Establishment and characterization of a melanoma patient derived xenograft model comprising three different sublines with distinct biological features

Kerstin Klingner, Dorothee Lenhardt, Elke Simon, Anne-Lise Peille, Julia Schüler
Charles River Discovery Research Services Germany, GmbH; Freiburg, Germany

1 INTRODUCTION

Patient-derived tumor xenografts (PDX) play a major role in the development of new cancer therapies and their strengths and weaknesses have gradually been elucidated. Large panels of solid cancer PDX are available to screen innovative compounds, identify new targets and study tumor biology.

In the current study we established a melanoma PDX from donor patient tissue. In addition, we were able to create two sublines from spontaneous metastases occurring in the murine host during the establishment phase of the original model. All three lines were characterized by tumor growth kinetics, antitumoral activity against standard of care Temozolomide (40 mg/kg/d; iv; twice weekly for three weeks) and patho-histological examination. Furthermore, molecular examination of primary PDX and its metastases is underway as well as cell line establishment.

2 RESULTS

Figure 1. Tumor growth behavior of melanoma PDX MEXF 2090P, 2090L and 2090S. All three in vivo lines depicted distinct growth kinetics. The doubling times varied significantly (Kruskal-Wallis, p< 0.0018) between 12.34 days (primary tumor, MEXF 2090P), 19.01 days for the liver metastasis model (MEXF 2090L) and 30.78 day (spleen metastasis, MEXF 2090S). MEXF 2090S showed the longest doubling time within the melanoma PDX panel.

Figure 2. Patho-histological features of melanoma PDX MEXF 2090 P, 2090L and 2090S. All three in vivo lines were diagnosed as well-differentiated melanoma. Stroma content determined by H&E stain as well as by PCR was 3-5% in all three lines.

Figure 3. PDX derived cell line from melanoma PDX MEXF 2090P. Cell line established from primary PDX MEXF 2090P. Human origin of cells was confirmed by PCR as well as STR analysis.

Table 1. Characteristics of the donor patient and the corresponding PDX

<table>
<thead>
<tr>
<th>Model name</th>
<th>Year of Surgery</th>
<th>Histology origin</th>
<th>Differentiation</th>
<th>Age at Surgery</th>
<th>Gender</th>
<th>Cell Line</th>
<th>Availability</th>
<th>Ethnicity</th>
<th>molecular analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEXF 2090P</td>
<td>2010</td>
<td>melanoma</td>
<td>Primary</td>
<td>68</td>
<td>female</td>
<td>yes</td>
<td>Caucasian</td>
<td>gene chip array; SNP-6; WES</td>
<td></td>
</tr>
<tr>
<td>MEXF 2090L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEXF 2090S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. STR analysis of melanoma PDX MEXF 2090P, 2090L and 2090S. Short tandem repeat analysis revealed the human origin as well as the unique profile of the PDX models and the cell line derived of 2090P in comparison to the cell line database of DMSZ.

Figure 5. Determination of sensitivity towards SoC compound Temozolomide. Temozolomide was applied to all three lines. 6 mice per group and line were treated either with Temozolomide (40 mg/kg/d, iv, twice a week for three weeks) or the control vehicle (10% DMSO, 90% NaCl). The primary tumor depicted statistically significant antitumoral activity with a T/C (test vs control) value of 36% (p< 0.05, t test, two-tailed) two weeks after the last treatment (experiment day 32). The two lines derived of metastases were resistant against the alkylating agent depicting T/C values of 76% (2090L) and 79% (2090S), respectively.

3 CONCLUSION

- Spontaneous metastases are a rare event in PDX models growing subcutaneously in NMRI nude mice.
- Shedding some light into the biology of the metastatic event in the current model will help to understand and influence the metastatic process in general.
- Further characterization of the PDX model and its different sublines will help to elucidate the resistance mechanism behind these data. Those lines can serve as indispensable tools in the oncology drug development pipeline.