Inflammation comes of age

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Innovacos Corp

Grand Seminar

Salt Lake City
November 14th, 2012
Inflammation Comes of Age

Inflammaging → Senescence

Age-related conditions → Aging

cause

consequence

cause
The Main Characters

• Aging
  - The process of becoming older, a process that is genetically determined and environmentally modulated

• Causes of aging
  - Multifactorial phenomenon
  - Reactive Oxygen Species (ROS) and “chronic” inflammation

• Inflammation
  - Acute, hours/days → UV-induced erythema
  - Chronic, micro or low grade, years → age-related conditions

• Senescence
  - A state of non-proliferation preventing cell tumoral progression
  - An adaptative response to stress
  - Senescent-associated secretory phenotype (SASP)
    → age-related conditions
Reactive Oxygen Species (ROS) and Inflammation

Aging

Age-related conditions
- neurological diseases
- osteoporosis
- diabetes
- obesity
- cancer

Inflammaging

Senescence

ROS

Inflammation
Senescence – A Cellular Commitment

• Complex mechanism(s) preventing proliferation of potential tumor cells
  - Cells lock themselves in a non-proliferative state

• Triggers
  - DNA damage response
  - Radiation, chemical stress
  - Inflammation
  - Oxidative stress (ROS) – Mitochondrial dysfunction

  Cells have sensed a critical level of damage

  Mechanism of protection against various forms of stress
Senescence-Associated Secretory Phenotype

• The signature of senescent cells is the acquisition of a senescent-associated secretory phenotype (SASP), which results in a striking increase in the secretion of pro-inflammatory cytokines
  - TNF-α
  - IL-6
  - IL-8
  - MMP-1
  - MMP-3

Transcription factor NFκB

Stress

Inhibitor - IκB

degradation

Catalytic “active” subunit

Inactive configuration
Senescence and NFkB Activation

Senescence trigger(s)
- Oxidative stress
- UV radiation
- DNA damage

Senescent state

NFkB

IkB

“active”

Oxidative damage

Inflammatory reactions

Degradation of skin macromolecules

Senescent cells remain metabolically active

- TNF-\(\alpha\)
- IL-6
- IL-8
- MMP-1
- MMP-3
Are there biological targets for cosmetic ingredients?
### Gene Expression in Aged Skin Cells – cDNA Array

<table>
<thead>
<tr>
<th>Gene</th>
<th>Aged cells vs Young cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>↓ 17%</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>↓ 61%</td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>↓ 54%</td>
</tr>
<tr>
<td>Collagen</td>
<td>↓ 42%</td>
</tr>
<tr>
<td>MMP-1</td>
<td>↑ 205%</td>
</tr>
<tr>
<td>MMP-3</td>
<td>↑ 2000% (20x)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>↓ 12%</td>
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**Reduced gene expression**

**Unregulated gene expression**
Gene Extract – *Laminaria saccharina*

- Rejuvenation of gene expression in aged fibroblasts

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<th>Functions</th>
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<tr>
<td>Catalase</td>
<td>↑163%</td>
<td>Scavenges (H_2O_2)</td>
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<tr>
<td>Glutathione reductase</td>
<td>↑739%</td>
<td>Recycles glutathione</td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>↑150%</td>
<td>Recycles thioredoxin</td>
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Recycling Antioxidants

Glutathione reductase

- Oxidized Glutathione
- Reduced Glutathione

- Neutralizes free radicals
- Reduces lipid peroxidation
- Recycles vitamin E

Thioredoxin reductase

- Oxidized Thioredoxin
- Reduced Thioredoxin

- Scavenges H$_2$O$_2$
- Over expression associated with increased longevity

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Gene Extract – *Laminaria saccharina*

- Rejuvenation of gene expression in aged fibroblasts

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**Targets of action**

- ROS
- Endogenous Antioxidant protection
- ECM Integrity
- Senescent cell

**TNF-α**
- IL-6
- IL-8
- MMP-1
- MMP-3

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Cellular Pathways Modulated by Senescence

