Comparative Evaluation of Chemotactic Factor Effect on Migration and Differentiation of Stem Cells of the Apical Papilla

Sara Fayazi, DDS, Koyo Takimoto, DDS, PhD, and Anibal Diogenes, DDS, MS, PhD

Abstract

Introduction: Cell homing strategies could potentially be used in regenerative endodontic procedures (REPs) to promote the progressive coronal migration of stem cells, including stem cells of the apical papilla (SCAPs), along with formation of a new vascular network without the need for intentional apical trauma and intracanal bleeding. Although many chemotactic factors have been investigated for different mesenchymal stem cells, their effect on SCAP migration and differentiation is not fully understood. This study aimed to comparatively evaluate the effect of stromal cell–derived factor 1 (SDF-1), transforming growth factor beta 1 (TGF-β1), platelet-derived growth factor, granulocyte colony-stimulating factor (G-CSF), or fibroblast growth factor 2 (FGF-2) on the migration and differentiation of SCAPs. **Methods:** A characterized SCAP cell line was fluorescently labeled with Vybrant DiO dye (Life Technologies, Grand Island, NY) and used in transwell migration assays. Cells were subjected to 1, 10, or 100 ng/mL of each factor or a combination of factors followed by detection in a fluorescent plate reader. Lastly, SCAP differentiation into a mineralizing phenotype was evaluated in the presence or absence of the tested factors by quantitative alizarin red staining and alkaline phosphatase activity. Data were analyzed with 1-way analysis of variance with the Tukey post hoc test. **Results:** Maximum migration was observed with G-CSF or FGF-2, which was significantly greater than the effects observed by the other tested factors. A combination of G-CSF with TGF-β1 significantly augmented both migration and differentiation into a mineralizing phenotype. **Conclusions:** G-CSF appears to be well suited to be further investigated as a key chemotactic factor in cell homing–based regenerative endodontic procedures. *(J Endod 2017;■:1–6)*

Key Words

Cell homing, chemotactic factor, chemotaxis, granulocyte colony-stimulating factor, growth factors, migration, regenerative endodontics, stem cells of apical papilla, transforming growth factor beta 1

Significance

Granulocyte colony-stimulating factor (G-CSF) could be used to recruit apical mesenchymal stem cells into disinfected root canals in the next generation of regenerative endodontic procedures using cell homing principles.

Regenerative endodontic procedures (REPs) are now recognized as a treatment alternative to apexification for the treatment of immature teeth with pulp necrosis (1). These procedures rely on adequate disinfection, transfer of mesenchymal stem cells (MSCs) from the apical region including stem cells of the apical papilla (SCAPs) into the canal system, and the placement of a coronal restoration that provides an adequate seal and bioactive signals (2–4). The transfer of stem cells is most commonly accomplished by intentional laceration of the apical tissues, releasing stem cells from apical niches becoming part of the blood clot that forms within the root canal (5). It has been documented that approximately 77% of all reported REPs rely on this intentional trauma of the apical tissue in order to recruit undifferentiated stem cells (4, 5).

The use of intracanal bleeding for stem cell transfer is not without procedural and biologic limitations. There is often difficulty in evoking sufficient bleeding to form a stable blood clot within canals, and vigorous attempts may damage both the apical papilla and the Hertwig epithelial root sheet interaction, decreasing or abolishing the chance of continued tooth formation (6, 7). Interestingly, the formation of an apical root section completely separate from the main root has been reported after REPs, suggesting that both the apical papilla and the Hertwig root sheath may remain vital after prolonged pulp necrosis and that they may become detached from the root because of procedural trauma (8). Furthermore, the delivery of cells to an environment that is completely devoid of vascularity in the most coronal parts of the canal poses a major biologic challenge. This lack of control over stem cell fate with the currently used techniques may underscore why, despite desirable reported clinical outcomes such as healing of apical periodontitis, the predictability of continued development and re-establishment of vitality responses are unknown (1). Furthermore, many histologic studies have shown that the formed tissues lack the organization of the native pulp-dentin complex (9–11). Therefore, it would be advantageous if MSCs progressively migrated, populating the pulpal space along with a newly established supportive blood supply without damaging the periradicular tissues. Signaling molecules that provide the chemotactic signal for this process to take place need to be further investigated.

From the Department of Endodontics, University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Address requests for reprints to Dr Anibal Diogenes, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900.
E-mail address: diogenes@uthscsa.edu

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Stem cell homing strategies require the departure of stem cells from their niche, migration, and nesting of the stem cells into a new site. In this approach, cells are attracted to either exogenous or endogenous factors (12, 13). Although some chemotactic factors such as stromal cell–derived factor 1 (SDF-1), transforming growth factor 1 (TGF-β1), platelet-derived growth factor (PDGF), granulocyte colony-stimulating factor (G-CSF), or fibroblast growth factor 2 (FGF-2) have been shown to promote the migration of certain MSCs (14–16), their effect on the recruitment and differentiation of SCAPs is largely unknown. Thus, this study sought to comparatively evaluate the effect of known chemotactic signals on the recruitment of SCAPs. In addition, the chemotactic factors with the 2 greatest migratory effects, namely, G-CSF and FGF-2, were further evaluated for their effect on SCAP differentiation into a mineralizing phenotype and modulation by the presence of TGF-β1.

Materials and Methods

SCAP Culture

A previously characterized SCAP cell line was used (17); cells of passage 4 to 8 were maintained in culture at 37°C and 5% CO_2 in basal culture media composed of alpha-minimum essential medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 1 L-glutamine (20 μL/mL; Gemini, West Sacramento, CA), penicillin (100 U/mL, Gemini), and streptomycin (100 mg/mL, Gemini) on 10-cm cell culture uncoated polystyrene dishes. Cells were allowed to expand in culture to 70%–80% confluency followed by treatment with 0.05% trypsin (Gibco) and passing the culture to subsequent culture plates or used in experiments.

