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· Mesenchymal Stem Cells and Responses to Cutaneous Injury

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Despite significant advances in medical and surgical wound care, treatment of wounds that are slow to heal due to either diabetes mellitus or burn injury remains challenging. Conventional treatments of chronic wounds include topical antibiotics, compression bandages and debridement with or without grafting; advanced therapies include application of bioengineered skin substitutes and growth factors. However, resistance of chronic wounds to these therapies is not uncommon. In the event of burn injury, pressure garments, silicone sheeting and steroid injections have shown only limited success in the reduction of hypertrophic scarring. Clearly, new therapies are urgently needed. The long-term goal of our research is to develop stem cell-based therapies that enhance cutaneous responses to injury while promoting regeneration rather than scar formation. Our current objectives are: 1) to determine the impact of the diabetic metabolic environment of high glucose and fatty acids on adult stem cell regulation of the local cellular responses to injury; and 2) to determine whether therapeutically administered adult stem cells reduce hypertrophic scarring by releasing soluble factors that regulate fibroproliferative responses to cutaneous injury.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent emerging cell-based therapies to ameliorate tissue damage due to injury and disease. These adult stem cells are commonly referred to as mesenchymal stem cells, multipotent mesenchymal stromal cells or stromal progenitor cells. They were first isolated from the bone marrow but have now been found in almost all adult tissues. There is now abundant evidence demonstrating the therapeutic potential of MSCs for repair and regeneration of damaged tissue in almost all of the major organs of the body including heart, brain, lung, liver, kidney, eye and skin. These studies reporting efficacy and broad applicability have motivated the rapid development of MSC-based therapies as indicated by the ninety clinical trials currently listed in the U.S. National Institutes of Health registry of clinical trials. Differentiation and paracrine signaling have both been implicated as mechanisms by which MSCs improve tissue repair. MSC differentiation contributes by regenerating damaged tissue, whereas MSC paracrine signaling regulates the local cellular responses to injury. Current data suggest that the contribution of MSC differentiation is limited due to poor engraftment and survival of MSCs at the site of injury. Given these limitations, it has been proposed that MSC paracrine signaling is the primary mechanism accounting for the beneficial effects of MSCs on responses to injury such as inflammation, angiogenesis, and fibroproliferation. This hypothesis is further supported by the observation that MSC-conditioned medium also enhances tissue repair.

MSC Paracrine Signaling Regulates Local Cellular Responses to Cutaneous Injury

Cutaneous wounds treated with bone marrow-derived MSCs exhibit enhanced wound repair. Administration of MSCs to either acute or diabetic wounds in rodents accelerates wound closure. Decreased wound size was also observed when autologous MSCs were applied to human chronic wounds. Subsequent focused analyses of wound histology have indicated that treatment with MSCs results in accelerated epithelialization, increased granulation tissue formation and increased angiogenesis *in vivo*. Growing

evidence indicates that MSC paracrine signaling is the predominant mechanism responsible for this enhanced wound repair. MSC-conditioned medium has an effect similar to that of MSCs on wound repair *in vivo*. In addition, we and others have reported that MSC paracrine signaling regulates the responses to injury by dermal fibroblasts, epidermal keratinocytes and endothelial cells. We have recently determined that MSC paracrine signaling induces dermal fibroblasts to proliferate and migrate in response to injury. MSCs also secrete a chemoattractant for dermal fibroblasts. Further investigation showed that MSCs also regulate dermal fibroblast gene expression. Collectively, the combined *in vivo* and *in vitro* data demonstrate that MSC treatment impacts all phases of wound repair including inflammation, epithelialization, granulation tissue formation and tissue remodeling.

Our central hypothesis for this project is that chronic exposure to elevated levels of fatty acids induces MSCs to alter expression of angiogenic and inflammatory mediators, and that these fatty acid-induced changes affect the ability of MSCs to regulate cellular responses to injury. This hypothesis is supported by our recent work showing that exposure to elevated levels of unsaturated fatty acids inhibits MSC proliferation and increases MSC release of both angiogenic and inflammatory mediators. Current aims for this project are: 1) to determine whether exposure to elevated fatty acid levels induces changes in MSC regulation of cellular responses to injury; 2) to determine whether the Toll-like receptor 4 (TLR4) is required for fatty acid-induced changes in MSC paracrine signaling; 3) to determine whether the peroxisome-proliferator-activated receptor γ (PPARγ) mediates the effects of omega-3 polyunsaturated fatty acids on MSC paracrine signaling. These studies will provide insight into the efficacy of autologous MSC-based therapies for patients with type 2 diabetes.

The long-term goal of our research is to develop stem cell–based therapies that enhance cutaneous responses to injury while promoting regeneration rather than scar formation.

Mesenchymal Stem Cells and the Diabetic Metabolic Environment

Type 2 diabetes affects almost 200 million people worldwide and increases the risk of heart disease, stroke, kidney failure, limb amputation due to non-healing foot ulcers, and blindness. Currently there is significant scientific and clinical interest in the promise of MSCs to treat diabetes mellitus and diabetic complications. However, critical to the development of MSC-based therapies for patients with type 2 diabetes is an understanding of how their metabolic environment, which consists of high levels of glucose and fatty acids, impacts MSC biology. To date, most studies have investigated the effect of hyperglycemia on MSCs; in contrast, little is known about the impact of elevated plasma fatty acid levels. It remains to be determined whether chronic exposure to elevated levels of fatty acids affects MSCs' ability to: 1) home to sites of tissue injury; 2) release trophic factors that regulate local cellular responses to injury; and 3) differentiate to replace damaged tissue. Our research objective is to address these gaps in the knowledge in order to optimize MSC-based therapies for patients with type 2 diabetes.

MSC-based Therapies to Prevent or Reduce Hypertrophic Scarring

A new direction in our laboratory is to determine the potential of MSC-based therapies for prevention of hypertrophic scar formation after deep dermal injury. Despite studies in the heart, lung and kidney demonstrating that MSCs reduce fibrotic responses to injury, almost nothing is known about the effect of therapeutically administered MSCs on hypertrophic scar formation. In this project, we will determine whether MSC signaling to dermal fibroblasts inhibits TGF-b1 mediated fibrotic responses to injury such as proliferation, myofibroblast formation, and extracellular matrix homeostasis. We will also determine whether MSC treatment of deep dermal wounds ameliorates fibroproliferative scar formation in the Duroc pig, a validated animal model of hypertrophic scarring. We will assess the effect of MSCs on scar thickness, collagen fiber organization, and numbers of mast cells and myofibroblasts in the healed wound.

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