



Mesenchymal Stem Cell Conditioned Media Induces Hair Regeneration in Alopecia Areata: A Case Study

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Abstract

Alopecia Areata (AA), a hair loss condition caused primarily by autoimmune disorder, affects significant population globally. The disease hampers quality of life causing anxiety and low self-esteem in patients. This case reports discuss about condition of an alopecia areata patient and her subsequent treatment with Mesenchymal Stem Cell derived Conditioned Media (MSC-CM). The severity of disease was assessed by calculating Severity of Alopecia Tool (SALT) score which was 6%. The topical treatment for two months resulted in visible hair growth in vertex scalp area with SALT score approaching 0%. MSCs secrete number of growth factors, cytokines, lipid vesicles, and paracrine molecules in surrounding media which may regulate growth of hair follicles, vascularization, and morphogenesis. This report indicates that topical treatment of MSC-CM can alleviate alopecia condition and promote hair growth. This may be mediated through passive diffusion of paracrine factors and exosomes.

Keywords: Mesenchymal stem cells; Conditioned media; Alopecia areata; Hair regeneration; Hair regrowth

Introduction

Alopecia is characterized by hair loss in one or more regions of scalp or body. The prevalence rate is 2.1% in USA with global incidence risk rate of 2%. Several factors including stress, hereditary, nutritional disorders, and thyroid functional abnormalities, psychological, genetic, and immune disorders have been held responsible for triggering of disease. Though, the disease is not life threatening, it is associated with aesthetic values and self-dignity. The commencement of disease in early age can cause psychological concerns like low self-esteem, depression, or anxiety, affecting the quality of life. Alopecia Areata (AA), specifically, is an autoimmune disorder caused due to targeting of immune-privileged Hair Follicles (HF) by T cells. AA is characterized by patchy scalp hair loss which may progress to complete scalp hair loss (alopecia totalis) or complete body hair loss (alopecia universalis). The incidence of AA is associated with co-occurrence of other autoimmune disorders like psoriasis, vitiligo, systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease. Further, thyroid related abnormalities are frequently found in AA patients. Diabetes mellitus co-occurrence with AA has been reported in patients with increased insulin resistance [1]. The current available interventions include topical minoxidil, topical anthralin, intralesional corticosteroids injections; topical corticosteroids contact sensitizers, and Psoralen plus Ultraviolet an Irradiation (PUVA) therapy. These therapeutic approaches are either partially successful or are associated with significant side effects. HF transplant is mediated by advanced surgical procedures involving implantation of hair follicles to region without hair. The success of procedure is limited due to poor availability of donors, low survival of transplants, painful procedures, and expensive treatment. Further, the results achieved are often temporary [2].

Strategies based on stem cells are under investigation for their ability to regenerate the lost hair follicle and stimulate hair growth. Mesenchymal Stem Cells (MSCs) particularly have drawn attention of researchers and clinicians owing to ease of availability, immune-privileged nature and regenerative capacity. MSCs are multipotent cells present in multiple tissues including adipose, umbilical cord, bone marrow, hair follicle, dental pulp and liver. These are characterized by high proliferative capacity, plastic adherence, and spindle shape morphology. Some studies have demonstrated the therapeutic efficacy MSCs in HF regeneration [3,4]. However, the regenerative potential of MSCs has been attributed to the growth factors they secrete. It has been reported that up

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Received Date: 24 Feb 2020

Accepted Date: 25 Mar 2021

Published Date: 29 Mar 2021

Citation:

Mahmood A, Seetharaman R, Kshatriya P, Patel D, Mishra R, Srivastava AS. Mesenchymal Stem Cell Conditioned Media Induces Hair Regeneration in Alopecia Areata: A Case Study. *Ann Stem Cell Res Ther.* 2021; 5(1): 1041.

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to 80% of stem cell mediated regeneration is contributed by secretory factors of their secretome [5]. In this report, we have investigated the therapeutic effect of MSC derived Conditioned Media (CM), in a patient with alopecia areata, on observational basis.

Case Presentation

Patient

A 45-year-old female patient suffering from alopecia areata for 5 months approached our center. The preliminary examination of scalp region showed presence of small bare non scarring patch in vertex area. The severity of disease was assessed by calculating Severity of Alopecia Tool (SALT) score. The SALT score was assessed to be 6%. The SALT score calculation involves the percentage of hair loss in effected area in particular scalp region [6]. The patient was under mild anxiety and nervousness due to the persistent disease.

Preparation of MSC-conditioned media

Before procedure informed consent of patient was collected. The detailed procedure has been discussed previously [7]. Briefly, 100 ml of adipose tissue was collected from the patient by lipoaspiration from waist area by a trained plastic surgeon under the aseptic environment. The cells were processed in an ISO 9001: 2015 certified and DSIR (Department of Scientific and Industrial Research) recognized laboratory. The lipoaspirate was processed in a biosafety laminar airflow chamber. MSCs were isolated from adipose tissue by mechanical agitation including vertexing. After centrifugation, the resulting pellet was cultured in DMEM medium (Invitrogen, Paisley UK). Supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, at 37°C in humidified atmosphere containing 5% CO₂. The media were changed after every 3 days. Approximately 5 × 10⁶ MSCs of passage 1 were seeded in each T175 culture flask (n=5) containing 30 ml of DMEM medium supplemented with 10% FBS. MSCs were analyzed for spindle shaped morphology and any contamination using a phase-contrast microscope. When cells reached 70% confluence at passage 2, the culture media was replaced with serum-free DMEM. Post 72 h of incubation, the culture media was separated and collected. The culture media was then centrifuged at 2000 rpm for 5 min to remove the cell debris, filtered through 0.22 µm filter, and then concentrated by ultra filtration using centrifugal filtering units with a cut-off value of 3 kDa (Amicon Ultra-15; Millipore, MA), according to the manufacturer's instructions. The concentrated 30% MSC-CM in cream base was prepared for topical application. The cream was applied on affected area of scalp once

daily for a period of two months. The patient was monitored for development of any adverse events or severity of disease. During this period, patient did not receive other treatment for alopecia.

