Glyoxalase enzymes prevent skin ageing

Researchers at Dior Science and the Université Pierre et Marie Curie (UPMC–Sorbonne Université) in France confirm that skin indeed possesses its own natural protection system.

Skin ageing is one of the starkest reminders of the passage of time. It is a complex process resulting from both chronoageing, which is intrinsic, and photoageing, which is extrinsic and caused by exposure to ultraviolet rays from the Sun. As skin ages it loses its elasticity, the epidermis and dermis progressively thin and cell turnover rate dramatically slows down. The dermal-epidermal junction also flattens. Skin ageing not only produces wrinkles, it also impairs many physiological functions – wounds take longer to heal, for example.

All these changes are mainly caused by damage to biomolecules such as proteins that can be modified by oxidative processes occurring in the body. This oxidation occurs every day and is a natural consequence of breathing, eating and living, but in skin, it can be accelerated by exposure to UV rays and all other forms of oxidative stress (such as pollution and cigarette smoke).

One example of a damage process is when the dicarbonyl compounds glyoxal (GO) and methylglyoxal (MGO), which are produced during various metabolic pathways, react with proteins. These compounds are highly reactive and modify cells and their components, such as DNA and proteins. Over time, such modifications produce irreversible cellular damage.

The good news is that there exists a natural line of defence against both GO and MGO in form of the glyoxalase (GLO) system, which is composed of the two intracellular enzymes glyoxalase 1 and 2 (GLO1 and GLO2). These enzymes work together to detoxify GO and MGO.

Researchers from the UPMC-Sorbonne Université and Dior Science have now shown in experiments for the first time that this glyoxalase system indeed exists in human skin. “This result is remarkable since it shows that dicarbonyl stress-induced glycoxidation does not only affect extracellular proteins, such as elastin and collagen, but also intracellular proteins,” explain team leaders Isabelle Petropoulos of the UPMC and Dior researcher Carine Nizard. “This intracellular glycation produces changes in the function of cells that make up the ‘living’ part of skin, not only the ‘architectural’ part (elastin and collagen). The GLO system is thus fundamental for preventing these changes and thus preserving skin homeostasis.”

The GLO system is found in the heart of skin cells – the keratinocytes of the epidermis (which are responsible for tissue renewal) and the fibroblasts of the dermis (the layer just above subcutaneous tissue). It detects and neutralises GO and MGO as soon as they are produced and thus prevents them from attacking cells and their components. It thus limits protein modification by GO and MG, which causes cell and, ultimately, tissue ageing. GO and MGO are eliminated and transformed first by GLO1 and then by GLO2, producing molecules that are not toxic, such as glycolate (in the case of GO).

There is a problem, however, say the researchers in that the GLO system appears to weaken with time and becomes less effective. This loss of activity is even more pronounced in photo-aged skin.

WHERE ARE THE GLYOXALASES LOCATED IN SKIN?

In their *in vitro* experiments, the researchers used antibodies against GLO1 and GLO2 to study how these glyoxalases are expressed in model primary keratinocytes and fibroblasts in both young and old (or senescent) human skin cells. They also looked at how active the enzymes were in these samples. They found that GLO expression was not modified in fibroblasts as skin cells age but that the activity and expression of GLO1 decrease. They also found that GLO2 was expressed in primary keratinocytes and that it also decreases with photo-exposition.

“The GLO system in skin appears to protect protein homeostasis in skin, especially in the basal layer of the epidermis, where stem cells are found,” explain Petropoulos and Nizard. “We have also obtained preliminary results showing that glyoxalases might play an important role in how keratinocytes proliferate and differentiate.

“The *in vitro* studies of GLO in senescent keratinocytes show that GLO1 activity decreases significantly, but without any modifications in its expression,” they add. “This change leads to an accumulation of proteins that have been modified by GO and MGO. These modified proteins also accumulate in samples of photo-exposed old skin, particularly in the dermis. However, they do not accumulate as much in non-photo-exposed skin samples.”

“In contrast, in the basal layer of the epidermis, where GLO1 is strongly expressed, the level of damaged proteins does not increase. The system thus appears to protect proliferating cells against damage.”

In *ex vivo* studies on slices of young and old human skin, the researchers found that GLO1 is expressed in the superficial layers of the epidermis and that this expression actually increases with age. Exposure to UV rays lowers this expression, however, regardless of the age of the skin sample. Skin containing lots of age spots also has a lowered GLO expression as does skin cells that have been artificially “stressed” by dicarbonyl molecules for 24 hours.

