

PDT WITH A GENETICALLY-ENCODED PHOTSENSITIZER MINISOG BY CONTINUOUS WAVE OR PULSED LASER IRRADIATION ON TUMOR SPHEROID MODEL

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Introduction

A genetically encoded photosensitizer miniSOG is a powerful instrument for investigation of cellular mechanisms of photodynamic therapy (PDT) [1, 2]. **The purpose** of this study was to investigate the phototoxicity of miniSOG in HeLa Kyoto 3D tumor spheroids upon CW and pulsed periodic laser irradiation and develop an effective regimen of PDT.

Materials and Methods

3D tumor spheroids: Human cervical carcinoma HeLa Kyoto, cultivation of 5×10^5 cells per well in the 96-well ultra-low attachment round bottom plates during 4 days.

Bright-field and fluorescence microscopy: DM IL LED (Leica, Germany), Axio Zoom.V16 (Carl Zeiss, Germany) ex. 450 - 490 nm, reg. 500 - 550 nm.

PDT: diode laser Epistar LED (Taiwan); 470 nm; 65, 35 or 120 mW/cm²; 20 min; CW or pulsed periodic modes (50 Hz; 10, 2.5, or 1.25 ms).

Cell viability assay: Apoptosis/Necrosis detection kit (Abcam, UK).

Tumor spheroid growth was estimated.

Results

Photobleaching of miniSOG

Maximum fluorescence decrease (60% or 75%) without temperature effects was reached at 120 mW/cm² in CW mode or pulse periodic mode with pulse duration 2.5 ms, respectively (Fig. 1).

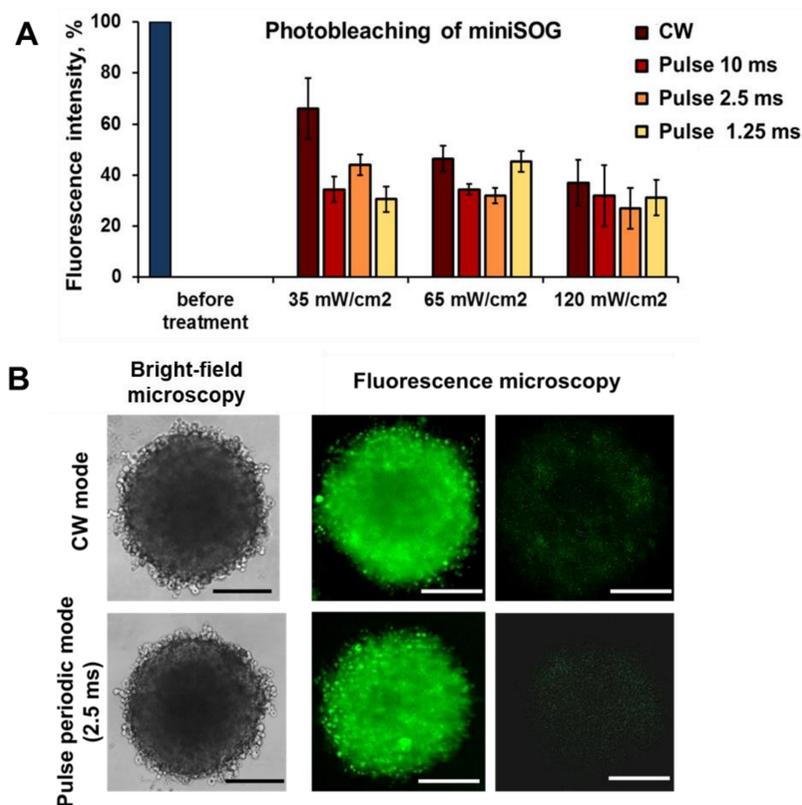


Fig. 1 Photobleaching of miniSOG in HeLa Kyoto tumors spheroids. A - Quantification of the fluorescence signal. B - Bright-field and fluorescence microscopy of tumor spheroids. Bar is 170 μ m

Phototoxicity of miniSOG

PDT in CW mode demonstrated a moderate expansion of central necrotic core of tumor spheroids, while pulse periodic mode provided an extreme increase in number of apoptotic cells, including late stages of apoptosis, in all zone of the spheroid. (Fig. 2).

