Prolonged activation of ERK contributes to the photorejuvenation effect in photodynamic therapy in human dermal fibroblasts

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Abstract

Photodynamic therapy (PDT) is known to be effective in the photorejuvenation of photoaged skin. However, the molecular mechanisms of rejuvenation by PDT remain elusive. In this study we aimed to understand the molecular events occurring during the photorejuvenation after PDT in dermal fibroblasts in vitro. First, we found that PDT conditions resulted in an increased fibroblasts proliferation and motility in vitro. Under this condition, cells had increased intracellular reactive oxygen species (ROS) production. Importantly, PDT induced a prolonged activation of extracellular-signal-regulated kinase (ERK) with a corresponding increase in matrix metalloproteinase (MMP)-3 and collagen type Iα mRNA and protein. Moreover, inhibition of PDT-induced ERK activation significantly suppressed fibroblast proliferation and expression of MMP-3 and collagen type Iβ following PDT. In addition, NAC (an antioxidant) inhibited PDT-induced fibroblast proliferation and ERK activation indicating that prolonged ERK activation and intracellular ROS contribute to the proliferation of fibroblasts and the dermal remodeling process for skin rejuvenation. We also identified increased collagen volume and decreased elastotic materials which are used as markers of photorejuvenation in human skin samples using histochemistry. Results from this study suggest that intracellular ROS stimulated by PDT in dermal fibroblasts lead to prolonged activation of ERK, and eventually fibroblast proliferation and activation. Our data thus reveal a molecular mechanism underlying the skin rejuvenation effect of PDT.

Result

Figure 1. Low level PDT treatment enhanced proliferation of human dermal fibroblasts. (A) ALA (5 mM) had cytotoxic effect on primary dermal fibroblasts. Cells were treated with different concentrations of ALA (up to 5 mM) for 12 h and cell cytotoxicity was measured by MTT assay. Results are mean ± SD. (B) The effect of irradiation dose of light and distance of the light source on fibroblasts. Cells were treated with different irradiation dose of the light and distance from the light source for 12 h and cytotoxicity was measured by MTT assay. Results are mean ± SD. (C) PDT treatment had no morphological change. Fibroblasts were treated with ALA (5 mM) and an incandescent light (30 mW/cm², 1 min) for different time points (30 min, 1 h, 2 h, 4 h). (D) Prolonged activation of ERK contributed to the proliferation of fibroblasts. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (E) PDT-induced fibroblast proliferation. Cells were treated with PDT for different time points and measured ROS generation by FACS using the DCFH-DA. As a control, cells were treated with ROS (100 μM) alone followed by PDT for 1 min. (F) PDT-induced fibroblast proliferation. Cells were treated with PDT for different time points and measured ROS generation by FACS using the DCFH-DA. As a control, cells were treated with ROS (100 μM) alone followed by PDT for 1 min. (G) Prolonged activation of ERK contributed to fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (H) PDT-induced fibroblast proliferation. Cells were treated with different concentrations of ALA (up to 5 mM) for 12 h and cell cytotoxicity was measured by MTT assay. Results are mean ± SD. (I) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (J) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (K) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (L) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (M) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h). (N) PDT-induced fibroblast proliferation. Cells were treated with ALA (5 mM) and irradiation for 1 min followed by treatment with PDT for different time points (30 min, 1 h, 2 h, 4 h).

Figure 3. PDT-induced prolonged ERK activation contributes fibroblasts proliferation and motility. (A & B) PDT-induced prolonged ERK activation. Fibroblasts were treated with ALA only (light only) and ALA and light (PDT) in (a) and (b) for 1 min. Images (c) and (d) for 30 min and cell frames were analyzed by western blot. (C) TNS-induced activation of MAPK in dermal fibroblasts. Cells were treated with TNS (50 μg/ml) for 30 min and cell frames were analyzed by western blot as a control of MAPK activation. (D) Nuclear localization of ERK is induced by PDT treatment in fibroblasts. Cells were treated with indicated conditions and immunofluorescence analysis were done by phase-contrast microscopy.

Figure 5. MMP-3 and collagen type Iα mRNA and protein levels after PDT was up-regulated in fibroblasts. (A) Cells were treated with PDT for indicated time points and mRNA was analyzed and analyzed by RT-PCR. (B) Cells were treated with PDT for indicated time points and collagen type Iα was analyzed by western blot. (C) PDT-induced regulation of mRNA and protein levels of MMP-3. Cells were treated with PDT for indicated time points and mRNA level was analyzed by real-time PCR and protein level was analyzed by western blot. (D) Inhibition of ERK activation by PD98059 suppressed up-regulation of MMP-3 and collagen type Iα mRNA after PDT treatment. Cells were pretreated with PD98059 (20 μM) for 30 min followed by treatment with PDT (20 J/cm²) and collagen type Iα mRNA levels were analyzed by real-time PCR. (E) PDT-induced ERK activation caused collagen in dermal fibroblasts. Cells were treated with PDT for 30 min followed by treatment with PDT for 24 h. Cell lysates were analyzed by western blot.

Figure 6. PDT resulted in histological changes indicating reformation of photoaged human skin. Skin biopsy specimens before and 1 month after the PDT were examined with histological-staining. Results from this study suggest that intracellular ROS stimulated by PDT in dermal fibroblasts lead to prolonged activation of ERK, and eventually fibroblast proliferation and activation. Our data thus reveal a molecular mechanism underlying the skin rejuvenation effect of PDT.

Conclusion

- Photodynamic therapy (PDT) is known to be effective in the photorejuvenation of photoaged skin. However, the molecular mechanisms of rejuvenation by PDT remain elusive.
- In this study, the authors aimed to understand the molecular events occurring during the photorejuvenation after PDT in dermal fibroblasts in vitro.
- They found that PDT conditions resulted in increased fibroblasts proliferation and motility in vitro.
- The fibroblasts had increased intracellular reactive oxygen species (ROS) production.
- Importantly, PDT induced prolonged activation of extracellular-signal-regulated kinase (ERK) with a corresponding increase in matrix metalloproteinase (MMP)-3 and collagen type Iα mRNA and protein.
- Inhibition of PDT-induced ERK activation significantly suppressed fibroblast proliferation and expression of MMP-3 and collagen type Iβ following PDT.
- NAC (an antioxidant) inhibited PDT-induced fibroblast proliferation and ERK activation, indicating that prolonged ERK activation and intracellular ROS contribute to the proliferation of fibroblasts and the dermal remodeling process for skin rejuvenation.
- The study also identified increased collagen volume and decreased elastotic materials as markers of photorejuvenation in human skin samples using histochemistry.

Reference