Laser Capture Microdissection in 2-D Co-Culture Models as a Tool to Study Tumor-Stroma Interactions

BioTechniques 33:474-475 (September 2002)

Cellular adhesion, migration, and invasion are essential processes in tumor progression (5). These processes include actions by tumor cells as well as by the stromal microenvironment. In the last few years, it has become evident that tumor cell behavior and progression are affected by tumor stroma, including peritumoral fibroblasts (4,8).

In vitro, tumor cell-stromal fibroblast interaction can be studied by 2-D and 3-D co-culture models (9). In conventional 2-D co-culture systems, the different cell types are grown together in glass-slide-based chambers with medium. Immunohistochemical detection at the protein level can be easily performed; however, for protein and gene expression analysis, the cell compartments have to be dissociated and sorted, for instance, by flow cytometry or magnetic cell separation. These procedures could possibly result in stress-induced alterations in RNA expression.

Here we present a novel technique for co-culturing and separating fibroblasts and carcinoma cells. It is based on cell co-cultivation on a 1.35-µm-thin membrane combined with laser capture microdissection (LCM) of the cell compartments after rapid immunolabeling. LCM permits the rapid and reliable procurement of populations of cells from tissue sections by infrared laser beam power, resulting in samples that are well suited for subsequent molecular analysis (2,6).

We show that our technique of co-culture-LCM allows the extraction of intact RNA from a limited number of co-cultivated cells separated without cell cross contamination after immunostaining as a tool for expression profiling and genetic analysis.

The tumor cell line PE/CA-PJ34 (ECACC accession no. 97062513), as a model of an invasive tumor, was established in our laboratory from a surgically removed squamous cell carcinoma of the oral cavity as described previously (1). Tumor differentiation and matrix protein expression in this cell line were characterized immunohistochemically after culturing on 4-well cell culture slides (FALCON®/CBP; BD Biosciences, Heidelberg, Germany). We used the human fibroblast cell line hTERT-BJ1 (BD Biosciences Clontech) as an artificial stroma compartment.

hTERT-BJ1 fibroblasts (5 × 10^4), Invitrogen GmbH, Karlsruhe, Germany) were allowed to adhere to 2 × 2 cm membrane fragments floating in cell culture medium (IMDM, 10% FCS, and 80 µg/mL gentamycin). This special 1.35-µm-thin membrane (P.A.L.M. GmbH, Bernried, Germany) was originally developed for the mounting of histological tissue sections on regular glass slides for the so-called MicroBeamMOMeNT (microdissection of membrane mounted native tissues) technique, mainly to hold the microdissected area or specimen together during the catapulting procedure with the PALM LCM System (P.A.L.M. GmbH). The fibroblasts were cultured until subconfluent on the upper side of the membrane, subsequently overlayed with 5 × 10^4 carcinoma cells and co-cultured for 6–10 days.

The membrane does not influence cell growth. Differences in growth behavior, cell morphology, and laminin-5 immunostaining between cells cultivated on slides and on the membrane were not observed (data not shown).

The co-culture membranes were fixed in 70% ethanol for 1 min and transferred to RNase-free glass slides. After flattening and air-drying, the membranes were fixed with rubber cement. For identifying the tumor cell compartment, immunolabeling for the laminin γ2 chain was performed, a marker that is expressed only in epithelial tumor cells. We used a modified immunostaining protocol based on the ChemMate™ Detection Kit AP/red (Dako A/S, Glostrup, Denmark) that allows the identification of the tumor cells and maintains the DNA and RNA integrity for subsequent analysis. Membranes were incubated for 10 min at room temperature with the anti-γ2 chain antibody GB3 (Harlan Sera-Lab Ltd., Leicestershire, UK) at a concentration of 60 µg/mL, diluted in Antibody Diluent Solution (ZYMED® Laboratories, San Francisco, CA, USA) supplemented with 1 U/µL RNase inhibitor (Roche Applied Science, Mannheim, Germany). After rinsing three times in RNase-free PBS, detection of the primary antibody was performed using the ChemMate Detection Kit AP/red according to the

Figure 1. Co-culture-LCM. Panel a represents a survey of the 2-D membrane co-culture model. The tumor cell areas are immunostained with the laminin γ2 chain antibody GB3 (arrows). For better visualization, fibroblasts are counterstained with hematoxylin (bar = 100 µm). Immunostained tumor cell areas (b) or unstained fibroblasts (c) are microdissected with the laser beam. The laser tracks are clearly visible (arrowheads) and the precision of the procedure enables the dissection of the area of interest without cross contamination (bars = 100 µm).
The microdissected cells can be performed with standard procedures (data not shown). Analysis of RNA molecules has been more troublesome and can be a major limiting step for downstream applications because of loss of RNA quality and quantity during RNA sample preparation from a limited number of immunostained cells. Previously, we developed a total RNA extraction protocol for limited amounts of starting material that combines a guanidinium thiocyanate-based denaturation with a modified RNA precipitation and purification (3). This protocol also worked perfectly under the immunostaining conditions described here. After total RNA extraction, reverse transcription with 1 μL 25 μM Oligo (dT)₁₅ primer was performed using the SUPER-SCRIPT™ Choice System according to the manufacturer’s guidelines (Invitrogen GmbH). For each sample, a control reaction was performed without the addition of reverse transcriptase to prove the absence of amplifiable DNA in the RNA extractions. cDNA amplification was carried out with 30–40 amplification cycles with primers (ams biotechnology GmbH, Wiesbaden, Germany) for the housekeeping gene transcript cyclophilin and for the laminin γ2 chain as a marker for epithelial tumor cells (7). Eight microliters of amplification product were run on a 2% agarose gel. We examined the RNA quality from the microdissected co-cultured cells by RT-PCR for a housekeeping gene transcript and for the laminin γ2 chain gene transcript used in immunostaining the tumor cell compartment (Figure 2). We show in this study that laser capture-microdissected tumor and stromal cells from the membrane-based co-culture model can be used for gene expression profiling and DNA based analysis.

REFERENCES


This study has been supported by the grant no. B307-01028 from the Center for Clinical Research of the University of Jena. Address correspondence to Dr. Regina Dahse, Klinikum der FSU Jena, Institut für Humangenetik und Anthropologie, Kollegiengasse 10, D-07740 Jena, Germany. e-mail: rdah@mti-n.uni-jena.de

Received 7 March 2002; accepted 23 May 2002.

R. Dahse, A. Berndt, K.M. Haas, P. Hyckel, F.D. Böhmer, U. Claussen, and H. Kosmehl†
Friedrich Schiller University Jena
†Helios-Clinic Erfurt Germany

For reprints of this or any other article, contact Reprints@BioTechniques.com