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

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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

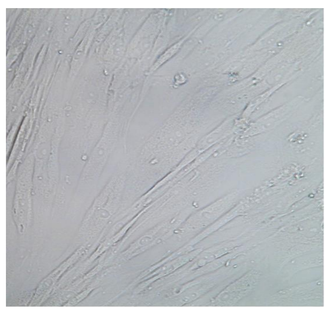
- ❖ Morphology of HDFa line was assessed by:
  - light microscopy.
  - immunofluorescence.
- ❖ Flow cytometry was used for the evaluation of the following surface markers: CD34, CD45, CD90, CD73, CD44, CD29, CD117, HLA-A2, CD105, CD109, CD26, IL-10R, HLA-DR, and TGF-βRIII.
- ❖ Mitochondrial bioenergetics 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.

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

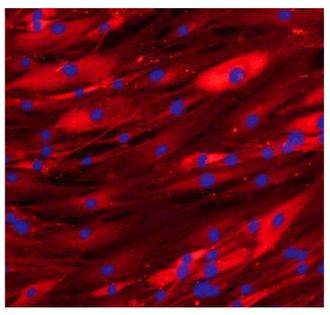
XF Protocol

RESULTS

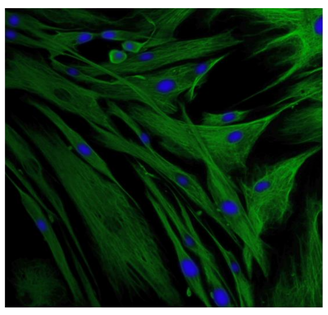
Morphology of normal HDFa cells



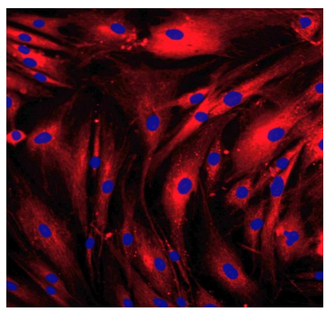
HDFa (Ob. 20x)



CD105



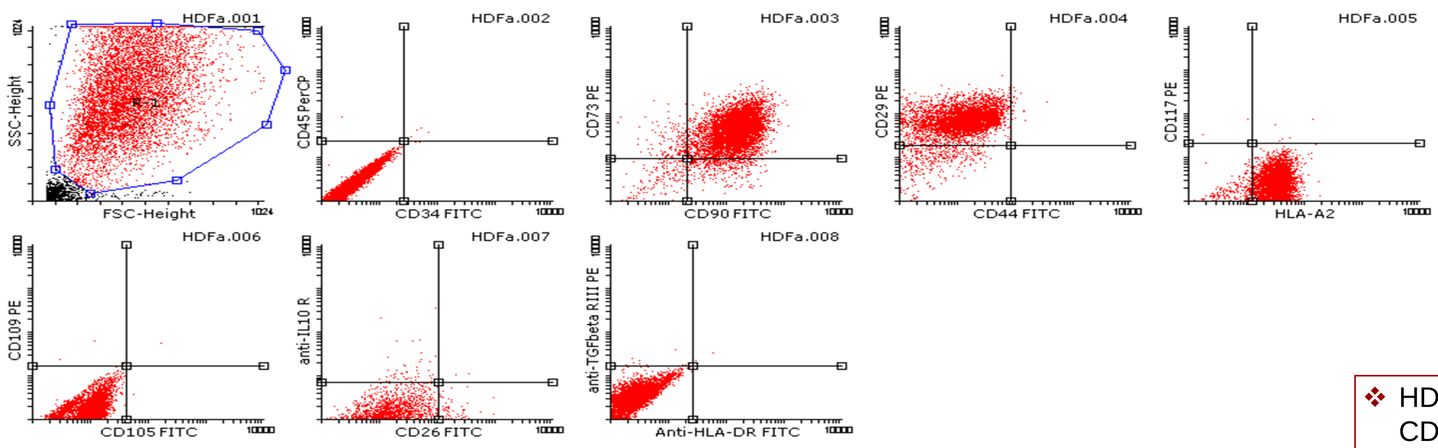
Vimentin



Ki67

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

Molecular markers expression of normal HDFa cells



	Events	% of Vis
All events	8,260	100.00
Left Bottom	8,251	99.89
Right Bottom	3	0.04
Left Top	2	0.02
Right Top	4	0.05

