed it in the context of pancreatic cancer models, although in principle it is applicable to any anatomic structure or tumor in the retroperitoneal or IP space. This noninvasive technique is repeatable and can be used not only to provide image guidance for tumor dose planning but also to accurately monitor tumor burden and regrowth after intervention.

Methods and Materials

Mouse and tumor models

All animal studies were conducted under approved guidelines set forth by the Institutional Animal Care and Use Committee in a protocol approved by the Memorial Sloan Kettering Cancer Center (MSKCC) Animal Research Center. For dose-limiting toxicity and orthotopic xenograft studies, female Balb/c nu/nu mice of 6 to 8 weeks of age were purchased from Harlan Laboratories (Indianapolis,