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Review Article

Surgical thyroparathyroidectomy prevents progression of 5TGM1 murine multiple myeloma *in vivo*

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A B S T R A C T

The 5TGM1 multiple myeloma transplanted C57BL6/KaLwRij model recapitulates many disease features including monoclonal paraprotein production as well as the development of osteolytic bone lesions. Since a significant association between serum parathyroid hormone PTH variations, bone anabolism and myeloma progression in patients receiving proteasome inhibitors exists, this study investigated the effect of the PTH axis on murine myeloma development *in vivo*. C57BL6/KaLwRij myeloma-bearing mice underwent thyroparathyroidectomy (TPTX) before and after 5TGM1 cell transplantation. TPTX significantly and permanently inhibited 5TGM1 myeloma cell engraftment and prevented multiple myeloma growth and progression. These data support the hypothesis that the PTH axis is an important mediator of myeloma bone disease.

1. Introduction

A variety of murine models have been used over the last several years to study myeloma biology including the SCID-hu model and the 5TMM model [1–4]. In the SCID-hu model, human myeloma cells are engrafted into a human fetal or rabbit (SCID-rab) bone, implanted in a SCID mouse. In these xenograft models, the rapidly growing tumor cells effect the engrafted bone microenvironment inducing significant osteolysis and angiogenesis [5]. In the syngeneic 5TGM1 mouse model *de novo* myeloma developed spontaneously in elderly C57BL/KalwRij mice [6]. Interestingly, approximately 0.5% of these mice develop myeloma when older than 2 years of age [7]. The 5TMM cell lines were established by transplanting these primary myeloma cells into syngeneic recipients, and the lines propagated by intravenous injection of the tumor cells into young syngeneic mice [4]. Several 5TMM lines are now widely available and utilized [8,9] with the 5T2MM and the 5T33MM the most studied [8,9].

While 5T2MM cells model a form of human multiple myeloma with moderate growth rates and the development of osteolytic lesions [3], the 5TGM1 model produces an aggressive form of myeloma with rapid growth and extensive osteolytic disease, reminiscent of the disease and the extensive bone involvement typical of the disease in humans [4]. As myeloma progresses in 5TGM1 myeloma-bearing animals they typically experience extramedullary tumor growth in the spleen as well as subcutaneous masses and eventually paraplegia [8,9]. Importantly, there are numerous and important similarities between both mouse models and the human disease; the tumor cells have a predominant localization in the marrow; serum polyclonal immunoglobulin concentration progressively decreased with a parallel expansion of monoclonal protein producing cells that is correlated with myeloma tumor burden.

We have previously demonstrated a close interaction between the parathyroid hormone (PTH)/PTH receptor 1 (PTHR1) system and myeloma progression *in vitro* and *in vivo* [10]. Specifically, a close parallelism between rapid and transient spikes in PTH levels following proteasome inhibitor therapy and the control of myeloma progression [11]. These profound effects are significantly linked to the subsequent anabolic osteoblastic activity resulting in increased mineralization rates and bone volume/total volume (BV/TV) in sequential human bone biopsy specimens [11]. In murine models, we demonstrated that 5TGM1 myeloma cells express the PTHR1 [10] and have reported the loss of PTH-mediated effects when animals are concomitantly treated with a potent PTH receptor antagonist, PTH(7 – 34) [10]. Therefore, the purpose of this study was to define the behavior of murine myeloma progression *in vivo* in the presumptive absence of endogenous PTH following complete thyroparathyroidectomy (TPTX).

2. Material and methods

5TGM1 cells were received from University of Texas Health Science Center, San Antonio and maintained in RPMI 1640 (high glucose) (Gibco BRL, Gaithersburg, MD) medium + 15% fetal bovine serum (FBS) (Gibco BRL) + 1 × penicillin/streptomycin (Gibco BRL) at 37 °C

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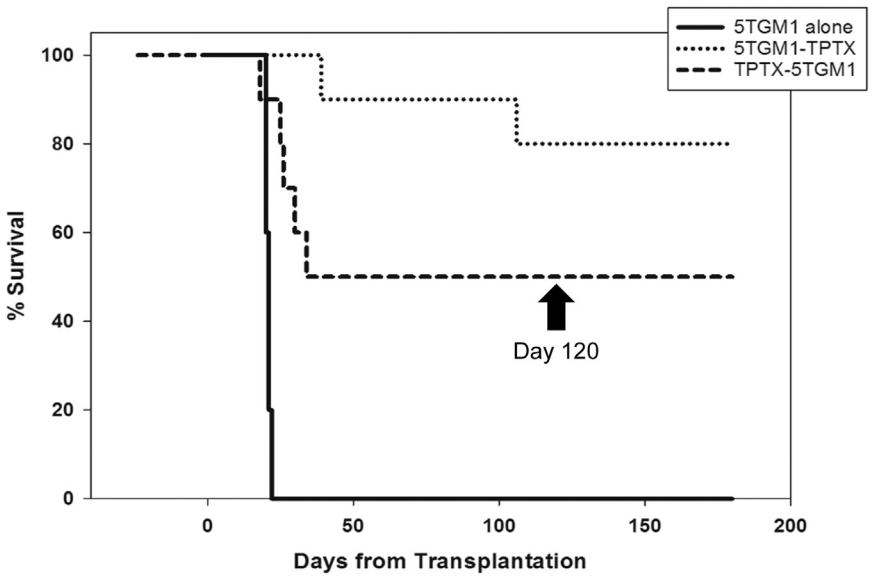

