

Review

Microdissection Techniques for Cancer Analysis

(microdissection / cancer / microarray)

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Abstract. One difficulty in studying molecular changes of tumours has been the inability to isolate DNA and RNA from a homogeneous cell population. The combination of several new technologies should help overcome these hurdles. Microdissection is a technique for rapid and easy procurement of a pure cellular subpopulation away from its complex tissue milieu. Laser-assisted microdissection has recently been identified as a quick, simple and effective method by which microdissection of complex tissue specimens can be routinely performed for molecular analysis. With the advent of laser microdissection, cDNA libraries can be developed from pure cells obtained directly from stained neoplastic tissue, and microarrays of thousands of genes can now be used to examine gene expression in microdissected tumour tissue samples. This review will concentrate on the application of different microdissection techniques in the area of cancer research.

There have been dramatic advances in the last two decades in our understanding of the molecular processes involved in cancer. This understanding has revealed numbers of exciting new targets for the development of effective therapies, some of which have already entered clinical practice. There is a pressing need to integrate this knowledge with structural and architectural data derived from conventional morphological approaches (Surivatanauksorn et al., 1999). Molecular analysis of cells in their native tissue environment provides the most accurate picture of the *in vivo* disease state. However, accomplishing this goal is more difficult than just grinding up a piece of tissue and applying the extracted molecules to a panel of assay.

Tissues are complicated three-dimensional structures, composed of a large number of different types of

interacting cell populations. The cell subpopulations of interest might constitute a tiny fraction of the total tissue volume. For example, a biopsy of breast tissue harbouring a malignant tumour usually contains the following types of cell populations: 1) fat cells in the adipose tissue surrounding the ducts; 2) normal epithelium and myoepithelial cells; 3) fibroblasts and endothelial cells in the stroma and blood vessels; 4) premalignant carcinoma cells in the "in situ" lesions and 5) clusters of invasive carcinoma. Analysis of a tumour or preneoplastic genomic alteration can, therefore, be compromised by the presence of surrounding normal cells. If the goal is to analyse the genetic changes of premalignant cells, this subpopulation usually occupies less than 5% of the tissue volume (Simone et al., 1998). The accurate analysis of molecular changes associated with tumours and their precursor lesions requires precise isolation of the specific cell types from a heterogeneous background of non-neoplastic elements such as normal epithelium, desmoplastic stroma, inflammatory cells and blood vessels (Dean-Clower et al., 1997).

Culturing the cell population is one approach to reducing contamination. However, cultured cells might not accurately represent the molecular events taking place in the actual tissue they were derived from. The cell culture condition can never duplicate the environment of the cells in the actual tissue from which they were derived. Cell sorting techniques have also been used as a means of cell selection. They can be easily applied to tumours amenable to the formation of a suspension but cell sorting techniques are rarely applicable in solid tissue, in which intercellular adhesion prevents the disaggregation of cells. The problem of cellular heterogeneity has thus been a significant barrier to the molecular analysis of normal and diseased tissue. It is obvious that the selective isolation of tumour cells and their precursor lesions requires a technique by which the cells of interest can be isolated from the primary lesion itself, without any intervening step. This problem can now be overcome by new developments in the field of tissue microdissection.

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Abbreviations: LCM – laser capture microdissection, LMM – laser microbeam microdissection, LPC – laser pressure catapulting.

Microdissection Techniques

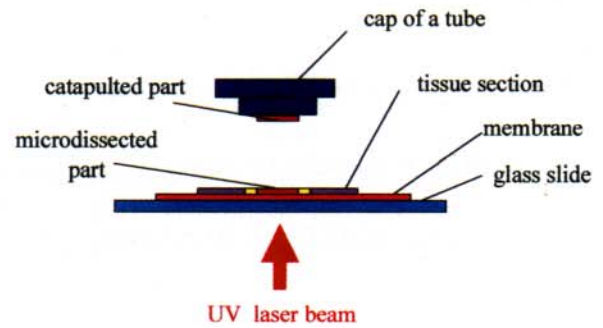
Tissue microdissection techniques enable the isolation of specific subpopulations from a diverse background of cell types, usually obtained under direct visual inspection. Microdissection techniques can be divided into three categories: 1) selective ablation of unwanted regions; 2) manual extraction of desired foci and 3) use of laser pulses to capture cells of interest.

