IL NUOVO CIMENTO **44 C** (2021) 142
DOI 10.1393/ncc/i2021-21142-2

COMMUNICATIONS: SIF Congress 2020

A novel targeting approach for melanoma cancer treatment based on photodynamic therapy

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received 15 January 2021

Summary. — Photosensitizing molecules have been at the basis of photodynamic therapy (PDT) since its early development in the 1950s. The therapy is a selective treatment method based on the administration of a photosensitizer to diseased cells: when irradiated with visible light of suitable wavelength, the photoactivated molecule starts a cascade of molecular transitions leading to cytotoxic effects in the targeted cells. The achievement of effective bio-compounds with photosensitizing capabilities and increased selectivity towards specific tumors is at the heart of the current research in the field. The aim of the herein project is to create an all-in-one multifunctional bio-molecule to be used in PDT treatments for melanoma cancer cells and holding targeting, imaging and photosensitizing potential.

1. – Introduction

Despite the remarkable scientific improvements of the last decade, cancer is still one of the leading causes of death worldwide [1].

The complexity of this multifaceted disease is reflected in the large number of therapies that have been introduced over the years. Indeed, current clinical treatments include chemotherapy, radiation therapy, surgery, immunotherapy and other targeted therapies, along with various combinations of these treatments [2].

However, in the majority of cases these approaches induce highly weakening side effects on the body. For example, chemotherapy is frequently associated with systemic discomforts such as nausea, mucositis and alopecia [3], and the impossibility to accumulate a high radiation dose often limits radiotherapy-based treatments [4].

In this context, photodynamic therapy (PDT) has been introduced as a modern and less invasive form of therapy, effective as a standing-alone technique, when possible, or in combination with other therapeutic protocols [5]. In addition to this, PDT is ideal for the treatment of superficial tumors such as skin cancers, which often do not necessarily require surgical excision [6] and can be easily reached by light irradiation.

One of the advantages of PDT lies in its limited toxic effects to the biological tissue [2]. Indeed, the photosensitizing molecule, also called photosensitizer (PS), is preferentially accumulated in the diseased cells and its cytotoxic action is triggered only when irradiated with light, thus limiting its action to the interested area only [7].

1˙1. *PDT mechanism.* – Antitumor effects of PDT are obtained with the combination of a photosensitizer, visible light and oxygen [8]; these three components are separately non-toxic but when together they initiate a photochemical reaction culminating in the formation of reactive oxygen species, including singlet oxygen ($^1O_2$).

After being administered to the cell, the photosensitizer is irradiated with light of suitable wavelength and promoted into the excited singlet state S^1 upon photon absorption [5]. In this state, the molecule is liable to return to the ground state by emitting light (fluorescence) or through non-radiative decay (heat emission); in addition to these two processes, the molecule can also shift to the excited triplet state T^1 through a process known as intersystem crossing [9] (fig. 1).

The triplet state is the therapeutic form of the compound: in this level, the PS can reacts with the cell by following two different pathways, type I and type II, respectively. Type-I mechanism leads to the production of reactive oxygen species (ROS) resulting in oxidative stress and consequent death of the cancer cells.

In type-II mechanism, the PS in the excited triplet state transfers its energy directly to the molecular oxygen naturally occurring in the biological environment: in this way, singlet oxygen is generated. The interaction of singlet oxygen with the cell membrane and other organelles induces an extensive damage leading to cell death.

Despite both mechanisms culminate in the destruction of cancer cells, type-II mechanism is the process which conditions the efficiency of PDT [9, 10].

