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Human adipose tissue-derived stem cells inhibit the activity of keloid fibroblasts and fibrosis in a keloid model by paracrine signaling

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ABSTRACT

Background: Human adipose tissue-derived mesenchymal stem cells (ASCs) have potential utility as modulators of the regeneration of tissue that is inflamed or scarred secondary to injuries such as burns or trauma. However, the effect of ASCs on one particular type of scarring, keloidal disease, remains unknown. The absence of an optimal model for investigation has hindered the development of an effective therapy using ASCs for keloids.
Objective: To investigate the influence of ASCs on angiogenesis, extracellular matrix deposition, and inflammatory cell influx in keloids.
Methods: We analyzed the proliferation, migration, and apoptosis of human keloid-derived fibroblasts treated with a starvation-induced, conditioned medium from ASCs (ASCs-CM). This was achieved by Brdu proliferation assay, a validated co-culture migration assay, and flow cytometry, respectively. To assess the change in phenotype to a pro-fibrotic state, fibroblasts were analyzed by real-time PCR and contraction assay. A keloid implantation animal model was used to assess the paracrine effect of ASCs histochemically and immunohistochemically on scar morphology, collagen deposition, inflammatory cell composition, and blood vessel density. In tandem, an antibody-based array was used to identify protein concentration in the presence of ASCs-CM at time point 0, 24, and 48 h.

Abbreviations: NF, normal fibroblasts; KF, keloid fibroblasts; DMEM, Dulbecco's modified eagle medium; CM ASCs-CM, conditioned medium from nutrient poor, adipose tissue-derived mesenchymal stem cells; BSA, bull serum albumin; Brdu, 5-bromo-2′-deoxyuridine; TGF, transforming growth factor; ILs, interleukins; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; COL-I, type I collagen; COL-III, type III collagen; α-SMA, alpha smooth muscle actin.

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Results: ASCs-CM inhibited the proliferation and collagen synthesis of human keloid-derived fibroblasts. ASCs-CM was associated with reduced inflammation and fibrosis in the keloid implantation model. Thirty-four cytokines were differentially regulated by ASCs-CM at 24 h. These included molecules associated with apoptosis, matrix metalloproteases, and their inhibitors. The same molecules were present at relatively higher concentrations at the 48 h timepoint.

Conclusion: These results suggest that ASCs are associated with the inhibition of fibrosis in keloids by a paracrine effect. This phenomenon may have utility as a therapeutic approach in the clinical environment.

1. Introduction

Mesenchymal stem cells (MSCs) have an intrinsic ability to renew, proliferate continuously, and differentiate down pluripotential lineages including those of adipose, cartilage, and bone tissue. Recently, MSCs have been postulated as a therapeutic agent to prevent fibrosis. They have been administered in many fibrotic diseases to modulate local cell groups and regulate stem cell turnover in a paracrine manner [1–4]. This signaling requires the secretion of factors that influence, amongst other processes, growth and immune function [1,5,6]. MSCs are thought to exist in almost all postnatal tissues, and better-investigated examples include Wharton's jelly-derived MSCs (WJ-MSCs), bone marrow-derived MSCs (BMSCs) and adipose tissue-derived MSCs (ASCs). BMSCs and ASCs are probably the best characterized and most commonly used in clinical practice [7]. ASCs, with an abundant supply of fat tissue in adult patients, relative ease of isolation *ex vivo*, and less ethical restrictions on clinical usage, make them a more facile choice for clinical applications. There is an increasing body of work indicating that ASCs have a potential therapeutic use as allogeneic products for tissue engineering because they can maintain a non-immunogenic and/or immunosuppressive profile after implantation *in vivo* [8–10]. Within injured or inflamed tissue, the secretory properties of ASCs may be greatly influenced by the surrounding local microenvironment. One such environmental factor is serum starvation of ASCs to mimic hypoxic/ischemic conditions. It is one of the most frequently used paradigms to study the paracrine effect of ASCs [11–15]. Further, the ASC-conditioned media (ASCs-CM) from nutrient deprivation is thought to contain many signaling molecules. Previous research has demonstrated that serum-starved BMSCs-conditioned medium (BMSCs-CM) could alleviate peritoneal fibrosis in a rodent model through the secretion of tumor necrosis factor-inducible gene 6 (TSG-6) [14]. Despite the potential therapeutic significance of ASCs-derived secreted proteins, the identity of these molecules and their effects on keloid disease remain indeterminate.

Cutaneous keloids are a significant clinical problem as they grow slowly but progressively and have a high rate of recurrence despite treatment. They are considered as benign neoplasms with excessive proliferation of fibroblasts and extracellular matrix deposition. Keloidal development and fibroblast activation may be stimulated by soluble signals from neighboring cells such as macrophages, lymphocytes, and mast cells [16]. Fat grafting has triggered global interest as the stem cells derived from the adipose tissue have been postulated as playing a role in tissue regeneration. With the ready availability of adipose cells from fat harvest and transfer – now a commonplace procedure in plastic surgery– there is a pressing need to know whether ASCs can have any therapeutic role clinically for keloid disease. As an example, Piccolo et al. used fat grafting to treat 87 patients with problematic wounds and other clinical problems including hypertrophic burns scars and keloids [17]. All patients demonstrated visible improvement.

ASCs-conditioned media is considered to be a useful paradigm with which to study the paracrine effect of ASCs on many different cell types such as dermal fibroblasts [11], melanocytes [18], and cancer cells [19]. Keloid fibroblasts are pivotal in the production of excessive connective tissue within the keloid tissue. It is not known how adipose tissue and ASCs media can modulate keloid fibroblasts. Recently, it has been reported that ASCs-CM can inhibit TGF-β1-induced differentiation of human normal fibroblasts and keloid scar-derived fibroblasts [13]. Furthermore, BMSCs-CM attenuated the proliferation and extracellular matrix production of human hypertrophic scar (HS) and keloid fibroblasts *via* the activation of a TGF-β3-dependent pathway [20,21]. Conversely, other work has demonstrated that human WJ-MSCs produce paracrine signals that enhanced the proliferation and fibrosis of keloid fibroblasts [22]. Given the role that ASCs have been shown to play in regulating scar deposition, the current study sought to investigate the role of ASCs in keloid disease. However, the lack of a suitable animal model for keloid research and an overreliance on the investigation of mechanistic effects on the cellular level have hampered progress in developing new treatments for *in vivo* keloid disease.

Therefore, to determine the effect of local signals from hASCs on the keloid tissue, hASCs were exposed to serum-free media to generate a stress-response that was hypothesized to trigger the production of modulatory cytokines into the surrounding medium (ASCs-CM). Both keloid-derived fibroblasts were exposed to ASCs-CM. In tandem, an established keloid implantation model was used to investigate the effects of human ASC paracrine signaling on keloid disease [23]. Finally, the conditioned media derived from hASCs was analyzed by an antibody-based protein array as an assay for the presence of multiple cytokines and growth factors.