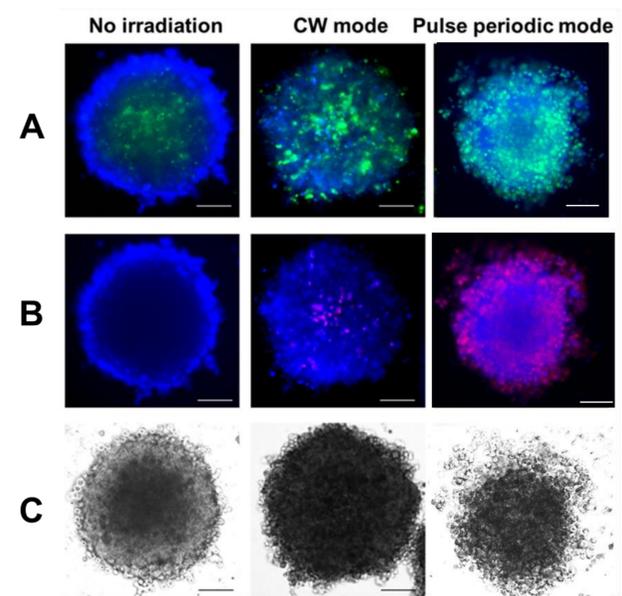


Fig. 2 The viability assay of HeLa Kyoto miniSOG-H2B tumor spheroids 4 h after PDT. (A, B) Fluorescent images. Blue channel - live cells, green channel - necrotic and late apoptotic cells, red channel - apoptotic cells. (C) Bright-field microscopy. Bar is 170 μ m.

PDT with miniSOG in CW mode slightly inhibited spheroids growth, while pulse periodic mode led to complete suppression of spheroid growth (Fig. 3).

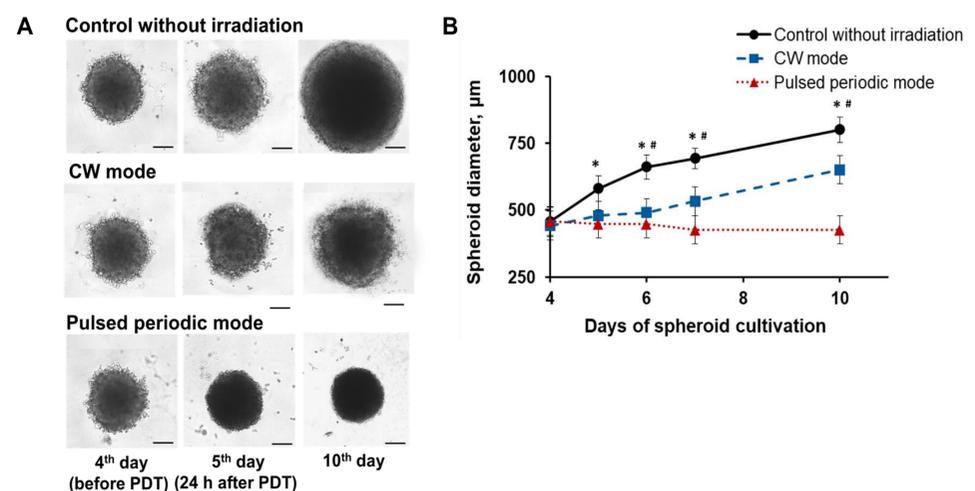


Fig. 3 Effect of PDT on tumor spheroids growth. A - Bright-field microscopy. Bar is 160 μ m, B - Growth rates of HeLa Kyoto miniSOG tumor spheroids. Mean \pm SD (n=5).

Conclusion

In summary, we report for the first time on an effective regimen for PDT with miniSOG in a tumor spheroid model upon pulsed periodic laser irradiation.

Acknowledgements

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References

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2. A.P. Ryumina et al, BBA, 2013, 1830(11):5059-5067.