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Noninvasive visualization of tumor growth in a human colorectal liver metastases xenograft model using bioluminescence *in vivo* imaging

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ABSTRACT

Background: Bioluminescence imaging (BLI) is an ideal tool for noninvasive, quantitative monitoring of tumor progression/regression in animal models. The effectiveness of different treatment strategies is displayed by an altered intensity of bioluminescence, demonstrating a change of the tumor burden. The aim of this study was to establish a reliable, reproducible colorectal hepatic metastases cancer animal model.

Methods: Cells of the human colon carcinoma cell line HCT-116 Luc^{pos} expressing the firefly luciferase enzyme gene were used. HCT-116 Luc^{pos} cells (2.5×10^6) were injected through the portal vein into the liver of immunoincompetent nude mice. BLI was used to analyze intrahepatic tumor burden and growth kinetic.

Results: HCT-116 Luc^{pos} cells demonstrated a progressive and reproducible growth in the liver after intraportal injection. Four days after injection, the animals were analyzed for tumor growth by BLI, and mice without or too low bioluminescence signals were excluded (between 10% and 20% animals). HCT-116 Luc^{pos} intrahepatic tumors responded successfully to different dosages (5 and 10 mg/kg) of 5-fluorouracil.

Conclusions: BLI is an important tool with many potential advantages for investigators. The measurement of intrahepatic tumor growth by imaging luciferase activity noninvasively provides valuable information on tumor burden and effectiveness of therapy. Thus, the

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presented intrahepatic metastases model based on the growth of HCT-116 Luc^{pos} cells is suitable for *in vivo* testing of different cancer therapy strategies.

1. Introduction

Colorectal carcinoma (CRC) is the third leading cause of cancer-related deaths worldwide. Although great progress has been made in diagnosis and treatment, still 40%–50% of colorectal cancer patients succumb to the disease or direct consequences within 5 y. Most patients are either diagnosed with synchronic metastases or develop metachronic metastases in the liver during the course of the disease. Surgery is the primary treatment option for isolated metastases, but only 20% to 25% of patients displaying hepatic metastases are suitable for resection, and recurrence after surgical therapy is common [1]. Due to improvements in surgical and medical therapy strategies, the median overall survival increased to almost 30 mo during the last years [2]. Besides further development of surgical techniques [3], new medical treatment modalities are needed to improve the clinical outcome of patients with hepatic colorectal metastases. Reproducible and biologically suitable animal models are needed to test novel alternative immunologic, chemotherapeutic, or antiangiogenic therapies. The requirements for successful animal models have been emphasized and should include dependable reproduction of the biology of human cancer, specific locations of metastases, reliability, reproducibility, and objective endpoints of therapeutic responses [4].

The easiest model of human CRC is subcutaneous implantation or injection of tumor cells. This model, however, does not reflect the pathophysiological conditions of CRC growth, thus being inappropriate for mimicking interactions between CRC and therapeutic agents. The orthotopic implantation or injection of tumor cells into the murine colon or cecal wall has been considered to be the most realistic model of human colorectal cancer growth in a murine model. In several reports, it has successfully created hepatic metastases [5,6]. Other authors, in contrast, report an unsatisfactory controllability of the induction of liver metastasis following orthotopic implantation [7].

Models of liver metastases have been developed using splenic [8] or portal [9,10] routes of tumor cell inoculation. In these models, however, analysis of metastatic tumor growth relies on post mortem analysis. With regard to therapy studies, individual differences in tumor growth make it difficult to assess the therapeutic effects in these models. Different *in vivo* imaging techniques in animal cancer models have been described that provide an alternative to *post mortem* analysis of tumor burden. One of the most sensitive imaging techniques in animal cancer models is based on bioluminescence imaging [11]. Basically, in *vivo* bioluminescence imaging (BLI) is the conversion of the substrate luciferin by luciferase into the light-emitting product oxyluciferin. After inoculating cancer cells stably transduced with a luciferase gene and administering the substrate luciferin *in vivo*, the number of viable cancer cells can be measured by quantification of the bioluminescence activity.

The objectives of this study were the establishment of an appropriate, reliable animal model of colorectal hepatic metastases and the validation of the intrahepatic tumor burden *in vivo* using BLI. In addition, the therapeutic effect of the clinically relevant chemotherapeutic 5-fluorouracil (5-FU) on HCT-116 derived liver metastases was monitored noninvasively.

2. Materials and methods

2.1. Cell lines

The human colorectal cancer cell lines HCT-116 and HT-29 were purchased 2010 from the Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures (DSMZ; ID: ACC 581 and ACC299, respectively). The cell line SW-620 was obtained 2010 from the European Collection of Cell Cultures (ECACC, Salisbury, UK; ID: 87051203). The CD133-positive HCT-116 cells were lentivirally transduced using the calcium-phosphate method [12]. The expression vector FUGLW was co-transfected with three helper plasmids pRSV-Rev (Addgene plasmid 12253) [13], pMDLg/pRRE (Addgene plasmid 12251) [13], and pMD2.G (Addgene plasmid 12259) into the packaging cell line. After 48 h, the virus containing supernatant was harvested and added to HCT-116 cells in the presence of 8 μg/mL polybrene (hexadimethrine bromide; Sigma-Aldrich, Taufkirchen, Germany). The FUGLW vector expresses eGFP and firefly luciferase (luc) under the ubiquitin promoter. The vector uses the FUGW backbone (Addgene plasmid 14883) [14] where eGFP was replaced with a luc/eGFP fusion construct (pJW.GFP-yLuc, a kind gift from Dr. Michael H. Bachmann, Stanford University School of Medicine, Stanford, CA). The transduced HCT-116 cells (HCT-116 Luc^{pos}) were enriched by FACS sorting with a purity >90% and cultured in McCoys 5A medium (GIBCO/Invitrogen, Darmstadt, Germany) supplemented with 10% fetal calf serum and 100 IU/mL penicillin/0.1 mg/mL streptomycin. The HCT-116 Luc^{pos} cells were routinely tested for mycoplasma contamination to ensure that only negative cells were used.

2.2. Animals

Six- to 10-wk-old female NMRI (Foxn1^{nu}) nude mice (Harlan Laboratories GmbH, www.harlan.com), weighing 18–20 g at the time of surgery, were used. The animals were maintained in a specific pathogen-free environment at a temperature of 22 ± 2°C, a humidity of 45% ± 10%, and a 12 h light/12 h dark cycle. Autoclaved chow pellets (Altromin GmbH, Lage, Germany) and autoclaved tap water were provided *ad libitum*. Housing and all procedures involving animals were in accordance with protocols approved by the university's animal care committee and in compliance with the guidelines on animal welfare of the National Committee for Animal Experiments.