Membrane proteins of keratinocytes protection by the cannabidiol applied before and after UVB irradiation

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Abstract:

The continuous increase in daily exposure to ultraviolet radiation, which influence on the redox state of skin cells, may contribute to the damage to the structure and function of cellular macromolecules, which favors the search for protective compounds. One promising compound is cannabidiol (CBD), phytocannabinoid which has antioxidant and anti-inflammatory properties. Therefore, the aim of this study was to compare the effect of CBD applied after (treatment) as well as before and after (pretreatment+treatment) keratinocytes irradiation with UVB on the proteomic profile of membrane proteins. Obtained data showed that both UVB radiation and CBD treatment significantly modified the proteomic profile of keratinocyte membranes. UVB was shown to dramatically increase the expression of proteins involved in the regulation of cell translation and proliferation (S3a/L13A/L7a ribosomal proteins), calcium ion homeostasis and inflammatory response (S100/S100-A6 proteins) and cellular redox state (peroxireoxin-1). Long action of CBD (pretreatment + treatment) was more effective in preventing changes caused by UVB, compared to the action of CBD used only after UVB irradiation. The strong activity of CBD applied before and after UVB irradiation suggests that this phytocannabinoid is effective in protecting skin cells against UVB-induced changes, in the keratinocyte proteome.

Keywords: Cannabidiol; UVB; Skin cells; Keratinocytes;
Oxidative stress; Membranes; Proteomic analysis

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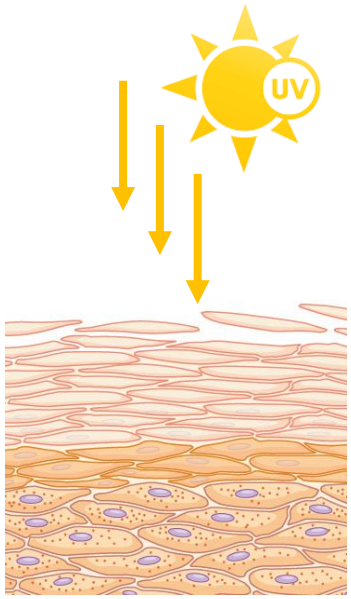
Introduction



skin keratinocytes

Overproduction of **ROS**

alterations in
cell signaling and **cell metabolism**

Inflammation
Apoptosis

skin injuries

- **Oxidative modifications** of lipids, nucleic acids, and proteins
  - ✓ *changes in functioning of redox-sensitive transcription factors, Nrf2 and NFκB, which control the cellular response to UV radiation*
- Generation of oxidative fragmentation and cyclization products **– lipid peroxidation**
  - ✓ *oxidative fragmentation products (4-HNE and MDA)*
  PUFA cyclization products (isoprostanes)
- changes in expression of membrane receptors

redox imbalance

✓ **There is still need a cytoprotective compound against UV-induced metabolic changes in skin cells.**

