SOLID TUMORS

458

PROMISING OUTCOMES WITH TANDEM AUTOLOGOUS STEM CELL RESCUE IN 'LATE' WILMS TUMOR RELAPSE

Chaudhury, S., Merchant, N., Tse, W., Duerst, R., Schneiderman, J., Morgan, E., Kletzel, M. Childrens Memorial Hospital, Northwestern University's Feinberg School of Medicine, Chicago, IL

Introduction: Optimal management of relapsed Wilms' tumor (WT) patients remains unclear. Modern second-line treatment consists of either salvage chemotherapy±radiation therapy or chemotherapy followed by high-dose chemotherapy and autologous hematopoietic stem cell rescue (HD-ASCR).
Methods: Fifteen consecutive patients with relapsed/ persistent WT from 2001-09, enrolled on IRB protocol with planned two tandem cycles of HD-ASCR. Myeloablative chemotherapy regimen for first cycle consisted of Etoposide 2400 mg/m2, Carboplatin 2000 mg/m2, Cyclophosphamide 3600 mg/m2; and Melphalan 180 mg/m2 and cyclophosphamide 4500 mg/m2 for the second.
Results: There were 6 males and 9 females with a median age at diagnosis of 4.6 y(range 3-16 y), and median time from diagnosis to relapse 1 y (range 0.1- 7.5 y). Median time from relapse to HD-ASCR was 5 m (range 3-31 m). Histology was favorable in 12 and anaplastic in three. Five of 15 patients received HD-ASCR for early relapse/ refractory disease within 6 months from diagnosis. Ten patients received planned two cycles of HD-ASCR while the remaining 5 received only 1 course of HD-ASCR due to disease progression or toxicity. Disease state at the time of HD-ASCR was CR in 7, VGPR in 5 and PR in 3. Post- ASCR, median time to neutrophil engraftment and unsupported platelet count >20 K was 12 and 21 days, respectively. Regimen related toxicity included infections (1 aspergillus, 1 klebsiella), hemorrhagic cystitis (1) and one fatal case of acute renal failure. At a median follow-up of 60 months, nine patients are alive and disease free. Six patients died from disease relapse in five and renal failure prior to engraftment in one. Five patients with refractory disease/early relapse (≤6 months from initial diagnosis) before undergoing HD-ASCR had significantly worse outcomes from relapse. ($p = 0.001$). The 5 year estimated event-free survival (EFS) and the overall survival (OS) in the remaining 'late' relapsed patients are 55% and 75% respectively. There was no correlation of outcome with disease stage at diagnosis, site of relapse, disease state at ASCR-HD.
Conclusions: Tandem HD-ASCR remains an effective, non-toxic treatment for patients with relapsed Wilms' tumor especially if greater than 6 months from diagnosis. Patients with persistent disease or early relapse within 6 months from diagnosis likely have different biological characteristics and should receive a novel modality of therapy.

459

AUTOLOGOUS STEM CELL TRANSPLANT FOR ADVANCED STAGE PEDIATRIC SOLID TUMORS

Hutspardol, S.[1], Pakakasama, S.[2], Sirachainan, N.[2], Anurathapan, U.[2], Hongeng, S.[2] [1] Srinakharinwirot University, Nakorn Nayok, Thailand; [2] Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Autologous stem cell transplant (ASCT) potentially promotes the survival in high-risk pediatric solid tumors. Individual preparative regimen for each primary solid tumor is not yet determined owing to various conditions of disease status and previous treatment. We have transplanted 17 patients with advanced stage solid tumors using two different preparative regimens. Primary diagnosis included 10 patients (58.9%) with neuroblastoma stage 3 and 4, 2 patients (11.7%) with Wilms tumor stage 4, 2 patients (11.7%) with germ cell tumor stage 3 and 4, one each patient with stage 4 rhabdomyosarcoma (5.9%), Ewing's sarcoma (5.9%), and retinoblastoma (5.9%). Indication for ASCT was tumor recurrence in 7 patients (50%) and residual disease in 7 patients (50%). Eleven patients (65%) with previously treated high-dose cyclophosphamide received regimen 1 consisting of carboplatin, etoposide, and melphalan during conditioning. Regimen 2 (carboplatin, etoposide, cyclophosphamide) was chosen for 6 patients (35%) without previous treatment of high-dose cyclophosphamide. Most patients achieved engraftment within a median time of 11 days (range 7-18 days). There were 5 patients who alive and disease-free at the end of study. Median follow-up among survivors was 4.1 years (range 1.2-6.3 years). Disease progression-free survival (PFS) at 1- and 3-year post-ASCT was 47% and 32%. Overall survival (OS) at 1-, 3-, and 5-year post-ASCT was 71%, 36%, and 18%. There was no significant difference in PFS and OS between two different transplant regimens in univariate analysis ($p = 0.36$ and $p = 0.62$). Likewise, there was no significant difference in PFS and OS between two indications of ASCT (residual disease and tumor recurrence; $p = 0.37$ and 0.50), time from diagnosis to ASCT (< 1 year and ≥ 1 year; $p = 0.72$ and 0.24), and primary diagnosis (neuroblastoma and non-neuroblastoma; $p = 0.75$ and 0.87). No severe transplant-related toxicity and mortality was observed herein. Due to limited number of patients in both regimens, continuing investigation of ASCT role as salvage therapy for advanced solid tumors should be carried on.