The first category requires the use of an ablative technology, such as ultraviolet radiation, to destroy the unwanted regions, and the islands of tissue that are left behind are manually scraped off for molecular analysis. While ablative technologies are readily applicable to formalin-fixed archival material and can be used to isolate microscopic lesions with reasonable precision, the surrounding tissue is by definition unsuitable for PCR and cannot be reused.

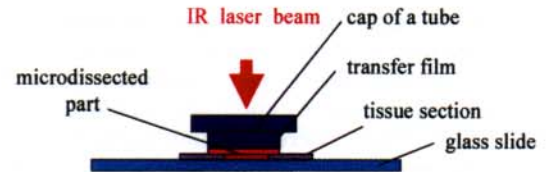
The early microdissection techniques, involving manual or micromanipulator guidance of the needle to scrap off the cells of interest under a microscope, have been applied for some years to isolate a few hundred cells from heterogeneous tissue samples. One of the earliest examples of this technique was described by Goelz et al. (1985), who removed areas of interest from the paraffin block itself for molecular analysis, using before and after histological section to assess the purity of the DNA extraction. Manual methods of microdissection allow the extraction of DNA from lesion less than 1 mm in size under direct inspection (Zhuang et al., 1995). Since the number of premalignant cells is usually small, this method is not suitable for premalignant lesion analysis. Precision, avoidance of contamination and efficiency of the procedure are the most important parameters in tissue microdissection. Manual methods are rather tedious, operator-dependent and require considerable manual dexterity to prevent contamination with unwanted surrounding tissue elements.

The most recent advance in the field of microdissection has been the advent of laser-based microdissection techniques. Laser-assisted microdissection has been developed to procure precisely the cells of interest in a tissue specimen, in a rapid and practical manner. The current prototypes of these techniques include laser microbeam microdissection (LMM) coupled with laser pressure catapulting (LPC) and laser capture microdissection (LCM) (Fig. 1).

The LMM with LPC technique uses tissue that has been mounted on a 6 μ m polyethylene membrane and placed on a glass slide, onto which the operator directs an ultraviolet laser beam under direct visualization, by a light microscope. The membrane supports the section and is of good optical quality, non-stretchable and it sinks in extraction buffers (Bohm et al., 1997). The laser beam burns the rim of the membrane and ablates the underlying unwanted tissue around the area of inter-



A: LMM-LCP



B: LCM

Fig. 1. Principle of A: LMM coupled with LPC and B: LCM, IR – infrared, UV – ultraviolet.

est, leaving the desired cell population intact. The latter is then isolated by catapulting under pressure onto an overhanging cap (Figs. 2A, B, C). A great advantage is the well-preserved morphology of the transferred cells, which can be readily visualized under the microscope (Figs. 2D, E). Laser isolation and cell pick-up procedures are quick and easy to perform.

The LCM technique uses transparent ethylene vinyl acetate thermoplastic transfer film containing a near-infrared absorbing dye, attached to a 6 mm-diameter rigid, flat cap that is placed in contact with the tissue. An infrared laser, focused to the size of the desired target, melts the film directly above the target cells. The selected cells become adherent to the melted film on the cap, which absorbs the energy of the laser pulse. Removal of the cap from the tissue section effectively procures the targeted cells. The cap is placed directly into a fitted tube containing the buffer for molecular processing (Pappalardo et al., 1998). Up to 3000–5000 cells can be isolated onto a single cap in this fashion. The greatest advantage of this method is that it avoids any intricate operator-dependent step and the procurement of the material is in a non-contact manner, which minimizes the risk of contamination.

To positively identify the specific cells desired for microdissection, histochemical fixation must be used to preserve the tissue morphology. In general, existing methods of tissue processing focus mainly on morphological preservation for pathological diagnosis, not molecular analysis. Procedures that involve manipulation of tissue, including fixation, dehydration, clearing, embedding, staining and sectioning are all factors that could adversely affect the quality of the desired