Polymerase Chain Reaction for Detection of Melanoma in Peripheral Blood: Too Early to Assess Clinical Value

Antonio C. Buzaid, Charles M. Balch*

The traditional approach to the study of tumor markers has been based on the detection of substances either induced by or released by cancer cells. In high-risk patients with melanoma, we and others (1-4) have evaluated several potential tumor markers including lipid-associated sialic acid (in plasma), neuron-specific enolase, and circulating S100 protein (a marker of neuroendocrine tumors). Preliminary results using these markers have indicated that they have limited value in the clinical setting. The development of new molecular biology techniques, particularly the polymerase chain reaction (PCR), has provided a means by which molecular markers present at low copy numbers can be detected with a sensitivity significantly higher than that achieved using antibody-based techniques. In 1991, Smith et al. (5) proposed for the first time that melanoma cells could be detected in the peripheral blood using coupled reverse-transcription (RT) and PCR (RT-PCR) to target tyrosinase messenger RNA (mRNA). Tyrosinase, the first enzyme in melanin biosynthesis, is a mono-oxygenase that catalyzes the conversion of tyrosine to dopa and of dopa to dopaquinone. Thus, tyrosinase is one of the most specific markers of melanocytic differentiation. Tyrosinase expression can be detected not only in melanocytes and melanoma cells but also in Schwann cells (6). Nonetheless, because these cell types are not known to circulate, the detection of tyrosinase transcripts in the peripheral blood of patients with melanoma has been considered evidence of circulating melanoma cells. Since the original report from Smith et al. (5), several other groups have pursued this lead (7-11). The results are summarized in Table 1.

As clearly shown in the table, the results are extremely variable. The most striking discrepancies were seen in patients with stage IV disease, in whom the sensitivity rates ranged from 0% to 100%. For instance, in the series of Brossart et al. (7), 100% of the patients with stage IV disease had marker-positive RT-PCR findings regardless of response to therapy, whereas in the current report by Kunter et al. (12), only 28% of the patients with stage IV disease had positive RT-PCR. Furthermore, in the series of Kunter et al., positive RT-PCR was observed only in patients whose disease progressed during therapy. Significant discrepancies were also observed in patients with stage I and stage II disease, for whom the positive RT-PCR frequency ranged from 0% to 71%. Interestingly, the expected disease recurrence frequency for patients with stage I or stage II disease is on the order of 20%-40% for the whole cohort. Assuming that false-positive results did not occur, the data reported by Hoon et al. (8) suggest that circulating melanoma cells may be present in the blood of patients in whom recurrent disease does not develop, perhaps because many circulating tumor cells may not have metastatic potential.

The causes of such diverse results are probably multifactorial. The most important differences relate to the processing of the blood samples and to the RT reaction. For instance, Smith et al. (5), Brossart et al. (7), Foss et al. (10), and Kunter et al. (12) extracted total RNA from whole blood (after centrifugation and discarding the plasma), whereas Hoon et al. (8) and Battayani et al. (9) used density-gradient methods to separate the peripheral mononuclear cell fraction from whole blood. Furthermore, differences in the RNA extraction process and in the PCR method itself also may have affected the sensitivity of the technique. Another important factor is the possibility of unrecognized carryover contamination leading to false-positive results. The necessity for stringent PCR contamination controls cannot be overemphasized. Indeed, in their laboratories, Foss et al. (10) and Pittman et al. (11) recognized serious problems with false-positive results that took many months to overcome. As expected, the elimination of the possible carryover contamination resulted in a marked decrease in the sensitivity reported by both of these groups.

How useful are data that indicate that patients who are RT-PCR positive for tyrosinase mRNA have a shorter survival? To date, the clinical value of this information is somewhat limited because the management of RT-PCR-positive patients would be basically the same as for patients who are RT-PCR negative for the biomarker. Even as a marker of disease progression, the sensitivity of the RT-PCR technique as reported by Kunter et al. (12) was low: only nine of 21 patients whose disease progressed were RT-PCR positive. Furthermore, the investigators did not compare the prognostic value of RT-PCR for tyrosinase mRNA with other simpler but powerful prognostic markers such as serum lactic dehydrogenase. Finally, Kunter et al. (12) stated that this study demonstrates for the first time that the detection of tyrosinase mRNA in cells present in peripheral blood could serve as a marker of rapid tumor progression and poor clinical outcome. However, Battayani et al. (9) previously reported that patients with metastatic melanoma who tested positive with RT-PCR for tyrosinase mRNA were four times more likely to have rapid disease progression than were patients with negative RT-PCR results.

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Table 1. Studies using coupled reverse transcription and polymerase chain reaction to detect tyrosinase messenger RNA (mRNA) in the peripheral blood of melanoma patients

<table>
<thead>
<tr>
<th>Author/year (reference No.)</th>
<th>No. of patients positive for tyrosinase mRNA/No. of patients tested (%)</th>
<th>According to AJCC disease stage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brossart et al. /1993(7)</td>
<td>I and II 1/10 (10) / III intact ND / IV non-visc. 6/17 (35) / IV visc. 4/4 (100)</td>
<td>25/25 (100)</td>
</tr>
<tr>
<td>Hoon et al. /1995 (8)</td>
<td>12/17 (71) / III intact 5/6 (83) / IV non-visc. 25/29 (86) / IV visc. 46/48 (96)</td>
<td></td>
</tr>
<tr>
<td>Battayani et al. /1995 (9)</td>
<td>2/10 (20) / ND / ND / ND</td>
<td>14/33 (42) / 16/32 (50)</td>
</tr>
<tr>
<td>Foss et al. /1995 (10)</td>
<td>ND / ND / ND / ND</td>
<td>0/16 (0)</td>
</tr>
<tr>
<td>Pittman et al. /1996 (11)</td>
<td>0/16 (0) / ND / ND / ND</td>
<td>3/24 (12.5)</td>
</tr>
<tr>
<td>Kunter et al. /1996 (12)</td>
<td>0/16 (0) / ND / ND / ND</td>
<td>9/32 (28)</td>
</tr>
</tbody>
</table>

*AJCC = American Joint Committee on Cancer; ND = not done; NED = no evidence of disease; and visc. = visceral.
† Results were reported analyzing four tumor markers (tyrosinase, p97, MUC18, and MAGE-3).
‡ Thirty-six additional patients with localized ocular melanoma were studied, and all had negative polymerase chain reaction results.

PCR. Perhaps the greatest potential value of techniques such as RT-PCR for the detection of tyrosinase mRNA or other biomarkers of melanoma (including p97, MAGE-3, and MUC18) is in melanoma patients with disease stages I through III who are rendered disease free by surgery. If prospective studies with large numbers of patients demonstrate that those who test positive by RT-PCR for the presence of relevant biomarkers are significantly more likely to develop recurrent disease, then toxic forms of adjuvant therapy including bio-chemistry (combination of cisplatin-based chemotherapy with interleukin 2 plus interferon) or high-dose interferon could be restricted to this subset of patients.

References

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