Oxidative Stress and Protein Oxidation in UV Related Human Cancer

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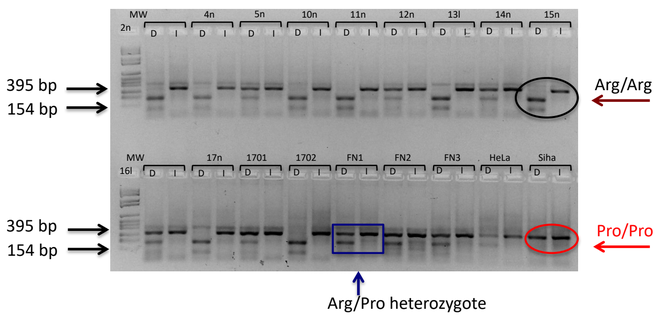
Introduction

- The UV component of solar radiation is the driving force of Skin Carcinogenesis .
- The incidence of Non Melanoma Skin Cancer (NMSC) is steadily increasing all over the world.
- New experimental data for improving the prevention and treatment of solar lesions are urgently needed.
- The role in skin carcinogenesis of UV damage to cell components other than the genome is largely underexplored.
- Here we report experimental data on the proteome oxidative damage in solar-induced skin neoplastic lesions and on the UV-B modulation cell phenotype in human primary fibroblasts.

Redox proteomic analysis of solar skin lesions

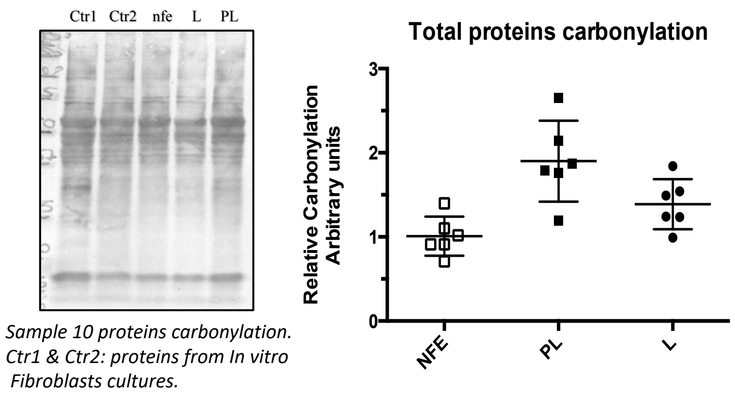
- A redox proteomic analysis was performed on solar skin neoplastic lesions from patients referring to the ISG IRCCS and requiring surgical treatment. This study was approved by the local ethical committee (approval release no. 745/17, 21-01-2016).
- Protein extraction and analysis from neoplastic areas, from peri-lesional areas and from a non photo-exposed region was performed as previously reported (De Marco F et al 2012).

The TP53 Pro72Arg Polymorphism is not associated with risk of solar skin lesions.



TP53 Exon 4 amplicons digested (D) or undigested (I) with 2.5 IU of *Bst*-UI. The ARG coding variant is cleaved into two 135 and 238 bp products. The PRO variant is unaffected. Both digested and undigested bands in D lanes indicate Pro/Arg heterozygousy.

