Letters to the Editor

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References


Different prognostic values of p15INK4b and p16INK4a gene methylation in multiple myeloma

We analyzed 61 multiple myeloma (MM) samples by methylation-specific polymerase chain reaction (PCR). Survival was significantly lower in patients with methylated p16INK4a gene, but was not different between patients with methylated and unmethylated p15INK4b gene, suggesting that p15INK4b and p16INK4a genes have a distinct influence on the outcome of MM.
Expression of the p15INK4b gene is induced by TGF-β and inactivation of p15INK4b by methylation suggests a possible mechanism of escape from regulatory signals provided by the bone marrow micro-environment. However, the role of p15INK4b methylation in the development of MM and progression of the disease remains unknown. In order to investigate the relative distinct influences of p15INK4b and p16INK4a methylation on the clinical outcome of MM, we analyzed p16INK4a and p15INK4b gene methylation using methylation-specific PCR (MSP) in 61 previously untreated MM patients. The median age of the patients was 65 years (range: 39-83). Clinical staging was: stage I, 11 (18%); II, 8 (13%); III, 42 (69%) and IV in 5 (8.2%). M-component was IgG in 36 cases (59%), IgA in 16 (26%), and Bence Jones in 9 (14.8%). The median percentage of BM plasma cells was 20% (SD 23.8; range: 6-96). Thirty-three patients (54.1%) were treated with melphalan-prednisone, eighteen (29.5%) with intensive protocols, and nine (14.8%) were not treated.

The median time of follow-up was 8.6 years (range 3.5-16.1). Methylation of p16INK4a was found in 16 cases (26.2%), of p15INK4b in 16 (26.2%), of p16INK4a or p15INK4b in 25 (41%), and of both genes in 7 (11.5%). p15INK4b and p16INK4a methylation was found to be independent ($\chi^2=2.3$, $p=0.13$). No correlation could be made between methylation of either p16INK4a or p15INK4b and gender, age, isotype, level of M-component, percentage of bone marrow plasmacytosis, stage of disease, renal insufficiency, serum levels of lactate dehydrogenase, albumin, calcium, β2-microglobulin, C-reactive protein, or response to treatment. However, patients with methylated p16INK4a gene showed significantly poorer overall survival (OS) ($p=0.01$, log-rank test) and progression-free survival (PFS) ($p=0.005$) (Figures 1 and 2). The only other initial characteristic which affected OS in univariate analysis was β2-microglobulin ($p=0.006$). Final models of multivariate analysis showed that methylation of the p16INK4a gene remained an independent prognostic factor for OS ($p=0.035$, relative risk = 2.86, 1.076-7.60) with β2-microglobulin ($p=0.003$, relative risk = 1.18, 1.08-1.3). In contrast, OS and PFS were not significantly different between patients with methylated and unmethylated p15INK4b gene ($p=0.59$ and 0.28, respectively) (Figures 1 and 2). When OS and PFS were compared between patients with either p15INK4b or p16INK4a gene methylation (n=25) and patients with both genes unmethylated (n=36), the difference was significant for PFS ($p=0.029$) but not for OS ($p=0.24$). Incorporation of p15INK4b methylation in the multivariate model with p16INK4a methylation and β2-microglobulin did not modify the results. Thus, the poor outcome of MM patients with methylation of CDK inhibitors seems entirely related to p16INK4a gene methylation. The prognostic impact of p16INK4a methylation has been previously described by other groups and recently Mateos et al. found a correlation between p16INK4a methylation in MM patients and the percentage of S-phase plasma cells. This group also reported a significant association between p16INK4a methylation and the stage of disease,
β₂-microglobulin serum levels, and high C-reactive protein values. We did not observe any correlation between p16\(^{ink4a}\) methylation and the initial characteristics of the patients. However we observed almost the same differences in OS and PFS as Mateos et al. did. Ng et al. reported similar incidences of p16\(^{ink4a}\) gene methylation in pre-treated and post-treated MM patients. These findings suggest that in spite of possible variations between techniques used, heterogeneity of patients, and other unknown factors, p16\(^{ink4a}\) methylation analysis in MM might provide interesting prognostic information and warrants future prospective studies.