Fig. 1. Kaplan-Meier analysis of myeloma-bearing mice. 5TGM1 myeloma cells were injected at time 0 (first injection) and at 120 days (second injection). Percent survival is plotted for 5TGM1 cell injection alone (group 1; 5TGM1, solid line), 5TGM1 cell injection followed by TPTX 10 days post tumor inoculation (group 2; 5TGM1-TPTX, dotted line) and TPTX followed by 5TGM1 cell injection 20 days after TPTX (group 3; TPTX-5TGM1, dashed line). Arrow shows time of second 5TGM1 cell injection into surviving group 2 and group 3 animals (Day 120).

in an atmosphere of 5% CO_2 /95% air. Cells were maintained in a range of 0.5–2 × 10^6 cells/ml. Before transplant, cells were washed with PBS 3 times and counted by Cellometer Mini (Nexcelom, Lawrence, MA) using a trypan blue exclusion method. Following centrifugation (300 × *g* for 5 min), 0.5 × 10^6 cells were resuspended in 100 µl PBS cell pellets and injected *via* the tail vein into C57BL6/KaLwRij mice.

2.1. Animal procedures

2.1.1. Thyroparathyroidectomy (TPTX) procedure

C57BL6/KaLwRij mice were housed and bred at the University of Arkansas for Medical Sciences (UAMS) Animal Facility. All animal procedures were reviewed and approved by the UAMS IACUC. TPTX was performed (as described) [12], 8–12 week old mice (both male and female). Briefly, after mice were anesthetized using 2–3% isoflurane they were placed on a surgical bed and a midline neck incision made. The salivary glands, and sternohyoideus muscle were separated from the midline and retracted. The thyroid and the parathyroid gland, located as a single pair laterally or posteriorly to the thyroid gland or on the lateral edge of the thyroid, were excised and the incision was later sutured with a wound clip. The removal of all parathyroid glands and approximately 70% of thyroid glands was confirmed by visual microscopic inspection. Sham operated controls underwent the same process and exposure without removal of the thyroid and the parathyroid glands. Post-surgery all mice fasted overnight and allowed to access to deionized water *ad libitum*. To prevent post-surgical hypocalcaemia, 1 M $CaCl_2$ solution in the drinking water was supplemented for the first week. Pharmacological pain control was provided as required according to the approved post-surgical protocol. 5TGM1 Cell infusion and blood collection: All mice received a 0.5 × 10^6 5TGM1 cell infusion by intravenous injection as described [10]. Three months after the initial TPTX procedure and first cell line infusion, all the surviving animals received a second 5TGM1 infusion of an additional 0.5 × 10^6 cell by intravenous injection [10]. Blood samples were collected *via* retro-orbital vein on day 1, 3 and then weekly. Serum levels of PTH and IgG2 levels were measured using a mouse PTH 1–84 ELISA Kit (Immutopics Inc Athens OH) and an ELISA kit for IgG2B (Bethyl Laboratories, Inc Montgomery, TX) according to the manufacturer's instructions.

2.1.2. Statistical methods

Survival was estimated using the Kaplan-Meier method. Kaplan-Meier curves were created using SigmaStat 2.03 (Systat Software Inc, San Jose, CA) and the log-rank test (Wilcox survival) was used to analyze survival data. $p < 0.05$ was considered significant.

3. Results

These studies were performed using three groups of mice (50% male) with a median age of 10 weeks. The animals were randomly assigned to three groups: Group 1 (Sham) consisted of ten animals who had sham surgery and received a 5TGM1 infusion at day 0. Group 2 (5TGM1-TPTX) consisted of 10 mice (5 animals were lost for post-surgical complications) which on day 10 post 5TGM1 cell infusion (underwent TPTX). Group 3 (TPTX-5TGM1) consisted of 13 mice (2 animals were lost for postsurgical complications) that were infused with 5TGM1 cells 20 days post-TPTX. In all, only 10 operated animals experienced post-TPTX complications and died in the first week after the procedure. These animals were excluded from the survival analysis such that the analysis included only mice that survived TPTX and that were successfully infused with 5TGM1 tumor cells.

Within the first 24 h post-TPTX, serum PTH 1–84 dropped below the assay detection limit (~ 0.6 pcg/ml) and remained low for 3 days. Serum PTH then progressively increased and returned to approximately 80% of pre-surgery baseline levels in approximately 3–4 weeks. Any animal that did not demonstrate the rapid drop in serum PTH levels following TPTX was excluded with the assumption of incomplete TPTX surgery. The baseline calcium level (5.5 – 6.2 mg/ml) did not significantly change (there was an overall 20% drop) after the procedure.

All ten (10) sham-operated control mice (group 1) developed myeloma and died within 3 weeks post-transplant. Two mice from group two (5TGM1-TPTX) and five mice from group three (TPTX-5TGM1) manifested significant multiple myeloma. All seven animal deaths were radiologically, pathologically and serologically attributed to the progression of myeloma. In all, thirteen mice underwent TPTX before or after transplantation and demonstrated significantly increased survival compared to the Sham control group ($p = 0.003$ and $p < 0.001$, respectively) (Fig. 1). As myeloma progressed, immunoglobulin IgG2 levels in the animals that was < 0.5 mg/ml increased up to 25 mg/ml after cell line infusion; such levels correlated with disease status (Fig. 2) as we and others have shown [4]. The mice in which disease control was apparent also had a parallel stabilization of serum immunoglobulin levels (Fig. 2).

All surviving group 2 and group 3 mice (n = 8 and 5, respectively) received a 2nd 5TGM1 cell (0.5 × 10^6 cells) infusion approximately 120 days after the first transplant (Fig. 1). These mice were monitored serologically for an additional 60 days and clinically for 6 months post