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Single component image guided 'On-demand' drug delivery system for early stage prostate cancer

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ABSTRACT

The early stage detection, diagnosis and treatment of prostate cancer are life-saving events in patients housing potentially invasive disease. The healthy human prostate accumulates the maximum amount of zinc compared to any soft tissue in the body. But in the contrary, an inability of cancer cells to accumulate zinc has been found with the development of malignancy resulting in a dramatic reduction in the zinc content of prostate tissue. In this current study, we designed and developed a dipicolylamine-coumarin-chlorambucil (Dpa-Cm-Cmbl) based system which can act as a sensor (for the prostatic zinc i.e. diagnosis) as well as photoresponsive drug delivery system (treatment). We demonstrated that Dpa-Cm-Cmbl being a single component system, first guided us to locate the diseased area by using cellular zinc concentration as a biomarker and in the next there was on-demand release of anticancer drug chlorambucil by employing the external stimulus light. In-vitro studies showed that Dpa-Cm-Cmbl presents excellent properties like detection of cancerous regions in the prostate, photoregulated drug delivery in controlled manner, biocompatibility and all together an efficient chemotherapy.

1. Introduction

Cancer is the most prevalent life-threatening disease at present and prostate cancer seemed to be the prime cause of death in men, preceded only by lung malignancy [1], and shows hardly any symptoms in its early remediable stage. Hence, the detection of prostate cancer prior to spreading beyond the confines of the organ could provide the only hope of a cure to patients. Although various approaches for diagnosis and staging of prostate cancer have been developed [2], still milestones to be achieved to get better control over the cancer and toxicity during the treatment.

Recent studies have revealed that mobile zinc is an excellent biomarker for early stage prostate cancer [3–9]. Mobile zinc is of the utmost important in prostate physiology. Zinc-rich prostatic fluid protects and helps to nourish sperm cells. The healthy prostate contains higher amount of zinc (3000 nmol/g) compared to other soft tissues in the body, and there is a clear and well established correlation between total prostatic zinc trafficking and cancer progression [2,10–12]. These zinc levels decrease during the development of cancer and this decrease is dramatic (6–9 fold drop) at the early stage [3,13,14]. Therefore, by monitoring the level of free zinc in prostatic cells we can determine the stage of cancer. Taking this advantage of the prostate cancer physiology, we wanted to develop a single component fluorescent probe based on coumarin platform that can serve the dual modalities; arresting the mobile zinc trafficking in prostatic cancer cells as well as controlled delivery of antitumor agent.

Photoremovable protecting groups have recently proved their potential as a very skilful tool in the field of biotechnology and biomedical applications [15,16]. Their specific photocleavable bonds enable biomolecule uncaging, purification of proteins, fluorescence activation, and drug delivery engaging extreme spatial and temporal control [17–19]. Though the externally triggered photocleavable protecting groups have several advantages but the main drawback of most of them is their fluorescent signal is not target specific leading to zero discrimination between healthy and tumor cells [20,21]. Absolute discrimination of tumor cells from normal cells followed by specific killing of cancer cells while leaving normal cells intact will lead to an efficient chemotherapy. To attain high selectivity, thus, an ideal drug delivery system (DDS) is necessary to control anticancer drug release only at the tumor.

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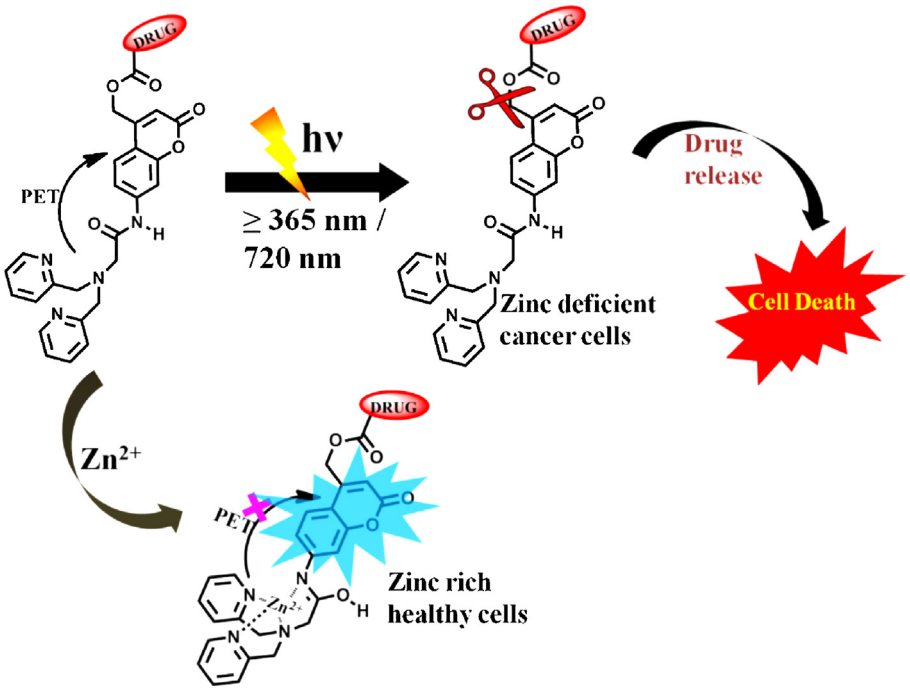

Scheme 1. Zinc activated turn-on fluorescence and monitoring of cellular mobile zinc followed by light triggered release of chlorambucil by Dpa-Cm-Cmbl.

