Co-regulation of Sirt1 and Pin1 Contributes to UV A/B-Induced Skin Photo Ageing

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BACK GROUND
• Skin ageing can be caused by many external and internal factors; UVRs are one of the main causes
• UV light promotes ageing by increasing ROS levels which interfere with critical pathways and cause DNA damage
• Resultant collagen breakdown and elevated levels of matrix metalloproteases leads to appearance of ageing marks like wrinkles and lack of elasticity
• Sirtuins; NAD+ dependent deacetylases are critical to many processes from inflammation to longevity.
• Sirt1 being most studied sirtuin for diversity of roles it plays in mammals was considered for plausible role in skin ageing and regeneration
• Combination of Sirt1 and Pin1 may be critical for regulation of oncogenesis because Pin1 is a ubiquitously expressed cis/trans-isomerase, and it is renowned for playing critical role in various cell death related diseases
• Pin1 is also known to regulate cell cycle by phosphorylation of cyclin D1 proteins
• Our study focused on Roles of Pin1 and Sirt1 along with their inhibitors and activators in ageing and rejuvenation of skin
• Moreover, mechanism of co-regulation of Pin1 and Sirt1 was also aimed in this study (Ref. Yaar M, Gilchrest BA, 2007. Langton et.al., 2010. Rache et.al., 2010. Finkel et.al., 2009. Wulf et. al., 2001)
METHODOLOGY
**In Vivo**

**Wound Healing Mice Models:** Mice were divided in four groups; normal, vehicle, KSY-PH1 0.1% and KSY-PH1 1% each with n=3.

**Wound Creation:** Wound was created in the posterior dorsal region of mice using a 6mm biopsy punch.

**Compound Treatment:** 0.1% and 1% solutions of KSY-PH1 in a mixture of Ethanol, Propandiol and distilled water were applied topically on the wounded skin everyday for 10 days. Vehicle group was treated with solvent only.

**Wound size and Physiological Factors:**
Wound diameter was measured in centimeters everyday using a scale and average was taken for plotting. Physiological factors of skin like erythema and melanin content were measured using combo system (DermaLab® Combo, CORTEX TECHNOLOGY, Denmark).
**In Vitro**

**Cells:** NHDF and HaCaT cells were purchased from Korean Cell line Bank (Seoul National University, Korea) whereas other cells were already being grown in the lab.

**UV Treatment:** Almost 80% confluent NHDF and HaCaT cells were treated with UVB light at a dose of 144 mJ/cm²

**Compound Treatment:** 1, 5 and 10 µM solutions of KSY-PH1 diluted in serum free media from 1mM stock were used for treatment.

**Flow Cytometry:** FACS Calibur® was used to measure ROS production after 24h of UVB and KSY-PH1 treatment.

**Wound Healing Assay:** Rate of cell migration after treatment of KSY-PH1 was checked by IncuCyte ZOOM (ESSEN BIOSCIENCE) in scratched cells every four hours of treatment.

**SDS-PAGE & Western Blot:** Expression of Sirt1 and Pin1 was checked by running total cell lysate on SDS-PAGE and western blotting.

**MTT Assay:** Cell viability after compound treatment and UVB exposure was checked by MTT assay.

**siRNA Knockdown:** Pin1 and Sirt1 specific siRNAs (BIONEER) were transfected to 30-40% confluent cell plates using Lipofactamine (Invitrogen). Cells were harvested for western blot analysis after 24-48 hours.
RESULTS
1. KSY-PH1 Decreases UVB Light-Induced ROS Production And Increases Cell Viability in NHDF Cells
2. KSY-PH1 Reduces Cell Apoptosis in UVB Irradiated NHDF Cells
3. KSY-PH1 Enhances Wound Healing \textit{In Vivo}

<table>
<thead>
<tr>
<th>Days After Skin Puncture</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSY-PH1 0.1%</td>
<td>![KSY-PH1 0.1% Image]</td>
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<td>KSY-PH1 1%</td>
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**Relative decrease in wound size**

