Photobiomodulation therapy is able to decrease *IL1B* gene expression in an *in vitro* cellular model of hidradenitis suppurativa

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Hidradenitis suppurativa (HS, Online Mendelian Inheritance in Man - OMIM#142690) is a chronic, autoinflammatory disorder affecting hair follicle [1]. This condition leads to the occlusion and rupture of the follicular epithelium generating subcutaneous nodules, dermal abscesses, fistulae, and sinus tracts [2]. HS usually manifests after puberty with a prevalence ranging from < 1 to 4% in general population [3].

Pathogenesis of HS is still quite unclear: environmental and genetic factors contribute to disease onset [4]. In familial cases, HS displays an autosomal dominant pattern of inheritance in up to 40% patients. To date, the most common mutations described are located in *NCSTN* gene, encoding for Nicastrin, one subunit of gamma-secretase [5, 6].

Combinations of antibiotics and immunosuppressant drugs are usually the first-line strategies in patients' treatment; however, there are severe cases that require surgery [7]. One of the main issues of patients that undergo these approaches is the critical wound care that impairs their quality of life. For these reasons in our previous work, we suggested the use of photobiomodulation (PBM) therapy as an adjuvant in order to accelerate healing process [8]. In fact, it has been demonstrated that laser light has properties of stimulation of wound healing, angiogenesis/vasodilatation, and anti-inflammatory and antimicrobial effects [8].

In this study, we analyzed the effect of PBM on *IL1B* gene (encoding for interleukin-1β [IL-1β]) expression in immortalized human keratinocyte cell line (HaCaT) using a wild-type line and a *NCSTN* knockout cell model mimicking genetic-driven HS, after treatment with bacterial lipoprotein Pam2CSK4.

Two irradiation protocols were selected using near-infrared (NIR) and Blue PBM based on our previous experience [9]. The protocol 970 nm, 0.3 W/cm^2, 20 J/cm^2, continuous wave (CW) was chosen in order to replicate the settings of our previous work [10], being the most effective protocol in terms of wound healing. Instead, the protocol 445 nm, 0.2 W/cm^2, 10 J/cm^2, CW was chosen after a viability assay using fluency at 10–30–50 J/cm^2, as the safest for cell lines (Fig. 1a, b).

We focused the attention on the gene expression of *IL1B*, a pro-inflammatory cytokine that participates in many inflammatory disorders, leading to an increased production of other inflammatory cytokines. Increase in IL-1β protein level was observed in HS keratinocytes in response to microbial product, and also in HS lesional skin biopsies [11]. According to this, we observed an increase of *IL1B* gene expression in HaCaT *NCSTN* knockout cells that became significant after Pam2CSK4 treatment, if compared with wild-type cells (Fig. 2a).

PBM alone is not able to induce *IL1B* expression, both in wild-type and in *NCSTN* knockout cells. On the contrary, both laser protocols (BLUE and NIR) after Pam2CSK4 treatment were able to significantly reduce *IL1B* expression leading us to hypothesize a PBM anti-inflammatory effect in each cell line (Fig. 2b, c).

PBM exerts a photochemical effect in which laser light interacts with photosensitive molecules such as chromophores present in mitochondria [8]; to date, the mechanism and the following modifications on cell signaling still need to be elucidated. It is known that the effect of laser light depends on the physiological state of cells, showing an evident impact especially on stressed cells [12], as the *NCSTN* knockout and Pam2CSK4-treated cells in our work.

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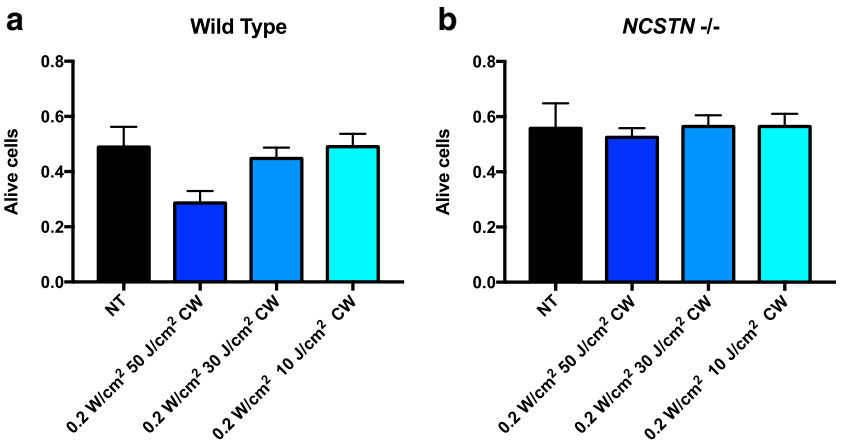