Transwell Migration Assay

For all the transwell experiments, cells were resuspended in serum-free basal media at the concentration of 5×10^3 SCAPs per 40 μL and labeled with Vybrant DiO (Life Technologies, Grand Island, NY) for 30 minutes following the manufacturer's instructions. After staining and the removal of unbound dye, cells (40 μL) were added to the upper chamber onto the membrane (3 μm pore size) of Flacon FluoroBlok 96 well HTS system placed fitted with 3 μm pore (Thermo Fisher Scientific, Waltham, MA). Before use, the membranes were coated with 1% poly-D lysine (Sigma-Aldrich, St Louis, MO) for 2 hours. After seeding, cells were allowed to attach to the membrane for 2 hours, whereas the lower chamber contained serum-free media to maintain a neutral migration drive. In order to establish the time course for maximum effect and concentration of FBS in the positive control, the lower chamber received media containing either 0%, 1%, 2%, 10%, or 20% FBS, and the fluorescent signal on the bottom surface of the membrane was measured with a Flex Station 3 multimode plate reader (Life Technologies) at 6, 12, 24, 48, and 72 hours of incubation. Empty wells with media only were used as blank for normalizing the autofluorescence signal.

Next, fluorescently labeled SCAPs were exposed to 1, 10, or 100 ng/mL human recombinant SDF-1 (Preprotech, Rocky Hill, NJ), human recombinant TGF-β1 (Sigma-Aldrich), human recombinant PDGF-BB (Preprotech), human recombinant G-CSF (Preprotech), or human recombinant FGF-2 (Preprotech). In these experiments, serum-free medium was used as a negative control, and medium containing 20% FBS was used as a positive control. Because the maximum fluorescence signal was detected at 24 hours of incubation, this time was used for the end point measurement of the fluorescence signal. Lastly, the 2 factors with the most effective chemotactic factors, namely, G-CSF and FGF-2, were tested. Also, TGF-β1 was added to each tested factor or in combination because of its prominent presence on dentinal walls and reported bioactivity after regenerative endodontic procedures. Thus, these experiments evaluated whether there is an additive or synergistic effect on migration among G-CSF, FGF-2, and TGF-β1.

Mineralization Differentiation

Cells were plated onto 24-well uncoated culture plates and allowed to grow to 80% confluency in basal media containing 10% FBS. Then, cells were exposed to mineralization differentiation media (StemPro Osteogenesis Differentiation Kit, Thermo Fisher Scientific) for 21 days in the presence or absence of 10 ng/mL of the most effective chemotactic factors, namely, G-CSF and FGF-2. In addition, because of the prominent role of TGF-β1 in odontoblastlike cell differentiation and its reported presence in conditioned dentin, combinations of these factors in the presence or absence of TGF-β1 were also tested to evaluate whether there is an additive or synergistic effect on differentiation.

At the end of the differentiation period, the medium was aspirated. Cells were fixed with 4% paraformaldehyde for 30 minutes followed by 3 washes with phosphate-buffered saline (5 minutes each), stained with alizarin red, and quantification of staining was performed using the osteogenesis quantification kit (Millipore, Billerica, MA) as recommended by the manufacturer. In addition, in parallel separate experiments, alkaline phosphatase (ALP) activity was quantified using the Alkaline Phosphatase Quantification Kit (Sigma-Aldrich) following the manufacturer's recommendations.

Data Analysis

All experiments were repeated at least 3 times (total of $n = 12–36$ group). Data from the control time course experiments were analyzed with 2-way analysis of variance and the Bonferroni post hoc test, whereas 1-way analysis of variance with the Bonferroni post hoc test were used for all other experiments with significance set at $P < .05$ using the Graph Pad Prism version 6.1 software (Graph Pad, La Jolla, CA).

Results

SCAP Migration

A kinetic migration experiment was first performed to optimize the time required for maximum migration and the concentration of the FBS to be used. The concentration of 20% FBS evoked the greatest migration observed followed by 10% FBS. On the other hand, 1% and 2% FBS did not evoke an increase in SCAP migration when compared with the negative control (Fig. 1). Importantly, the maximum migration was observed at 24 hours for all the tested groups with no further significant increase in migration with longer incubations (Fig. 1).

Of the tested chemotactic factors, G-CSF (100 ng/mL) evoked the greatest migration (299% ± 58%) compared with the control, which was not statistically different than the effect observed with 10 ng/mL but greater than 1 ng/mL G-CSF ($P < .05$, Fig. 2). FGF-2 was equally effective, evoking its maximum effect at the concentration of 10 ng/mL followed by 1 ng/mL evoking the migration of 282% ± 65% and 276% ± 62% compared with the control, respectively (Fig. 2). However, a decrease in migration was observed with the greater concentration of 100 ng/mL (230% ± 29% compared with the control, $P < .05$). An increase in migration was observed with increasing concentrations of SDF-1 evoking 168% ± 20%, 193% ± 31%, and 203% ± 26% for the concentrations of 1, 10, and 100 ng/mL, respectively (Fig. 2). Similarly, TGF-β1 also evoked the migration of SCAPs of 163% ± 17.7%, 165% ± 18%, and 174% ± 23% for the concentrations of 1, 10, and 100 ng/mL (Fig. 2). Lastly, PDGF evoked the migration of SCAPs of 134% ± 12%, 181% ± 28%, and 166% ± 20% compared with the control at the concentrations of 1, 10, and 100 ng/mL, respectively (Fig. 2). Importantly, SDF-1, TGF-β1, and PDGF promoted less