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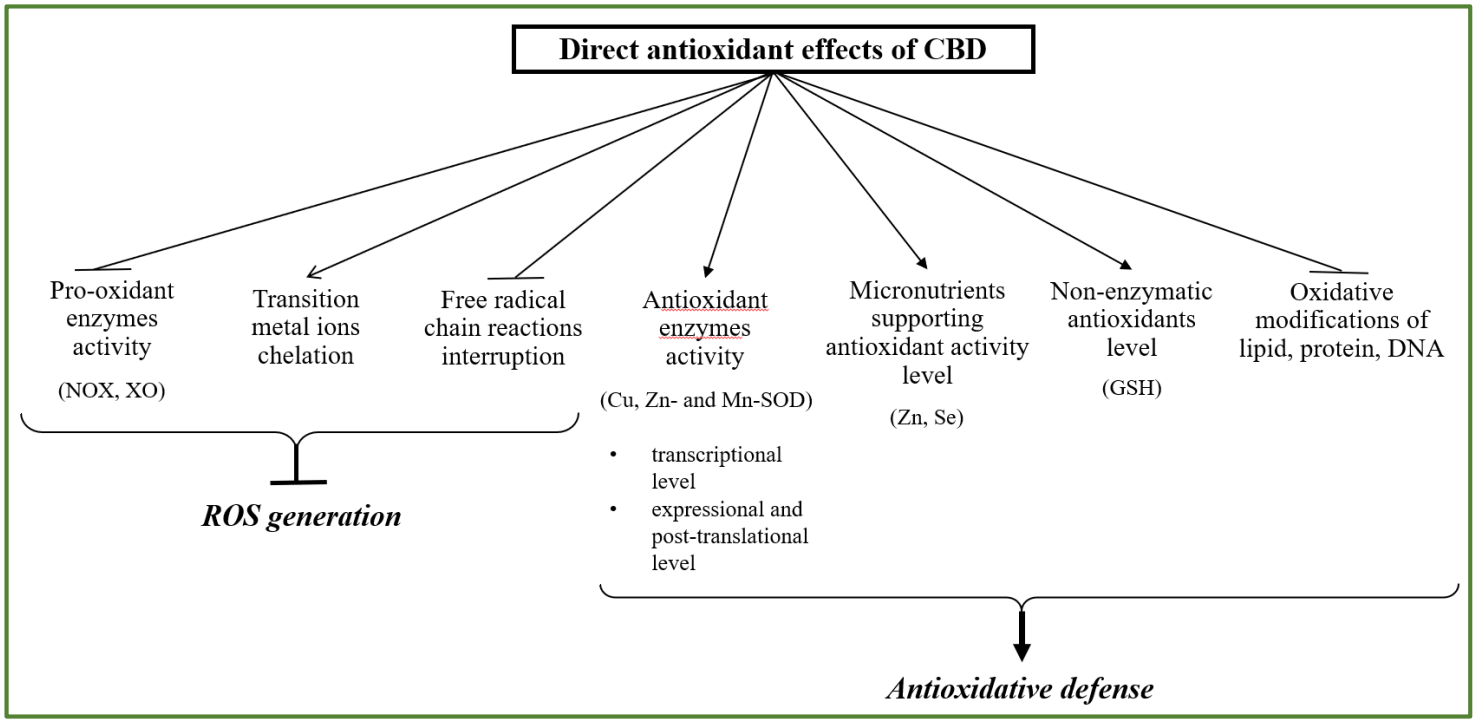
Introduction



Cannabis sativa L.

✓ **Cannabidiol**, non-psychoactive phytocannabinoid, has beneficial pharmacological effects including **antioxidative** and **anti-inflammatory** effects due to its chemical structure.

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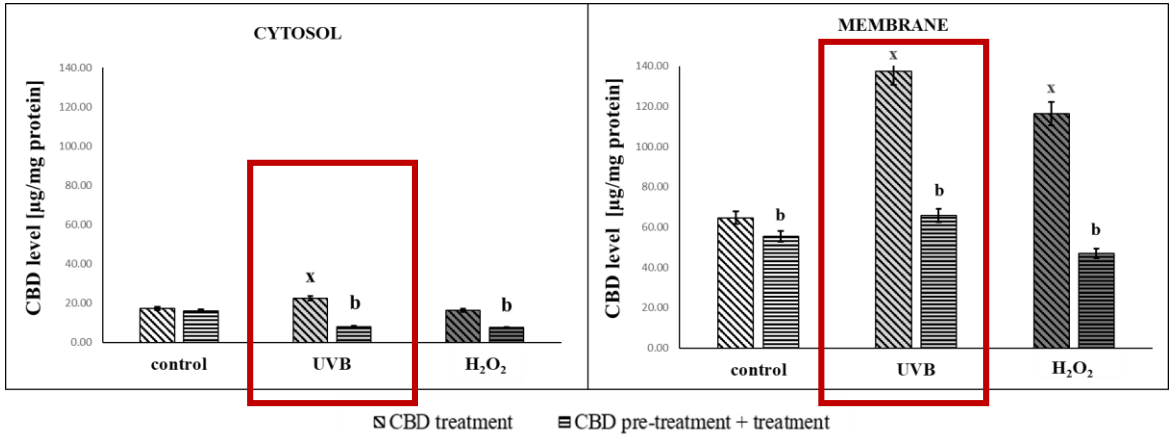
Atalay, S.; Jarocka-Karpowicz, I.; Skrzydlewska, E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants 2020, 9, 21.

Introduction

- ✓ CBD is able to **penetrate keratinocytes and accumulates within the cellular membrane**.

