Application of methacarn fixation for genetic analysis in microdissected paraffin-embedded tissue specimens

Makoto Shibutani, Chikako Uneyama, Naoya Masutomi, Hironori Takagi, and Masao Hirose
Division of Pathology, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

Summary. Recent advances in microdissection have succeeded the molecular biological approaches on specific cells of interest within tissue specimens by overcoming the obstacle of tissue complexity. Recently, we found that methacarn is suitable for analysis of RNA, protein, and genomic DNA in small tissue samples using paraffin-embedded tissue (PET) sections in conjunction with microdissection technique. By application of sensitive quantitation methods, such as those utilizing fluorescent dyes specific for RNA or protein, molecules of small quantity can be normalized between samples, and thus quantitative expression analysis for RNA or protein can be applied in microdissected small tissue specimens. In addition, methacarn-fixation extends its availability for genomic DNA analysis in terms of target fragment size and number of microdissected cells required. Paraffin embedding permits ease of handling tissues that extend the availability of methacarn fixation for genetic analysis in large-scale experiments. In addition, considering its advantages for immunohistochemistry, tissue embedding after methacarn-fixation should be recommended as a valuable approach for routine application possibly in combination with targeted genetic analysis of immunophenotypically defined cell populations. In combination with techniques such as expression library construction, microarray and subtractive hybridization or differential display, microdissection will permit the establishment of "genetic fingerprints" of specific cellular areas.

Key words. Microdissection, Methacarn fixation, Gene expression, Mutation analysis, Paraffin-embedded tissue

Introduction

Analysis of pathological tissue specimens in organ at the level of RNA, protein and DNA has contributed to the investigation of molecular events as well as the advance of diagnostics. However, the cellular heterogeneity composing
organs/tissues with an admixture of reactive cell populations may influence the outcome of analyzed results. Recent advances in microdissection have succeeded the molecular biological approaches on specific cells of interest within tissue specimens (Emmert-Buck et al. 1996; Schütze and Lahr 1998). Quantitative gene expression analysis in the pathologically altered cell populations can provide valuable information regarding the mechanism underlying biological phenomena, such as inflammatory cell response, cell growth, differentiation, and apoptotic cell death. Furthermore, mutation analysis of single cells becomes now essential for investigation of carcinogenetic mechanisms.

For histological assessment, tissue fixation and subsequent paraffin embedding are routinely employed because of the ease of handling tissues and subsequent staining, as well as the good preservation of tissue architecture. Until recently, formaldehyde-based fixatives, such as buffered-formalin, have been used for this purpose. However, with such cross-linking agents, there is limited performance in terms of the efficiency of extraction and quality of extracted RNA (Cooms et al. 1999; Foss et al. 1994), protein (Ikeda et al. 1998; Shibutani et al. 2000), and genomic DNA (Berger et al. 1999; Blomeke et al. 1997; Diaz-Cano and Brady 1997; Foss et al. 1994; Frank et al. 1996; Howe et al. 1997; Merkelbach et al. 1997; Ortiz Pallardo et al. 2000; Poncin et al. 1999; Scholte et al. 1997; Whittington et al. 1999), with consequent difficulty in the analysis of microdissected, histologically defined tissue areas. Therefore, unfixed tissue preparation now becomes the goldstandard for analysis of microdissected cells. However, preparation of cryosections from unfixed frozen tissue for the purpose of microdissection may not be suitable for routine samples because of the inconvenience in terms of tissue storage and the skill required for cryosection preparation and subsequent microdissection. As compared to unfixed frozen tissue, paraffin-embedded tissue (PET) retains benefits in terms of the performance in handling tissues of many samples and accessibility to histologically defined cells such as specific neuronal cell populations.

For molecular analysis of microdissected cells, extraction efficiency and quality of molecules are critical. Recently, we found that methacarn meets these critical criteria for analysis of RNAs, proteins and genomic DNAs in defined areas of PET sections (Shibutani et al. 2000; Uneyama et al. 2000), Methacarn is a non-cross-linking protein-precipitating fixative, and has been developed as a methanol-based Carnoy’s solution to reduce tissue shrinkage by ethanol (Puchtler et al. 1970). As the main focus of this article, we present procedures of methacarn fixation and following quantitative gene expression analysis and genomic DNA analysis applicable for microdissected small cell areas in PET sections.

**Fixation and Paraffin Embedding**

Methacarn solution consisting of 60% (vol/vol) absolute methanol, 30%