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Administration of human umbilical cord blood cells delays the onset of prostate cancer and increases the lifespan of the TRAMP mouse

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Abstract

Stem cell transplantation to improve the onset and survival of animals or humans with prostate cancer has not been studied adequately. In this study, we examined whether intravenous administration of human umbilical cord blood (HUCB) mononuclear cells into TRAMP (transgenic adenocarcinoma of the mouse prostate) mice can delay the onset of prostate cancer and improve survival of these mice before and after the development of cancer. Twenty TRAMP mice were randomly divided into 2 groups. One group of 10 mice received 200×10^6 HUCB mononuclear cells retro-orbitally into the venous plexus at the age of 6 weeks. Another group of 10 mice did not receive HUCB cells and served as control mice. The presence of tumor was detected by abdominal palpation, which was confirmed by biopsy. When 4 of the 10 control mice developed the tumor, they were treated with the same dose of HUCB cells. Either at the time of death or sacrifice, various tissues were examined for the presence of HUCB cell total RNA by reverse transcriptase PCR. Also, the tissues were examined histologically for the presence of metastasis and carcinoma. Kaplan–Meier survival plots were used to assess the lifespan of the mice. The data show that the control mice developed the tumor much earlier than the treated mice (control vs treated: 238 ± 38 vs 311 ± 40 days; $P<0.001$). Also, transplantation of HUCB cells either before or after the development of tumor significantly increased the life span compared to that of control mice. Persistence of human RNA either in blood or spleen was associated with prolonged survival. No graft vs host disease was observed in any of the mice. In conclusion, transplantation of HUCB mononuclear cells via intravenous administration into TRAMP mice retards not only the development of prostate cancer but also increases the lifespan of these mice.

Keywords: Prostate cancer; Human umbilical cord blood cells; Stem cell therapy; TRAMP mice

1. Introduction

Carcinoma of the prostate is the second leading cause of cancer-related death in American men [1],

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and the death seems to be due to failure to control metastatic disease. Although a variety of treatment modalities are available to improve survival in patients with metastasis, consideration of stem cell therapy has not received much attention. Nishiyama et al. [2] reported complete remission of hormone-refractory advanced carcinoma of the prostate by high dose chemotherapy and transplantation of peripheral blood stem cells. To our knowledge, no studies have been published to show an improvement in survival of animals or patients with prostate cancer by human stem cell transplantation.

The transgenic adenocarcinoma of the mouse prostate (TRAMP) model has been extensively used to study the prevention and regression of prostate cancer [3]. TRAMP mice treated from 12 to 30 weeks of age with E-7869 (R-flubiprofen) demonstrated reduced primary tumor incidence and metastasis [4]. Other therapies also demonstrated improvement in tumor incidence as well as metastatic relapse in this mouse model of prostate cancer [5–7]. Thus, the TRAMP mouse can serve as a useful animal model for therapeutic studies of human adenocarcinoma of the prostate.

Previous studies from our laboratory have shown that transplantation of human umbilical cord blood (HUCB) mononuclear cells via intravenous administration was found to improve survival of mice with lupus [8]), type 1 diabetes [9], type 2 diabetes [10] and other conditions [11]. Other investigators also demonstrated the efficacy of HUCB cells in rats with stroke [12], brain injury [13] and in mice with amyotrophic lateral sclerosis [14]. In this study, we examined whether transplantation of HUCB mononuclear cells into TRAMP mice would delay the onset of prostate cancer as well as prolong the lifespan of these mice with tumor burden. Also, the effect of HUCB mononuclear cells was investigated on survival before and after the development of the neoplasm.

2. Materials and methods

2.1. Animals

A total of 20 TRAMP mice (Jackson Laboratory, Bar Harbor, ME) were used for the study, and divided into 2 groups. One group of 10 mice received 200×10^6 HUCB mononuclear cells retro-orbitally into the venous plexus at the age of 6 weeks. Ten mice did not receive any cells and served as controls. Initially, when an abdominal mass was detected in a mouse, a biopsy of the mass was performed and malignancy confirmed. Subsequently, abdominal palpation was performed regularly to detect the onset of neoplasm. When 4 of the 10 control mice demonstrated detectable abdominal mass, they were given 200×10^6 HUCB mononuclear cells retro-orbitally to examine whether transplantation of these cells would prolong survival of these mice. Thus, the study finally included 3 groups of mice. All mice were fed Purina rodent chow 5001 ad libitum and allowed to drink tap water. Blood from orbital plexus was drawn every 3 weeks from all the mice after 180 days of age until death. At necropsy, both the abdomen and various organs were analyzed for metastases. This study was done without any prior or concomitant immunosuppression.

Procedure for the collection and use of HUCB for this study was approved by the Institutional Review Board of New Jersey Medical School, Newark, NJ. The mice were housed in an AAALAC-1 approved animal facility, and the project was approved by the Institutional Animal Review Committee.

