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Deciphering the action of (R)-10-hydroxystearic acid, on the secretome of dermal fibroblasts by mass spectrometry-based proteomics.

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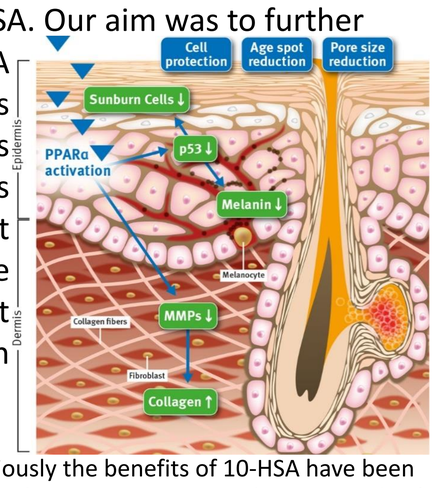
Introduction

Not only the presence of wrinkles is perceived as major sign of aging but also the presence of uneven skin tone, age spots and conspicuous skin pores are important concerns especially for Asian women. We have previously identified (R)-10-hydroxystearic acid (10-HSA) as novel active ingredient for anti-aging skin care applications. The molecule was selected from a reporter assay screening program on PPAR-alpha. PPAR-alpha agonists potentially have a wide variety of skin care benefits. We also showed in vitro stimulation of collagen I and III protein synthesis on dermal fibroblasts by 10-HSA and confirmed also clinical benefits of 10-HSA, on alleviating the appearance of age spots and conspicuous pores. The influence of secreted growth factors and cytokines from fibroblasts on melanogenesis and keratinocyte differentiation is well known and some of these may account for the clinical effects of 10-HSA. Our aim was to further the understanding of the clinical effects of 10-HSA by examining the secretome of dermal fibroblasts that have been exposed to 10-HSA using mass spectrometry-based proteomics. With this approach we could identify the protein levels that were modulated by 10-HSA and gained more insight to the biomolecular mechanisms that could account for the clinical improvements in age spots and conspicuous pores.



Previously the benefits of 10-HSA have been shown by in vitro, ex vivo and in vivo studies*

Materials and methods

In vitro cell culture: Human dermal fibroblasts (HDF) from a 50 years old female donor (Fb-D) were cultured for 24 hours in DMEM 10% FCS 1% penicillin/streptomycin before being starved in DMEM low glucose containing 0.2% FCS and 1% P/S for 2.5 days and then another 48h with test compounds. The preparation of the extracellular matrix proteins was according to the methods of Todorovic et al. 2010, in Mol Cell Proteomics.
Preparation for proteomic analysis: Extracellular matrix protein isolates were trypsin digested, reduced with DTT and alkylated (IAA). Peptides were then analysed by LC-MS/MS using C18 nanoLC ultra 2D+ (Eksigent) equipment and TripleTof 5600 (ABSciex) mass spectrometer according to Phylogene's protocol.

Results

The changes in the secretome of dermal fibroblasts induced by 10-HSA are shown in Table I to IV.

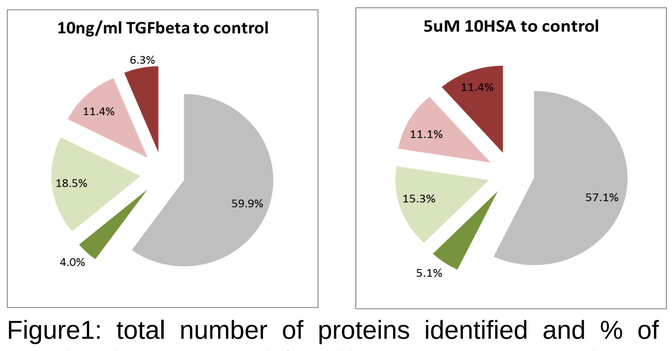


Figure1: total number of proteins identified and % of proteins that were modulated by 10-HSA and TGF-beta

