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Commentary Open Access

# Unexpected Traits In Diabetic Wound Healing. Emerging Role of Epigenetic Events: A comment

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## Commentary:

Type 2 diabetes mellitus (DM) is the most prevalent form of diabetes and recently acknowledged not as a single clinical condition, but importantly, as a group of metabolic disorders [1]. Lower extremities ulceration with a subsequent healing failure is a significant complication of DM associated to disability, morbidity and mortality [2].

More than 70 000 non-traumatic lower-limb amputations are performed every year in the United States in diabetic adults [3]. A more illustrative statement on the magnitude of this global problem is that every 20 seconds someone with diabetes is amputated [4].

Hyperglycemia is invoked as the proximal trigger for innate immunity's demise in diabetes. This has certainly been the case in describing the pathway to "chronification" of the diabetic foot ulcer [5]. These are the central pillars toward lower limb amputation. Acute exposure to high glucose concentrations exerts a detrimental metabolic and bio-energetic effect on cutaneous, healthy cultured fibroblasts [6]. However, the observation that fibroblasts replication is impaired not solely in cells harvested from diabetics, but in cells from people without diabetes, genetically predisposed individuals; fostered the hypothesis about the existence of a sort of systemic genetic or epigenetic imprinted predisposition for phenotypic expressions, even when the cells no longer remain in the diseased organism [7].

Within the broad works of Brownlee's unifying hypothesis, ROS were identified as the prime trigger in hyperglycemia-derived damages. This rendered support for the concept that ROS operates as the bridge between glycemia and the cells' transcriptional machinery. Mitochondrial excessive superoxide with the ensued ROS spillover stood as the core of the "hyperglycemic memory" in which chromatin remodeling and long-lasting epigenetic changes ensure the persistence of a myriad of molecular disorders involved in the pathogenesis of diabetic complications. Hyperglycemic memory for instance may explain why intensive glucose control does not improve vascular dysfunction even after glucose normalization.

Wound healing does not escape from the long arm of the metabolic memory. Recent evidences have emerged to fuel the concept that epigenetic changes are instrumental players for the diabetics' wound healing failure. The inflammatory process in diabetic ulcers is more a condition than a physiological reaction. Pro-inflammatory cytokines reduce fibroblasts and vascular progenitor cells migration, anchorage, activation, and most importantly "entice" these cells to commit suicide [8]. Via nuclear factor-kappaB (NFκB p65) and c-Jun N-terminal kinases signaling pathways, [9] inflammation also disrupts extracellular matrix synthesis not only by up-regulating matrix proteases [10] but also by dismantling anabolic pathways usually activated by the agonistic occupation of tyrosine kinase receptors, (including insulin receptor) which at the end, would turn to activate PI3K/Akt-mTOR axis [11]. Under conditions of poor growth factors availability and consequently reduced Akt activity, FOXO is activated and retained in the nuclear compartment, hence shutting down TOR anabolic activities [12]. Recent contributions by El-Osta's laboratory are pivotal to understand the perpetuation of inflammation in diabetes. They demonstrated that NFκB-p65 transcriptional up-regulation resulted from epigenetic marks which modified the nature of the histone methylation on the gene promoter region [13]. In other words, this study showed the existence of gene-activating epigenetic changes that may perpetuate a cellular behavior. Although epigenetic changes play an important role in regulating normal wound healing, particular epigenetic changes have shown to impact on the angiogenesis, and re-epithelialization processes in diabetic models [14]. This underscores that metabolic memory plays a broader pathogenic role which is not restricted to vascular cells. Furthermore, it is likely that the onset and accumulation of epigenetic modifications could be the driving force en route for the point-of-no return for end-organs complications or simply to irreversible insulin resistance [15].

Only by virtue of the existence of the metabolic memory it is possible to explain surprising traits shown by diabetic cells, and it is that these cells perpetually behave "as sick" even when glucose levels and other environmental (in vitro or in vivo) conditions are optimized. Cultured diabetic fibroblasts exhibit a reduced replicative lifespan even under "ideal" culture conditions [16]. These cells also exhibit a slow proliferative response and appear to be far more growth factors-demanding than non-diabetic counterparts in order to complete a mitogenic event [17]. Interestingly, the ability of some growth factors to up-regulate Akt and ERK1/2 to commit cells for proliferation is attenuated as a function of diabetes evolution data [18] suggesting the possible role of cumulative epigenetic events for the adoption of a loss-of-function phenotype. In an attempt to understand the molecular basis of diabetic wounds healing refractoriness, our group has systematically cultured primary granulation tissue fibroblasts from ischemic and neuropathic ulcers. We have confirmed the reduced replicative potential as compared to age-matched cells from non-diabetic, burn-injured donors. Again, the improvement of culture microenvironment including adequate oxygen availability does not ameliorate the proliferative arrest. We observed that this phenotype of arrest appears associated to the overexpression of activated forms of p53 and p21 along with a downregulation of the Akt/mTOR/cyclin D1 axis [19]. It is likely that somatic cells or at least

the cutaneous fibroblasts are endowed with the ability to recall the donor's metabolic status.

Recent observations from our group deserve special comment. Not because they are unprecedented, but particularly because only through the prism of transmissible epigenetic events they can be explained [20].

- Through the histological analysis of the granulation tissue biopsies from diabetic ischemic and neuropathic ulcers, we distinguished that the ulcer's major ethiopathogenic component imposes a particular histological pattern of granulation tissue, which is largely similar and privative for ischemic and for neuropathic classes.
- Micro vascular damages as the fibro-hyaline and proliferative arteriolar sclerosis, ordinarily of long term evolution, are found and completely recreated in neo-formed vessels within granulation tissues no older than two weeks. These observations incite to speculate that an aberrant driving force imposes over and impinge the morpho-functional organizational process during fibroangiogenesis.
- Following comparative RT-PCR studies using clinical biopsies of granulation tissue from pressure ulcers, and diabetics' ischemic and neuropathic ulcers; we detected a significant derangement in a group of well characterized glucose-metabolism related genes in the diabetic ulcers [21].

Diabetic ulcer cells express far less insulin receptor, hexokinase (isoforms 1 and 2), phosphofructokinase, pyruvate kinase (isoforms 1and 2), pyruvate dehydrogenase, and significantly more of its inhibitor enzyme pyruvate dehydrogenase kinase (isoform 4). We see with interest that granulation tissue which can be considered as a transient organ made up by "de novo" cells, reproduces the same transcriptional profile of those genes considered as insulin resistance / glucose intolerance markers, and predictors for type-2 diabetes onset, in the liver, skeletal muscle, and adipose tissue as has been broadly described [21-23]. This raises the questions about if the granulation tissue is an additional insulin-resistant organ?

It is likely that epigenetic, as the resultant governor of cell's phenotype upon its genome interaction with the environment, has provided an innovative research field and an era of hopes for the control of diabetic complications. Now we stand before the challenge to fully understand the phenomenon, so that in a near future we could teach cells to restore healthier metabolic memories again.

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