Chapter 1

TECHNICAL ASPECTS OF THE DETECTION OF DISSEMINATED TUMOUR CELLS BY MOLECULAR METHODS

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Abstract

The standard method for the detection of disseminated epithelial tumour cells is still immunocytochemistry despite some concerns such as relative low sensitivity and subjective evaluation. Several approaches have been made to develop sensitive and specific polymerase-chain reaction assays comparable to those in use for detection of minimal residual disease in haematological malignancies. The major problem is the absence of specific genetic aberrations in solid cancer. Thus, researchers focused on amplification of so-called tissue-specific expressed genes such as epithelial structure proteins or messenger RNA of tumour markers or tumour-associated proteins. Most assays were described as highly specific valuable tools by the developers, and subsequently as non-specific by investigators. This chapter describes the mechanisms leading to so-called 'false-positive' and 'false-negative' results, and discusses the strength and weakness of RT-PCR for detection of solid cancer cells. Furthermore, strategies are discussed for development of reverse-transcriptase polymerase-chain reaction systems and for using and increasing their specificity.

INTRODUCTION

Dissemination of solid tumours in the bone marrow or blood stream has been described for a variety of malignancies. The term 'disseminated tumour cells' or 'early tumour cell dissemination' usually means a very low amount of tumour cells in the marrow not detectable by routine microscopy of marrow or blood slides (1).

Molecular methods for the detection of minimal residual disease were first used in haematological malignancies such as non-Hodgkin's lymphoma or acute lymphocytic leukaemia (ALL) (2). Southern blot analysis detecting B-cell or T-cell specific rearrangements or genetic aberrations had a relative poor sensitivity between 1% and 5% (3, 4). The milestone was the description of the

K. Pantel (ed.), Micrometastasis, 1–18.

polymerase-chain reaction (PCR) technique for *in vitro* gene amplification in the mid-1980s. This technique allows a nearly exponential multiplication of a DNA-fragment with a pair of specific nucleotides called primers using a repetitive temperature profile for denaturation, primer-annealing and polymerization of DNA (5). The PCR-technique can be used for the sensitive detection of DNA-fragments, as well as for the detection of mRNA-templates after transcription into a cDNA in a reverse-transcriptase reaction (6).

Some haematological neoplasms bear optimal aberrations for PCR detection of minimal residual disease. The translocation T(14;18) is common in follicular lymphoma and can be amplified from DNA without reverse transcription. The second classic chromosomal aberration is the so-called Philadelphia-chromosome T(9;22) or bcr/abl-rearrangement in chronic myeloid leukaemia (CML). The molecular detection of T(9;22) requires necessarily transcription of mRNA into cDNA due to the varying size of the corresponding chromosomal DNA-segment. Both assays have been used with great success for the detection of minimal residual disease (6, 7). A positive signal in bcr/abl-PCR has become an indication for treatment of early relapse of CML after allogeneic stem cell transplantation by donor-lymphocyte infusions (8).

METHODS FOR THE DETECTION OF DISSEMINATED TUMOUR CELLS

The standard method for the detection of disseminated epithelial cancer is the immunocytochemical staining of epithelial-specific gene products commonly not expressed in haemopoietic cells such as cytokeratins or mucins (1). The sensitivity of this technique depends on the amount of cells examined and was initially quite poor due to the fact that most groups investigated not more than 2×10^5 mononucleated cells. This standard was increased to a minimum of 2×10^6 during the last years; however, RT-PCR offers sensitivity up to $1/10^7$ and its evaluation is nearly independent from investigator's bias (9).

In contrast to haematological malignancies specific chromosomal aberrations useful for detection of minimal disease by PCR-technique cannot regularly be found in epithelial tumour cells (10). The basic consideration in development of PCR assays for the specific detection of solid cancer cells in blood, bone marrow or peripheral stem cell aphereses was that epithel-derived cells do not usually occur in haemopoietic compartments. Thus, the development of PCR-techniques focused on the amplification of so-called lineage-specific transcribed genes obviously not expressed in haemopoietic cells (9). Knowledge from immunohistochemical tumour cell detection was transferred upstream to the mRNA-level. The easy upstream transfer carries a couple of pitfalls and may lead to 'false-positive' or 'false-negative' results of PCR assays. However, here it must be