460

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION ENHANCES ANTI-TUMOR ACTIVITY AGAINST RENAL CELL CANCER

Budak-Alpdogan, T., Sauter, C., Bailey, C., Biswas, C., Panis, M., Civriz, S., Alpdogan, O. Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

Allogeneic HSCT has been suggested as a treatment option for cytokine unresponsive, advanced renal cell cancer (RCC). However, tumor progression remains as the main problem post-transplant. We hypothesize that haploidentical (HI) HSCT might enhance graft versus tumor (GVT) activity against RCC and improve the outcome after transplant by tumor growth suppression. We first established a HI-transplant model using two different hybrid mouse strains as donor and recipient in the experiments. Lethally irradiated (CB6F1-(H2Kb/d) recipients were transplanted with T cell-depleted (TCD) bone marrow (BM) from B6CBAF1 (H2Kb/k). We found that B6CBAF1 TCD-BM cells engrafted well in recipients of HI-HSCT without graft failure when analyzed at varying time points after the transplant.

We then explored GVT activity in this HI-HSCT model. Lethally irradiated CB6F1 host were transplanted TCD-BM from the following donors; B6D2F1 (H2Kb/d), B6 (H2Kb), and B6CBAF1 (H2Kb/k). Animals received RENCA cells on the same day of the transplant. Low dose T cell ($1x10^5$) infusion was enough to elicit GVT effect but not GVHD and to provide murine RCC growth control and survival advantage in HI- HSCT. Recipients of HI-HSCT BM and T cells showed better anti-tumor activity than recipients of B6D2F1 or B6 BM + T Cells, respectively. Recipients of HI-HSCT BM and T cells did not reveal any tumor development in the first 50 days, and had a significantly better survival compared to other groups. Low dose haploidentical CD8+ T cells provided a better anti-tumor activity than CD4+ T cells but unseparated T cells resulted in significantly better survival than either subset alone. We concluded from this experiment that GVT activity against to RENCA cells is mainly driven by CD8+ T cells but they need CD4+ T cells help for optimal anti-tumor activity.

Our data suggested that HI-HSCT could provide substantial graft-versus-tumor effect against renal cell carcinoma that might suppress tumor growth and elicit survival advantage.

STEM CELL BIOLOGY

461

HUMAN UMBILICAL CORD BLOOD (HUCB) DERIVED STEM CELLS ENHANCES WOUND HEALING

Liao, Y.[1], Itoh, M.[2], Roberts, S.[1], Highet, A.M.[1], Yang, A.[1], Christiano, A.[2], Cairo, M.S.[1] [1] New York Medical College, Valhalla, NY; [2] Columbia University, New York, NY

Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited skin blistering disease caused by mutations in Col7a1gene, which encodes a major component in anchoring fibrils (Christiano et.al. *Nat Gen* 1993). An initial report has shown promises of Allogeneic (Allo) -SCT for the treatment of RDEB (Wagner et.al. *N Engl J Med* 2010). However no distinct anchoring fibrils were observed in the recipient skin and the functional cell populations are

