GUARDIAN OF GENOMIC INTEGRITY
GETTING OLDER WITHOUT SIGNS

- Nowadays, individuals live longer but pretend to look younger, which is directly linked to cellular health and vitality.

- The genetic code (DNA) contains the inherited information that is responsible for cellular functioning.

- The link between healthy aging and DNA has become evident in the latest launches.

The products associating aging and DNA has been increasing for years, recognising its tight relation.

Source: Mintel GNPD, April 2013
DNA REPAIR AND REJUVENATION

- Genetic damage accumulates when aging, which leads to cellular alterations.
- DNA integrity is crucial to maintain cells properly functioning as well as an efficient repairing system.
- As UV exposure is the main responsible for premature aging, these repairing mechanisms are especially interesting in sun care products.

More products are arising every day working on DNA to rejuvenate skin cells, being particularly abundant in the USA and UK.
DNA is a chemical entity subject to the damaging influences of endogenous and exogenous genotoxic agents that even lead to cell death.

- Oxidative agents: such as reactive oxygen species (internal/external sources)
- Heat or radiation exposure: may cause cyclobutane pyrimidine dimers (CPDs)
- Pollutants: such as benzo(a)pyrene diol epoxide (BaP)
- Diet: vitamin and minerals deficiency
- DNA replication errors

DNA structure is monitored by checkpoint pathways involved in:
- Transcriptional programmes (converting DNA into RNA, followed by the formation of the protein).
- Cell cycle arrest (giving time to fix the damage).
- DNA-repairing processes activation (fixing nucleotide errors and DNA strand breaks).
- Programmed cell death (in case of severe alteration).
FOXO FACTORS AND LONGEVITY

- The forkhead box (FOX) transcription factors are implicated in controlling the expression of genes involved in vital functions in many species.
- The FOXO subfamily regulates the genes involved in cell repair, renewal and longevity:

  - *Hydra* genus has an unlimited lifespan due to an indefinite self-renewal capacity of its stem cells.
  - In the *Caenorhabditis elegans* worm, DAF-16 (member of the FOXO family) is linked to raised resistance to oxidative stress and expanded lifespan.
  - In mammals, there are four FOXO genes: FOXO1, FOXO3 (or FOXO3a), FOXO4 and FOXO6, FOXO3a being a key factor.
FOXO3a AND DNA-DAMAGE RESPONSE

FOXO3a is clearly involved in lifespan and cellular damage response, its active form (non-phosphorylated):

- regulates cellular redox status by increasing antioxidants levels (catalase...).
- leads to cell cycle arrest (quiescent stage).
- induces the expression of genes involved in DNA-damage repair (Gadd45a).
- triggers apoptosis in case of severe damage.

FOXO3a is a key element in DNA-damage control
FOXO3a ACTIVATION AND ITS RESPONSIVE ELEMENTS

- FOXO activity is regulated by a pathway where insulin, phosphatidylinositol-3-kinase (PI3K) and protein kinase B/Akt are crucial.

- Insulin indirectly leads to the phosphorylation of FOXO3a, turning it inactive.

- In the nucleus, active FOXO3a binds to specific regions of multiple selected genes, known as forkhead responsive elements (FHRE).

- Thus, FOXO3a has the capacity to cause a wide range of different actions affecting cellular fate.

FOXO3a affects the expression of several genes involved in the DNA-repair mechanism.
FOXO3a, A KEY FACTOR IN LONGEVITY

- Senescent cells lose the ability to divide and accumulate errors, presenting a downregulation of FOXO3a activity as they have:
  - lower levels of the active form of FOXO3a.
  - higher levels of the phosphorylated FOXO3a (inactive form).
  - superior levels of the phosphorylated Akt (active form), which inactivates FOXO3a.

- In humans, a reduced FOXO3a activity accelerates cellular senescence and an increased activity is linked to longevity (proved on different ethnic populations).

FOXO3a is a crucial transcriptional factor that regulates DNA-damage response, stimulating the natural repairing systems and delaying signs of skin aging.
GUARDIAN OF GENOMIC INTEGRITY

- Active peptide that imitates the activity of FOXO3a by reinforcing the natural mechanisms to repair DNA damage, rejuvenating skin cells.

- Activates DNA-repair pathways.
- Protects DNA and cells from UV rays and pollutants.
- Reverts cellular senescence.
- Diminishes UV-induced damage in the DNA, *in vivo.*
1. Activation of FOXO3a responsive elements

- FOXO3a can interact with the forkhead responsive elements (small DNA fragments located in the promoters of different genes), regulating the expression of multiple genes as a consequence.
- A stably-transfected human epithelial FOXO3a-reporter cell line expressing the luciferase gene upon activation of such elements was used.
- One set of plates was used for luciferase activity detection (cells were washed and incubated with the peptide or the medium alone) and a second set was used for colorimetric analysis with crystal violet staining (normalizing luciferase units/cell).
- Cells incubated with the vehicle alone were used as the control.