	10ng/ml TGFbeta to control		5µM 10HSA to control	
	number	in %	number	In %
Total proteins	352	100%	352	100%
% Regulated proteins, p-value <0,05	141	40.1%	151	42.9%
% Non-regulated proteins, p-value >0,05	211	59.9%	201	57.1%
% Down-regulated proteins, p-value <0,05, fold change <0,5	14	4.0%	18	5.1%
% Down-regulated proteins, p-value <0,05, fold change 0,5 to 1	65	18.5%	54	15.3%
% Up-regulated proteins, p-value <0,05, fold change 1 to 1,5	40	11.4%	39	11.1%
% Up-regulated proteins, p-value <0,05, fold change >1,5	22	6.3%	40	11.4%

Table I

Paracrine/Autocrine growth factors and cytokines modulating keratinocyte differentiation & ECM production to influence pore wall elasticity and melanogenesis in age spots				
Name	Gene names	Fold Change	p-value	Potential function
Midkine	MDK MK1 NEGF2	15.74	0.0027	Involved in keratinocyte differentiation
Insulin-like growth factor-binding protein 2	IGFBP2 BP2 IBP2	15.07	0.0001	insulin-like growth factor I binding to improve pores, reduce sebum, decrease fibroblast migration and decrease melanocyte growth.
Isoform Delta of Stromal cell-derived factor 1	CXCL12 SDF1 SDF1A SDF1B	3.94	0.0002	involved in melanocyte/fibroblast migration and keratinocyte proliferation. Enhances wound healing.
Angiopoietin-related protein 4	ANGPTL4 ARP4 HFARP PGAR PP1158 PSEC0166 UNQ171/PRO197	2.90	0.0004	wnt signalling antagonist to reduce melanogenesis. Also improves keratinocyte differentiation.
Connective tissue growth factor	CTGF CCN2 HCS24 IGFBP8	2.29	0.0001	insulin-like growth factor binding (as above). Increases procollagen production to help pore wall structure.
Protein CYR61	CYR61 CCN1 GIG1 IGFBP10	1.45	0.0007	extracellular matrix binding. Inhibits melanocyte growth but increases MMP's
Transforming growth factor-beta-induced protein ig-h3	TGFBI BIGH3	1.41	0.0000	promotes fibroblast growth and keratinocyte differentiation.
Semaphorin-3A	SEMA3A SEMAD	1.35	0.0332	inhibits inflammation (reduces melanogenesis and ECM destruction) and decreases TEWL (keratinocyte differentiation) via neurophilin-1 receptor that protects against UVB apoptosis.
Insulin-like growth factor-binding protein 3	IGFBP3 IBP3	0.90	0.0384	Increased levels in conspicious pores
Growth/differentiation factor 15	GDF15 MIC1 PDF PLAB PTGFB	0.78	0.0078	transforming growth factor beta receptor binding [GO:0005160]. Involved in keratinocyte differentiation. GDF9 increases CTGF?
Dickkopf-related protein 1	DKK1 UNQ492/PRO1008	0.78	0.0050	low-density lipoprotein particle receptor antagonist activity and reduces melanogenesis
Secreted frizzled-related protein 1	SFRP1 FRP FRP1 SARP2	0.77	0.0050	frizzled binding reduces melanogenesis. Increased levels found in age spots
Fibroblast growth factor 5	FGF5	0.76	0.0483	fibroblast growth factor receptor binding and elevated levels in melanoma
Semaphorin-3B	SEMA3B SEMA5 SEMAA	0.72	0.0015	causes growth cone collapse of sensory neurons may help with itch
Netrin-1	NTN1 NTN1L	0.71	0.0329	proinflammatory and promotes melanoma invasiveness
Semaphorin-3D	SEMA3D UNQ760/PRO1491	0.63	0.0127	causes growth cone collapse of sensory neurons may help with itch
Gremlin-1	GREM1 CKTSF1B1 DAND2 DRM PIG2	0.48	0.0011	BMP binding and transient increases induces melanogenesis.
Adrenomedullin	ADM AM	0.36	0.0020	melanocyte dendrite branching factor, induces keratinocyte& fibroblast proliferation
Complement C1q tumor necrosis factor-related protein 3	C1QTNF3 CTRP3 UNQ753/PRO1484	0.34	0.0277	CTRP3 inhibits TGF-β1 induced collagen synthesis, proliferation and migration. Attenuates CTGF production. CTRP3 also attenuated TGF-β1-induced Smad3 phosphorylation, nuclear translocation, and interaction with p300