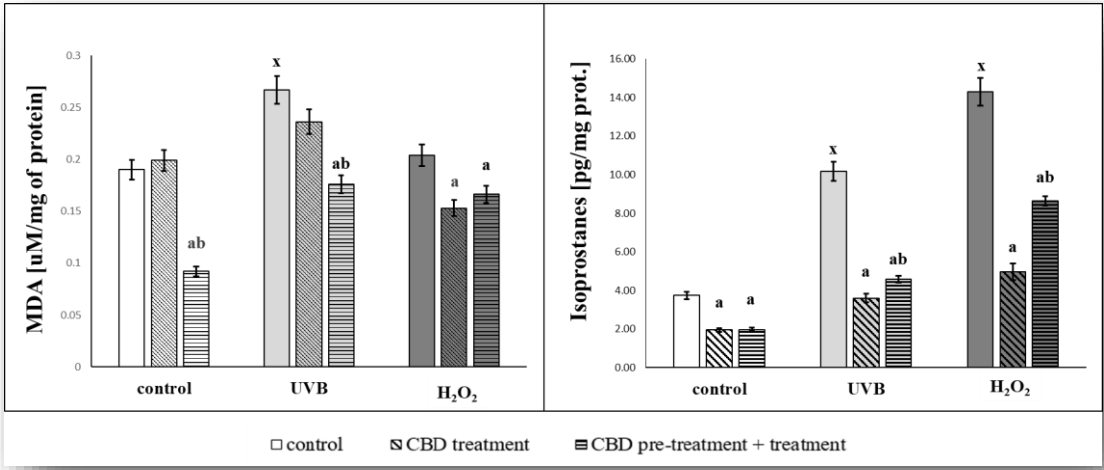


✓ **CBD protects keratinocytes** by **preventing changes in the composition of the cell membrane**: such as:

- increased levels of lipid peroxidation products (MDA and isoprostanes)
- reduced levels of polyunsaturated fatty acids

which were associated with UVB damage

Fatty acids µg/mg of protein	Control groups			Keratinocytes treated with UVB		
	Control	CBD treatment	CBD pretreatment + treatment	UVB	CBD treatment	CBD pretreatment + treatment
Oleic (C18:1n9c)	69.2±3.4	80.6±4.0^{a}	77.7±3.9^{a}	23.4±1.2^{x}	67.7±3.4^{ab}	77.3±3.9^{ab}
Linoleic (C18:2n6c)	21.6±1.1	25.3±1.3^{a}	22.5±1.1^{b}	7.9±0.4^{x}	22.4±1.1^{a}	18.4±0.9^{ab}
γ-Linolenic (C18:3n6)	7.44±0.37	8.86±0.44^{a}	7.16±0.36^{b}	2.70±0.13^{x}	7.57±0.38^{a}	6.89±0.34^{a}
Linolenic (C18:3n3)	0.10±0.01	0.12±0.01	0.11±0.01	0.03±0.01^{x}	0.09±0.01^{a}	0.11±0.01^{ab}
Arachidonic (C20:4n6)	8.48±0.42	9.98±0.50^{a}	9.15±0.46	2.97±0.15^{x}	8.61±0.43^{a}	8.72±0.44^{a}
cis-5,8,11,14,17-Eicosapentaenoic (C20:5n3)	4.40±0.22	5.16±0.26^{a}	4.44±0.22^{b}	1.51±0.08^{x}	4.30±0.22^{a}	4.50±0.22^{a}
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6n3)	2.60±0.13	3.23±0.16^{a}	3.05±0.15^{a}	0.91±0.05^{x}	2.67±0.13^{a}	3.11±0.16^{ab}



Atalay, S.; Dobrzyńska, I.; Gęgotek, A.; Skrzydlewska E. Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide. Redox Biol. 2020 Sep;36:101613.

