Identical T-cell Receptor Transcripts in Multiple Melanoma Metastases

David Schrama, Eva Fuchs, Eva-Bettina Bröcker, Per thor Straten, and Jürgen C. Becker

Department of Dermatology, Julius Maximilians-University, D-97080 Würzburg, Germany [D. S., E. F., E.-B. B., J. C. B.], and Tumor Cell Biology, Division of Cancer Biology, Danish Cancer Society, DK-2100 Copenhagen, Denmark [P. t. S.]

Abstract

Immune therapy for melanoma largely relies on preexisting T-cell responses. However, recent reports demonstrated the localized nature of such responses. Thus, we characterized the effect of immune therapy on the distribution of clonotypic T cells. To this end, we analyzed the T-cell repertoire of multiple metastases of differentially treated melanoma patients revealing oligoclonal T-cell responses and the occurrence of identical T-cell clones in several metastases. However, these findings were not limited to immune therapy but were also observed after chemotherapy, suggesting its similar impact on the distribution of T cells.

Introduction

The mainstay of immune surveillance is the circulation of cells, e.g., dendritic cells scan the body for the presence of new antigens and experienced T cells circulate to discover and eliminate appropriate antigen-bearing cells (1). In melanoma, however, the latter does not seem to be the case because clonotypic analysis of multiple metastases from untreated melanoma patients displayed a predominance of strictly localized T-cell clonotypes (2). Accordingly, it was demonstrated in a murine melanoma model that the reoccurrence of identical T cells in different metastatic lesions of the same mouse was virtually restricted to animals receiving IL-23 (3, 4). Hence, these findings raise the question of whether therapies modulating immune responses would also enhance the distribution of clonotypic T cells. Although the clinical and immunological effects of immunotherapy have been established (5), prior studies failed to address this question directly. Consequently, we analyzed and compared the TCR usage of TILs in multiple metastases from melanoma under therapy by a RT-PCR/DGGE-based method.

Patients and Methods

Patients. A total of 31 metastases used for the experiments were collected from six patients (two females and four males) suffering from stage IV melanoma. These patients received immune and/or cytostatic therapies. The details of the therapies applied to each patient are provided in Table 1. The patients gave informed consent for parts of their surgically removed metastases to be used for immunological monitoring.

TCR Clonotype Mapping by DGGE. The DGGE analysis used for clonotype mapping of the human TCR BV regions 1–24 has been described previously (6). Briefly, RNA was extracted using the “absolutely RNA RT-PCR” Kit (Stratagene, La Jolla, CA), and synthesis of cDNA was done with 1–3 μg of total RNA, oligo(dT), and SuperScript II reverse transcriptase (Invitrogen, Karlsruhe, Germany). cDNA was amplified by primers specific for BV families 1–24 and a constant region primer that contains a 50-bp GC-rich sequence at the 5′-end. DGGE analyses were performed in 6% polyacrylamide gels containing a gradient of urea and formamide ranging from 20% to 80% separating the amplicons due to their melting properties, which are based on their nucleotide sequence. To prove the identity of clonotypic T cells in different metastases, we applied comparable clonotype mapping. Amplicons of the same BV family of different metastatic lesions were analyzed next to each other in a denaturing gradient gel, and transcripts resolving at similar positions in the gel were subjected to sequence analysis.

Quantification of the TCR Repertoire. Quantification of the TCR repertoire of TILs was performed by real-time PCR with Taqman technology. The previously given BV primers served as forward primers, 5′-GGTGTTGGGAAGATCTCTGGCTTC-3′ served as reverse primer, and 5′-carboxyfluorescein-ATGGCCTCAAACACGCCGACCGCAGAGGCAGGCGG-3′ served as a probe for quantification of the TCR BV families. Primers and probe for the constant region of the TCRβ chain were designed with Primer Express software (Applied Biosystems, Weiterstadt, Germany) and are 5′-GGTGTTGGGAAGATCTCTGGCTTC-3′ (forward primer), 5′-CACTGACACGCAGCAGTACACGCAGG-3′ (reverse primer), and 5′-carboxyfluorescein-AGCAGACTGTCATACACCTCCAGATCGGCAGACCGCAGGCGG-3′ (probe). The primer efficiencies were determined as 78 ± 15%. The relative expression of each TCR BV family was calculated in relation to the expression of mRNA coding for the constant part of the TCR.

