Expression of vascular endothelial growth factor and angiopoietin-2 in myeloma cells

Angiogenesis is important for myeloma. To understand the mechanism of angiogenesis, we analyzed vascular endothelial growth factor and angiopoietins, new angiogenic molecules, in purified myeloma cells at mRNA and protein levels. Co-expression of these factors was observed. We suggest that these molecules may be significant as prognostic factors.

References

8. Otsuki K, Takahashi K, Takeda K, Nakayama M, Yoshizawa M, Fujita H, et al. Induction of heme oxygenase-1 by nitric oxide mediated by the other agents. This was shown in the present study. However, it did not offer any protection against apoptosis induced by oxidative stress. Theoretically, nitric oxide may increase the induced apoptosis, since it may react with the superoxides formed during BSO/etoposide exposure, leading to peroxinitrate radical formation, which itself is toxic for many proteins and enzymes.
9. In conclusion, HO-1 does not seem to confer etoposide resistance in AML cells and, its induction does not protect AML cells from etoposide-induced apoptosis.

Key words: AML, etoposide, BSO, NO, apoptosis, HO-1.

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References

We examined the expressions of VEGF-receptors, Flt-1 and KDR/Flk-1, by flow cytometry. However, these receptors were not expressed in both cell lines and fresh myeloma cells (data not shown). Tie-2, a receptor for angiopoietin-1 and angiopoietin-2, was also not expressed (Figure 1), indicating that VEGFs and angiopoietin-2 are not autocrine factors for myeloma cells but rather directly stimulate surrounding vascular endothelial cells.

Expression of VEGF121 was not a prognostic factor (data not shown). However, the survival rate at one year from diagnosis of cases expressing both VEGF121 and VEGF165 was 35.6 % (n=22) while that of cases expressing only VEGF121 was 87.5 % (n=8), suggesting co-expression of VEGF121 and VEGF165 is a prognostic factor (p<0.05, Cox-Mantel test). Survival rate at one year from diagnosis of cases with expression of angiopoietin-2 was 41.2% while that of cases without expression was 77.8 % (p<0.05, Cox-Mantel test), suggesting that expression of angiopoietin-2 may contribute to poor prognosis although analysis of more cases is needed to draw a conclusion.

To our knowledge, this is the first report directly describing expression of VEGF 165 and angiopoietin-2 in purified human myeloma cells. The finding of co-expression of VEGF 165 and angiopoietin-2 in myeloma cells provides useful information for the development of new strategies targeting angiopoietin-2 as well as VEGF. Therefore, it is anticipated that inhibition of angiopoietin-2 should improve the outcome of anti-VEGF receptor therapy, this latter already being evaluated in clinical trials for multiple myeloma.

Shima Uneda,* Fumihiko Matsuno,* Takashi Sonoki,* Izumi Tniguchi,* Fumio Kawano,° Hiroyuki Hata*

*Department of Internal Medicine II, Kumamoto University School of Medicine; °Department of Clinical Research, Kumamoto National Hospital, Kumamoto, Japan

Key words: angiopoietin, VEGF, multiple myeloma, angiogenesis.

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Correspondence: Dr. Hiroyuki Hata, MD, Ph D, Department of Internal Medicine II, Honjo 1-1-1, Kumamoto 860-8556, Kumamoto University School of Medicine, Japan.

Phone: international +81.96.3735156.
Fax: international +81.96.3635265.
E-mail: hata@kumamoto-u.ac.jp

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References
4. Iwasaki T, Hamano T, Ogata A, Hashimoto N, Kitano M, Kak-
The feasibility of reduced-intensity allogeneic hematopoietic stem cell transplantation from a related donor with HLA one-antigen with or without one-allele mismatch

It is still unclear whether reduced-intensity stem cell transplantation (RIST) from an HLA-mismatched related donor is feasible for hematologic malignancies. In the current study on the use of antithymocyte globulin (ATG) in 13 patients, we focused on this issue by evaluating regimen-related toxicities, engraftment, graft-versus-host disease (GVHD), infection, and overall survival. Our results suggest that this procedure may be acceptable for patients without a matched related donor.

