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Microchimerism in Salivary Glands after Blood- and Marrow-Derived Stem Cell Transplantation

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Blood- and marrow-derived stem cells (BMDSCs) provide disease-ameliorating effects for cardiovascular and autoimmune diseases. Microchimerism from donor BMDSCs has been reported in several recipient tissues. We hypothesized that this finding suggests a potential use of BMDSCs in the treatment of salivary dysfunctions. We investigated the presence of Y chromosome-positive cells in salivary gland biopsies of 5 females who had received a marrow or blood stem cell transplant from male donors. One to 16 years after transplantation, all recipients exhibited scattered Y chromosome-positive cells in the acini, ducts, and stroma of their salivary glands (mean of 1.01%). Potentially, these cells can be markers of transplantation tolerance, contribute to neoplastic epithelial tissues, or engraft at sites of injury. In addition, transplantation of BMDSCs could be used for treatment of Sjögren's syndrome and salivary glands damaged by therapeutic irradiation for cancers of the head and neck.

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INTRODUCTION

Recent reviews suggest transplantation of blood- and marrow-derived stem cells (BMDSCs) provide disease-ameliorating effects for cardiovascular and autoimmune diseases [1,2]. Microchimerism arising from BMDSCs and organ transplantations has been reported in a variety of recipient tissues (heart, liver, kidney, gastrointestinal [GI] tract, lung, endometrium, buccal epithelium) [3-12]. We hypothesized that this phenomenon has implications for the potential use of BMDSCs in the treatment of salivary dysfunctions (eg, Sjögren's syndrome and salivary glands damaged by irradiation) for which no suitable conventional treatments are currently available. Here we report, for the first time, evidence of microchimerism resulting from BMDSCs in salivary glands of recipients.

METHODS

This study was approved by the institutional review boards at McGill University, National Institutes of Health, and Veterans Affairs Medical Center. Labial salivary gland tissue was collected from 5 female subjects who had previously received either an allogeneic bone marrow or peripheral blood stem cell transplant from their brothers (Table 1). This gender-mismatched strategy allowed us to detect donor Y chromosomes in cells of the female-recipient salivary tissue. At the time of salivary gland biopsy, all patients were well, in hematologic remission, with full donor lymphohematopoietic engraftment. We chose to biopsy minor labial salivary glands because they share abundant similarities with the major salivary glands (ie, parotid, submandibular, and sublingual glands), they are routinely used to obtain information about all salivary tissue in patients with Sjögren's syndrome and they can be obtained with little discomfort and morbidity compared to biopsies from the major glands [13]. We performed colocalization techniques to genetic and protein markers as we described previously [10]. Using fluorescence microscopy

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Table 1. Characteristics of the Female Transplant Recipients

	#1	#2	Recipient #3	#4	#5
Characteristic					
Age at transplant (years)	31	36	37	52	28
Reason for transplant	CML chronic phase	MDS (RAEB)	AML CR1	Aplastic anemia	CML chronic phase
Type of transplant	BM	PBSC	PBSC	PBSC	BM
Conditioning	Cy 120 TBI 13 Gy	Cy 120 TBI 12 Gy	Cy 120 TBI 12 Gy	Cy 120, Flu 125, ATG 120	Cy 120, AraC 500 TBI 5.5 Gy
Time from transplant to salivary gland biopsy	57 months	50 months	40 months	13 months	201 months
History of pregnancy	Never	Never	1 daughter 2 miscarriages	1 daughter 1 son	Never
History of blood transfusion	Never	Yes	Yes	Yes	Yes
GVHD	COP	None	Liver	Skin, oral	Skin, oral
Active at time of biopsy	no	—	no	yes	yes
% of positive male cells in salivary gland	1.09%	0.95%	0.65%	0.92%	1.44%
% of positive male cells in buccal mucosa	11.3%	2.4%	12.7%	n/a	n/a

