Chapter 9

MINIMAL RESIDUAL DISEASE IN MELANOMA

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Abstract

A number of specific genes encoding for melanosomal proteins are selectively expressed in melanocytes and melanomas. For detection of circulating melanoma cells, the expression of the *tyrosinase* gene is most widely used. Several cohorts of melanoma patients from single institutions have been analyzed by various research groups for the presence of circulating melanoma cells in all stages of disease. The percentage of patients with evidence for occult tumor dissemination has been correlated with the stage of disease in several, but not all, reports. Two prospective analyses suggest that the PCR result is of prognostic value in melanoma. Several laboratories have found PCR evidence for circulating melanoma cells in the great majority of untreated patients with Stage IV disease, other groups have reported much lower frequencies. Taken together, there is a wide range of results. Methodological differences are likely to account for this discrepancy. With the availability of true quantitative real-time reverse transcriptase (RT)-PCR systems, accurate quantification of *tyrosinase* transcripts over a range of 1 to 10,000 tumor cells per milliliter of blood is possible. Quantitative real-time RT-PCR systems also dramatically improve quality control, since exact quantitation of housekeeping gene mRNA facilitates determination of sample quality. Two large clinical trials are currently under way within the EORTC and in the US to adequately determine the clinical usefulness of PCR detection of minimal residual disease in melanoma.

1. INTRODUCTION

Malignant melanoma accounts for 1% to 3% of all malignant tumors (1). Once disseminated beyond the regional lymph nodes, malignant melanoma is largely incurable, with a median survival of 4–6 months (2). Early identification of melanoma patients at risk for hematogenous spread of the disease would be desirable. Therefore, polymerase chain reaction (PCR) tests to detect circulating melanoma cells have been developed.

In principle, single tumor cells in the bone marrow, for instance, can be detected by immunohistological techniques. However, this method is not sufficiently sensitive to reliably monitor early metastasis or minimal residual disease,

K. Pantel (ed.), Micrometastasis, 173–183.
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since only a very limited number of cells can be assayed at one time. For the detection of occult metastasis from melanoma cells, several marker genes have been used, including *tyrosinase*, a key enzyme in the melanin biosynthetic pathway, melanA/MART-1, a melanosomal protein of unknown function or the melanoma-associated antigen A (MAGE-A) genes.

One limitation of those reverse transcriptase (RT)-PCR assays was the inability to quantitate the transcript amount accurately. The recently developed TaqMan and Light Cycler techniques combine amplification, detection and quantification, and are (a) easy to handle, (b) very rapid, (c) reproducible and (d) suited for high throughput screening applications. The novel possibility of real-time PCR is changing this whole field of investigation in two ways. First, real-time PCR provides quantitative data on minimal residual disease, which may be more informative. Secondly, real-time PCR allows much more detailed analysis of sample quality.

2. PRINCIPAL PCR DETECTION METHODS

This chapter describes the principles of occult tumor cell detection using PCR, and summarizes the currently available clinical data from trials utilizing PCR-based techniques. RNA-based methods require active transcription of the gene of interest. Fortunately, a high transcript number from the gene of interest is usually present in a tumor cell. RNA-based detection, therefore, has the advantage of high sensitivity and of detecting primarily viable cells, although detection of unviable cells in the early stages of apoptosis is theoretically possible. The number of RNA copies of a gene in any particular tumor cell may, however, vary during the cell's life cycle or as a result of de-differentiation.

2.1 Qualitative PCR Assay

RT-PCR is a highly sensitive method for detecting rare tumor-cell derived mRNA, allowing the diagnosis of tumor dissemination at early stages. This information may have important prognostic and therapeutic implications because residual tumor cells that are below the limit of detection using standard diagnostic techniques are nevertheless associated with increased risk for overt clinical relapse (3).

First, the RNA is extracted from the sample and the mRNA is reverse-transcribed into cDNA. The gene of interest is then amplified using primers specific for that gene. Ideally, these primers should not amplify genomic DNA, which often contaminates the cDNA preparation. Amplification of a cDNA sequence without amplification of the genomic counterpart of this sequence can be achieved if one primer is interrupted by an intron in the genomic DNA. The intron will have been deleted during RNA processing and, therefore, will not interrupt the primer sequence in the cDNA version of the gene. Alternatively, primers can be chosen that flank an intron in the genomic sequence, thereby