Original Study

Individual Susceptibility Analysis Using Patient-derived Slice Cultures of Colorectal Carcinoma

Rasmus Sönnichsen,[1,2] Laura Hennig,[1,2] Vera Blaschke,[1] Karsten Winter,[1] Justus Körfer,[1,2] Susann Hähnel,[1] Astrid Monecke,[3] Christian Wittekind,[3] Boris Jansen-Winkeln,[4] René Thieme,[4] Ines Gockel,[4] Kerstin Grosser,[5] Arved Weimann,[5] Christoph Kubick,[6] Volker Wiechmann,[6] Achim Aigner,[7] Ingo Bechmann,[1] Florian Lordick,[2] Sonja Kallendrusch[1]

Abstract

Patient-derived slice cultures bear the potential to investigate individual response to treatment and thus improve treatment stratification. We prepared slice cultures of colorectal carcinomas and investigated individual response to 5-fluorouracil-based treatment. We detected dose-dependent response dynamics and identified a possible nonresponder in our cohort. Based on these findings, the next step is the correlation with clinical outcome in larger cohorts.

Background: Nonresponse to chemotherapy in colorectal carcinoma (CRC) is still a clinical problem. For most established treatment regimens, no predictive biomarkers are available. Patient-derived tumor slice culture may be a promising ex vivo technology to assess the drug susceptibility in individual tumors. **Methods:** Patient-derived slice cultures of CRC specimens were prepared according to a standardized protocol and treated with different concentrations of 5-fluorouracil (5-FU) and an adapted FOLFOX regimen (5-FU and oxaliplatin) to investigate histologic response. Additionally, a semi-automatized readout using fluorescent stain-specific segmentation algorithms for Image J was established to quantify changes in tumor proliferation. Nonresponse to chemotherapy was defined as persisting tumor cell proliferation. **Results:** Slices treated with 5-FU showed lower tumor cell fractions and dose-dependent alterations of proliferating tumor cells compared with controls (1 μM, Δ +3%; 10 μM, Δ −9%; 100 μM, Δ −15%). Individual tumor samples were examined and differences in chemotherapy susceptibility could be observed. Untreated slice cultures contained an average tumor cell fraction of 31% ± 7%. For all samples, the histopathologic characteristics exhibited some degree of intratumoral heterogeneity with regard to tumor cell morphology and distribution. The original tumor matched the features found in slices at baseline and after 3 days of cultivation. **Conclusions:** Patient-derived slice cultures may help to predict response to clinical treatment in individual patients with CRC. Future studies need to address the problem of tumor heterogeneity and evolution. Prospective correlation of ex vivo results with the clinical course of treated patients is warranted.

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F.L. and S.K. contributed equally to this article.

[1]Institute of Anatomy, University of Leipzig, Leipzig, Germany
[2]University Cancer Center Leipzig
[3]Institute of Pathology
[4]Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Hospital Leipzig, Leipzig, Germany
[5]Department of General and Visceral Surgery
[6]Institute of Pathology, St. Georg Hospital, Leipzig, Germany
[7]Rudolf-Boehm-Institute for Pharmacology and Toxicology, Clinical Pharmacology, University of Leipzig, Leipzig, Germany

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Address for correspondence: Rasmus Sönnichsen, University Cancer Center Leipzig (UCCL), University Medicine Leipzig, Liebigstraße 20, Leipzig 04103, Germany
E-mail contact: rasmus.soennichsen@medizin.uni-leipzig.de

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Introduction

Colorectal cancer is the third most common cancer worldwide and accounts for almost 700,000 deaths per year.[1] Current treatment consists of chemotherapy combined with targeted therapy, depending on RAS mutation status, surgery, or a combination of both, depending on the specific disease stage.[2] Despite recent advances, patient stratification and individual response to treatment often remain insufficient. The majority of patients with colorectal carcinoma (CRC), who are treated with established chemotherapy protocols, do not achieve durable responses.[3,4] Predictive markers enable a better allocation of patients to specific treatment regimens and thus can improve response to treatment and ultimately survival outcomes. There is a gap between currently used ex vivo models for prediction of tumor response and applicability to clinical practice. In many models, important aspects such as the tumor microenvironment[5] and heterogeneity[6] are not optimally depicted.[7] In contrast, patient-derived, organotypic slice cultures allow for the investigation of human tumors in an autologous environment ex vivo. Slice cultures have been established for several cancer types.[8-13] This model can be used to study basic principles of tumor biology, novel drugs, and response to treatment in a pretherapeutic setting.[8-13] This may eventually help to choose the most promising drugs for individual patients. We here describe the development of a semi-automatized readout for patient-derived slice cultures and a standardized experimental protocol that permits fast assessment of effects of cytotoxic agents ex vivo.