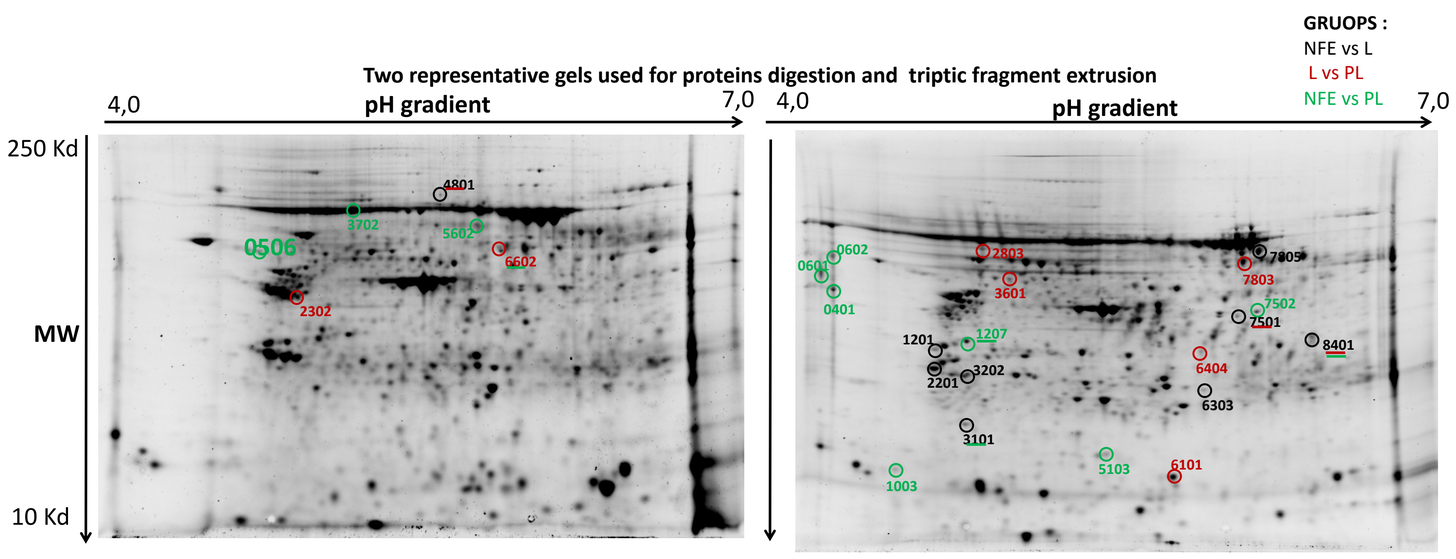
Peri-lesional (pre-neoplastic) tissues present high protein oxidation Neoplastic tissues appear to have a slightly increased protein oxidation.



Sample 10 proteins carbonylation. Ctr1 & Ctr2: proteins from In vitro Fibroblasts cultures.

The presence of Alpha and Beta Human Papillomavirus was evaluated according to Manos et al. (1989) and Berkhout et al. (1995) respectively. These highly oncogenic epitheliotropic viruses are well known modulators of tissue response to UV radiation (Perluigi M et al 2009) and to Oxidative Stress (De Marco F et al 2012) and may play a role in solar skin carcinogenesis. Only HPV negative samples were used for redox- proteomic assays.

Two representative gels used for proteins digestion and triptic fragment extrusion

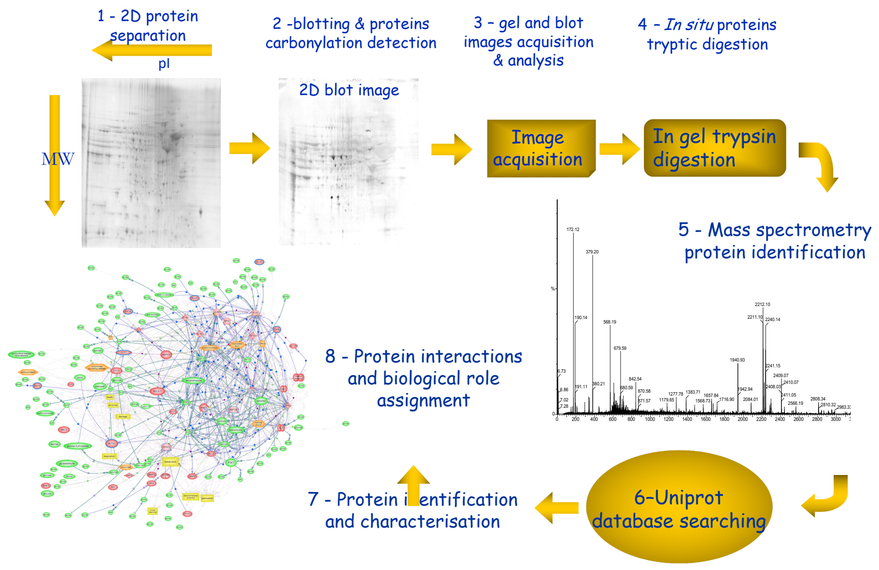


Differentially oxidised proteins areas can be assigned to a few functional groups