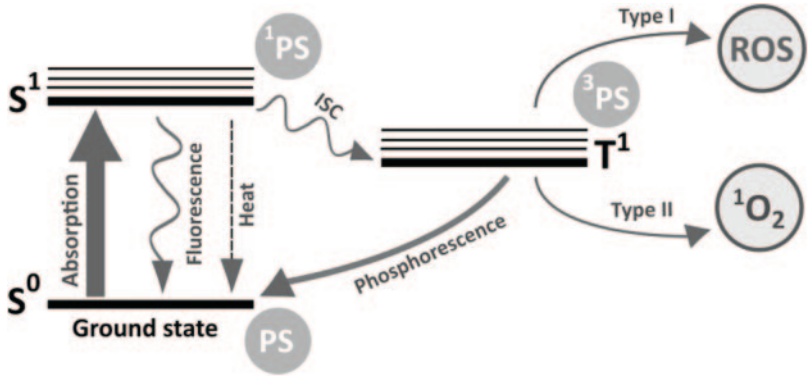
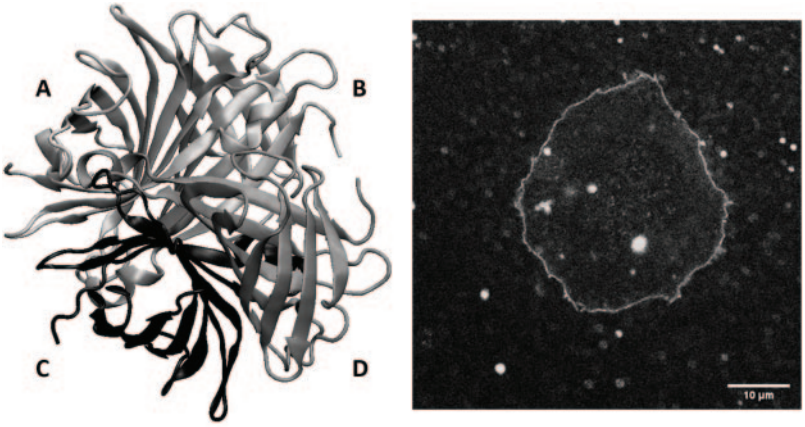


Fig. 1. – Mechanisms behind the photodynamic reaction. The scheme shows type-I and type-II photochemical mechanisms at the basis of PDT, where: PS is the ground state photosensitizer, ^{1}PS is the PS in first excited state, ^{3}PS is the PS in the excited triplet state, ROS are reactive oxygen species and $^1O_2$ is singlet oxygen.



(a) **(b)**

Fig. 2. – (a) A three-dimensional structure of streptavidin (PDB: *3wyp* [12]) with the letters indicating the 4 different biotin-binding sites. (b) A melanoma cancer cell showing the fluorescence emission of a streptavidin-FITC complex on its plasma membrane. The picture has been taken with a 100× oil immersion objective.

1˙2. *PS bioavailability*. – The majority of the existing photosensitizers are poorly soluble in water and tend to form aggregates in aqueous solutions [11], a condition in which their photophysics is dramatically impaired and their photosensitizing properties highly reduced. Aggregates also significantly reduce the bioavailability of the photosensitizing molecule at the cell entrance.

Furthermore, the lifetime of singlet oxygen is very short, $\sim 3.5\,\mu$s, thus limiting its action to only approximately 150 nm in cells [8]. This means that in order to be effective on cancer cells, singlet action must be formed in close their very close proximity.

For this reason, binding the photosensitizer to a carrier such as a protein [11] or incorporating it in nanostructured delivery system such as a nanoparticle [13] brings two important advantages: it significantly increases its solubility in physiological media and it allows its accumulation in the proximity of the targeted cells. In particular, proteins represents promising delivery systems thanks to their high biocompatibility.

2. – A novel approach

Our idea takes place from the need of developing a multifunctional carrier relying on the combination of three diversified properties which, when combined, can provide precise localization of the photosensitizer inside the cell environment.

In order to create such type of carrier, we selected a protein-based scaffold which can be extensively functionalized. Streptavidin is a tetrameric protein (fig. 2(a)) which shows extraordinary affinity for biotin, a water-soluble vitamin [14]; streptavidin-biotin interaction is among the strongest non-covalent affinity known ($\sim 1.3 \times 10^{-15}$) and the 4 subunits of the protein present single biotin-binding site each. This property can then be exploited to create a multifunctional carrier.

Indeed, besides having photosensitizing capabilities, the delivery system must have targeting capabilities in order to direct its disruptive photosensitizing action towards a specific target, such as melanoma cancer cells in our case (fig. 2(b)). More specifically, the targeting capabilities are established on the presence of a highly expressed membrane

protein. In addition to this, we decided to introduce a fluorescent probe: this can give imaging properties to the multifunctional delivery system, making it possible to spatially localize the compound inside the cell environment with an optical microscope before inducing the damaging effects of photodynamic therapy.

The overall advantage of such a kind of multifunctional system would be the introduction of a better localization of the compound in the biological environment, combining highly targeting capabilities with an effective photodynamic treatment.

3. – Conclusions and future outlook

A melanoma cell line has been selected for testing the effectiveness of this multifunctional compound. In conclusion, the preliminary work herein presented for introducing a multifunctional targeting-carrier, able to both efficently deliver a photosensitizer and to allow accurate spatial localization, will be followed by an extensive characterization of the compound.

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EU, SA and CV acknowledge support from Azienda USL di Piacenza, Italy, and Fondazione di Piacenza e Vigevano.

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