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The GSK-3β/Fyn/Nrf2 pathway in fibroblasts and wounds of type 2 diabetes

On the road to an evidence-based therapy of non-healing wounds

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A constitutively downregulated cytoprotective mechanism in response to oxidative stress and its constant companion, inflammation, may exist in clinical and experimental diabetes. The Nrf2 signaling pathway promotes the expression of a plethora of genes that regulate processes involved in protein stability, proteosome integrity, autophagy, senescence and protection against oxidative stress and inflammation. Nrf2 is held in the cytoplasm as an inactive complex bound to Keap1, which facilitates its ubiquitination. Dissociation of Nrf2 from its repressor Keap1 occurs in response to a stressful insult. Covalent modifications involving phosphorylation or acetylation of the free Nrf2 dictates its nucleocytoplasmic localization and henceforth the transcriptional activity of this pleiotropic protein. Bitar and Al-Mulla recently reported that an enhancement in the GSK-3β-Fyn signaling mechanism in wounds or fibroblasts of type 2 diabetes contributes to the diminution in Nrf2 nuclear accumulation and the concomitant aberration in the expression of Nrf2-dependent phase 2 antioxidant enzymes. This phenomenon was associated with a significant decrease in key fibroblast functions essential for wound healing, including cell migration and contraction. Overall, the authors newly identified defects in the GSK-3β-Fyn-Nrf2 signaling pathway during diabetes that may assist in placing us on the road for an evidence-based therapy of non-healing chronic wounds.

Keywords: diabetes, wound healing, oxidative stress, Nrf2, fibroblasts

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With the increase in prevalence of type 2 diabetes mellitus, a rise in the incidence of secondary co-morbidities, including impaired wound healing, cardiovascular disease, kidney failure and retinopathy, is anticipated. For example, approximately 15% of all individuals with diabetes will at some time have a non-healing wound despite insulin treatment and a meticulously controlled diet.[1] This unrelenting decline in tissue repair mechanisms means for many patients that the condition may progress to lower extremity amputations.[2] The life-long sustained effects of oxidative stress, electrophile toxicity, chronic low-grade inflammation and more recently premature senescence, appear to contribute in large to these chronic diabetic complications. To this end, there is an urgent need to develop an effective strategy of targeting a multi-factorial cytoprotective mechanism that mitigates the deleterious effects of these stressors and henceforth ameliorates the ravages of diabetes-related pathologies, including non-healing wounds.

An attractive and promising possibility is typified by a pathway that is mediated by the pleotropic transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2).[3] Nrf2, through binding to the antioxidant response element (ARE), regulates a diverse array of more than 200 gene encoding proteins, which enable cells to combat oxidative stress, resolve inflammation, maintain proteosome integrity, delay senescence and modulate autophagy.[4] Most of these potentially based therapeutic features of Nrf2 have been illustrated through the use of Nrf2 knockout mice or their fibroblasts.

Under physiological conditions, Nrf2 resides primarily in the cytoplasm in association with its repressor, Keap1, that

promotes rapid proteasome-mediated degradation via a Cul3-based E3 ubiquitin ligase complex.[5] However, in response to a stressful insult to the organism or cell itself, Nrf2 is stabilized by dissociation from Keap1, translocates into the nucleus and binds to cis-elements called ARE as a heterodimer with other transcription factors, such as Maf or jun.[6] This enhances the coordinated induction of a battery of cytoprotective genes. Another mechanism mediating the nucleocytoplasmic localization of free Nrf2, involves phosphorylation or acetylation. In this context, oxidative stress promotes the phosphorylation of Nrf2 at Tyr 568, possibly via a process involving the glycogen synthase kinase-3β (GSK-3β)/Fyn kinase-dependent pathway.[7-9] This results in a significant diminution in the nuclear accumulation of Nrf2 and the subsequent impairment in Nrf2-induced activation of ARE-driven gene promoters. In contrast, acetylation of Nrf2 by CREB-binding protein (CBP) enhances the DNA binding and the transcriptional activity of this pleiotropic protein.[10]