The absence of prognostic impact of p15\(^{ink4b}\) gene methylation is in marked contrast with the prognostic value of p16\(^{ink4a}\) methylation. We previously reported frequent methylation is in marked contrast with the prognostic value of p16\(^{ink4a}\) methylation. We did not observe any correlation between p16 INK4a methylation and the initial variations between techniques used, heterogeneity of patients, and other unknown factors, p16\(^{ink4a}\) methylation might play a role in the initial transplantation of plasma cells. However, p15\(^{ink4b}\) methylation might exert a lesser influence on subsequent tumor progression.

**Non-myeloablative stem cell transplantation with low-dose total body irradiation and fludarabine for metastatic renal cell carcinoma**

We evaluated the feasibility of non-myeloablative stem cell transplantation for metastatic renal cell carcinoma after a non-myeloablative conditioning regimen combining low-dose TBI and fludarabine. Seven consecutive patients were included. Initial engraftment occurred in all patients and 6/6 evaluable patients achieved sustained donor chimerism. One patient experienced a partial response but the other 6 progressed.

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Metastatic renal cell carcinoma (RCC) is largely insensitive to chemotherapy. In 2000, Childs et al. published the results of non-myeloablative stem cell transplantation (NMSCT) combining cyclophosphamide and fludarabine in 19 patients with metastatic RCC. Ten of the 19 patients enjoyed objective responses, including 3 with sustained CR. Although the conditioning regimen was non-myeloablative, the neutrophil count fell to less than 0.1 \times 10\(^9\)/L in all patients. The Seattle team has recently proposed an original approach to NMSCT with a conditioning regimen based on 2 Gy TBI + fludarabine, followed by post-transplant immunosuppression with cyclosporine A (CyA) and mycophenolate mofetil (MMF) that permitted the transplant to be performed in an ambulatory care setting. In the present study, we report our experience with 7 patients with RCC.

Seven consecutive patients with metastatic RCC were included (Table 1). Written informed consent was obtained from patients and donors and our institution's Ethical Committee approved the protocol. Four patients had HLA-identical siblings and three had alternative donors. Conditioning consisted in 90 mg/m\(^2\) fludarabine combined with 2 Gy TBI. The whole post-transplant procedure was carried out as outpatient except in the haemodialyzed patient. Post-transplant immunosuppression consisted in CyA and MMF. Disease responses were defined using the criteria of Childs et al. Stem cell mobilization and collection were carried out as previously reported. The protocol involved a prospective comparison of graft manipulation, so that patients #1–3 received unmanipulated PBSC, patients #4–6 CD8-depleted PBSC and patient #7 CD34-selected PBSC. Three patients without GVHD received additional DLI (per protocol) on days 40 and 80. Post-transplant DLI were unmanipulated in patient 2 and CD8-depleted in patients #5 and 7. Chimerism was assessed as previously reported.

None of the patients developed grade >2 regimen-related toxicity. The neutrophil nadir occurred on day 7 and was 0.97 \times 10^9/L (0.12–1.67). Two patients did not require hospitalization within the first 30 days following NMSCT, and the other five were hospitalized for a median of 9 (6–22) days. Total white blood cell (WBC) and CD3\(^+\) cell chimerisms were 91% (90–95) and 67% (20–89) on day 21 and 95 (95–96) and 83 (32–96) on day 100, respectively (Figures 1A and 1B).

We observed only 1 partial response. This response occurred in patient #1 who had extensive lung metastases. The disease remained stable for the first 150 days after transplantation (Figure 1C) but the tumor mass was markedly (> 50%) reduced on day 240. This patient experienced both acute and chronic GVHD. Response persisted until day 389 when a chest CT-scan showed elimination or major reduction of 80% of the metastases with stabilization of the others, with the exception of two lesions that progressed (Figure 1D). Unfortunately the patient subsequently relapsed in the liver and died of disease progression. All other patients progressed (Table 1). We show here that engraftment can be achieved in RCC patients with this low-intensity

**References**