to be characterized. Stem cells with pluripotent properties, including unrestricted somatic stem cells (USSCs) have been isolated from HUCB and may represent novel stem cells for the RDEB regenerative therapy (Liao/Cairo et al, *Exp. Hem*, 2011).
Goal: To determine the potential of USSCs in promoting wound healing and treating RDEB.
Method: USSCs were isolated from HUCB and characterized by flow cytometry, RT-PCR, bisulfate sequencing and immunocytochemistry. A $1cm^2$ full-thickness excisional wound was created at the dorsal of NSG mice, followed by intradermal injection of 1 million USSCs or PBS at a 1cm- distance from the margin of the wound.
Results: USSCs transcribe a low level of ES factors and have a mosaic DNA methylation pattern at the regulatory regions of Nanog and Oct4. USSCs express Col7a1 at a level that is comparable to human keratinocytes and fibroblasts. Through an initial treatment of ascorbic acid, EGF and BMP4 for 7 days followed by culture in defined K-SFM medium, USSCs were able to differentiate and express keratinocyte- specific genes. In the mouse wounding model, the wounds closed at a faster rate in USSC-treated mice as compared to PBS and a significant difference was observed between the two groups on days 6-10 post wounding ($F_{(1,168)} = 50.8$, $P<0.01$). USSCs promoted epithelialization and facilitated formation and remodeling of epidermis. More host-derived endothelial cells ($PECAM^+$) were recruited to the USSC-treated wounds as early as three days post wounding. Furthermore, the USSC-treated skin healed with skin appendages in the center of the wounded area. However human cells could be sporadically detected only in the basal membrane in skin sections without apparent differentiation. This suggests that the improved murine wound healing is due to a paracrine effect of USSCs.
Conclusion: HUCB- USSCs showed beneficial effects in cutaneous regeneration. The ability of USSCs to express Col7A1 and promote wound healing suggests their potential application in the RDEB therapy.

462

SERINE/THREONINE PIM KINASES PLAY AN IMPORTANT ROLE IN MAINTAINING THE NUMBER AND FUNCTION OF HEMATOPOIETIC STEM CELLS

An, N.[1], Lin, Y.-W.[2], Liu, A.[1], Lilly, M.[3], Mahajan, S.[1], Kraft, A.[1], Kang, Y.[1] [1] *Medical University of South Carolina, Charleston, SC;* [2] *Date Red Cross Hospital, Hokkaido, Japan;* [3] *University of California Irvine, Irvine, CA*

Pim (proviral insertion in murine lymphoma) kinases are a small family of constitutively active, highly conserved serine/threonine kinases, and have 3 members (Pim1, 2 and 3). The roles of Pim kinases in the regulation of hematopoietic stem cells (HSCs) are currently unknown. In the current study, we used transgenic (Tx) and knockout (KO) mouse models to characterize the roles of Pim kinases in hematopoiesis. We generated Pim1 Tx mice bearing human Pim1 kinase under the vav- hematopoietic regulatory elements (vav-hPim1). Pim1, 2 and 3 single, double and triple KO mice were also used in this study. We found that: 1). Vav-hPim1 Tx mice developed lymphoid, but not myeloid, malignancies. About 10% of vav-hPim1 Tx mice developed lymphoid leukemia/lymphoma. 2). Vav-hPim1 Tx mice had increased hematopoietic stem/progenitor cell (HSPC) population. In those vav-hPim1 Tx mice that were free of leukemia/lymphoma, a more than 2 fold increase in the number of c-Kit+Sca-1+Lin- (LSK) HSPCs were noted when compared to age-matched, wild-type littermates (p less than 0.05). Vav-hPim1 Tx mice exhibited enlarged spleen, higher CXCR4 surface expression and increased colony forming units (CFUs). Additionally, lethally irradiated mice transplanted with splenocytes from vav-hPIM1 Tx mice had better survival compared to those transplanted with splenocytes from wild-type control mice. 3). Pim1, 2, 3 single KO mice had relatively unchanged HSPCs, while Pim1/2, Pim2/3 double KO and Pim1/2/3 triple KO mice exhibited decreased HSPCs, suggesting potential overlap in function of Pim1, 2, 3 kinase. When compared to wild-type littermate controls, Pim double or triple KO mice exhibited lower number of LSK HSPCs, lower CXCR4 surface expression, and reduced CFUs. Furthermore, lethally irradiated mice transplanted with marrow cells from Pim double or triple KO mice had much slower hematopoietic recovery, compared to those receiving marrow from normal control mice. 4). IL-3, IL-6 and Thrombopoietin upregulated Pim1 expression in Lin- marrow cells. Stem cell factor and Flt-3 had no effect on Pim 1 expression. 5). Over-expression of Pim1 kinase led to up-regulation of transcription factor genes important in the sonic Hedgehog signaling, illustrating a potential mechanism through which Pim1 enhances hematopoiesis. In conclusion, our studies demonstrate a novel role of Pim serine/threonine kinases in hematopoiesis, and provide a novel molecular mechanism in the regulation of HSCs.

