A NEW *VITREOSCILLA FILIFORMIS* EXTRACT GROWN IN SPA WATER-ENRICHED MEDIUM ACTIVATES ENDOGENOUS CUTANEOUS ANTIOXIDANT AND ANTIMICROBIAL DEFENSES THROUGH A POTENTIAL TOLL-LIKE RECEPTOR 2 /PROTEIN KINASE C, ZETA TRANSDUCTION PATHWAY

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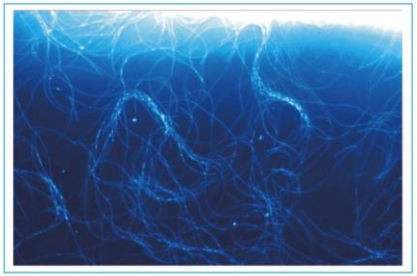
INTRODUCTION

Vitreoscilla filiformis (VF) biomass (VFB) has been used in cosmetic preparations and shown to modulate the major inducible free-radical scavenger mitochondrial superoxide dismutase in skin cells. By adding La Roche-Posay thermal spring water (LRP-TSW) to the *VF* culture medium, we obtained a biomass (LRP-VFB) with a similar mitochondrial superoxide dismutase activation capacity to *VF*. However the new biomass more powerfully stimulated mRNA expression and antimicrobial peptides in reconstructed epidermis. Interestingly, a predictive computer model that analyzed transducing events within skin epidermal cells suggested that this protective activity may involve the Toll-like receptor 2/protein kinase C, zeta transduction pathway. Protein kinase C, zeta inhibition was effectively shown to abolish VFB-induced gene stimulation and confirmed this hypothesis. This discovery opens new avenues for investigation into the improvement of skin homeostatic defense in relation to the control of its physiological microbiota and innate immunity.



***Vitreoscilla filiformis* culture**

OBJECTIVE

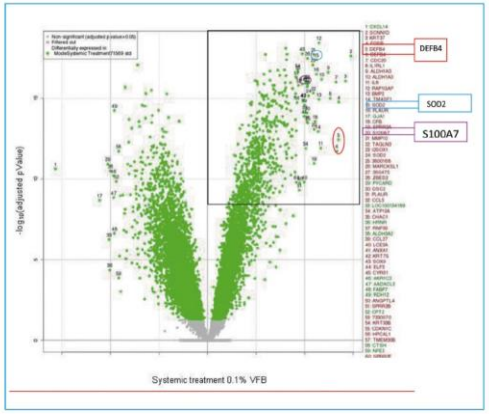
The LPS of this Gram negative bacteria reproduces a part of VFB activities once isolated. Interestingly, it not only stimulates endogenous mitochondrial antioxidant defenses but also endogenous antimicrobial defenses such as ß defensins and S100 calcium-binding protein A7 (S100A7). To investigate the possible signaling endogenous pathways linking TLR2 to the strongly up regulated chemokine (C-C motif) ligand 20 (CCL20) and interleukin 8 (IL8) that play a major role in the regulation of skin homeostasis, we tested the potential of the PROPAGAPATH in silico model. PROPAGAPATH is a dynamic and oriented signaling propagation pathway database, developed by Helios Biosciences, originally developed, validated, and enriched with immunological and neural cell propagation data. We wanted to evaluate the potency of this model to detect minimal propagation pathways when confronted with data from human skin epidermal keratinocytes. We used a combination of full genome expression studies and PROPAGAPATH analysis to understand which endogenous pathways the keratinocytes trigger in response to *VF* stimulation.

MATERIAL AND METHODS

Reconstructed human epidermis (RHE) samples were obtained from SkinEthic Laboratories. They were used at day 17 after the beginning of the reconstruction process. It contains normal human epidermal keratinocytes (NHEK) cultured on an inert polycarbonate filter at the air-liquid interface, in a chemically defined culture medium. The RHE samples were incubated in a 5% CO2 atmosphere at 37°C overnight.

