Report

Chromosome in situ hybridization on formalin-fixed mammary tissue using non-isotopic, non-fluorescent probes: technical considerations and biological implications

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Summary

Fluorescent in situ hybridization techniques have provided an important tool for interphase cytogenetic studies of human neoplasms. However, these techniques are difficult to use on formalin-fixed archival tissue sections. We describe here a non-fluorescent, non-isotopic in situ hybridization (ISH) approach that is easily applicable to paraffin-embedded breast tissue sections. The technical steps that must be monitored and individualized to optimize signal generation and detection are discussed. This ISH technique has several advantages over fluorescent detection methods. The signal obtained can be viewed using an ordinary light microscope and does not fade with time. More importantly, the signal is observed and analyzed in the context of tissue morphology. The technique permits detection of numerical chromosomal abnormalities not only in malignant but also in apparently normal and potentially premalignant mammary tissue. This may allow identification of focal genetic abnormalities as well as field-defects and enable analysis of their evolution during the multistep transformation to mammary neoplasm. This technique is also suitable for analysis of tumor heterogeneity and the correlation of numerical chromosomal aberrations with histologic, immunocytochemical, and clinical features of breast tumors.

Introduction

Major advances have been made over the last three decades in understanding the molecular basis of human neoplasia. The identification of specific karyotypic abnormalities associated with specific diseases, e.g. the Philadelphia chromosome in chronic myelogenous leukemia [1], provided initial leads which were followed up with molecular characterization of the genomic regions involved in several hematologic cancers [2]. However, elucidation of the molecular pathogenesis of solid tumors has lagged behind, in part because of the lack of repeatedly accessible tissue and the resulting inability to obtain karyotypes and consistently identify cytogenetic abnormalities [3]. Although solid tumors account for the majority of human malignant neoplasms, less than 10% of described cytogenetic analyses have been derived from solid tumors [4]. Detection of karyotypic abnormalities in normal and premalignant epithelial cells is even more difficult and has seldom been performed.
Even when aneuploid cells are detected in cultures of mammary tissue, it is impossible to determine whether these are derived from ‘normal’, prema-
ignant, or malignant tissue. To understand the multi-step evolution of mammary neoplasms we must have means of detecting specific genetic changes occurring at the various stages.

The limitations of conventional cytogenetic analysis of solid tumors have led to the development of fluorescent in situ hybridization (FISH) techniques which can circumvent some of these problems by allowing chromosome-specific hybridization on small numbers of cells [5, 6]. Because the procedure can be applied to interphase cells, it is often referred to as ‘interphase cytoge-
netics’ [5–10]. The technique involves fluorescent detection of the hybridized probes complementary to the specific genomic regions of interest. The probes used most often are those that recognize the repetitive α-satellite sequences in the centromeric regions of the chromosomes. Use of whole chromosome libraries or region-specific probes on condensed chromosomes or interphase nuclei permits analysis of tumor-specific translocations in inter-
phase nuclei [11, 12].

Centromeric probes are now available for most human chromosomes. Several studies using these probes for detection of numerical chromosomal abnormalities in different tumor types have recently been published [7–9, 13]. These studies have provided information about the distribution of copy numbers of different chromosomes in individual nuclei. Most investigators have utilized tu-

cor cells or nuclei disaggregated from fresh tu-
mors or tissue blocks. However, disaggregating nuclei from tissue blocks leads to loss of tissue ar-
chitecture. Preservation of cellular spatial relation-
ships is especially important for breast tissue, which because of its unique histological organi-

zation presents problems of its own. Unlike mucosal tissue, normal breast epithelium exists in the form of relatively small ducts and lobules scattered throughout the large amount of fibro-fatty tissue; this complicates analysis by techniques requiring whole-tissue extracts or disaggregated cells. Of-
ten, a substantial fraction of breast tumor tissue is composed of diploid stromal cells. Some investi-
gators have prepared touch preparations from hu-
man tumors to preserve architectural relationships. Touch preparations, however, are limited by the differential ease with which different cell types at-
tach to slides on direct contact.

Use of paraffin-embedded sections is an obvi-
ous alternative to circumvent these problems. However, performance of ISH on paraffin-embed-\nded sections and correlation of specific chromoso-
mal anomalies (genotype) with tissue morphology (phenotype) has been done only to a limited extent. The principal obstacles to successfully using FISH techniques directly on paraffin-embedded sections are background tissue fluorescence and the relatively weak intensity of the signal [14]. In addition, signal detection requires the use of specialized flu-
orescence microscopes and a rather strenuous pro-
cess of counting the signal in the dark room, and the signal obtained fades with time. Therefore, at-
ttempts have been made to use non-fluorescent chromogens for detecting the hybridization signal. Such modifications have allowed detection of the human papilloma virus [15, 16] and cytomegalovi-
rus DNA sequences [17] in human tissue, and de-
tection of chromosome-specific sequences in hu-
man germ-cell tumor xenografts [18] and, more re-
cently, human tumor tissue [19].

We describe here the adaptation of the ISH tech-
nique for examination of human mammary tissue using a peroxidase-based detection system that can be applied to formalin-fixed archival tissue sec-
tions and that generates a permanent signal visible under a light microscope. We also demonstrate the potential of this technique for elucidating the ge-
netic evolution of breast epithelium during multi-
step carcinogenesis, for understanding the ‘field-
defects’ in patients with a neoplastic or ‘high-risk breast’, and for analyzing tumor heterogeneity.

Materials and methods

Tissue acquisition

Tissue blocks were obtained from mastectomy