Ultimately, the Dior and UPMC scientists would like to harness these new results and find ways of maintaining the activity of the GLO system at its youthful level. This might be achieved by identifying antioxidant technologies capable of efficiently targeting the system and boosting it.■

www.dior.com/beauty/en_us/ds-discover-dior-science.html

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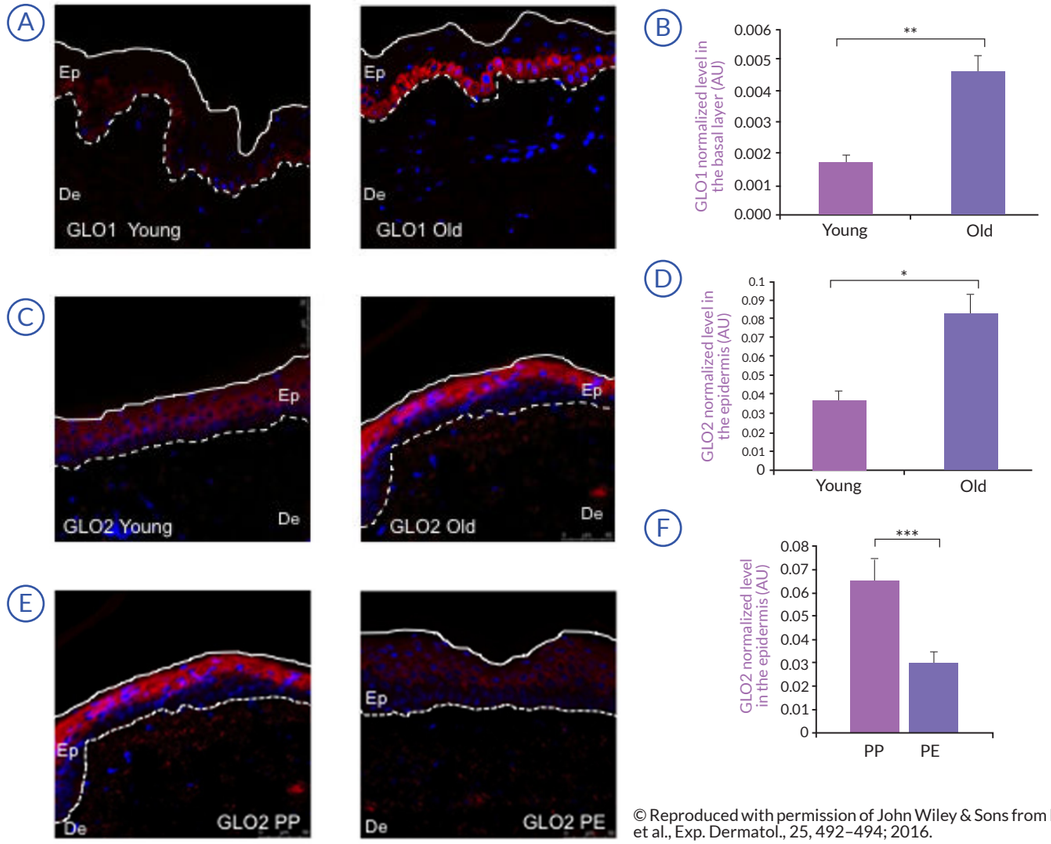
AGE, PHOTOEXPOSITION AND GLYOXALASE EXPRESSION

Localizing where glyoxalases are found in chronologically aged skin, quantifying their amount and looking at the effects of photoexposure during ageing



The researchers stained the nuclei of keratinocytes and fibroblasts with DAPI, a fluorescent stain used extensively in fluorescence microscopy, in human skin biopsies and detected glyoxalases with specific polyclonal antibodies. (A) and (C) The images show skin biopsies of 10 young (average age 27.5 +/- 1.7 years) and 10 old (average age 63.2 +/- 1.6 years) donors. Images were taken with a confocal laser microscope and the relative expression of glyoxalases quantified and standardized to basal layer area and number of cells of the basal layer for GLO1 and to the epidermis surface for GLO2. (E) Representative pictures of photo-protected (PP) and photo-exposed (PE) skin biopsies of 10 old (average age: 63.2 ± 1.6 years) donors. Images were again taken with a confocal laser microscope and the relative expression of GLO2 standardized to the epidermis surface. Ep: epidermis; De: dermis. The solid line indicates the outermost layer of skin and the dashed line indicates the basal lamina. (B) and (D) Quantifying GLO1 level in the basal layer of the epidermis and GLO2 in the whole epidermis. (F) Quantifying GLO2 expression in the epidermis.