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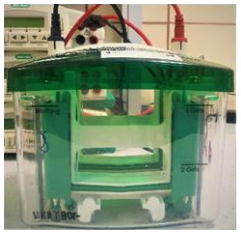
Method

✓ Main experimental steps for proteomic analysis of keratinocyte membranes
SDSPAGE/nanoHPLC/QexactiveOrbiTrap

CBD
+
Keratinocytes
(CDD 1102 KERTr)

Membrane Fractionation

Protein extraction
from membrane fractions



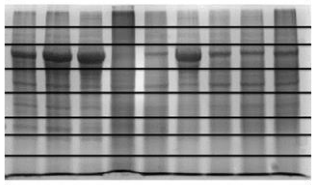
Protein separation
10% Tris-Glycine
SDS-PAGE gel

(peptide mixture)

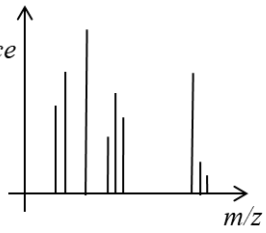
In-gel digestion
(with trypsin)

Reduction (with DTT) and
Alkylation (with iodoacetamide)

Gel – cutting
Entire lines were cut from the gels and sliced into eight sections



Relative abundance



Proteomic examination
nanoHPLC/QexactiveOrbiTrap
(LC-MS/MS)

Protein identification And LFQ
Proteome Discoverer 2.0

Statistical analysis (one-way ANOVA, post-hoc analysis) and **visualization** (PCA, Heatmap, Volcano plot),
MetaboAnalyst 4.0, Perseus 1.6.5.0, STRING

Data interpretation

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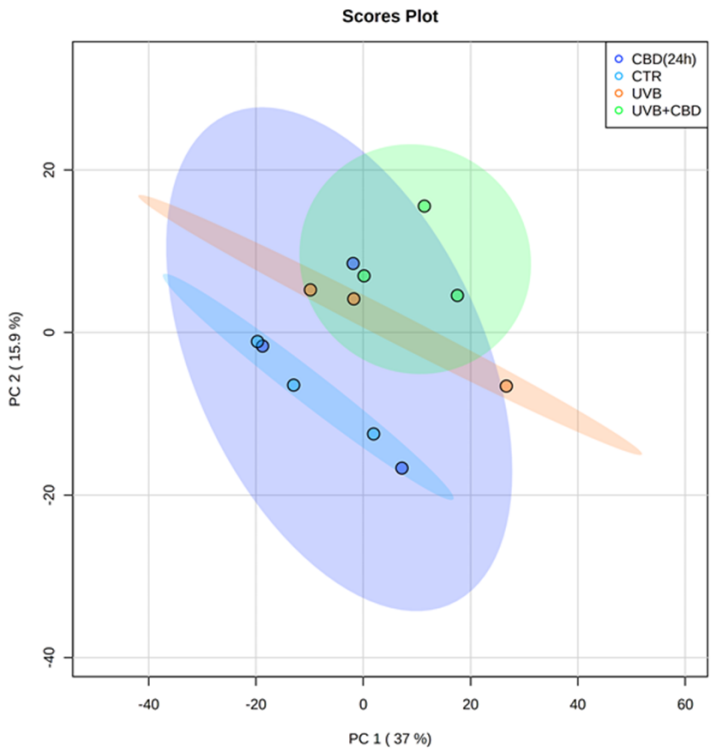
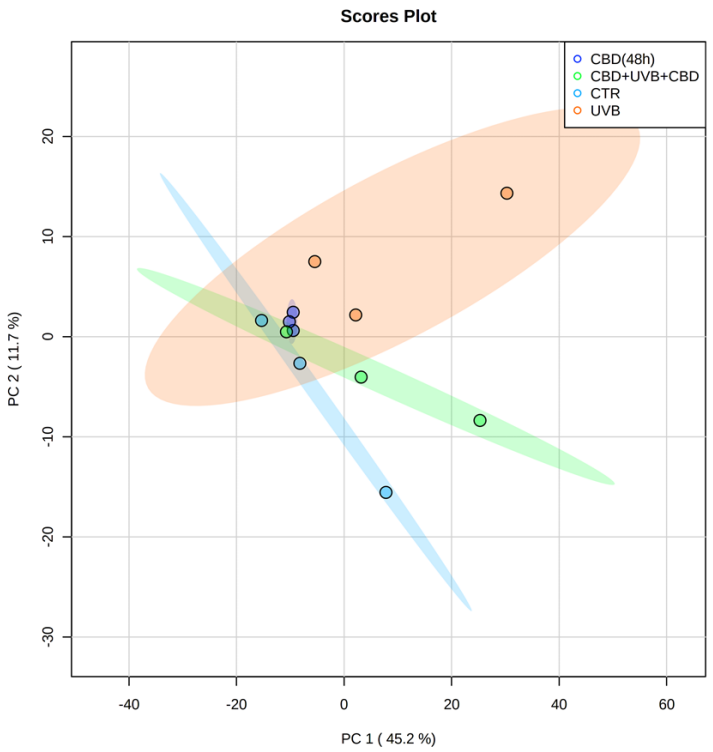
Results and Discussion

UVB+CBD



CBD + UVB +CBD



✓ The PCA demonstrated that CBD pre-treatment brought along clustering of experimental groups more in themselves.

