Technical Advance

Microbeam MOMEiNT

Non-Contact Laser Microdissection of Membrane-Mounted Native Tissue

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The analysis of tissue-specific genetic alterations depends on the selective procurement of homogeneous cell populations. Microbeam microdissection of membrane-mounted native tissue (MOMEiNT) permits the rapid, selective, and low-contamination procurement of tumor or other cells from histological sections by non-thermic non-contact laser microdissection. Tissue sections are mounted on a specifically designed ultratwin transparent supporter membrane. Tissue together with the membrane are then dissected with an ultraviolet (337-nm) pulsed laser microbeam coupled into a robot-stage microscope. The ultraviolet laser causes dissection by cold photolysis due to the high photon density of the microbeam rather than by local heating. The track of the laser microbeam can be preselected freely on a video screen, and the size and form of the dissects can thus be adapted to the histological features of the section with a delineation accuracy in the micron range. Polymerase chain reaction amplification of DNA from the dissects is not impaired, and tumor-specific loss of heterozygosity of the APC gene as well as homozygous deletion of the MTS1 gene are demonstrated in bladder carcinomas. Taken together, microbeam MOMEiNT is a novel technique that utilizes membrane-based microdissection by an ultraviolet laser microbeam, thus providing a flexible, easy-to-use high-performance tool for the molecular pathologist. (Am J Pathol 1997, 151:63–67)

Polymerase chain reaction (PCR)-based analysis of genomes has become a widely used diagnostic tool that is transforming the medical clinical laboratory. Nonhuman pathogen DNA or RNA is currently being routinely detected in tissues or body fluids by PCR or reverse transcription (RT)-PCR. The analysis of tumor-specific genomic alterations can, however, be compromised by the presence of surrounding or infiltrating normal cells that contaminate the PCR template. Microdissection has proven an effective tool to selectively procure the tumor cells, because it allows the direct comparison of histopathological with molecular findings. Microdissection is of particular value when looking for tumor-specific allelic or homozygous deletions. Homozygous tumor-specific deletions, eg, have been demonstrated in native tumors for the first time using a membrane-based microdissection technique.

Some microdissection methods are, however, time consuming or hampered by inadequate delineation accuracy or difficult transfer of the dissects, or they require a great deal of manual dexterity (reviewed in Ref. 14). Here, a robust method, termed microbeam microdissection of membrane-mounted native tissue (MOMEiNT), is presented that permits...
the rapid, selective, and low-contamination procurement of tumor cells from membrane-mounted tissue sections.

Materials and Methods

Membrane-mounted sections of formalin-fixed, paraffin-embedded normal kidney and freshly frozen bladder carcinomas were prepared as described, except that a specifically designed 6-μm polyester membrane was used to support the sections for subsequent laser microdissection (Figure 1). This membrane is of good optical quality, is nonstretchable, sinks in proteinase K buffer, and does not interfere with the PCR. The membrane-covered glass slides were handled as ordinary glass slides, and routine hematoxylin and eosin (H&E) staining was performed. After turning the membrane upside down on the glass slide, distilled water, ethanol, glycerol, or mineral oil was used as an embedding medium. The slides were then placed on a robot-stage microscope equipped with infinity optics into which a 337-nm pulsed laser microbeam had been coupled through the epifluorescence path (P.A.L.M., Wolfbrathausen, Germany), and areas of interest were dissected from the membrane-mounted sections with the laser microbeam by moving the slides with a digitally controlled motor-driven microscope stage (20-nm steps along the x and y axis). The ultraviolet laser microbeam causes dissection by local photolysis of the supporter membrane and tissue section due to the high photon density of the microbeam rather than by local heating or coagulation. The dissection track was selected with a computer mouse and followed on a video screen, and the dissectates (ie, the dissected piece of the supporter membrane with the attached area of the tissue section) were pierced with a 30-gauge needle or taken with a small forceps and transferred into a PCR tube. Cell lysis, PCR, and gel electrophoresis were performed as described.

