Dynamic Photosensitizer-Antibody Conjugates for Detection and Therapy of Cancer

A Thesis submitted for the fulfillment of

Ph.D. Degree of Science in Biochemistry

By

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Declaration

I declare that, this thesis has not been submitted to this or any other university.

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من

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Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Jemal *et al*., 2011 & Xu *et al.,* 2012). Developing targeted therapies that are selectively toxic to cancer cells while sparing normal cells may lead to more effective treatment options; of these therapies photodynamic therapy (PDT) which is a promising non-invasive treatment and has been approved by the U.S. Food and Drug Administration for the treatment of localized tumors (Lee and Baron, 2011). PDT can be defined as the administration of a non-toxic drug or dye known as a photosensitizer (PS) either systemically, locally, or topically to a patient with a lesion/tumor. After an incubation period, the lesion/tumour is targeted with a light of specific wavelength determined by the PS used. In the presence of oxygen, this leads to the generation of reactive oxygen species (ROS), singlet oxygen is the main and the primary phototoxic species generated by the most of the photosensitizers, its accumulation leads to the oxidative stress of tumors which results in necrotic and/or apoptotic cell death.

The use of PDT as a cancer therapy is particularly attractive owing to its specificity and selectivity as the PS concentrates specifically within the malignant tissue. For this reason, PDT is becoming a major subject of intense investigation as a possible treatment modality for various forms of cancer. One considerable advantage is fact that PDT is minimally invasive, much cheaper and has less harmful side-effects than conventional chemotherapy, radiotherapy or surgery (Agostinis *et al.,* 2011). Three main processes by which reactive oxygen species contribute to the destruction of tumors by PDT; (i) direct cellular damage, (ii) indirectly through the damage to tumor vasculature and (iii) by the activation of the immune response against tumor cells (Solban *et al.,* 2006 & Pizova *et al.,* 2012).

Skin cancers are ideally suited for PDT, Conventional treatments for skin cancer include surgical excision, ionizing radiation, and topical chemotherapy but PDT presents a novel alternative that is noninvasive, well tolerated by patients, and can be repeated without cumulative toxicity. PDT is the treatment of choice for patients with large or multicentric lesions, especially on facial areas. Also, PDT is preferred for patients with multifactorial health risks that prohibit surgical intervention (Zeitouni *et al.*, 2003 & Hönigsmann, 2012).

Indocyanin green (ICG), a tricarbocyanine dye known to emit fluorescence in the infrared range and is commonly used as an angiographic agent, exhibits characteristics of an ideal photosensitizer, in particular its strong absorption in the near infrared part of the visible spectrum (between 700–800 nm) allowing deeper tissue penetration without causing significant heating (Skrivanová *et al.*, 2006). The main two disadvantages of using ICG in PDT are that ICG easily binds to lipoprotein, which leads to the drug's rapid clearance from the body (a plasmatic half-life of around 2–4 min) and the in vitro and in vivo instability of ICG. Developing a biocompatible and biodegradable nanoparticulate system entrapping ICG molecules can provide efficient aqueous-stability, photo-stability and thermal stability to these ICG molecules (Kim *et al.*, 2010 & Sharma *et al.*, 2012). The protective effect of nanoparticles seems to be due to the polymeric-envelop, which protected the entrapped ICG by isolating it from the surrounding environment (Saxena *et al.*, 2004 & Kim *et al.*, 2012).

Kim *et al.*, (2007) presented a dual-function, nanosize agent for both early-stage cancer detection by photoacoustic imaging and localized cancer treatment by PDT. The agent is designed by encapsulating ICG dye in a biocompatible matrix. These nanoparticles were developed based on (photonic explorers for biomedical use by biologically localized embedding) PEBBLE technology. PEBBLE is a generic term to describe nanofabrication

techniques that utilize biologically inert polymers to manufacture nanometer-sized spherical optical sensing devices. PEBBLEs are specifically designed to be minimally invasive, facilitating analyte monitoring in viable single cells or cell cultures without perturbing normal cellular functions (Lee and Kopelman, 2012).

Although photosensitizers accumulate in cancer cells, the tumor specificity ratios are low; this can result in severe normal tissue damage after PDT of large surface areas. To improve specificity, photosensitizers have been coupled to targeting elements such as monoclonal antibodies directed against tumor associated antigens (Bhatti *et al.,* 2008). The obvious advantage of using an antibody as a vector for tumor localization of a photosensitizer is the property of antibody to bind specifically to a marker which is more abundant in tumor than in normal tissue. Another advantage of the photosensitizer-antibody conjugate strategy is that the photosensitizer can be selected on the basis of its optimum photochemical properties and not its tumor localizing capacity, since the tumor selectivity is provided by the antibody specificity (Milgrom, 2008).

Epidermal growth factor receptor (EGFR) is type-1 tyrosine kinase, it is a transmembrane glycoprotein found primarily on cells of epithelial origin. It plays a central role in numerous aspects of keratinocyte biology. EGFR is important for promotion of cell survival, increasing epidermal thickness and regulation of cell migration. Abnormal EGFR function has been described in epithelial tumors including those induced by two-stage chemical carcinogenesis in mice skin (Kiguchi *et al.,* 1998 & Cichocki *et al.,* 2012). A majority of squamous cell carcinomas have high EGFR expression. Overexpression of the EGFR by malignant cells is associated with poor prognosis and resistance to therapy. Because of the relationship between overexpression of EGFR and aggressive behavior of tumor cells, the antibody directed against EGFR potentially blocks activation of this receptor and

prove to have anti-tumor activity (Dlugosz *et al.*, 1997 & Burtness, 2005). Conjugation of a photosensitizer to the anti-EGFR monoclonal antibody can be used for increasing the tumor selectivity of this photosensitizer.

The polymeric nanoparticles containing ICG (ICG-PEBBLE) can be modified superficially using special targeting moieties (such as antibodies) for site specific action. ICG containing nanoparticles can be coated by a single layer of anti- EGFR antibody, resulting in nanocapsules that can bind specifically to EGFR overexpress cells (Kim *et al.*, 2007).

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