We develop a new single component drug delivery system by integrating the two factors: first, the internal control, prostatic zinc level, which is used for the signal (fluorescent) output by the fluorophore and second, the external control employing the photorelease of antitumor drug chlorambucil either by visible light (one-photon) or NIR light (two-photon) (Scheme 1). In this system both the signaling subunit and the phototrigger are the same. Xu et al. first reported a coumarin derived transformable sensor (CTS) [22]. We employed the built-in S_N1-photocleavage of coumarin to construct a single component target specific photoresponsive drug delivery system by borrowing the design from Xu et al. The fluorescence of the system is quenched by photoinduced electron transfer (PET) from the binding site to the fluorophore. The cascade will start by complexation of the intracellular prostatic zinc leading to the termination of PET, fluorescence output and clear discrimination of tumor cells. Then, in the subsequent step the specific localized area will be irradiated by light resulting in the 'on-demand' release of the antitumor agent.

2. Experimental section

2.1. Materials and methods

2.1.1. Synthesis of dipicolylamine-coumarin-chlorambucil (Dpa-Cm-Cmbl) conjugate

2.1.1.1. Step (i). 7-amino-4-bromomethylcoumarin (**1**) (100 mg, 0.39 mmol) was treated with chlorambucil (120 mg, 0.39 mmol) in presence of potassium carbonate (K_2CO_3, 1.2 eqv.) in dry *N,N*–dimethylformamide (DMF) at 60 °C temperature for a period of 6 h. Then the reaction mixture was extracted with ethyl acetate (3 × 30 mL) and the solvent was evaporated under reduced pressure. The crude reaction mixture was purified by silica gel column chromatography using 20% ethyl acetate in pet ether to afford the caged conjugate **2** in 96% yield as a brown colored solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.81 (t, J = 7.8 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.478 (t, J = 7.8 Hz, 2H), 3.69 (m, 8H), 5.24 (s, 2H), 5.94 (s, 1H), 6.44 (s, J = 1.8 Hz, 1H), 6.56 (dd, J = 8.4, 1.8 Hz, 1H), 6.66 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 26.9, 33.1, 33.7, 41.6, 52.6, 61.5, 99, 105.3, 106.3, 111.8, 112.3, 125.9, 129.8 (2C), 144.9, 151.3, 153.7, 156.1, 161, 172.8.

2.1.1.2. Step (ii). A solution of 456 mg (2.2 mmol, 5 eqv.) of 2-bromoacetyl bromide in 5 mL of dry CH_2Cl_2 was added dropwise to a solution of 216 mg (0.45 mmol) **2** in 30 mL of dry CH_2Cl_2 stirred at room temperature. After stirred for 2 h at 40 °C, the solvent was removed under reduced pressure to give product **3** as a pale-yellow solid without further purification in 97% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.82 (t, J = 7.2 Hz, 2H), 2.47 (m, 4H), 3.69 (m, 8H), 4.05 (s, 2H), 5.34 (s, 2H), 6.30 (s, 1H), 6.66 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 7.67 (m, 2H), 7.92 (d, J = 1.2 Hz, 10.18 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 26.9, 33.1, 33.7, 41.6, 61.4, 62.4, 106.6, 110.7, 112.3, 112.7, 116.2, 125.6, 129.8 (2C), 142.6, 144.9, 150.6, 154.1, 160.3, 172.3, 172.7.

2.1.1.3. Step (iii). Finally, Dpa-Cm-Cmbl conjugate (**4**) was afforded by refluxing **3** (132 mg, 0.22 mmol) with di-(2- picolyl)amine (DPA) (42 L, 0.23 mmol), *N,N*-diisopropylethylamine (DIPEA) (0.4 mL) and potassium iodide (20 mg) in dry acetonitrile (30 mL) for 4 h. After coming to room temperature the solvent was removed under reduced pressure to obtain a yellow oil, which was purified by neutral alumina column chromatography (CH_2Cl_2:MeOH = 100:2) to afford **4.** ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.98 (t, J = 6 Hz, 2H), 2.46 (t, J = 6 Hz, 2H), 2.59 (t, J = 6 Hz, 2H), 3.57 (s, 2H), 3.61 (m, 8H), 4 (s, 4H), 5.27 (s, 2H), 6.37 (s, 1H), 6.63 (d, J = 12 Hz, 2H), 7.06 (d, J = 6 Hz, 2H), 7.31 (d, J = 6 Hz, 2H), 7.45 (d, J = 6 Hz, 1H), 7.68 (t, J = 6 Hz, 2H), 7.84 (s, 1H), 7.91 (d, J = 6 Hz, 1H), 8.65 (s, 2H), 11.44 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 26.5, 33.3, 33.9, 40.5, 53.6, 59, 59.8, 60.9, 107.4, 111.1, 112.2, 112.8, 116.1, 122.9, 123.6, 123.9, 129.7, 130, 134.7, 142.5, 144.4, 148.8, 149, 154.4, 157.3, 160.9, 170.3, 172.7.