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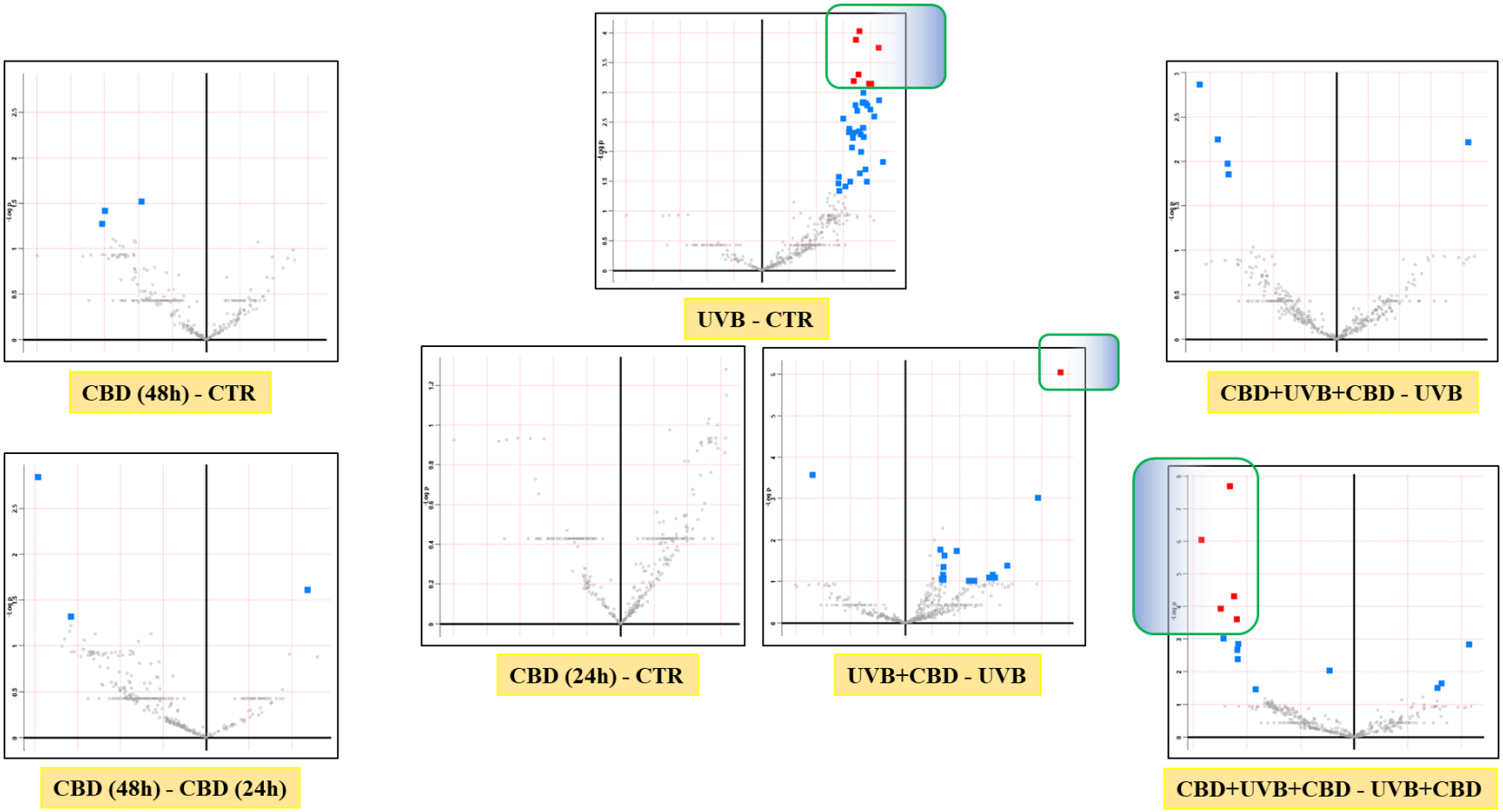
Results and Discussion



(blue was used for proteins changed significantly with p-value<0.05;
red was used for proteins changed significantly with FDR-adjusted p-value <0.05)

✓ The volcano plots comparing the groups:

- control group and UVB exposed group,
- pre-treated/treated with CBD before + after or only after UVB irradiation
- between CBD pre-treated and CBD treated groups

show that the keratinocyte membrane proteomes were significantly different between the groups.