AML CR1 indicates acute myelogenous leukemia in first complete remission; AraC, Cytarabine 500 mg/m^2; ATG, antithymocyte globulin 120 mg/kg; BM, bone marrow transplant; CML, chronic myelogenous leukemia; COP, cryptogenic organizing pneumonia; Cy, Cyclophosphamide 120 mg/kg; Flu, Fludarabine 125 mg/m^2; MDS (RAEB), myelodysplastic syndrome with excess of blasts; PBSC, peripheral blood stem cell transplant; TBI, total-body irradiation; GVHD, graft-versus-host disease.

on 10-μm-thick frozen salivary tissue sections: (1) cells from the male donors were identified using fluorescence in situ hybridization (FISH) using a human Y chromosome probe conjugated with digoxigenin and visualized with Tyramide-FITC, and in the same sections, (2) salivary epithelial cells were identified by the presence of epithelial markers (cytokeratins; CK) using fluorescence immunohistochemistry (FIHC). The CK antibodies (BioGenex, San Ramon, CA) stain the salivary gland parenchymal cells while leaving nonepithelial cells (such as endothelial cells, stromal cells, and blood cells) unstained. We further determined whether male donor BMDSCs had fused with female recipient cells using FISH with X and Y chromosomal probes (Vysis, Downers Grove, IL). The sensitivity and specificity of the Y and X chromosomal probes used was between 97% and 100% [10]. As negative and positive controls, the DNA probes and antibodies were tested on labial salivary glands of normal healthy male and female volunteers.

RESULTS AND DISCUSSION

One to 16 years after male-to-female BMDSC transplantation, all 5 female recipients had Y chromosome-positive salivary cells (mean of 1.01%, range: 0.65%-1.44%) in the gland parenchymal tissue (Table 1). Our Y chromosome probe used in FISH has a false-positive rate between 0% to 0.2%, and a false-negative rate between 1.5% to 3.1% (ie, we were using a Y chromosome probe that underestimated the true frequency of Y chromosome-positive cells, but was unlikely to overestimate it). These Y chromosome-positive cells expressed CK-8, -9, -13, -18 (markers for salivary cells) (Figure 1A-C). Many of these cells were identified in each of the parenchymal components of the glands, that is, acini and intercalated, striated, and excretory ducts. We also used the following markers to further characterize the phenotypes of the Y chromosome-positive cells (Figure 1D-F): Na^+-K^+-$2Cl^-$ cotransporter (NKCC1, a marker for acini; donated by R.J. Turner), claudin-1 (a tight junction protein in salivary ducts; Zymed, San Francisco, CA), and von Willebrand factor IV (a marker for endothelial cells; Novocastra, Newcastle, UK). Our salivary gland specimens had few foci of lymphocytic infiltration. However, in situations where these lymphocytic infiltrations were detected by H&E staining in proximity to salivary cells, we immunostained an additional slide combining a hematopoietic lineage (CD45) marker, an epithelial marker (NKCC1) and the Y chromosome probe. This modification of the technique described earlier [14] using different fluorescent colors, showed scattered Y^+/$CD45^+$ cells in the stroma, but these were exceedingly rare in the epithelial structures (Figure 1G). Therefore, we conclude that the vast majority of the Y chromosome-positive cells in the intercalated ducts and acini of our specimens were of epithelial phenotype. In more than 1000 cells examined per patient, we detected no XXXY-positive cells (no cell fusion events). We previously reported in our studies of buccal epithelial cells in a female recipient that DNA genotyping had excluded the possibility that all observed Y chromosome-positive cells were transferred in utero by her son during pregnancy (a process referred to as fetal microchimerism) [10].

Our results indicate that a small percentage (about 1%) of cells derived from marrow or granulocyte-colony stimulating factor (G-CSF) mobilized blood cells can migrate into the salivary glands. Higher rates of microchimerism seem to occur in tissues with high cell turnover rates and/or that are sites of frequent injuries, for example, buccal (oral) epithelial cells (~10%) [10], endometrial glands [12,15], skin (~20%) [16], and liver (25%; an organ exposed to