Fig. 1 Viability assay in wild-type (**a**) and a *NCSTN* knockout (**b**) HaCaT cells. HaCaT cells were irradiated 24 h after seeding with a diode laser device (class IV, K-Laser Blue series, K-Laser d.o.o., Sezana, Slovenia) with three blue laser protocols (Blue photobiomodulation (PBM)—445 nm, 0.2 W/cm2, 50–30–10 J/cm2, continuous wave (CW)). Metabolic activity was determined 24 h after irradiation by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) cell proliferation assay (M-6494, Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instructions. Absorbance was measured in a LT4000 plate reader (Labtech, Sorisole, Bergamo, Italy). The results are expressed as percentage of alive cells respect to not irradiated cells

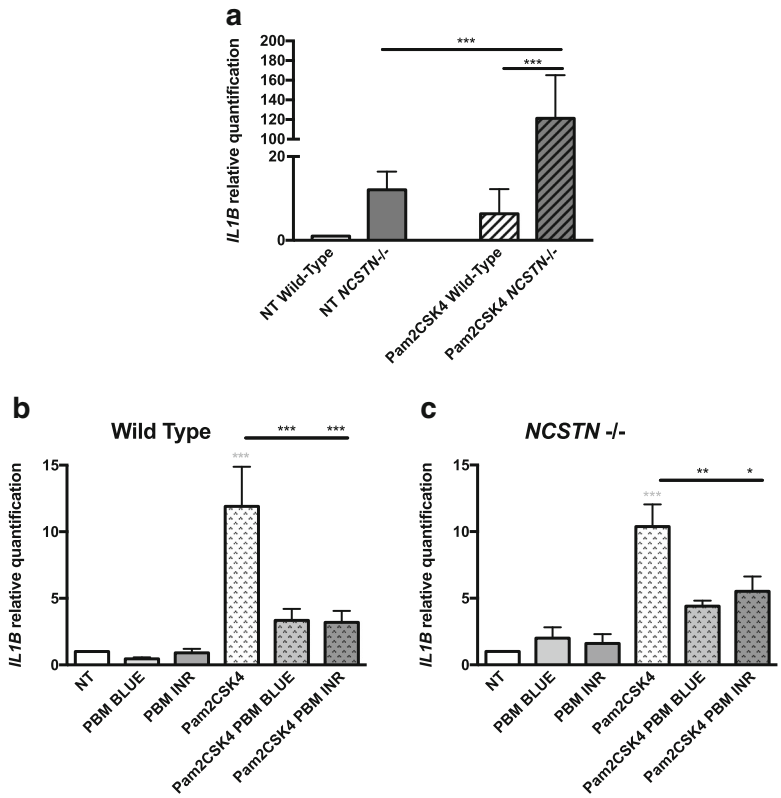

Fig. 2 *IL1B* gene expression in wild-type and *NCSTN* knockout HaCaT cells. *IL1B* gene expression after only Pam2CSK4 treatment in wild-type and *NCSTN* knockout HaCaT cells (**a**). *IL1B* gene expression after Pam2CSK4 treatment and PBM in wild-type (**b**) and *NCSTN* knockout (**c**) HaCaT cells. Cells were cultured on a 24-well plate at a density of 80,000 cells/well. After 24 h, cells were treated with 100 ng/μl of Pam2CSK4 (tlrl-pm2s-1, InvivoGen, San Diego, CA, USA) to induce an inflammatory state. The following day, cells were irradiated with two different laser protocols (Blue photobiomodulation (PBM)—445 nm, 0.2 W/cm^2, 10 J/cm^2, continuous wave (CW); and near-infrared (NIR) PBM—970 nm, 0.3 W/cm^2, 20 J/cm^2, CW). Total RNA was extracted 24 h after irradiation using TRIzol reagent (Euroclone, Pero, Milan, Italy) and retro-transcribed using High-Capacity cDNA Reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA). To quantify mRNA, TaqMan™ probes for IL-1β (*IL1B* Hs01555410_m1) and β-actin (as calibrator and reference, ACTB: Hs99999903_m1) gene expressions were employed on Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific) platform. Raw fluorescent data were converted in fold-increase with the Relative Quantification (RQ) manager software (Thermo Fisher Scientific) using the ΔΔCt method. The results are reported as RQ with respect to untreated cells (ANOVA test followed by Bonferroni's correction: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; in black bold is reported the p value for the comparison between Pam2CSK4 treated cells and the cells treated with Pam2CSK4 and irradiated; in gray is reported the p value for the comparison between not treated and Pam2CSK4-treated cells)

Hotz and collaborators hypothesized that there is a vicious circle of inflammation development, linked to an increase of proinflammatory cytokine productions that could foster a dysbiosis of skin-resident microbes contributing to perpetual skin inflammation in HS lesions [13]. The effect of PBM on the decrease of *IL1B* expression could help to block this vicious mechanism; in fact, PBM set at low irradiance does not generate any damage on cells [6] and it could be an additional tool in management and treatment of skin lesions in HS patients, considering also its ability to stimulate wound healing.

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Compliance with ethical standards

Conflict of interest G.O. has a part-time employment in K-Laser d.o.o. (Sežana, Slovenia). The other authors have no conflict of interest to declare.

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