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Enhancement of lysine acetylation accelerates wound repair

Francesco Spallotta,[1,†] Chiara Cencioni,[1,†] Stefania Straino,[2] Gianluca Sbardella,[3] Sabrina Castellano,[3] Maurizio C Capogrossi,[2] Fabio Martelli[4] and Carlo Gaetano[5,*]

[1]Laboratorio di Biologia Vascolare e Medicina Rigenerativa; Centro Cardiologico Monzino; IRCCS; Milan, Italy; [2]Laboratorio di Patologia Vascolare; Istituto Dermopatico dell'Immacolata; IRCCS; Rome, Italy; [3]Dipartimento di Scienze Farmaceutiche; Università degli Studi di Salerno; Salerno, Italy; [4]Laboratorio di Cardiologia Molecolare; Policlinico San Donato; IRCCS; Milan, Italy; [5]Division of Cardiovascular Epigenetics; Department of Cardiology; Goethe University; Frankfurt am Main, Germany

[†]Current affiliation: Division of Cardiovascular Epigenetics; Department of Cardiology; Goethe University; Frankfurt am Main, Germany

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In physiopathological conditions, such as diabetes, wound healing is significantly compromised and chronic complications, including ulcers, may occur. In a mouse model of skin repair, we recently reported that wound treatment with Sirtuin activators and class I HDAC inhibitors induced keratinocyte proliferation and enhanced healing via a nitric oxide (NO) dependent mechanism. We observed an increase in total protein acetylation in the wound area, as determined by acetylation of α-tubulin and histone H3 Lysine 9. We reasoned that this process activated cell function as well as regulated gene expression to foster tissue repair. We report here that the direct activation of P300/CBP-associated factor (PCAF) by the histone acetylase activator pentadecylidenemalonate 1b (SPV-106) induced Lysine acetylation in the wound area. This intervention was sufficient to enhance repair process by a NO-independent mechanism. Hence, an impairment of PCAF and/or other GCN5 family acetylases may delay skin repair in physiopathological conditions.

Wound healing is a combination of cellular and molecular processes aimed at restoring the original architecture of damaged tissues. In the last few years several epigenetic events have been identified as involved in the regulation of the healing process in different organs.[1,2] Among these, the repair process of skin is probably the best characterized. It can be divided in 4 phases: (1) the immediate-early response activated by the mechanical stimuli and bio-chemicals released at the site of damage; (2) the inflammatory response; (3) proliferation and migration of wound-edge ephitelium; and (4) the healing closure.[3] All these phases are orchestrated by mechanisms, at least in part of epigenetic origin,[4] aimed at activating the repair machinery regulated at transcription and post-transcription level.[4] Understanding these molecular mechanisms is of utmost relevance in physiopathological conditions, such as diabetes, where the skin repair mechanism is inefficient often leading to chronic ulcers formation.[4]

Interestingly, at an early healing stage the involvement of mechanisms associated to epigenetic processes has been recently described.[5] The introduction of changes in the level of histone methylation,[5] in fact, has been found to be associated with a loss of global inhibitory histone methylation, such as the trimethylation of histone H3 lysine 27 (H3K27me3). The downregulation of Polycomb group proteins paralleled by upregulation of histone demethylases has been reported at the basis of this process determining a wide enrichment in potential accessible chromatin in skin fibroblast and keratinocytes,[5] that it is supposed to positively contribute to skin repair during the early healing stages. The presence of an accessible chromatin, together with post-transduction modifications introduced in transcription factors and structural proteins may, in fact, provide specific signals required by an active tissue repair process. Remarkably, it is this specific condition that could be of interest for the identification of drugs relevant to the amelioration of ulcer healing.

A simple model to investigate skin repair at molecular level is represented by the skin punch biopsy realized in rodents. A full-thickness punch of about 3 mm is surgically created on the back of mice or rats and its spontaneous healing process can be followed until closure, which typically occurs in about 2 weeks. This simple model allows the comparison of different treatments directly applied on the site of the wound.[6-8] By using this approach, we recently demonstrated the functional cross-talk between class I HDACs and Sirtuins, mediated by nitric oxide (NO), and necessary for skin repair.[9] A body of evidence, in fact, assigns to NO a functional relevance in all 4 phases of wound repair[10,11] and several therapeutic approaches for chronic ulcers were recently focused on devices able to release constant NO levels in the wound site.[12,13] The mechanism of NO action, however, is still poorly characterized and the pleiotropic properties of this gaseous molecule make it difficult to clearly understand its effect at cellular and molecular level. In our prior work, we reported

