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Soluble GnT-V shift toward M2 macrophage in transgenic mouse skin

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Oligosaccharides are involved in a variety of human diseases. Glycosyltransferases are present in the Golgi apparatus and are released from cells after cleavage in pathological conditions. N-acetylglucosaminyltransferase V (GnT-V) is involved in the biosynthesis of beta1-6GlcNAc branching on N-glycans and has been implicated in tumor progression and metastasis. Recently, we have found that GnT-V is also involved in skin homeostasis. In contrast, a secreted type of GnT-V (soluble GnT-V) itself is reported to promote angiogenesis, which is completely different from its original function as a glycosyltransferase (JBC 277, 2002), and this might also play a role in tumor invasion, metastasis, and wound healing. In this study, we further explored the novel function of soluble GnT-V (sGnT-V) by analyzing the skin phenotype of sGnT-V transgenic mice (sGnT-V Tg). We found that the early stage but not mid and late stage wound healing was delayed in sGnT-V Tg mouse skin. Inflammation is a feature of early stage wound healing. As a result from delayed wound healing, mRNA expressions of proinflammatory cytokines such as TNFα, IL-6, and IL-1β were decreased on day 2 wound skin in sGnT-V Tg mice. Recently it is reported that M1 macrophage plays a role in the early stage of inflammation and M2 macrophage is important in mid to late stage tissue proliferation during wound healing. The expression of arginase I, the marker of M2 macrophage were increased on day2 wound skin and the ratio of (arginase I positive macrophage / total macrophage) were higher in normal skin and day2 wound skin in sGnT-V mice. These results suggest that decrease in early stage wound healing might be the result of dominant M2 macrophage in sGnT-V Tg mouse skin. Increased M2 macrophage might also contribute to tumor expansion in the tumor-microenvironment.

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MALDI mass spectrometry imaging reveals a significantly altered proteome during wound-induced scar formation

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Scar formation is the inevitable and currently untreatable consequence of tissue damage, and misregulation can lead to the development of pathological scarring such as keloids. In developed countries alone, it is estimated that ~100 million people each year will be left with a scar following surgery; moreover, there are ~ 11 million people with keloid scars. Despite the high incidence and debilitating nature of both normal and keloid scars, we still lack effective treatments. The lack of understanding about exactly which molecules are contributing to, or are responsible for, scar formation is impeding progress in developing targeted therapeutics for their treatment. This project used MALDI Mass Spectrometry Imaging to identify a signature of proteins expressed during scar formation. Scars resulting from excisional skin wounds made to the dorsal skin of adult male mice were collected at 7 or 14 days post wounding, freshly frozen, cryosectioned, coated with matrix optimised for the detection of proteins in the 2-20kDa range, and irradiated with a nitrogen laser beam at 80 micron intervals across the tissue sections. The resulting mass spectra revealed a significantly and reproducibly altered proteomic signature in scarred versus normal dermis. Specifically, proteins having mass-to-charge values (m/z; ~equivalent to mass in Daltons) of ~2797, ~4743, ~6284 and ~6657 were expressed at higher intensities in scar vs normal tissue on Day 7, and the up-regulation of 3 had resolved by Day 14. Peaks at m/z ~4970 and ~6284 showed a significant up-regulation in scar tissue on Day 14 post wounding. On-going work is focused on: 1) identification of the protein(s) represented by the differentially-expressed peaks; 2) using MALDI imaging and our established "scar signature" to evaluate the anti-fibrotic potential of novel therapies, and; 3) comparison of the "scar signature" observed in skin to other fibrotic tissues. The results of this work are hoped ultimately to be of prognostic, diagnostic, or therapeutic value.

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Decline of vascular adjustment to pressures in the early stages of aging

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In healthy skin, local application of low pressures induces a protective pressure-induced vasodilation (PIV) that is lost in advanced aging. When PIV is absent, skin blood flow directly decreases under low pressures, leading to an early ischemia. Aging is associated with high risk of pressure ulcers (PU) that partly depends on the intrinsic skin properties to withstand mechanical stress, including vascular capacities. Our aim was to test the vascular capacities of the skin to adapt against low and high pressures in the early stages of aging (12-month mature rats compared to 6-month young Wistar rats). Using laser Doppler flowmetry, we measured skin blood flow in response to low pressures applied at 11.1 Pa/s (PIV) and the post-ischemic reactive hyperemia following a 3-min application of 28.8 kPa. Since PIV requires an intact endothelial function, we also evaluated the endothelium-dependent and –independent responses to acetylcholine (Ach) and to sodium nitroprusside (SNP), respectively. PIV was reduced in mature rats compared to young rats, indicating a defective ability to resist to very low pressures. As a consequence, the statistical decrease in skin blood flow was observed for local pressure as low as 2.3 kPa for mature rats, whereas it occurred at 6.3 kPa for young rats. At higher pressures, the post-ischemic reactive hyperemia was also reduced in mature rats compared to young rats, indicating a defective ability of the blood vessels to resume and maintain blood flow when the cause of ischemia is removed. These vascular alterations to pressures were associated with a reduced vasodilation to Ach without changes in vasodilation to SNP, indicating an endothelial dysfunction. In conclusion, the vascular adjustments of the skin to low and high pressures decline in the early stages of aging. It would be interesting to study how this age-related decline of the microvascular adjustments to pressures could participate to the increased PU incidence largely reported in elderly people.