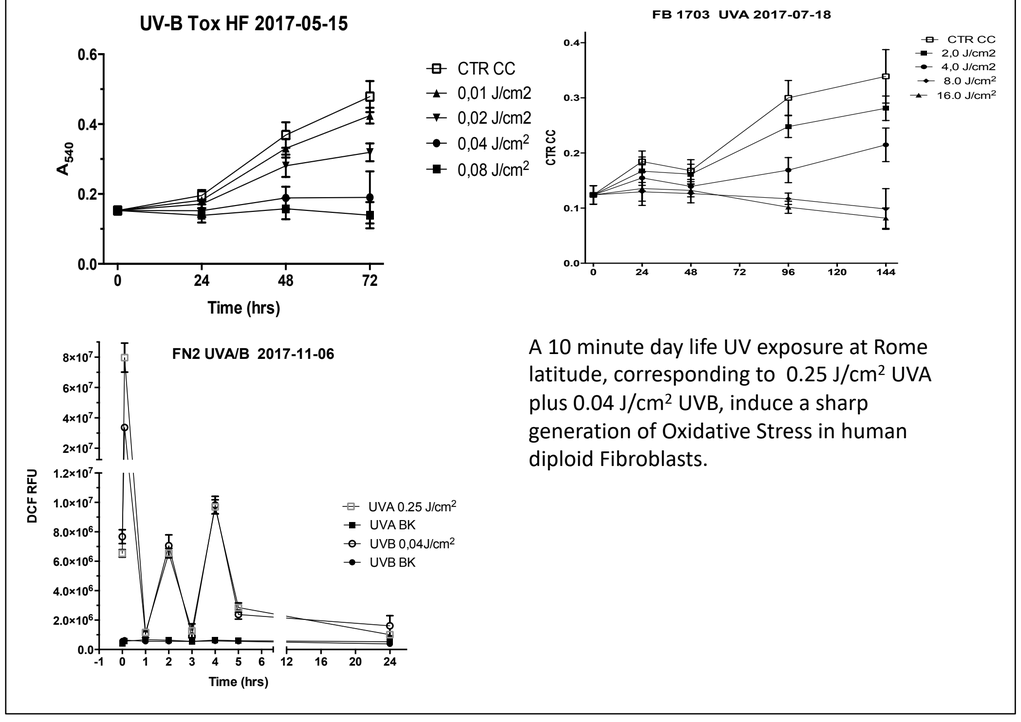
Chaperoning & Stress Response	PDI-A1 Protein Disulfide Isomerase A1; HS90B (Heat shock protein HSP 90-beta); HSP72 (Heat shock-related 70 kDa protein 2); HSP-90B1 (Endoplasmin); CRT Calreticulin; Endoplasmic Reticulum Chaperone BiP (GRP-78); HIP (Hsc70-interacting protein); UV excision repair RAD23B; Transitional Endoplasmic Reticulum ATPase; PRDX-2 Peroxiredoxin 2; Protein/Nucleic Acids deglicase DJ-1; Serpin B5; Nucleolin;
Protein folding/refolding & Protein qualitity control	PDI-A1 Protein Disulfide Isomerase A1; HS90B (Heat shock protein HSP 90-beta); HSP72 (Heat shock-related 70 kDa protein 2); HSP-90B1 (Endoplasmin); CRT Calreticulin; Endoplasmic Reticulum Chaperone BiP (GRP78); HIP (Hsc70-interacting protein); Transitional Endoplasmic Reticulum ATPase;
Protein phosphorilation	Serine/threonine-protein kinase SIK3
Proteasome function	UV excision repair RAD23B; UBP5 Proteasome subunit beta type-6 Proteasome activator complex subunit 1
DNA Damage Repair	UV excision repair RAD23B; Transitional Endoplasmic Reticulum ATPase; Protein/Nucleic Acids deglicase DJ-1;
Proteins and vescicle trafficing	HSP72 (Heat shock-related 70 kDa protein 2); Kinectin; Spectrin alpha
Cell architectural, cell adhesion & motility, ECM interaction	PDI-A1 Protein Disulfide Isomerase A1; HSP-90B1 (Endoplasmin); CRT Calreticulin; Myosyn-9; MAP-4 (Microtubule-associated protein); Dermatopontin; Vinculin; Collagen Alpha; Nucleolin;
Cell proliferation	Nucleolin; TD-52 TD-54 Serpin B5
Apoptosis/survival	HS90B (Heat shock protein HSP 90-beta); HSP72 (Heat shock-related 70 kDa protein 2); HSP-90B1 (Endoplasmin); Annexin A5; Nucleolin; UBP5; Apoptosis-associated spek-like proteins containing a CARD
Inflammation and Immunity	PDI-A1 Protein Disulfide Isomerase A1; CRT Calreticulin. Proteasome subunit beta type-6; Kinectin; Myosin -9;