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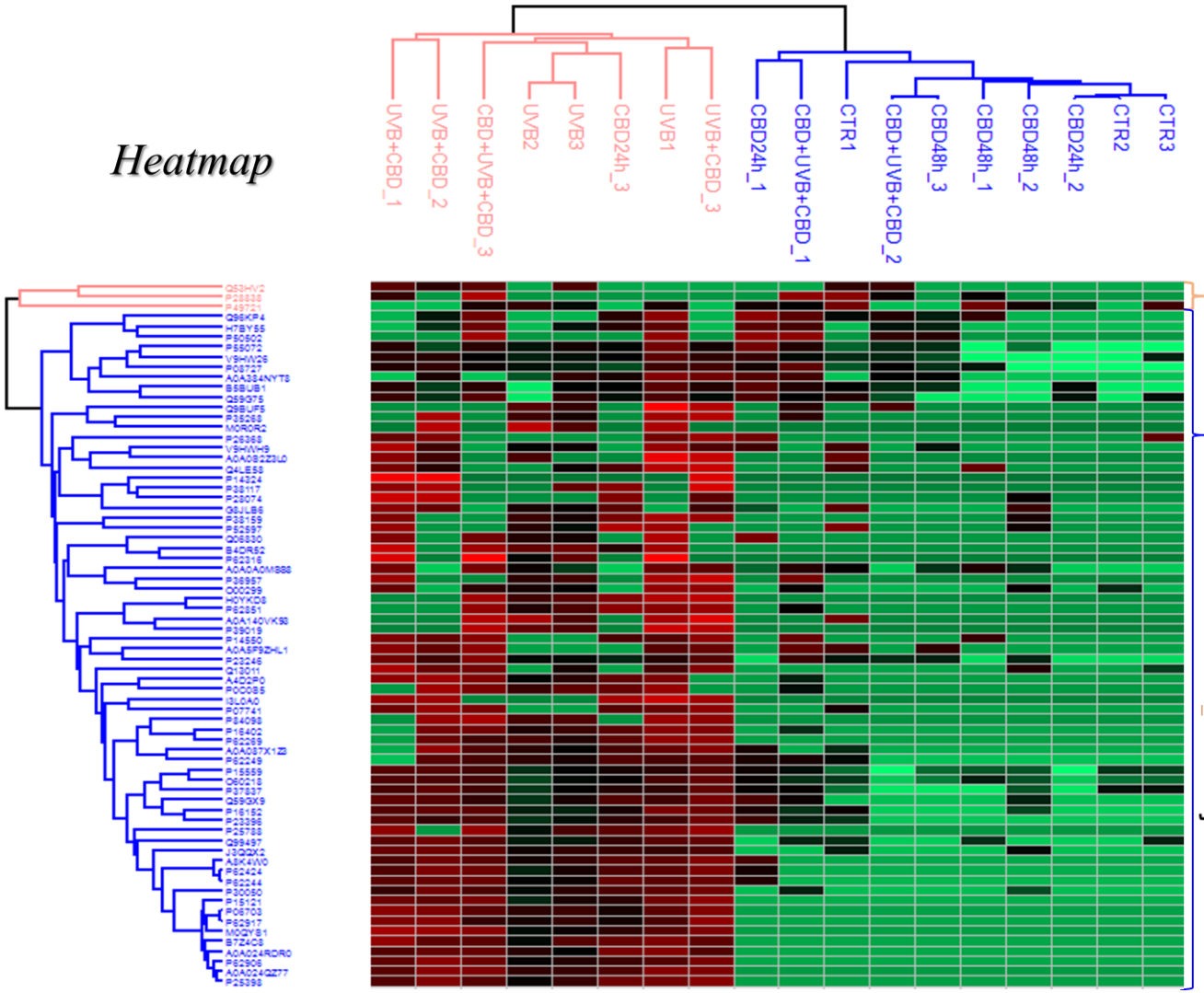
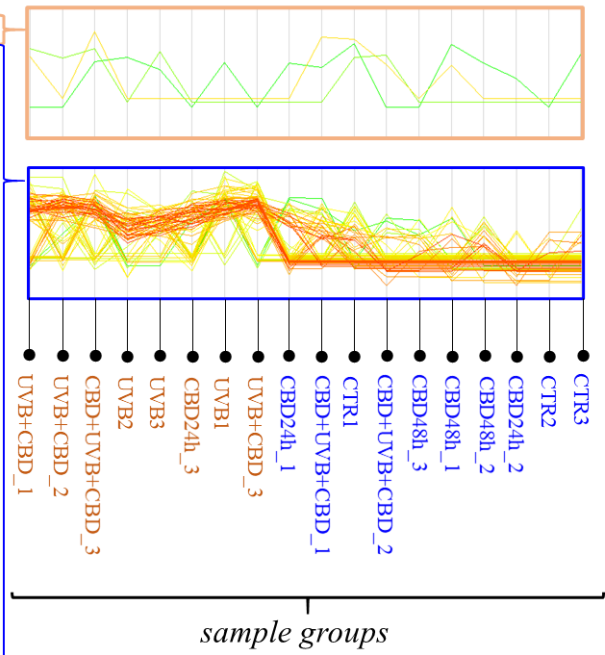
Results and Discussion