463

ENDOTHELIAL PROGENITOR CELL MOBILIZATION IN C57BL/6 MICE FOLLOWING TREATMENT WITH SINGLE AGENT OR COMBINATION NEUPOGEN (G-CSF), PLERIXAFOR (AMD3100), AND VEGF

Frank, R.R.[1], Jagan, S.[1], Paganessi, L.A.[1], McNulty, M.A.[2], Sumner, D.R.[2], Fung, H.C.[1], Gregory, S.A.[1], Christopherson, K.W.[1,2] [1] *Rush University Medical Center, Chicago, IL;* [2] *Rush University Medical Center, Chicago, IL*

Introduction: Bone marrow (BM) derived endothelial progenitor cells (EPC) can differentiate to form vasculature. It is thought that EPC have a potential role in homeostasis of the endothelium as well as repair after ischemia or other endothelial injury by inducing neovasculatization. Successful mobilization of EPC from the BM into the peripheral blood (PB) would allow for relative ease of collection and use for cell therapy.
Methods: A screen of 10 agents in C57BL/6 mice identified G-CSF, AMD3100, and VEGF as agents that resulted in a $\geq$ 2-fold increase in circulating EPC. Subsequently, neupogen (G-CSF, 50g/kg SC twice a day for 4 days), plerixafor (AMD3100, 5mg/kg SC, once 1 hr prior), and VEGF (50g/kg SC twice a day for 4 days) were assessed in individual mice (N = 5) as single agents or in combination. PB and BM were collected and plated for colony formation at 5%CO_2 5% O_2. After 14 days, total EPC and EPC subtypes corresponding to endothelial colony-forming cells (ECFC) and colony-forming units (CFU-EC), as previously reported by Yoder, et al. *Blood* 2007, were scored. In parallel, hematopoietic progenitors (CFU-GM, BFU-E, & CFU-GEMM) were evaluated after 7 days in culture. Data is presented as colonies /mL PB or /femur $\pm$SEM and analyzed using Mann-Whitney *U* test.
Results: Analysis of data indicates, as compared to saline, that treatment with single agent G-CSF, AMD3100, or VEGF results in significant mobilization of total EPC into the PB (3.7 ± 2.1, 61.0 ± 7.7, 60.4 ± 3.8, or 37.9 ± 6.7 CFU/mL respectively, $p<0.05$). In addition, the total EPC in the PB increased further with the combination G-CSF+AMD3100 (95.3 ± 9.4 CFU/mL, $p<0.05$) but not G-CSF+VEGF, VEGF+AMD3100, or G-CSF+VEGF+AMD3100 (65.3 ± 15.7, 56.1 ± 7.7, 54.9 ± 18.9 CFU/mL respectively). In all cases the majority of EPC mobilized into the PB were CFU-EC. Only AMD3100 mobilized significant numbers of ECFC ($p<0.05$). In the BM, total EPC and ECFC decreased in response to G-CSF but not VEGF or VEGF+AMD3100. G-CSF and AMD3100 containing regimens but not single agent VEGF mobilized CFU-GM, BFU-E, and CFU-GEMM into the PB ($p<0.05$).
Conclusion: Treatment with single agent or combination G-CSF, AMD3100, and VEGF mobilize significant numbers of EPC into the PB. However, only AMD3100 significantly mobilizes the ECFC subtype of EPC that has been reported to form capillary-like structures and micro-vessels. Further studies are warranted to assess potential therapeutic outcomes of mobilized EPC in disease model systems.

464

MESENCHYMAL STEM CELLS ENHANCED METASTASIS OF NEUROBLASTOMA VIA SDF-1/CXCR4 AND SDF-1/CXCR7 SIGNALLING

Ma, M., Chan, G.C.-F. The University of Hong Kong, Hong Kong, Hong Kong

Background & Objective: Bone marrow is a frequent metastatic site for neuroblastoma. The SDF-1/CXCR4 axis has long been proposed as an important pathway during metastasis of various cancers, including neuroblastoma (Zhang L, *et al.* 2007). But the expression and function of CXCR7, the other known receptor for SDF-1, in metastatic neuroblastoma has yet to be defined. We investigated the chemotactic effects of neuroblastma cells towards human bone marrow mesenchymal stem cells (MSCs) induced by the above two pathways.