THE GLYOXALASE SYSTEM

The GLO system is the main enzymatic system that detoxifies dicarbonyl compounds in biological cells and it catalytically converts reactive alpha-oxoaldehydes into their corresponding alpha-hydroxyacids. It is composed of two enzymes: GLO1 and GLO2 and requires glutathione (GSH) as a cofactor to work. GLO1 is a dimeric enzyme that has been passed down to us through evolution and is found in bacteria, fungi, plants, yeasts and mammals. It catalytically isomerises hemithioacetal, which forms spontaneously when the glutathionyl group of GSH reacts with aldehydes such as MGO and GO. Then, GLO2 (a thiolesterase that is active as a monomer) hydrolyses S-(2-hydroxyacyl)glutathione derivatives to regenerate GSH and produces hydroxyacids. MGO is transformed into D-lactate and GO into glycolate. GLO2 replenishes cells with GSH but GLO1 consumes it.

CARINE NIZARD AND ISABELLE PETROPOULOS

Glyoxalases and their protective role in skin

Interview with Carine Nizard, Dior Science researcher, and Isabelle Petropoulos, associate professor at the Université Pierre et Marie Curie (UPMC-Sorbonne Université).

The collaboration between UPMC and Dior Science began 18 years ago and was instigated by UPMC laboratory director Bertrand Friguet and research scientist Carine Nizard of LVMH laboratories. We talk to Carine Nizard and long-time colleague Isabelle Petropoulos of the UPMC about the group's work.

BEL DUMÉ: Could you explain the background to your work?

Carine Nizard and Isabelle Petropoulos: All the cells in our body rely on precise mechanisms that regulate protein homeostasis to maintain a stable and functional proteome. Cells find it more and more difficult to preserve the stability of their proteome with time, which leads to progressive protein alteration and subsequently normal skin ageing.

There are many systems in skin that eliminate unfolded and/or non-functional proteins and we began our research by studying one of the most important of these, the proteasomes, which exist in cells and the nuclei of cells. This system is modified during ageing, exposure to ultraviolet rays and all types of oxidative stress (such as pollution and cigarette smoke to name but two). LON protease, which is similar to the proteasomes found in cells, exists in mitochondria.

There are only a few systems to repair proteins (in contrast to the many that repair DNA) since there are only two amino acids that can be repaired once they have been oxidized. One of these systems is known as PMSR or MSR (Methionine Sulfoxide Reductase). This system exists in skin and protects it against oxidative stress.

BD: Could you describe your work on MSR and how it led to your research on the glyoxalase system?

CN and IP: We studied skin cells that had been exposed to UV rays in vitro and found that MSR expression and activity decline with age and photo-exposition. It thus becomes less efficient and protects cells less well. We also studied models of reconstructed skin and found that when it was oxidized it reflects less light (that is, its reflectance is reduced - as measured with a radiance meter).

Based on these results, we developed a cosmetic product that makes use of an active ingredient called lipochroman, which is a powerful antioxidant analogue of vitamin E and a potent scavenger for reactive oxygen species (ROS) and reactive nitrogen species (RNS), produced by environmental elements like pollution or UV rays. Lipochroman boosts the activity of MSR.

The GLO system is another, well-known, detoxification and anti-glycoxidation system in the body, similar to proteasomes and MSR, but it acts on a different level since its action precedes these to eliminate certain components toxic to proteins. Recently, researchers also discovered that it plays an important role in animal longevity, so we thought it was important to study how this system behaves in human skin.

BD: Could you briefly explain your research on glyoxalase and the most important results you have obtained so far?

CN and IP: We are looking at how glyoxalase (GLO) acts to eliminate certain products of metabolism and oxidative stress, like dicarbonyls such as glyoxal (GO), which are toxic for proteins and cells. Until now, researchers were mainly familiar with the role this system plays in protecting against diabetic vascular complications or cardiovascular disease but its importance in detoxifying dicarbonyls inside skin cells themselves was less known.

GLO eliminates dicarbonyls and so protects cells from further oxidation. As mentioned, it is a detoxification system but until now, we did not know whether it existed in skin. Our work is the first to show that it indeed exists in normal human skin, proving that dicarbonyl stress does affect keratinocyte proteins as well. The intracellular glycation and glycoxidation induce alterations in cell function. The GLO system is thus crucial for preventing these alterations and preserving skin's homeostasis.■

Read the full interview at
https://dior-skin-research.nature.com