MORPHOLOGIC, IMMUNOPHENOTYPIC AND BIOENERGETIC
CHARACTERIZATION OF
A HUMAN DERMAL FIBROBLAST CELL LINE

IOANA ZINUCA PAVEL¹, OANA DUICU¹, FLORINA BOJIN²,
ANDREEA PRIVISTIRESCU¹, DORINA CORICOVAC³, CRISTINA DEHELEAN³, DANINA MUNTEAN¹

¹Department of Pathophysiology ²Department of Immunology, ³Department of Toxicology,
"Victor Babes" University of Medicine and Pharmacy Timisoara

BACKGROUND

Ultraviolet-B (UVB)-induced morphological changes in cultured fibroblasts have been systematically studied in the past decades in relation with photoprotective strategies.

AIM

The present study was aimed at providing a comprehensive characterization of a human dermal fibroblast cell line (HDFa) in terms of:

- ❖ morphological and phenotypical aspects
- ❖ mitochondrial function and bioenergetics.

MATERIAL AND METHODS

- ❖ Morphology of HDFa line was assessed by:
 - light microscopy.
 - immunofluorescence.
- ❖ Flow cytometry was used for the evaluation of the following surface markers: CD34, CD45, CD90, CD73, CD44, CD29, CD117, HLA-A2, CD105, CD109, CD26, IL-10R, HLA-DR, and TGF-βRIII.
- ❖ Mitochondrial bioenergetics was measured by using Seahorse Bioscience XF24 extracellular flux analyzer.
 - 10K, 20K, and 30K cells were seeded in order to obtain the optimal seeding density
 - OCR (oxygen consumption rate) and ECAR (extracellular acidification rate) were evaluated at 37°C
 - OCR was reported in units of nmoles/minute and ECAR in mpH/minute.
 - Cells were metabolically perturbed by 3 successive compound additions that shifted the bioenergetic profile of the cells: Omy (**LEAK**), FCCP (**ETS**), and Ama (**ROX**).
 - Mitochondrial respiratory rates were corrected for oxygen flux due to instrumental background and ROX.
 - Respiratory control ratio (RCR) was calculated as the ratio between ETS and LEAK state.

RESULTS

Morphology of normal HDFa cells

❖ HDFa cells have elongated shapes, are adherent, and expand rapidly in culture.

Molecular markers expression of normal HDFa cells

- ❖ HDFa express CD90, CD73, CD29 and HLA-A2;
- ❖ HDFa are negative for CD34, CD45, CD44, CD117, CD105, CD109, CD26, IL-10R, HLA-DR and TGF-βRIII.

Mitochondrial function and bioenergetics of normal HDFa cells

The Seahorse Extracellular Flux Analyzers

GROUP Summary

Group	Injection	Pretreatment	Media	Cell Type
1	Inj. Strategy	No Pretreatment	XFAM +	HDFa 1000X
2	Inj. Strategy	No Pretreatment	XFAM +	HDFa 2000X
3	Inj. Strategy	No Pretreatment	XFAM +	HDFa 3000X

Mitochondrial respiration rates corrected to ROX: pmols/(min*no cells)

No of cells	ROUTINE	LEAK	ETS	RCR
10K cells/well	22,81± 0,6	8,68± 0,6	32,21± 0,9	3,71± 1
20K cells/well	33,26± 2,1	8,32± 1,8	58,45± 1,1	7,02± 0,4
30K cells/well	44,21± 2	3,29± 1,3	76,42± 3,8	23,17± 1,9

Command	Time (min)
Calibrate	
Equilibrate	
Mix x4	1
Wait x4	2
Measure x4	3
Inject Port A	
Mix x3	1
Wait x3	2
Measure x3	3
Inject Port B	
Mix x3	1
Wait x3	2
Measure x3	3
Inject Port C	
Mix x3	1
Wait x3	2
Measure x3	3

XF Protocol

- ❖ Oligomycin (Omy, Complex V inhibitor): OCR decreases; since ATP synthesis via OXPHOS is blocked, energy production shifts to glycolysis → ECAR significantly increases: **maximum glycolytic capacity** of the cells
- ❖ FCCP (uncoupling agent): OCR significantly increases as more O₂ is consumed to pump the excess protons back across the mitochondrial membrane, while ECAR slightly increases due to cells attempt to maintain their energy balance by using glycolysis to generate ATP
- ❖ Antimycin A (Ama, Complex III inhibitor): the cease of electrons flow at the ETC drastically reduces OCR, while ECAR is not modified, since cells are shifted to a glycolytic state, in order to maintain their energy balance.

- ❖ With increasing cell density, basal OCR values are linearly increasing;
- ❖ For both basal and FCCP stimulated rates, the ECAR value increased from 20K to 30K cells;
- ❖ With 30K cells the optimal responses of OCR and ECAR occurred as in our hands this density was found to be within the linear response range .

CONCLUSION

Characterization of HDFa cell line was performed in order to further report on their changes in response to different protocols of UVB exposure in the presence vs. the absence of protective phytochemicals.