An indolent non-healing wound is a characteristic feature of clinical and experimental diabetes, although its exact causes are unknown. Employing cultured dermal fibroblasts and a 7-d full thickness circular wound of type 2 diabetes, Bitar and Al-Mulla presented intriguing evidence linking this diabetic phenotype to heightened states of oxidative stress and inflammation in addition to a dysregulation in the Nrf2 signaling pathway.[11] The authors showed that the generation of reactive oxygen species (ROS) by NADPH oxidase and mitochondria, together with the cellular contents of protein-bound carbonyls and lipid peroxidation, were augmented as a function of diabetes. Similarly, an increase in key indices of inflammation (e.g., TNF-α, IL-1β, MCP1 and fractalkine) was also evident in fibroblasts[11] and wounds[12] of type 2 diabetes. This diabetes-related increase in pro-oxidant/inflammatory capacity was associated with a significant decrease in total levels of Nrf2, an abnormality that appeared to stem from an upregulation in the Keap1-Cul3-dependent Nrf2 degradation. Consistent with these data, Bitar and Al-Mulla also demonstrated that the nuclear accumulation of Nrf2, its transcriptional activity and the levels of expression of Nrf2-dependent phase 2 antioxidant enzymes were also reduced as a function of diabetes. The authors proposed that diabetic cells or wounds not only face states of oxidative stress and inflammation, but are also likely to have an impaired adaptive response to these stresses. In advancing this notion, they subjected fibroblasts derived from control and type 2 diabetic animals to endogenous and exogenous oxidative stress using oligomycin and tetra-butylhydroquinone, respectively. Their findings confirmed that control fibroblasts or wounds (unpublished observations) exhibited an adaptive induction in the Nrf2-dependent signaling pathway, a response that was severely impaired as a function of diabetes. More intriguingly, they documented that this diabetes-related deficit in both the cellular redox state and the Nrf2-based adaptive response to oxidative stress stem from an augmentation in GSK-3β/Fyn regulatory mechanism. Credence for this proposition is the authors' elegant finding showing that chemical inhibition (e.g., lithium, thiadiazolidinone TDZD), or genetic knockout of GSK3-β, mitigated the deficit in Nrf2 signaling during diabetes. Interestingly, knocking out Nrf2 in control fibroblasts recapitulated most of the aforementioned phenotypic features of diabetes. In view of the above documentation of heightened states of oxidative stress and inflammation, the main culprits driving wound healing impairment during diabetes, one can predict that antioxidants or Nrf2 activators, with the inherent properties of suppressing ROS and inflammation, could potentially have therapeutic values.

The authors' current data are reminiscent of previously published results documenting that Nrf2 knockout mice showed prolonged inflammation during cutaneous wound healing,[13] increased oxidative stress and impaired liver regeneration.[14] Moreover, the Nrf2-deficient animals are more prone to chronic kidney failure[15] and also appear to exhibit high susceptibility to a variety of oxidative stressors, including glucose-induced cardiomyocyte damage, acetaminophen toxicity and hyperoxic lung injury.[16,17] Indeed, even the protective effect of caloric restriction against tumor was virtually ablated in $Nrf2^{-/-}$ mice.[18] Finally, the anti-inflammatory potencies of a number of chemical classes appear to correlate with the ability of these agents to induce phase 2 enzymes, which require the functional integrity of Nrf2.[19]

Overall, the present study viewing Nrf2 signaling in the context of wound healing, inflammation, oxidative stress and diabetes comes on the heels of recent findings implicating Nrf2 first, in tissue regeneration and Notch 1 signaling,[20] and second, in preserving proteasome integrity and delaying senescence.[21] The latter phenomenon has been shown to contribute to chronic non-healing venous ulcers.[22] To this end, the findings of Bitar and Al-Mulla,[11] together with those garnered from the literature, open up the tantalizing possibility of targeting Nrf2 signaling, or its GSK-3β/Fyn regulatory mechanism, to specifically suppress the pernicious effects of oxidative stress and its constant companions, inflammation and senescence, on tissue repair mechanisms, henceforth preventing or retarding the development of non-healing diabetic ulcers. A case in point in this regard is a recent study published by the same authors revealing that the α-lipoic acid, a scavenger of ROS and an enhancer of Nrf2-dependent phase 2 antioxidant enzymes, ameliorated the defect in IGF-1 ability's to promote wound healing during diabetes. In addition, this Nrf2 activator also accelerated key elements of the reparative process both in vitro and in vivo models of wound healing during diabetes.[23] Similar data were obtained with the antioxidant EUK 134, a superoxide/catalase mimetic. Of course, much remains to be done to further understand the role that Nrf2 signaling plays as a function of diabetes, but the floodgates have been opened and Nrf2, Keap1 or GSK-3β is emerging as an increasingly attractive target for small molecule activators (Nrf2) or inhibitors (GSK-3β or Keap1) to ameliorate some of the chronic diabetic complications, including the indolent non-healing ulcers.

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