Results

Microdissection of membrane-mounted tissue sections using the ultraviolet laser microbeam was ideal at approximately 10 μJ/pulse and 18 Hz. Cavitation occurred at the dissection track when ethanol or distilled water was used as an embedding medium but was reduced or absent when glycerol or mineral oil was used. The width of the dissecting track could be narrowed to approximately 2 to 7 μm depending on the power of the laser microbeam and the embedding medium (Figures 2 and 3). Thus, infiltrating nontumor cells could be selectively destroyed with the laser microbeam (data not shown; see also Ref. 16). Dissection with the laser microbeam took approximately 10 to 30 seconds, and transfer of the dissectates into a PCR tube took another 2 to 5 minutes. Transfer was eased by electrostatic attraction between the polyester supporter membrane and the transfer needle and PCR tube, respectively. The size of most dissectates was in the range of 0.5 mm; many dissectates from the bladder carcinomas were even bigger than 1 mm. Thus, their transfer into the PCR tubes could be followed with the naked eye. PCR amplification of DNA from microbeam-dissected areas was not compromised compared with manually microdissected areas, both for fresh-frozen and for paraffin-embedded sections. Tumor-specific loss of heterozygosity of the APC gene (Figures 2
Figure 2. Frozen section of a transitional cell carcinoma of the bladder. H&E; original magnification, ×400. A: Before microdissection. B: Beginning of the microbeam microdissection at the top of the preselected area to be dissected (arrowhead). C: Dissection completed. D: After removal of the dissectate. The oval area in the middle is caused by light refraction of the mineral oil. Because the supporter membrane is not completely flat on the glass slide, not all areas of the section are in the focus.

Figure 3. Frozen section of a transitional cell carcinoma of the bladder. H&E; original magnification, ×400. A: Before microdissection. B: After microdissection. The irregularly shaped area of the tumor islet would have been difficult to procure by manual MOMeNT or laser capture microdissection.
and 4) and homozygous deletion of the MTS1 gene (Figures 3 and 4) were detected in two independent laser microbeam-microdissected bladder carcinomas.

Discussion

Membrane-based microdissection techniques, such as manual MOMeNT,6,7,14 laser capture microdissection (LCM),8 and microbeam MOMeNT, as well as selective ultraviolet radiation fractionation (SURF),2,3 meet two key requirements of a microdissection technique that is to be used in a routine setting. First, the quality of the dissection can be documented continuously in every single case, and second, there is a low risk of contamination by errant tissue flakes from neighboring areas of the section. To achieve this, the membrane in microbeam MOMeNT serves several purposes: 1) as a cover slip, it seals both the dissecate and the surrounding section and thus prevents contamination; 2) the membrane serves as a backbone of the tissue section and thus facilitates the handling of the dissecate during the transfer into the PCR tube; 3) the membrane also prevents tissue flakes to detach from a section and contaminate other dissecates and PCRs; and 4) the dissecate remains histologically intact and can be documented photographically.

The possibility to cut out irregular lesions is probably the strongest feature of microbeam MOMeNT (Figure 3). This is both due to the short wavelength of the ultraviolet laser and to the ultrathin supporter membrane (6 µm as compared with 100 µm in LCM and 1 mm in SURF) that causes only little dispersion of the laser energy within the membrane. The use of an ultraviolet rather than an infrared laser allows, due to the shorter wavelength, better focusing of the laser light. This results in a high photon density and small diameter of the microbeam focus of approximately 1 µm, which results in a delineation accuracy of 2 to 7 µm under working conditions. Moreover, the non-thermal ablation by the ultraviolet laser17 avoids the problem of energy dispersion by heat conduction that is encountered when infrared lasers are used. The delineation accuracy compares to microdissection with micromanipulators, which have been used for single-cell preparations from patients with hematological disorders.5,19 Single-cell preparations are, however, not necessary in most solid tumors, where the procurement of cell islets that contain several up to some hundred cells appears to be ideal for robust routine PCRs.

Both LCM and microbeam MOMeNT allow the mechanized microdissection by computer mouse and video screen, whereas the actual microdissection (gliing of the selected cells to the membrane in LCM and local ablation of a narrow track of supporter membrane and tissue surrounding the selected cells in microbeam MOMeNT) involves no physical contact with the tissue section. These features may prove indispensable in a routine setting, because the need for constant manual dexterity of the dissecator is greatly reduced and limited to the process of transferring the membrane/tissue dissecates into the PCR tube, which is, however, not a quality-determining factor. SURF and manual MOMeNT, in contrast, require a great deal of manual dexterity during the microdissection (manual MOMeNT) or inking (SURF), which also involves physical contact with the cells to be dissected.

Great care has been taken to use commercially available components for microbeam MOMeNT to render it user friendly and to cut maintenance cost. The use of a highly focused ultraviolet laser microbeam to cut histopathological sections mounted on a specifically designed ultrathin supporter membrane provides the flexibility and accuracy that may be critical in many irregular or infiltrated neoplastic lesions or in the procurement of specific cell clusters in developmental biology.

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References