Material and Methods

Specimens

Tumor specimens were obtained from patients treated at 2 academic hospitals in Leipzig, Germany. A total of 7 patients with CRC were included in the study (see Supplemental Table 1 in the online version). This study was approved by the ethics committee of the Medical Faculty, University of Leipzig (AZ 370-1316122013, AZ 370-13-ff). All patients provided their informed written consent to this study.

Preparation of Tissue Slice Culture

The preparation protocol was described previously[13] and was applied with some modifications. In brief, immediately after surgical resection and first macroscopic pathologic assessment, tumor samples were cut into slices of 350 µm using a tissue chopper (McIlwain TC752; Campden Instruments, Leicestershire, England). Tissue slice diameter was then standardized by using a 3-mm coring tool (kai Europe, Solingen, Germany). Three tissue slices were randomly pooled, placed on membrane inserts (Millipore Corporation, Billerica, MA), and cultivated in 6-well plates. Slices were incubated under standardized conditions of 37°C and 5% CO_2. Medium was changed 2 hours, 24 hours, and 48 hours after preparation unless stated otherwise. Slices fixed on the day of preparation (baseline samples), and untreated, cultivated slices (controls) served as controls. Tissue was fixed overnight using 4% paraformaldehyde.

Experimental Setup

After 24 hours of cultivation, slice triplets were treated with cytotoxic drugs. Slice cultures of 5 specimens were treated with 1, 10, and 100 µM of 5-fluorouracil (5-FU) (Medac, Wedel, Germany) for 48 hours. Drug-containing medium was prepared on the day of treatment and changed after 24 hours. Control conditions were cultivated as indicated above without cytotoxic supplement. One specimen (#27) was treated with an adapted FOLFOX-4 (oxaliplatin, folinic acid, and 5-FU) regimen. Slice cultures were incubated with folinic acid 10 µM (Teva Pharmaceutical Industries, Petach Tikva, Israel) and oxaliplatin 20 µM (Sanofi-Aventis, Paris, France) for 2 hours, followed by a treatment with 5-FU 10 µM for 22 hours. Subsequently, slices were treated with folinic acid 10 µM for 2 hours, followed by 22 hours of 5-FU 10 µM. Concentrations were adapted from experiments with 5-FU (Figure 3A) and oxaliplatin (data not shown). Controls and FOLFOX treated conditions were doubled to strengthen the informative value of this single proof-of-concept experiment. Untreated replicates of specimen #25 were cultivated to investigate the reproducibility of tumor cell distribution (Figure 2D).

Staining Procedure

Paraformaldehyde (4%) fixed slices were embedded in paraffin and processed to 5-µm sections. Hematoxylin and eosin staining (HE) was performed to assess histopathologic aspects and tumor cell proportion. Overall cell count, tumor cell count, and proliferation were analyzed by immunofluorescent staining. In brief, paraffin sections were deparaffinized. After antigen retrieval, sections were washed with 0.3% phosphate buffered saline/TritonX and blocked with 5% normal goat serum (Jackson ImmunoResearch, Suffolk, UK) for 30 minutes. Primary antibodies against cytokeratins (AE1+3; BioGenex, Fremont, CA, mouse, 1:100) (See Supplemental Figure 3 in the online version) and Ki67 (DCS, Hamburg, Germany, rabbit, 1:200) were diluted in 0.5% bovine serum albumin and incubated at 4°C overnight. Sections were rinsed with 0.3% phosphate buffered saline/TritonX and labeled with secondary antibodies respectively (goat-anti-rabbit 568, goat-anti-mouse 647, Alexa Fluor, Invitrogen, Eugene, OR). Nuclei were stained with Hoechst 33342 (Sigma-Aldrich, St. Louis, MO).

Analysis

Tumor cell-containing area was analyzed in HE sections (Figure 2C) by a pathologist (AM) using slide scans (Pannoramic SCAN and Pannoramic Viewer, 3D Histech, Budapest, Hungary) to investigate varying tumor cell fractions. Slices that contained more benign epithelial cells than neoplastic epithelial cells were excluded from analysis. Slices that did not contain tumor cells were excluded from analysis of proliferating tumor cell fraction but included in analysis of tumor cells per condition. For further analysis, 5 pictures (20×) per tissue slice were taken manually from fluorescent-stained sections using an Olympus BX51 fluorescent microscope (Olympus Deutschland, Hamburg, Germany). The positive pixel count was determined for Hoechst 33342, cytokeratin, and Ki67 stains with stain-specific segmentation algorithms for Image J.[14] Proliferating tumor area was calculated by analyzing pixels of Ki67 positive nuclei surrounded by cytokeratin-positive pixels.

Statistical Analysis

For every picture, the total cell count (Hoechst-positive), tumor cell count (Hoechst- and cytokeratin-positive), and proliferating