Table II

Structural proteins of ECM and DEJ to influence pore wall elasticity and melanogenesis in age spot				
Name	Gene names	Fold Change	p-value	Potential function
Fibronectin type III domain-containing protein 1	FNDC1 FNDC2 KIAA1866 MEL4B3	19.64	0.0000	ECM protein
Vitronectin	VTN	5.33	0.0000	extracellular matrix binding [GO:0050840]; heparin binding [GO:0008201]; integrin binding [GO:0005178]; polysaccharide binding [GO:0030247]; scavenger receptor activity [GO:0005044]
Hyaluronan-binding protein 2	HABP2 HGFAL PHBP	5.09	0.0345	glycosaminoglycan binding [GO:0005539]; serine-type endopeptidase activity [GO:0004252]
Proteoglycan 4	PRG4 MSF SZP	2.91	0.0290	polysaccharide binding [GO:0030247]; scavenger receptor activity [GO:0005044]
Pentraxin-related protein	PTX3 TNFAIP5 TSG14	1.93	0.0014	Involved in wound healing.
Tenascin	TNC HXB	1.75	0.0004	syndecan binding [GO:0045545]
Gremlin-2	GREM2 CKTSF1B2 DAND3 PRDC	1.74	0.0005	inhibits BMP signaling to reduce melanogenesis
Matrilin-2	MATN2 UNQ193/PRO219	1.47	0.0000	calcium ion binding [GO:0005509]
Versican core protein	VCAN CSPG2	1.38	0.0012	calcium ion binding [GO:0005509]; carbohydrate binding [GO:0030246]; extracellular matrix structural constituent [GO:0005201]; glycosaminoglycan binding [GO:0005539]; hyaluronic acid binding [GO:0005540]
Collagen alpha-2(VI) chain	COL6A2	1.31	0.0041	
EMILIN-1	EMILIN1 EMI	1.30	0.0001	extracellular matrix constituent conferring elasticity [GO:0030023]
Collagen alpha-3(VI) chain	COL6A3	1.22	0.0103	serine-type endopeptidase inhibitor activity [GO:0004867]
CD44 antigen	CD44 LHR MDU2 MDU3 MIC4	0.66	0.0066	collagen binding [GO:0005518]; hyaluronic acid binding [GO:0005540]; hyaluronan-glucosaminidase activity [GO:0004415]
Latent-transforming growth factor beta-binding protein 2	LTBP2 C14orf141 LTBP3	0.66	0.0007	Assists TGFbeta signaling for matrix production and melanogenesis
Collagen alpha-2(I) chain	COL1A2	0.59	0.0103	extracellular matrix structural constituent [GO:0005201]; identical protein binding [GO:0042802]; metal ion binding [GO:0046872]; platelet-derived growth factor binding [GO:0048407]; protein binding, bridging [GO:0030674]
Isoform 4 of Elastin	ELN	0.59	0.0018	extracellular matrix structural constituent [GO:0005201]
Vimentin	VIM	0.53	0.0002	double-stranded RNA binding [GO:0003725]; glycoprotein binding [GO:0001948]; identical protein binding [GO:0042802]; protein C-terminus binding [GO:0008022]; scaffold protein binding [GO:0097110]; structural constituent of cytoskeleton [GO:0005200]; structural constituent of eye lens [GO:0005212]
Prelamin-A/C	LMNA LMN1	0.06	0.0100	structural molecule activity [GO:0005198]