- **Vehicle**
- **KSY-PH1 0.1%**
- **KSY-PH1 1%**

**Average Wound Diameter (mm)**

**Days After Skin Puncture**

- **0**
- **2**
- **4**
- **6**
- **8**
- **10**

- **Vehicle**: ![Vehicle Graph]
- **KSY-PH1 0.1%**: ![KSY-PH1 0.1% Graph]
- **KSY-PH1 1%**: ![KSY-PH1 1% Graph]
4. KSY-PH1 Enhances Wound Healing In Vitro

- **KSY-PH1 (μM)**
  - 0, 1, 5, 10

- **Time Points:**
  - 0h, 8h, 16h, 24h

- **Western Blot Analysis:**
  - α-Pak, Cdc42, Rac1, Tubulin

- **MTT Assay:**
  - Cell Viability (% of Control)
  - 0, 0.5, 1, 2.5, 5, 7.5, 10, KSY-PH1 (μM)
5. KSY-PH1 Restores Pin1 and Sirt1 Expression in UVB Treated Skin Cells

**Graphs:**

- **Pin1 expression upon UVB and KSY-PH1 treatment**
  - CTL: 100%
  - UVB: 150%
  - UVB + KSY-PH1: 100%

- **Sirt1 expression upon UVB and KSY-PH1 treatment**
  - CTL: 100%
  - UVB: 70%
  - UVB + KSY-PH1: 100%
6. KSY-PH1 Up-regulates Sirt1 in Skin Cells

**NHDF**

<table>
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<tr>
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<th>Sirt1 expression (%) of control</th>
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<tbody>
<tr>
<td>CTL</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1 KSY-PH1 (μM)</td>
<td>90</td>
<td>110</td>
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<tr>
<td>5 KSY-PH1 (μM)</td>
<td>80</td>
<td>120</td>
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<tr>
<td>10 KSY-PH1 (μM)</td>
<td>70</td>
<td>130</td>
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<td>1 Resveratrol (μM)</td>
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<td>5 Resveratrol (μM)</td>
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**HaCaT**

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7. KSY-PH1 is More Effective Than Its Structural Analogues

![Graph showing cell viability](image)

- **Pin1**
- **Sirt1**
- **Tubulin**

Different concentrations of SA-1 and SA-2 were tested to compare their effectiveness.
8. KSY-PH1 Acts As Sirt1 Activator Predominantly in Skin Cells

**BV-2 Cells**

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**KSY-PH1 (µM)**

**SA-1 (µM)**

**SA-2 (µM)**

**HUVE VC Cells**

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**KSY-PH1 (µM)**

**SA-1 (µM)**

**SA-2 (µM)**
9. Sirt1 Suppresses the Level of Pin1 in NHDFs
9. Sirt1 Suppresses the Level of Pin1 in NHDFs

**Figure:**

- **Pin1 profile after Pin1 & Sirt1 knock-down and KSY-PHI treatment**
  - Bars represent Pin1 expression (% of control) across different treatments.
  - Graph shows a decrease in Pin1 expression with treatments.

- **Sirt1 profile after Pin1 & Sirt1 knock-down and KSY-PHI treatment**
  - Bars represent Sirt1 expression (% of control) across different treatments.
  - Graph shows an increase in Sirt1 expression with treatments.

Below the graphs, treatments include:
- **siScramble**
- **siPin1**
- **siSirt1**
- **KSY-PHI (μM)**

Sample concentrations: 1, 5, 10 μM
• KSY-PH1 is a natural compound found in some plants which can act as a protective substance against ageing in skin cells
• KSY-PH1 promotes wound healing and rejuvenation *In Vitro* and *In Vivo*
• Being a novel activator of Sirt1, KSY-PH1 can have many important implications yet to be discovered
• Sirt1 can directly or indirectly suppress the expression of Pin1 which turns out to be its novel target
ACKNOWLEDGEMENTS

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