*Correspondence to: Carlo Gaetano; Email: Gaetano@em.uni-frankfurt.de
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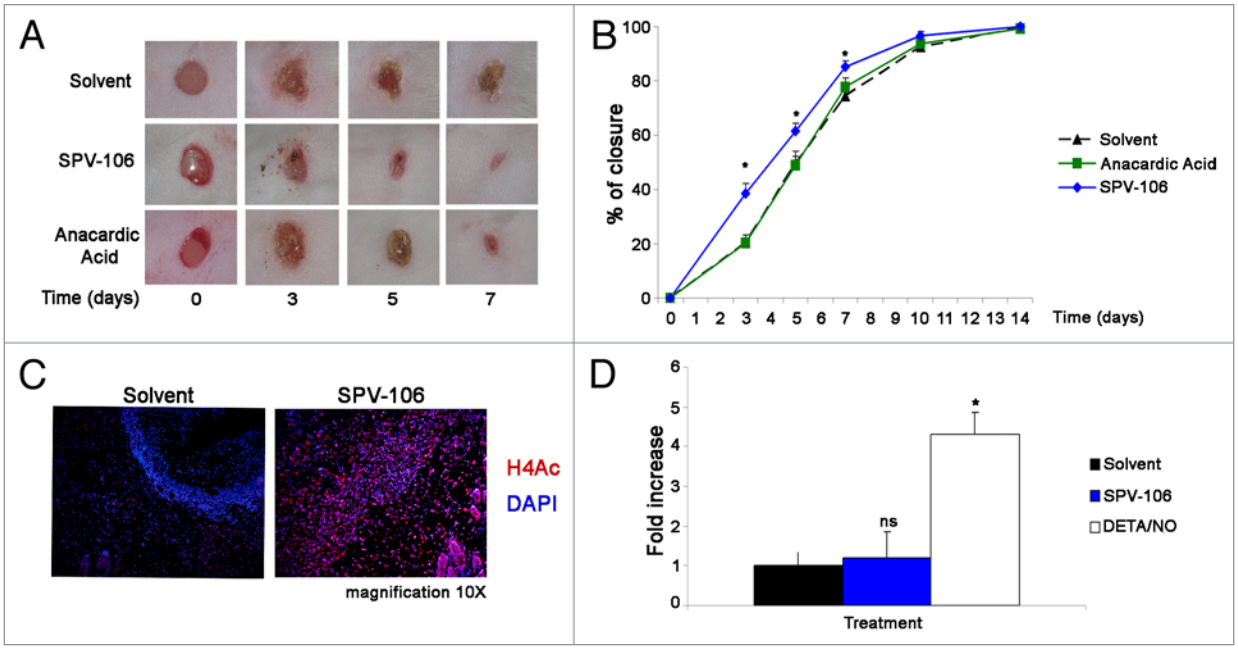

Figure 1. Histone acetylation is crucial determining wound healing. (**A**) Representative pictures of mouse wound healing. CD1 mice were treated topically with solvent (DMSO) the HAT activator SPV-106 (25 μM) and the HAT inhibitor Anacardic Acid (25 μM). Compounds were applied daily directly in the wound area at the indicated concentration in a total final volume of 20 μl of PBS solution and 0.1% DMSO. Another group of mice was treated with 20 μl of PBS solution and 0.1% DMSO alone. (**B**) Kinetic of skin repair in CD1 mice treated with SPV-106 (n = 10), Anacardic Acid (n = 10) and Solvent (n = 12). To calculate the kinetic of wound closure, three independent photograms were taken at each time point (days 0, 3, 5, 6, 7, 10, and 14) after treatment. Representative pictures were processed digitally, and wound areas calculated using the KS300 system (Zeiss). For each sample, the rate of the healing process was represented as a ratio of the wound area at each time point, divided by the total wound area at time 0. *p ≤ 0.05 vs. solvent. (**C**) Immunofluorescence analysis showing the histone 4 acetylation (H4Ac) in a wound treated with SPV-106 compared with solvent. Red: H4Ac; blue: nuclei counterstained with DAPI (magnification 10X). (**D**) Quantification of NO release by DAF-2DA staining in the human keratinocytes derived transformed cell line HaCaT after 1 h of treatment with solvent, SPV-106, or the NO donor DETA/NO (500 μM) used as positive control. * p ≤ 0.05 vs. solvent. Statistical analysis: data represent the mean of 3 independent experiments ± SE. Variables were analyzed by 2-sided Student's t-test and 2-way analysis of variance (wound healing experiments). A p-value ≤ 0.05 has been accepted as significant. All experimental procedures complied with the Guidelines of the Italian National Institutes of Health and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences) and were approved by the Institutional Animal Care and Use Committee.

that local applications of Thricostatin A (TSA), a pan HDAC inhibitor (HDACi), significantly enhanced lysine acetylation in activated keratinocytes resident in the wound.[9] This effect led to an accelerated wound closure, which was obtained also by using the class I selective HDAC inhibitor MS275.[14] On the other hand, Sirtuin activators accelerated wound closure, as well, suggesting that different types of epigenetic molecules involved in the regulation of lysine acetylation could be relevant for the amelioration of the repair process.[9] Hence, an increase in total protein acetylation, including that of histones, transcript factors, and structural protein, may be necessary to promote those changes in gene expression, cell growth and motility that occur during the epigenetic reprogramming that follows wound healing. The acetylation of α-Tubulin for instance seems involved in the regulation of cell motility and uptake of external substances, which may be important during the early phases of keratinocyte and/or other skin cell responses to acute damage. Nonetheless, changes in the structure of chromatin as those determined by histone Lysine acetylation may have an important inference on gene expression as described in our prior work.[9]

In this light, experiments were performed in which the p300/CREB binding protein-associated factor (PCAF) activator pentadecylidenemalonate 1b (SPV-106),[15] a HAT activator, was directly applied on wounded mouse skin. This treatment significantly accelerated skin repair compare to vehicle-treated animals and those exposed to the HAT inhibitor Anacardic Acid (**Fig. 1A and B**).[16] Intriguingly, the SPV-106 compound induced in vivo histone acetylation (**Fig. 1C**) without modification of NO levels, as determined by the fluorescence probe 4,5-diaminofluorescein (DAF-2 DA) in the human keratinocytes-derived HaCaT cells (**Fig. 1D**). These results suggest that lysine acetylation occurs downstream of NO signaling and point out that epigenetic molecules able to activate HATs could be important in tissue repair.

Although little is known about the role of acetylases during wound healing, and specifically that of PCAF, this enzyme seems important for keratinocyte activation and differentiation by a mechanism which involves Rb.[17] In conclusion, our work reports the unprecedented evidence that HAT activators, similarly to HDAC inhibitors, promote acetylation in the wound site and accelerate repair.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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