Table III

Proteases and sulphatases that degrade extracellular matrix and modulate melanogenesis via protease-activated receptors to influence pore wall elasticity and melanogenesis in age spot				
Name	Gene names	Fold Change	p-value	Potential function
Prothrombin	F2	7.46	0.0000	serine-type endopeptidase activity
A disintegrin and metalloproteinase with thrombospondin motifs 1	ADAMTS1 KIAA1346 METH1	2.28	0.0064	metalloendopeptidase activity
A disintegrin and metalloproteinase with thrombospondin motifs 5	ADAMTS5 ADAMTS11 ADMP2	1.78	0.0134	metalloendopeptidase activity and modulates proteoglycan synthesis
Carboxypeptidase Z	CPZ	1.39	0.0232	metallocarboxypeptidase activity
Serine protease 23	PRSS23 ZSIG13 UNQ270/PRO307	1.35	0.0149	serine-type endopeptidase activity
Serine protease HTRA1	HTRA1 HTRA PRSS11	1.20	0.0033	serine-type endopeptidase. Regulates availability of IGF by cleaving IGFBP. Processes LTBP, facilitates TGFbeta signaling
Neurotrypsin	PRSS12	0.68	0.0250	serine-type endopeptidase activity
Matrix metalloproteinase-14	MMP14	0.65	0.0048	metalloendopeptidase activity
Extracellular sulfatase Sulf-1	SULF1 KIAA1077	0.65	0.0011	N-acetylglucosamine-6-sulfatase activity
Thrombospondin type-1 domain-containing protein 4	THSD4 UNQ9334/PRO34005	0.64	0.0055	metalloendopeptidase activity
Calpain-2 catalytic subunit	CAPN2 CANPL2	0.10	0.0038	calcium-dependent cysteine-type endopeptidase activity

Table IV

Protease inhibitors to prevent ECM destruction and melanogenesis to influence pore wall elasticity and melanogenesis in age spot				
Name	Gene names	Fold Change	p-value	Potential function
Alpha-2-antiplasmin	SERPINF2 AAP PLI	3.90	0.0202	serine-type endopeptidase inhibitor activity. Plasmin involved in age spot formation.
Antithrombin-III	SERPINC1 AT3 PRO0309	1.75	0.0029	serine-type endopeptidase inhibitor activity
Metalloproteinase inhibitor 3	TIMP3	1.66	0.0011	metalloendopeptidase inhibitor activity
Tissue factor pathway inhibitor	TFPI LACI TFPI1	1.55	0.0012	serine-type endopeptidase inhibitor activity
Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2 IGHEP2	1.44	0.0020	serine-type endopeptidase inhibitor activity
Serpin H1	SERPINH1 CBP1 CBP2 HSP47 SERPINH2 PIG14	0.80	0.0266	serine-type endopeptidase inhibitor activity

Discussion

The secretome of fibroblasts treated with 10-HSA might influence the fibroblasts in autocrine fashion or keratinocytes, melanocytes or sebocytes in a paracrine fashion.
352 secreted proteins were annotated and increased concentrations of fibroblast-derived proteins that inhibit the wnt pathway (e.g. Insulin-like growth factor-binding protein 2 and angiopoietin-related protein 4 etc.) together with decreased concentrations of those that increase the wnt pathway (e.g. secreted frizzled-related protein-1) were observed. As the wnt pathway increases melanogenesis these results could account for the alleviation of age spots by 10-HSA.
Also, we observed increased levels of proteins that modify dermal fibroblast activity and keratinocyte differentiation such as transforming growth factor-beta-induced protein etc. which could account for the alleviation of conspicuous pores by 10-HSA. Moreover, we observed changes in the IGFBP proteins that control the levels of IGF1 that is associated with conspicuous pores and sebum production. Many proteins were increased that would be associated with a strengthening of the pore wall dermal matrix and protease inhibitors that would prevent its destruction.
In conclusion we have determined the effect of 10-HSA on the fibroblast secretome and gained more insight to the biomolecular mechanisms that could account for the clinical improvements in age spots and conspicuous pores.

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PHYLOGENE

DSM

* see DSM product documentation on 10-HSA