Results and Discussion

Specific T-cell responses to melanoma, albeit not sufficient to control this tumor, have been repeatedly reported to occur spontaneously (7, 8). Therefore, a major branch of therapies to treat melanoma is based on improving and/or eliciting immune responses to fight the tumor (9). Despite some encouraging successes, the overall response rate to these therapeutic interventions remained generally low. Several mechanisms have been proposed to explain the lack of efficiency of cellular immune responses, e.g., loss of target antigens on tumor cells, signal transduction defects in effector cells, or impaired tumor homing of specific T cells. The latter hypothesis could be readily addressed by analysis of the T-cell repertoire usage of TILs. Nevertheless, only a few research groups have investigated the T-cell clonality of TILs, and most of them focused on overexpressed TCR variable regions (10). Only recently, our research group analyzed the complete T-cell repertoire of multiple metastases of two untreated stage IV melanoma patients, revealing the predominance of strictly localized T-cell clonotypes within the different metastases (2). This finding suggested a limited capacity of tumor-specific T cells to recirculate and infiltrate different tumor manifestations. Thus, we asked ourselves whether this limitation could be improved by therapies stimulating the immune response. To study the effect of immune modulation on the distribution of identical T cells, we analyzed multiple metastases by the same highly sensitive technique based on RT-PCR and DGGE that was used by us previously (6). This technique allows us to distinguish between oligoclonal infiltrates, represented by distinct bands in the denaturing gel, and polyclonal infiltrates, indicated by a smear. To this end, a total of 25 metastatic lesions from four patients receiving different forms of immune therapy were investigated. The summary results from these analyses and details of the therapies administered are provided in Table 1. However, to allow a clear presentation of the...
obtained data, we restricted the comprehensive description to one exemplary patient (Fig. 1). This patient was vaccinated with peptide-pulsed dendritic cells and received additional IL-2 to boost the vaccination-induced T-cell response (Fig. 1A). Due to disease progression after 6 months the therapy was changed to dacarbazine. The patient suffered from multiple skin metastases, which were excised over the course of the treatment to palliate symptoms. Fig. 1B depicts a representative example for all metastatic lesions analyzed, indicated by a clonotype map obtained from sample 3-1, of the oligoclonality of the T-cell infiltrate in all cases. The number of detectable T-cell clones among TILs in the nine samples ranged from 2 to 17. This range was comparable with that observed in the other three patients receiving immune therapy, with an average number of T-cell clonotypes/metastasis of 20.3, 12.1, and 18.6 (range, 2–33 T-cell clonotypes/metastasis; Table 1).

To scrutinize the distribution of T cells, we performed a comparative TCR clonotype mapping of multiple metastases. Because identical TCR transcripts possess the same melting temperature, they should be retained at the same position in the denaturing gel; subsequently, the DNA coding for the complementarity determining region 3 of the TCR obtained from the appropriate bands can be tested for identity by sequence analysis. As depicted in Fig. 1C, a BV 9 and a

---

**Table 1: Patient characteristics and summary of clonal composition of analyzed samples**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>DOB</th>
<th>Therapy</th>
<th>No. of samples</th>
<th>Clone range</th>
<th>Average clone no.</th>
<th>Occurrence of identical T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>1943</td>
<td>DC/IL-2; DTIC</td>
<td>9</td>
<td>2–17</td>
<td>7.4</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>1932</td>
<td>Fotemustine/IFN-α, IL-2; DTIC/DNCB</td>
<td>3</td>
<td>14–25</td>
<td>20.3</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>1932</td>
<td>DTIC/DNCB; IFN-β, DMS/IL-2, IFN-α</td>
<td>8</td>
<td>2–21</td>
<td>12.1</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>1941</td>
<td>Temozolomide</td>
<td>5</td>
<td>9–32</td>
<td>18.6</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>1955</td>
<td>Fotemustine; DMS</td>
<td>3</td>
<td>9–32</td>
<td>17.7</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>1942</td>
<td>Temozolomide</td>
<td>3</td>
<td>12–19</td>
<td>15.3</td>
<td>4</td>
</tr>
</tbody>
</table>