2.2. Collection and preparation of human cord blood cells

HUCB samples were obtained from placentas of healthy full-term neonates. Each cord blood sample was collected into a 50 ml sterile polypropylene test tube containing 5 ml of citrate phosphate dextrose as an anticoagulant. The volume collected varied from 20 to 40 ml, and the samples were kept at room temperature until they were sent to the blood bank for storage. The samples were then transferred into a polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL) that allows gaseous transfer and were stored at 4 °C in a blood bank refrigerator. Donor specimens were combined according to their blood type (ABO). After storage for 10–13 days, units were placed in a 15 ml disposable centrifuge tube and the mononuclear cells were separated from the whole cord blood by Ficoll Hypaque (Sigma, St. Louis, MO) density gradient centrifugation. The cells were then washed

twice with phosphate buffered saline (PBS) and centrifuged for 10 min at 1000 rpm. One ml of PBS was added to the pellet for counting. After the viability and counting were determined, the mononuclear cells were centrifuged for 10 min at 1000 rpm, then 0.2 ml of PBS solution was added for final dilution and injection into the mouse (retro-orbital). This process was repeated the next day to bring the total number of mononuclear cells given to the animals up to 200×10^6. This large dose of cells was selected because of previous studies that showed a dose-dependent survival of mice [11].

2.3. Analysis of human growth hormone (HGH) expression by RT PCR

In order to identify the distribution of HUCB cells in transplanted mice, total RNA was isolated from blood, heart, lungs, brain, liver, spleen, pancreas, kidney and bone marrow tissues of control and treated mice as well as from HUCB cells by guanidine isothiocyanate method [15]. The extracted RNAs were purified by RNAeasy columns (Qiagen Inc., Valencia, CA). cDNA synthesis was performed by reverse transcription using Superscript (Invitrogen) or MMLV or AMV (Promega) reverse transcriptase enzyme. HGH gene was amplified by PCR (Perkin Elmer, Foster city, CA) using 5′-TGT CTT CCC ACC AT TCC TCC CTT A-3′ and 5′-CCA CTC ACG GAT TTC TGT TGT GTT TC-3′ primers to produce 434 bp fragment. Similarly HPRT house keeping gene was amplified using HPRT specific primers as positive control for PCR amplification. The PCR reactions were carried out in GeneAmp 9600 PCR system (Applied Biosystems) with initial denaturation at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 5 min. PCR products were run on 1.5% agarose gels on a horizontal electrophoresis apparatus, and visualized with ethidium bromide on a flourimager (Molecular Dynamics, Sunnyvale, CA). Band densities were analyzed using Image Quant software.

2.4. Histology

The necropsy material and biopsy specimens were formalin fixed. Routine sections were prepared (2–3 μ thick) and stained with hematoxylin and eosin.

2.5. Statistical analysis

All data are expressed as mean ± SD. To evaluate the onset of cancer in mice by palpation, a one sample *t*-test of significance was used with a *P*-value <0.001. Survival curves were plotted using the Kaplan–Meier method.

3. Results

Fig. 1 shows the onset of detectable abdominal mass in control and transplanted mice. As evident, the tumor was palpated at 238 ± 38 days in control mice and at 311 ± 40 days in transplanted mice ($P<0.001$). The control mice survived a mean of 21 days following the detection of mass compared to a mean of 69 days in transplanted mice ($P<0.001$). The 4 control mice that received HUCB cells, after an abdominal mass was detected, survived on an average of 50 days following transplantation.

Kaplan–Meier survival curves for control and transplanted mice are shown in Fig. 2. All control mice ($N=6$) were dead by 340 days of age, whereas 8 of 10 transplanted mice were dead by 450 days of age. Of the remaining 2 transplanted mice, one mouse had an abdominal mass and the other one without a mass. Both mice were sacrificed at 470 days of age. The 4 mice that received HUCB cells following the detection of neoplasm survived an average of 337 (range: 310–365) days.

At necropsy, all 20 animals were dissected and examined for the extent of the neoplasm. In general, the neoplasm was found spreading throughout the abdominal cavity in 18 animals, involving the spleen

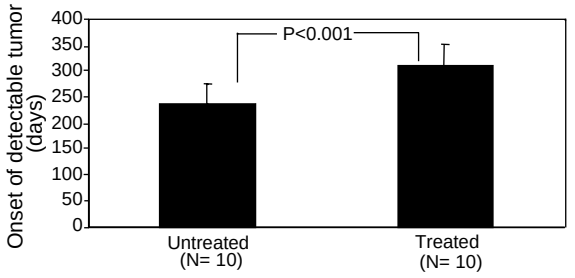

Fig. 1. Age of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice when tumor was detected. Each bar represents the mean ± SD.