Heatmap



Trendlines of protein levels



✓ The primary split in the upper hierarchical dendrogram showed that:

the samples which are treated with CBD for 48 h and pretreated+treated with CBD before and after UVB radiation (48h) clustered closer to each other majorly, independently than UVB exposed samples.

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Results and Discussion

✓ The data obtained from SDS-Page/nanoHPLC/QExactiveOrbiTrap show that membrane proteome of keratinocytes is critically changed after UVB radiation [UVB], while

CBD pretreatment+treatment (48h) [CBD+UVB+CBD]

as well as

CBD-treatment [UVB+CBD] modulate these changes

		Fold Change		
protein ID	**protein name**	**UVB/CTR**	**CBD+UVB+CBD/UVB**	**UVB+CBD/UVB**
A8K4W0	40S ribosomal protein S3a	58.92	1.65	2.59
M0QYS1	60S ribosomal protein L13a	14.16	4.35	5.46
P62424	60S ribosomal protein L7a	44.59	3.27	3.49
Q06830	Peroxiredoxin-1	148.92	0.76	1.09
V9HWH9	Protein S100	1.68	0.02	1.84
P06703	Protein S100-A6	40.81	1.53	3.31

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Results and Discussion

		Fold Change		
protein ID	protein name	UVB/CTR	CBD+UVB+CBD/UVB	UVB+CBD/UVB
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P06703	Protein S100-A6	40.81	1.53	3.31

- UVB radiation induces the level of **anti-apoptotic** ribosomal protein **S3a**, but also other ribosomal proteins **L13a and L7a** which have **pro-apoptotic** effect.
- CBD long-action [CBD+UVB+CBD] increases UVB-induced level of **S100-A6**, but it is 2 times less than after CBD treatment [CBD] and even CBD long-action [CBD+UVB+CBD] decreases UVB-increased level of Protein S100.
- Although UVB-induces level of **peroxideroxin-1** maintained by short time action of CBD (maybe as a cellular response to UVB-induced stress), long-action of CBD decreases UVB-induced level of peroxiredoxin-1.

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Conclusions

In summary,

Our study indicates that,

- ✓ UVB radiation significantly changes membrane proteome of keratinocytes.
- ✓ CBD therapies can effectively modulate the above changes, resulting mainly in cell proliferation and inflammation induced by UVB.
- ✓ Long-acting CBD may be more effective in counteracting membrane changes.

The protective effect of CBD against UVB-induced stress and its effects may be due to the

induction of pro-apoptotic ribosomal proteins

and/or

changes in crosstalk between NF-κB and Nrf2.

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This research was conducted within the project which has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754432 (and the Polish Ministry of Science and Higher Education, from financial resources for science in 2018-2023 granted for the implementation of an international co-financed project). ***Sinemyiz Atalay is supported by above project****.*

Thank you…

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