*a DOB, date of birth.
*b Therapies are listed in chronological order. All therapeutic regimens have been described previously in detail: DC, dendritic cell vaccination (15); DMS, Dartmouth regimen; DTIC, cisplatin, carmustine, and tamoxifen (16); DTIC and DTIC/DNCB, 2,4-dinitrochlorobenzene (17); fotemustine (18); temozolomide (19); and treosulfan (20).
*c Calculated as total clone number divided by number of samples.
*d The sum of all T-cell clones present in more than one metastasis.
BV 22 T-cell clone were detected in five of nine metastases, and two different BV 17 T-cell clones were observed in two metastases. In all four patients, identical T cells could be observed in multiple metastases. Notably, the extent of the distribution of identical T cells was much higher than that found previously in untreated melanoma patients (2), a finding strengthened by the notion that in mice the occurrence of identical T cells in multiple metastases is greatly increased after IL-2 treatment (3, 4).

To further evaluate the putative role of identical T-cell clones within the TILs, i.e., whether the clone belongs to an overrepresented family or rather to a small fraction of the total T cell repertoire, we performed semiquantitative analysis in selected cases. To this end, many of the T-cell clones were members of highly or moderately overrepresented TCR BV families, and only a few belonged to families expressed at rather low levels (Fig. 1D). Whereas TCR BV 9 was moderate to overexpressed in all samples analyzed, overexpression of TCR BV 17 and BV 22 was restricted to one sample. This pattern of TCR BV usage was found in all patients (data not shown). In general, the quantitative analysis revealed a trend within the respective patient, i.e., high or low relative expression of a TCR BV family was mostly consistent for the analyzed samples.

Because the distribution of identical T cells was not limited to metastases excised while patients underwent immune modulation, e.g., sample 6 of patient 1 (Fig. 1), we extended our study to two patients receiving solely cytostatic therapies to test the hypothesis that this form of therapy may also alter the distribution of clonally expanded T cells. The summary of this analysis is shown in Table 1, whereas the comprehensive description of the data obtained is again restricted to one patient. Fig. 2A depicts the detailed characteristics of patient 5. In accordance with previously published data (11, 12) and our findings regarding patients receiving immune therapy, all metastases were characterized by an oligoclonal T-cell infiltrate (Fig. 2B). The number of clones ranged from 9 to 32, with a clone number/metastasis of 17.7 (Table 1). The extent of distribution of identical T cells in multiple metastases was analyzed by comparative clonotype mapping. Surprisingly, identical T cells were detected in multiple metastases to the same extent as that seen in patients receiving immune therapy (Fig. 2C; Table 1). Semiquantitative analysis demonstrated that the identical T-cell clones were members of overrepresented TCR BV families (Fig. 2D). Again, as observed for the metastases obtained from the patients receiving immune therapy, the usage of different TCR BV families was similar in each of the samples analyzed if they were derived from one individual. Thus, immune-modulating and cytostatic therapies are likely to have the same effect on the distribution of identical T cells.

These overlapping characteristics of immune therapy and chemotherapy may be explained by immune-modulating effects exerted by chemotherapeutic operands, which can increase heat shock protein 70 transcription by activating heat shock transcription factor 1 (13). This, in turn, may lead to induction of immune-regulatory molecules, such
as the MIC genes, that are regulated by promoter heat shock elements similar to those of the heat shock protein 70 genes (14). Hence, cytostatic therapies may directly influence relevant molecules that help to induce and sustain cellular immune responses. Moreover, cytotoxic drugs are likely to release antigens from tumor cells and create “danger” signals that attract T cells.

The present work is, to the best of our knowledge, the most extensive study evaluating the complete TCR repertoire of TILs. The results obtained demonstrate that oligoclonal T-cell infiltrates of different cutaneous melanoma metastases are partially comprised of identical T cells. Notably, the extent of the distribution of identical T cells in multiple metastases is greater than that reported previously for untreated patients. The partial uniformity of T-cell responses in different metastases was confirmed by quantitative analysis, demonstrating a trend toward the usage of defined TCR BV families in the individual patients. In summary, our findings suggest an extended distribution of identical T-cell clonotypes in response to therapy. This effect, however, was not restricted to immune therapies but also holds true for chemotherapy, suggesting an immune-modulating function of the latter.

Acknowledgments

We thank Katrin Müller-Blech and Claudia Siedel for excellent technical assistance.

References

Identical T-cell Receptor Transcripts in Multiple Melanoma Metastases

David Schrama, Eva Fuchs, Eva-Bettina Bröcker, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/20/5664

Cited articles
This article cites 20 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/20/5664.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/62/20/5664.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.