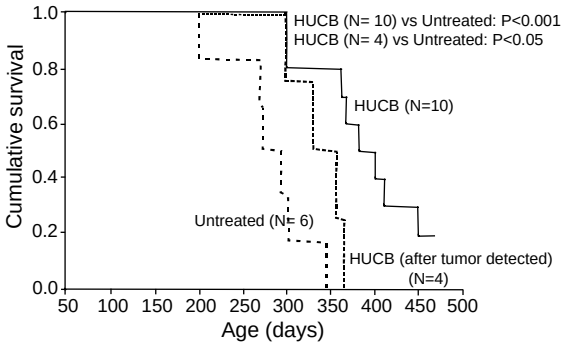

Fig. 2. Kaplan–Meier survival curves for untreated and human umbilical cord blood cells-treated TRAMP (transgenic adenocarcinoma of the mouse prostate) mice.

and liver. Gross metastasis was not observed in the lungs. In the remaining 2 animals that were killed at 470 days of age, one animal had an enlarged seminal vesicle with neoplasm and the other had a grossly appearing normal prostate gland. Histologically, the neoplasm was a cellular and poorly differentiated adenocarcinoma (Fig. 3). There was no clinical or histologic evidence of either acute or chronic graft-vs-host disease in any of the transplanted mice.

Total RNA for HGH was detected in peripheral blood of 4 of the 10 transplanted animals. Of these 4, one mouse that did not have any gross malignancy of the prostate had persistent RNA in the blood until sacrifice at 470 days of age. In addition, 2 of the 10 transplanted mice had detectable RNA in the spleen, and these mice survived 304 and 383 days, respectively.

Of the 4 mice that were treated with HUCB cells after the detection of neoplasm, human RNA was found in the spleen of only 1 mouse, and this mouse lived the longest (364 days). In general, the mice with demonstration of RNA either in blood or spleen lived longer than the mice without the presence of RNA.

4. Discussion

The major findings of the study were: (1) transplantation of HUCB mononuclear cells into the systemic circulation significantly delayed the onset of malignancy in TRAMP mice; (2) mice that received HUCB cells before or after the onset of malignancy had significantly increased survival; and (3) mice that demonstrated the presence of HUCB cells either in blood or spleen, as shown by human RNA, survived the longest as compared to those transplanted mice without the presence of human RNA.

Transplantation of HUCB, which is rich in hematopoietic progenitor/stem cells [16,17], is being evaluated as an alternative to bone marrow transplantation to treat metabolic [18] and malignant as well as nonmalignant diseases [19,20]. To our knowledge,

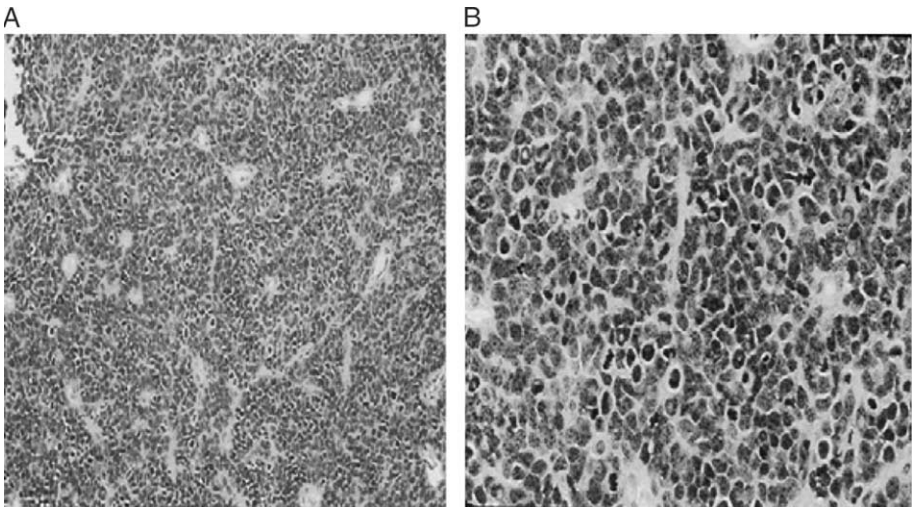

Fig. 3. Photomicrographs of the prostate adenocarcinoma. Hemotoxylin and eosin, 80× (A), and 210× (B).

this is the first report to demonstrate that the administration of HUCB mononuclear cells without immunosuppression prolong both the onset of the neoplasm and lifespan of mice with prostate cancer. The mechanisms by which these improvements occurred remain unknown.

It is interesting to note that transplantation of HUCB mononuclear cells prolonged survival of mice even after the detection of malignancy. If this observation is confirmed by additional studies, it is possible that human survival can be considerably prolonged with transplantation of HUCB cells in the presence or absence of concomitant immunosuppression.

It is also interesting to note that the transplanted mice did not demonstrate any clinical or histologic evidence of either acute or chronic graft-vs-host disease. No other evidence of adverse effects was noted in transplanted mice. All mice died from tumor burden.

In conclusion, transplantation of HUCB mononuclear cells into TRAMP mice retards not only the development of neoplasm but also prolongs the lifespan of these mice even after the development of neoplasm. The mechanisms underlying the benefit of this transplantation remain largely unknown, and require further study.

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