**JUVELEVEN™ peptide** is able to imitate the activity of FOXO3a

Both values were statistically significant.
2. DNA-repair pathways activation

- A host cell reactivation assay was performed on human epidermal keratinocytes (HEKα).
- Cells were transfected with an UVC-damaged plasmid constitutively expressing luciferase. Then, medium was removed and cells were incubated with JUVELEVEN™ peptide.
- Transfected HEKα with the irradiated plasmid have the luciferase gene disrupted, showing no luminescence.
- Transfected cells only treated with medium were used as the control.

**JUVELEVEN™ peptide activates natural DNA repair pathways involving FOXO3a**
3. Photoprotection test

- Pre-incubated human dermal fibroblasts (HDFa) with JUVELEVEN™ peptide were irradiated with simulated solar light (~60 J/cm²) for 210 min.
- Cell viability was determined after 24 h by neutral red uptake (NRU) method, measuring the optical density of the NR extracts at 540 nm in a spectrophotometer.
- Non-treated non-irradiated cells and non-treated irradiated cells were used as the controls.

**JUVELEVEN™ peptide** had a noticeable photoprotective effect.

Both values were statistically significant.
IN VITRO EFFICACY (IV)

4. DNA-protective effect against photoactivated BaP (I)

- Human normal fibroblasts, keratinocytes and melanocytes were incubated with BaP (40 µM) alone or together with JUVELEVEN™ peptide (10 µg/mL) for 2 h.
- Then, cells were irradiated (90 KJ/m²) with UVA/visible light (320-800 nm) for no more than 2 min at 4 ºC.
- DNA damage induced by photoactivated BaP was analyzed by the alkaline comet assay, expressed as olive tail moment (OTM).
- Non-treated irradiated cells and irradiated cells treated with BaP were used as the controls.

All three types of human skin cells were protected by JUVELEVEN™ peptide against BaP

A statistically significant DNA protective effect of 84.3%, 99.1% and 90.8% was obtained in fibroblasts, keratinocytes and melanocytes respectively.
IN VITRO EFFICACY (V)

4. DNA-protective effect against photoactivated BaP (II)

- FIBROBLASTS
- KERATINOCYTES
- MELANOCYTES

Non-treated cells  BaP  JUVELEVEN™ peptide + BaP
5. Reverting cellular senescence

Cellular senescence is reverted with JUVELEVEN™ peptide

The morphology of fibroblasts was recovered to that of 10 years prior.

• HDFa from a 55-year-old donor were treated with JUVELEVEN™ peptide and incubated for 24 h.
• A histochemical staining kit was used and β-galactosidase activity was determined (biomarker of cell aging).
Repair of UV-induced DNA damage (I)

- 21 volunteers between 25-45 years old, four test areas (two in the inner site of each forearm).
- Three areas were irradiated with 2 MED UV-light, two irradiated sites were treated with either a cream containing 2% JUVELEVEN™ peptide solution or a placebo cream.
- A suction blister biopsy of each area was collected to analyze CPD presence 6 h after irradiation and only three suction blisters (irradiated sites) were collected after 24 h.

A statistically significant reduction of pyrimidine dimers was induced by JUVELEVEN™ peptide, while placebo did not.

JUVELEVEN™ peptide diminishes UV-mediated DNA damage
IN VIVO EFFICACY (II)

Repair of UV-induced DNA damage (II)

Images showed less DNA damage when the peptide was applied (red spots)
COSMETIC BENEFITS

Guardian of genomic integrity

Cell rejuvenation by over 10 years

Key element in preserving the vital code

Decreases DNA damage in vivo

Key element in preserving the vital code
FINAL BENEFITS/FINAL PRODUCTS CLAIMS

- Stimulates the skin’s DNA-repair mechanisms to self-correct and eliminate DNA errors induced by daily agents (UV exposure, pollutants...)
- Bodyguard of genomic integrity
- Delays cellular senescence, improves cellular longevity
- Slows down visible signs of aging, elongating the youthful appearance of the skin
- Rejuvenates skin cells, moving 10 years backwards

juveleven peptide
DESCRIPTION
Hexapeptide that mimics the activity of FOXO3a (member of the Forkhead box transcriptional factors), which is involved in cell repair, renewal and longevity. JUVELEVEN™ peptide protects DNA from damage, stimulates its natural repair pathways and reverts senescence in fibroblasts.

APPEARANCE
Transparent solution containing 0.05% active ingredient.

INCI
Butylene Glycol, Water (Aqua), Acetyl Hexapeptide-51 Amide.
Preservative free.

PROPERTIES
JUVELEVEN™ peptide maintains genomic integrity by protecting and repairing DNA damage induced by several agents, and delays cellular senescence to ensure a longer and healthier aging.

APPLICATIONS
JUVELEVEN™ peptide can be incorporated into daily skin care formulations to protect the skin from DNA-damaging elements and enhance cellular longevity and vitality, helping to younger looking skin.

DOSAGE
2%

pH
Recommended pH range between 3.0 and 8.0.
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