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A total of 13 patients underwent RIST from a serologically HLA one-locus mismatched related donor between March 2000 and September 2002. The characteristics of these patients are shown in Tables 1 and 2. Both HLA antigen and allele matching were generally evaluated, since any disparity in HLA allele

The conditioning regimen consisted of cladribine (0.66 mg/kg) or fludarabine (180 mg/m²), busulfan (8 mg/kg), and rabbit anti-thymocyte globulin (ATG: 5 mg/kg in 2 patients, and 10 mg/kg in 16 patients). Infectious prophylaxis procedures have been described previously. Prophylaxis against GVHD was performed with cyclosporine (CSP) alone in the initial 7 patients. Thereafter, short-term methotrexate (MTX) was added to CSP in the subsequent 6 patients because of the observation of severe acute GVHD in the earlier group. Patients who developed grade II-IV acute GVHD were treated with methylprednisolone at a dose of 1-2 mg/kg/day iv. Infections disease was defined as an illness associated with symptoms and signs consistent with an infection, with microbiological documentation of a pathogen. Microbiological documentation consisted of the isolation of a pathogen by culture from a sterile or non-sterile site, or by histologic or immunohistochemical evidence. The primary endpoint of this study was the evaluation of engraftment, defined as >0.5×10⁹/L absolute neutrophil count (ANC) or >1.0×10⁹/L white blood cell count (WBC), and the toxicities associated with the procedure. The secondary end-points included evaluation of the extent of GVHD and infectious episodes. Differences in incidence were evaluated using Fisher's exact test. Actuarial overall survival was estimated by the Kaplan-Meier method.

We found that all of the patients tolerated our RIST regimen and organ toxicities were limited to less than grade II hepatic and stomatitis/gastrointestinal toxicity, except in one patient (UPN 389) who developed a subdural hematoma. The median number of CD34+ cells infused was 3.6×10⁶/kg (range, 2.2 to 7.3×10⁹/kg, Table 1) and the median duration of neutropenia was 12 days (range, 7-20, Table 1). Chimerism analysis was performed on days 30, 60, 90, 120, 180, 240, 300 and 360, and we confirmed that 11 of the 13 patients achieved engraftment from this HLA-mismatched transplantation. This result further suggests that our regimen, incorporating ATG, enables successful engraftment by overcoming the HLA barrier that is limited to HLA one-antigen with or without one-allele, as recently reported by Gajewski et al. One patient developed primary graft failure and the rapid emergence of recipient-type hematopoiesis on day 17, suggesting that our regimen is not truly myeloablative, and that the RIST procedure, relative to conventional transplantation with a myeloablative regimen, saves patients by retaining the ability of the marrow space to be repopulated by the recipient’s own cells.

Table 1. Patients’ characteristics.

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex</th>
<th>Age</th>
<th>Disease</th>
<th>Status at transplant</th>
<th>Regimen</th>
<th>HLA mismatched locus</th>
<th>RRT grade</th>
<th>CD34</th>
<th>Duration of neutr.</th>
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<tr>
<td>267</td>
<td>M</td>
<td>29</td>
<td>AML</td>
<td>NR</td>
<td>2CdA/Bu ATG</td>
<td>DRB1 (antigen)</td>
<td>0</td>
<td>2.23</td>
<td>15</td>
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<tr>
<td>295</td>
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<td>27</td>
<td>AML</td>
<td>NR</td>
<td>2CdA/Bu ATG</td>
<td>DRB1 (antigen)</td>
<td>1(hepatic)</td>
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<tr>
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<td>MDS</td>
<td>NR</td>
<td>2CdA/Bu ATG</td>
<td>B (antigen) + A (allele)</td>
<td>2(stomatitis)</td>
<td>4.38</td>
<td>11</td>
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<tr>
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<td>NR</td>
<td>Flu/Bu ATG</td>
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<td>2.58</td>
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<tr>
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<td>426</td>
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<td>NR</td>
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<td>434</td>
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<td>B (antigen) A (allele) + DRB1 (allele)</td>
<td>1(hepatic)</td>
<td>4.03</td>
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<tr>
<td>446</td>
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<td>A (antigen)</td>
<td>2(stomatitis)</td>
<td>3.57</td>
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</tr>
</tbody>
</table>

UPN: unique patient number; RMS: rhabdomyosarcoma; MDS: myelodysplastic syndrome; ARCC: adrenal cortical carcinoma; CR: complete remission; NR: no remission; CD34: CD34 cell dose×10⁹/kg; neut: neutropenia.