MSC-CM reinstates hair growth

The patient was evaluated at 1 month and 3 months post 1st application of MSC-CM (Figure 1). No adverse events associated with MSC-CM application were recorded. Before treatment single bare hairless oval region was observed at vertex of scalp. At 1month follow-up, the hair growth was observed with marked reduction in circumference of hairless patch. Post 3 months, significant difference was detected with visible increased hair count and density in effected scalp region. The SALT score reduced to approximately 0% with continued improvement and follow up to 6 months. After hair growth restored, patient was out of alopecia related anxiety, improved confidence level and led enhanced quality of life.

Discussion

Alopecia areata is marked by patchy hair loss caused primarily due to autoimmune disorder. The overactive immune cells abolish hair follicles causing non-scarring hair loss. Dermal papilla cells are the key regulator of initiation and regulation of hair growth. They control surrounding niche and mediate cross-talks with stem cells. Hair growth is primarily regulated by Erk activation and Wnt signaling. The stimulation from dermal papilla cells, activate hair follicles through activation of Wnt-β catenin pathway [8]. In patchy Alopecia, though progenitor cells are lost, hair follicle stem cells are preserved. This kind of Alopecia can be reversed through application of multipotent stem cells [9]. MSCs are the most investigated multipotent stem cells, owing to immune-privileged character, for their role in tissue regeneration and wound healing. MSCs promote angiogenesis, inhibit cell apoptosis, immunomodulate host immune system and demonstrate antifibrotic activity. The functional roles of MSCs are facilitated through secretory cytokines and growth factors. In the case discussed here, adipose derived MSCs were used to generate conditioned media (MSC-CM) comprising of secretory molecules. MSC-CM consists of cell free system enriched with growth factors, chemokines, cytokines and exosomes. The interventions subside requirement of immune-compatibility matching and exclude the possibility of teratoma formation. Moreover, the treatment procedure involved topical application of MSC-CM mixed with cream base.

The patient had received several treatments, without any noticeable change, before considering MSC-CM based intervention. Significant



Figure 1: Effect of MSC-CM on Alopecia areata. (a) Vertex area of scalp of patient showing hairless patch; (b) Partial recovery of hair on scalp post one month of follow-up; (c) Nearly complete covering of hairless patch with hairs post three months of follow-up of treatment.

improvement was observed in hair growth, after one month of MSC-CM topical application. The size of hairless patch was reduced, with visible increase in hair count. Three months of treatment resulted in effective resolution of alopecia. The SALT score reduced to 0% from initial 6%. The notable recovery was continued for 6 months follow up duration. Based on previous reports, it can be hypothesized that scalp hair augmentation might have mediated through paracrine factors secreted in MSC-CM. Vascular Endothelial Growth Factor (VEGF) reported in MSC-CM, augments hair regeneration by promoting perifollicular vascularization. Similarly, Hepatocyte Growth Factor (HGF) induces follicular growth by stimulating β catenin expression. The secretion of Insulin like Growth Factor -1 (IGF-1), has been correlated with migration, survival, proliferation, tissue remodeling and growth cycle of HF. IL-6 and M-CSF were reported to mediate hair growth in wounds. Another key secretory protein, Platelet-Derived Growth Factor (PDGF), trigger growth by promoting hair cycle to enter anagen phase [10,11]. Overall, growth factors through complex mechanism, in interdependent manner, induce proliferation of dermal papillae cells by modulating cell cycle *via* up regulation of cyclin D1 and CDK2. The factors activate Erk and Akt signaling pathway [12].

Though, pre-clinical and clinical studies have investigated effect of MSC-CM on hair growth and demonstrated its positive effect; mainly intradermal injections were used, which is invasive in nature [13]. Shin et al. used micro needle roller prior to topical application of conditioned medium of adipose tissue derived stem cells [14]. Frequent usage of intradermal injections is challenging for patients as it requires regular clinic visits, cause pricking discomfort, and impose fear of infection. This report highlights simple approach of topical application of MSC-CM for alopecia patients in self applicable mode without distress and possibility of infection. The microfiltration procedure used to filter MSC-CM make peptides size even, remove aggregates and improves diffusibility across the scalp [15]. Further, MSC-CM contains lipid bound exosomes which are enriched in growth related proteins, DNA and RNA. These exosomes can integrate with lipid membrane of hair scalp cells and facilitate the transfer of liposomal materials. The exosomal content play significant role on regulating key signal pathways affecting hair growth. Exosomes carry Wnt proteins on their surface which can induce activation of β -catenin signaling pathway involved in hair growth [16,17].

Conclusion

The report discussed here, demonstrate induction of hair growth in alopecia patient through topical treatment of MSC-CM. Though, experimental investigations have indicated towards success of intradermal applications of MSC-CM, inadequate data is available to provide conclusive evidence on topical application. Route of administration significantly influences efficacy of biological molecules. This report provides an early clue on possible passive diffusion of signaling molecules through scalp. Detailed investigation involving randomized placebo control studies are required to provide insight on efficacy and tolerability of treatment. Besides, other variables including source of MSCs, culture conditions (hypoxic or normal/3D or 2D culture) and formulations, can potentially alters the efficacy and require detailed analysis.

Funding

This study is funded by the Global Institute of Stem Cell Therapy and Research, San Diego, California, USA.

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