How can we target those pathways?
→ Proteomics
Proteomics – Protein analysis

- Proteins are the main effectors of cell biological activity
  - Growth factors
  - Peptides (short proteins)
  - MMPs (enzymes that breakdown collagen and elastin)
  - Pro-inflammatory cytokines (IL-1,-6,-8)

- Proteomic is a complex method used to separate (charge and size) and identify (fingerprint) thousands of proteins

- Proteomic can be used to quantitatively assess the increase, or decrease, of the expression of specific proteins upon stresses or treatments
Proteomics – Protein analysis

1° Separating proteins based on their overall charge (isoelectric point, pI)
2° Separating proteins based on their molecular size (Daltons)
Plant Cell Vacuole Extract – *Vigna radiata*

- Selective extraction of the vacuolar content

*Vigna radiata* plant cell vacuole extract

- Minerals
- Nutrients
- Proteins
- Helps in plant growth
Plant Cell Vacuole Extract – *Vigna radiata*

- Modulation of protein expression (proteomic) in UV-stressed skin fibroblasts

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<th>Function(s)</th>
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<tr>
<td>IL-6</td>
<td>↓ 1.7-fold</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induces MMP-1</td>
</tr>
<tr>
<td>MMP-1</td>
<td>↓ 1.4-fold</td>
<td>Collagen degradation</td>
</tr>
<tr>
<td>MMP-9</td>
<td>↓ 3.4-fold</td>
<td>Elastin degradation</td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Collagen type I</td>
<td>↑ 5.4-fold</td>
<td>ECM integrity</td>
</tr>
<tr>
<td>Prolyl hydroxylase</td>
<td>↑ 1.7-fold</td>
<td>Collagen maturation</td>
</tr>
<tr>
<td>Integrin-beta</td>
<td>↑ 2.0-fold</td>
<td>Cell survival</td>
</tr>
</tbody>
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Targets of action

- TNF-α
- IL-6
- IL-8
- MMP-1
- MMP-3

Senescent cell
Inhibition of IL-8 Secretion

Vigna radiata inhibits the secretion of IL-8

- Control cells
- Cells stimulated with PMA
- Cells stimulated with PMA in the presence of VRSC

* p<0.01
Plant Cell Vacuole Extract – *Vigna radiata*

- **Vigna radiata**
  - Collagen type I
  - MMP
  - IL-6, IL-8
  - Cellular viability - ENERGY -

- **Prolyl-4-hydroxylase**
- **Stabilizes the collagen triple helix**
NFkB Activation – Role of IkB

NFkB

Activation

Inhibitor - IkB

degradation

Inactive configuration

Catalytic “active” subunit
Fermented Leaf Extract (FLE)

- Inhibition of NFkB activation

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<tr>
<th>LPS</th>
<th>FLE</th>
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<tr>
<td>Negative Control</td>
<td>Positive Control</td>
</tr>
</tbody>
</table>

IκB degradation inhibition
Action of FLE on NFkB Activation Inhibition

NFkB

"active"

IL-6
IL-8
MMP-1
MMP-3

NFkB

"inactive"

TNF-α

FLE

IkB

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Conclusions

• Oxidative stress and chronic inflammation
  - Aging process
  - Age-related conditions – cutaneous impact
• ROS, inflammation, DNA damage and UV
  - Cellular senescence – protection mechanism
  - Secretory phenotype – pro-inflammatory → NFkB

  Senescence participates to skin aging

• It is cosmetically possible to mitigate the deleterious side effects of senescence

  Laminaria saccharina algae extract – rejuvenating aged cell

  Plant cell vacuole extract – rebalancing UV-stressed cells metabolism

  Fermented leaf extract – preventing NFkB activation
THANK YOU

Inflammation comes of age

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