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Microcirculatory and clinical effects of a new Evodia rutaecarpa fruit extract after topical treatment of human skin

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The aim of this study was first, to modulate the human skin microcirculation with a new plant extract and then, to measure the possible benefits on skin condition, in vivo. Recorded to the Chinese Pharmacopoeia, Evodia rutaecarpa (Rutaceae) is a fast-growing shrub of Asian origin. Its fruit is known to hold small amounts of indolequinazoline and quinolone alkaloids. Among them, rutaecarpine and evodiamine seems interesting for their vasorelaxant properties, described after oral intake. Nitric Oxide (NO) is the most important factor involved in the endothelial regulation of vascular function. Using DAF-FM specific probe, we measured a significant extracellular NO release by human dermal microvascular endothelial cells, after a 24h treatment with the Evodia rutaecarpa fruit extract tested at 1% (+ 169.2%, p=0.01). Blood vessels relaxation was then studied on human skin explants, after a 24h topical treatment with the plant extract formulated at 1%. After histological preparation, microscopy coupled to digital image analysis revealed a statistically significant increase of the vessels lumen diameter, in papillary and upper part of reticular dermis. This vasodilation was measured either in basal conditions (+ 275%, p=0.0002) or after preliminary noradrenalin-induced vasoconstriction (+ 252%, p=0.004). These results illustrated the expected effect of NO on smooth muscle vascular cells. Finally, in a monocentric, randomized, double-blind, dermatologically-controlled clinical trial, the extract formulated at 3% has been compared to its placebo, after twice daily application for 28 days. High resolution Laser Doppler blood perfusion Imager evidenced the increased blood flow (+11.5%, p=0.02) on the cheekbones. Skin texture fineness, brightness and complexion enhancement were also clinically assessed. To our knowledge, that the first time that the use of an Evodia rutarcarpa fruit extract is reported for its efficacy on skin microcirculation and consequently on skin condition

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Lactobacillus Rhamnosus GG increases the re-epithelialization rate of model wounds by stimulating keratinocyte migration

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Many studies have demonstrated the importance of probiotics and their potential therapeutic effects within the gut (Maria, 2008).Recently, the possible therapeutic effects of probiotics in other tissues has also begun to be investigated. Comparatively few studies have evaluated the use of topical probiotics in relation to the skin. In this study, we have conducted preliminary investigations into whether a well known probiotic, Lactobacillus Rhamnosus GG (LGG), can increase the rate of re-epithelialization in a model wound. Primary human keratinocytes (NHEK) were grown in proprietary medium until confluent. A scratch was then made in the monolayer using a sterile 1000-µl-pipet tip. The rate of re-epithelialization of the scratch was compared in untreated monolayers and monolayers treated with and LGG lysate made using108CFU/ml bacterial cells. The LGG lysate significantly accelerated re-epithelialisation of the scratches such that by 18 and 24hours, 92±1.6% and 97±1.5% respectively (P=0.02, n=3) of the scratch area was re-epithelialized compared with 71±1.1% and 81±1.3% respectively in control monolayers. In vitro proliferation and migration assays using MTT assay and Trans-well migration assay respectively demonstrated that LGG lysate significantly increased NHEK proliferation and migration rates relative to controls. However, the dominant mechanism was migration because scratches in cell cultures treated with the inhibitor of proliferation, mitomycin C still re-epithelialized significantly faster in the presence of the LGG lysate than controls (p=0.02). These data demonstrate that lysates from Lactobacillus Rhamnosus GG increase re-epithelialization by stimulation of keratinocyte migration.The use of probiotic lysates potentially offers new options to develop treatments that could improve wound healing.

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Transcriptome profiling in a delayed wound healing skin-humanized mouse model revealed genes implicated in extracellular matrix remodeling and collagen deposition

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Chronic ulcers management remains a major clinical challenge since pathogenic mechanisms responsible for healing impairment are not fully understood. Alterations in the granulation tissue formation appeared as a major cause of the delayed closure in a diabetes-induced delayed humanized wound healing model recently established in our laboratory. Furthermore, the treatment of such diabetic wounds with bioengineered dermis improved the healing response by triggering granulation tissue maturation. Aiming to dissect the molecular mechanisms responsible for the delayed healing in the model, global gene expression studies rendered a total of 49 differentially regulated transcripts. Most of them were related to extracellular matrix remodeling and collagen deposition, as revealed by functional enrichment analysis. Remarkably, microarray data supported granulation tissue alterations observed in the humanized diabetic wound healing model that were in turn, rescued after bioengineered dermis treatment. In conclusion, our data may shed light on the mechanisms involved in wound healing impairment. Therefore, these findings may have a relevant impact in the design of innovative therapeutic approaches for the treatment of diabetic wounds that could also be extended to the management of hard-to-heal wounds of other etiologies