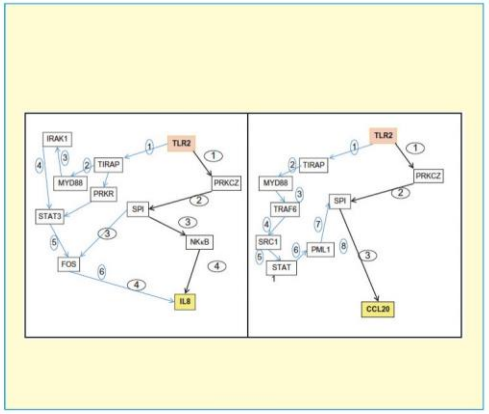
On day 18, heat-inactivated (120°C, 30 minutes) VFB, grown on media prepared either with osmosed water (VFB) or LRP-TSW (LRP-VFB), was added at either a concentration of 1% when applied topically to the RHE samples, or at a concentration of 0.1% when incubated systemically with the culture medium. All the RHE samples were then incubated in a 5% CO2 atmosphere at 37°C for 18 hours prior to mRNA extraction.

RESULTS

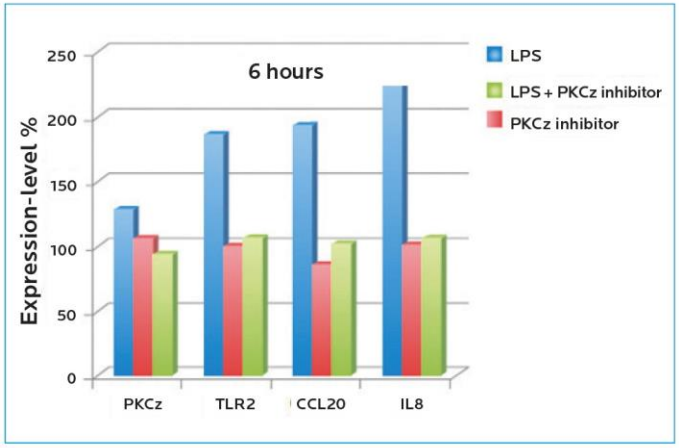


Full genome expression studies using reconstructed human epidermis (RHE) stimulated for 18 hours with 0.1% VFB.

Abbreviations: Defensin ß-4 (DEFB4): S100A7, S100 calcium-binding protein A7; SOD2, superoxide-dismutase 2; std, standard; VFB, *Vitreoscilla filiformis* biomass.



PROPAGAPATH analysis indicates that PKCζ is a potential transducer of the VFB activity of Toll-like receptor 2 (TLR 2) in skin included in the minimal signaling pathway connecting TLR 2 activation to interleukin 8 (IL8) and chemokine (C-C motif) ligand 20 (CCL20) induction.



Protein Kinase C, zeta (PKCz) transduces VFB - derived lipopolysaccharide (LPS) (10 µg/mL) signaling into normal human epidermal keratinocytes.
Expression-level fold changes in percentage change (100% seady-state level; 200% double expression) for PKCz,TLR 2, CCL 20, and IL8 in keratinocytes in response to VFB-derived LPS.

Abbreviations: CCL20, chemokine (C-C motif) ligand 20; IL8, interleukin 8; TLR 2, Toll-like receptor 2.

Comparative modulation of mRNA coding for antioxidant and antimicrobial proteins using VFB or LRP-VFB.

	VFB	LRP-VFB
SOD2	10.02	11.67
HMOX1	0.75	0.75
DEFB4	2.64	18.77
S100A7	4.65	16.77

Abbreviations: HMOX1, *heme oxygenase 1*.

CONCLUSION

PROPAGAPATH allowed us to identify the Toll-like receptor 2/protein kinase C, zeta transduction pathway for the first time in reconstructed skin. The VFB-derived LPS fraction stimulated gene expression and was inhibited with a PKCz inhibitor, suggesting that this specific fraction is indeed a key component of the protective action of VFB on skin. We also showed that cultivating *Vitreocilla filiformis* in selenium- and strontium- rich La Roche-Posay thermal spring water produced a biomass with an improved capacity to stimulate innate skin defense biomarkers, namely superoxide dismutase 2 and Defensin ß-4 .

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