	Events	% of Vis
All events	7,233	100.00
Left Bottom	189	2.61
Right Bottom	404	5.59
Left Top	160	2.21
Right Top	6,480	89.59

	Events	% of Vis
All events	6,304	100.00
Left Bottom	459	7.28
Right Bottom	1	0.02
Left Top	5,823	92.37
Right Top	21	0.33

	Events	% of Vis
All events	6,430	100.00
Left Bottom	651	10.12
Right Bottom	5,772	89.77
Left Top	2	0.03
Right Top	5	0.08

	Events	% of Vis
All events	6,428	100.00
Left Bottom	6,420	99.88
Right Bottom	2	0.03
Left Top	4	0.06
Right Top	2	0.03

	Events	% of Vis
All events	7,968	100.00
Left Bottom	6,529	81.94
Right Bottom	1,384	17.37
Left Top	52	0.65
Right Top	3	0.04

	Events	% of Vis
All events	6,613	100.00
Left Bottom	6,594	99.71
Right Bottom	4	0.06
Left Top	13	0.20
Right Top	2	0.03

- ❖ 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.

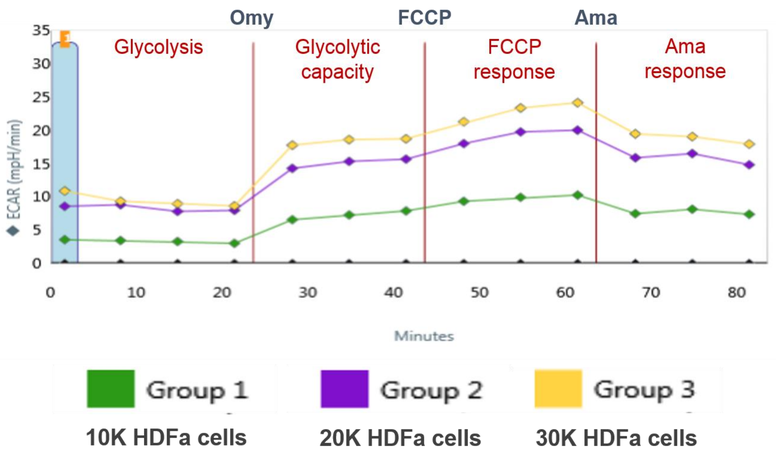
Mitochondrial function and bioenergetics of normal HDFa cells

The Seahorse Extracellular Flux Analyzers

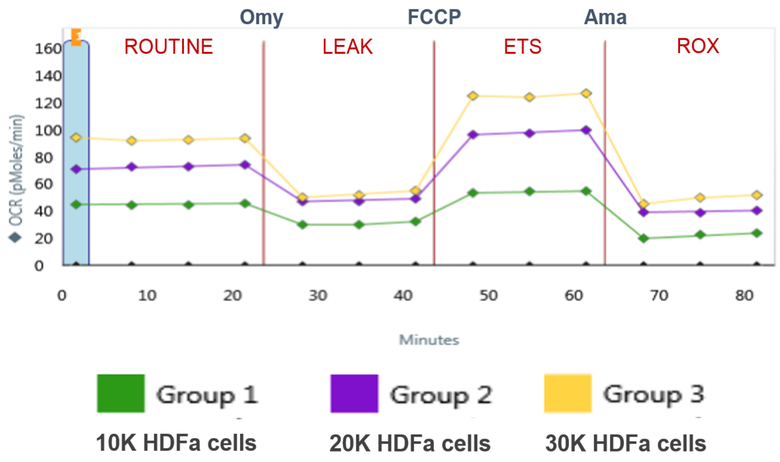
Mitochondrial respiration rates corrected to ROX: pmols/(min*no cells)

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

ECAR vs. time



OCR vs. time



- ❖ 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 O_2 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.

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