A Redox proteomics outline

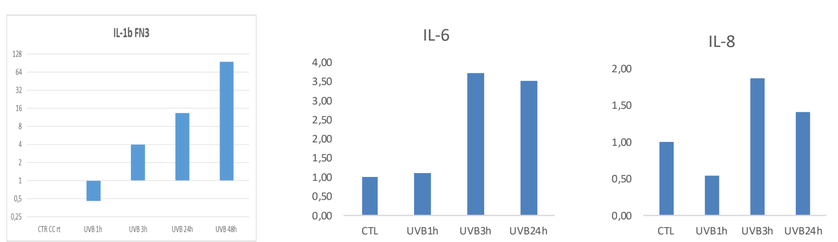
The combination of proteomics with Oxidative Stress biochemical related techniques enables the assessment of the proteome oxidative burden.

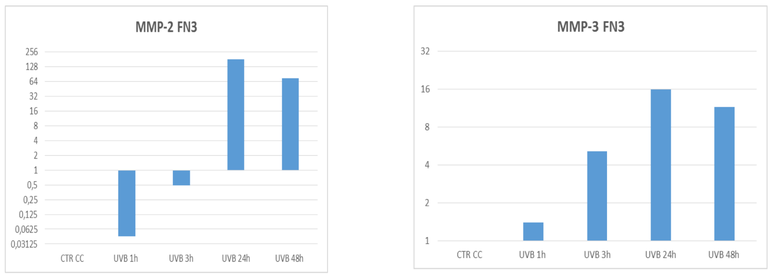


UV radiation generates Oxidative Stress and modulates fibroblast phenotype

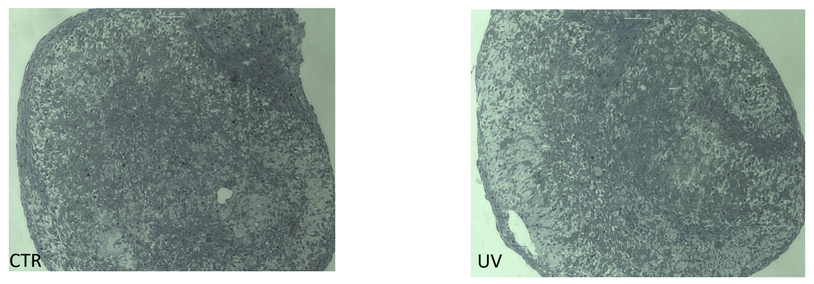


A 10 minute day life UV exposure at Rome latitude, corresponding to 0.25 J/cm^2 UVA plus 0.04 J/cm^2 UVB, induce a sharp generation of Oxidative Stress in human diploid Fibroblasts.





UV exposure induce an elevation of mRNA expression of inflammatory cytokines and of Matrix Metalloproteases.



3D spheroid cultures of human primary Fibroblasts.

TEM imaging reveals a marked increase of extracellular matrix upon UV irradiation.

Conclusions:

Protein oxidation in peri-lesional areas is consistently higher than in non photo-exposed areas.

Unexpectedly, proteins oxidation in peri-lesional areas is consistently higher than in lesional areas too!

Through Mass Spectrometry analysis, more than 40 differentially oxidised proteins were identified.

Oxidative damage mostly affects critical mechanisms in cancer promotion.
Namely: damage protection and repair of both proteome and genome.

UV irradiation of human Fibroblasts generates a sharp increase of endocellular Oxidative Stress coupled with the elevation of inflammatory cytokines, increased expression of Matrix Metalloproteases and an increased deposition of extracellular matrix, three distinct hallmarks of the Cancer Associated Fibroblasts (CAFs).

UV radiation, in addition to the well known mutagenic effect, perturb the proteome repairing activity and generates microenvironmental changes conducive to cancer. The UV component of solar radiation can act as both a cancer initiating and cancer promoting agent.

References

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