BIOCONJUGATION OF THIOPHENE-BASED MOLECULES WITH PROTEINS: A POWERFUL TARGETING STRATEGY FOR PHOTODYNAMIC THERAPY

Soraia Flammini[1], Mattia Zangoli[1], Nicol Spallacci[1], Matteo Di Giosia[2], Roberto Saporetti[2], Alberto Danielli[3], Angela Tino[4], Claudia Tortiglione[4], Matteo Calvaresi[2], Francesca Di Maria[1].
[1]CNR-ISOF, Via Piero Gobetti 101, Bologna, 40129, Italy, soraia.flammimi@isof.cnr.it. [2]Dipartimento di Chimica "Giacomo Ciamician", UNIBO, Via Selmi 2, Bologna, 40126, Italy. [3]FaBiT, UNIBO, Via Belmeloro 6, Bologna, 40126, Italy. [4]CNR-ISASI, Via Campi Flegrei, 34, Napoli, 80078.

Photodynamic therapy (PDT) is a non-invasive treatment for different types of cancer. It relies on the use of photosensitizer agents (PS) able to generate reactive oxygen species (ROS) upon light irradiation to locally kill cancer cells [1]. Thiophene-based materials are characterized by a wide range of interesting properties such as controllable tuning of the absorption and emission, easy synthetic modification, geometrical adaptability, biocompatibility, and much more. These characteristics make them suitable candidates as light-activated ROS generators for PDT [2]. Nevertheless, due to their inability to target specific cells, a biological carrier is required. Recently, we showed that an oligothiophene-based PS, namely ECB04, upon conjugation with human serum albumin (HSA) to improve its cellular uptake, is able to promote the complete eradication of HeLa cancer cells [3]. However, with the employed protocol, just one or two ECB04 molecules are linked to an HSA protein strand and the obtained bio-conjugate shows killing activity only at a micromolar concentration (IC50 ≈ 2 μM). Here, we propose a novel bioconjugation strategy for ECB04 involving an engineered bacteriophage M13. This genetically modified virus can be used as a carrier to target specific receptors, particularly, the epidermal growth factor receptor (EGFR). Remarkably, the resulting bio-conjugate is decorated with more than 400 ECB04 molecules. This significantly enhances the cellular uptake in cells overexpressing EGFR and drastically reduces the half-maximal inhibitory concentration (IC50) down to picomolar concentrations (IC50 = 160 pM), which is one of the lowest concentrations ever observed for PDT treatment. This effect has been consistently observed both *in vitro* and *in vivo.* Finally, we conclude with an outlook on the design and synthesis of even more efficient ECB-like oligothiophenes, their bioconjugation with HSA, and some preliminary tests of the adducts, that demonstrate the enhanced killing capability of these new compounds.

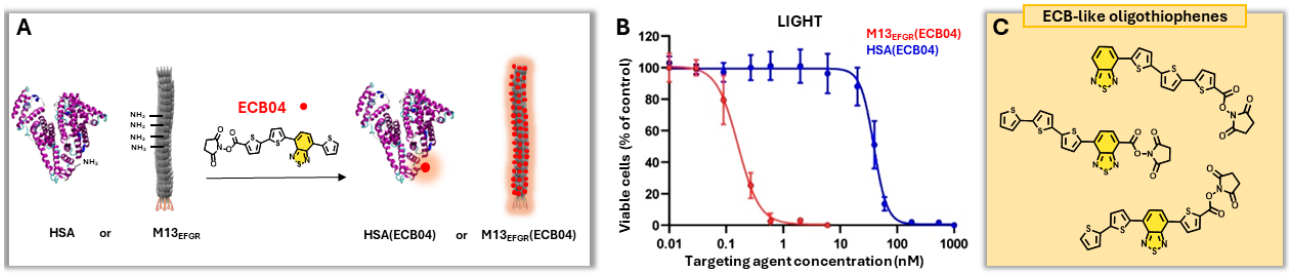

Figure 1. A) Chemical modification of HSA and M13 bacteriophage. B) Photo-dependent cytotoxicity on A431 cancer cells treated with $M13_{EGFR}$(ECB04) or HSA(ECB04) bioconjugates. C) ECB-like oligothiophenes molecular structures.

Key words: *Thiophene, ROS, Human serum albumin, Photodynamic therapy, Bacteriophage.*

REFERENCES

[1] X. Li et al., "Clinical development and potential of photothermal and photodynamic therapies for cancer", Nature Reviews Clinical Oncology, vol. 17, pp. 657–674, 2020.
[2] G. Barbarella et al., "Synthesis and Applications of Thiophene Derivatives as Organic Materials" in Advances in Heterocyclic Chemistry (E.F.V. Scriven, C.A. Ramsden, eds.), vol. 123, pp. 105–167, 2017.
[3] A. Cantelli et al., "Human Serum Albumin–Oligothiophene Bioconjugate: A Phototheranostic Platform for Localized Killing of Cancer Cells by Precise Light Activation", JACS Au, vol. 1, no. 7, pp. 925–935, 2021.