How can nano-delivery systems selectively kill cancerous cells?

“...the future design of nanoparticles should contain a set of key locks each of them releasing an action upon the presence of the appropriate key.”

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Treating cancer & its side effects

Cancer is one of the most widespread diseases and one of the biggest causes of death in the present day. Recent statistics have shown that there are now 2.5 million people living with cancer in the UK with a thousand new cases being diagnosed every day. Despite ground-breaking scientific advances in cancer research, mortality rates are still high due to the low efficiency of modern day chemotherapeutic drugs [1].

Nowadays, most forms of cancer can be treated with various chemotherapy agents. However, their high potency and toxic mechanism of action result in undesirable side effects. While formulations diffuse and distribute freely throughout the patient's body targeting and killing cancer cells, rapidly dividing healthy cells such as hair, skin or gastrointestinal cells are also affected leading to their death and an overall decrease in patient's well-being. Therefore, the ability to direct drugs *en masse* to a specific site of interest avoiding nonspecific distribution remains one of the biggest challenges to drug developers [1].

Recently developed nanoparticle-based drug delivery systems are able to overcome the pharmacokinetic limitations associated with conventional drug formulations. For example, they can be designed to detect overexpression of proteins (e.g., HER-2, HIF-1 and vimentin) while delivering therapeutic drugs. These drugs are able to interfere with the overexpressed proteins blocking proliferation of cancer cells and ultimately leading to targeted cell death [2].

Advanced nanoparticle designs

Current research is focusing on the development of advanced types of nanoparticles to overcome the major drawbacks associated with therapeutic drugs. For example, iron oxide nanoparticles have shown to be effective as heat mediators under an alternate magnetic field leading to complete apoptosis-mediated death of cancer cells [3]. On the other hand, gold nanoparticles (AuNPs) have emerged as candidates for targeting cancer cells [4–6].

Because of their small size, AuNPs, are easily taken up by cells and can be specifically functionalized to interact with biomolecules of interest, whilst displaying low cytotoxicity. Gold nanospheres (AuNSs) are especially promising due to their fast and facile synthesis, as well as ease of bioconjugation [7]. For example, it was shown that the conjugation of AuNSs with functional peptides results in highly active antiangiogenic therapeutics, owing to the increased local reactivity of the active peptides on the nanoparticle surface [8].

A very promising surface modification agent for AuNSs, is DNA [9–11]. AuNSs functionalized with a shell of oligonucleotides (SNAs) have been shown to traverse the charged cell membrane and enter a large variety of different cell types despite their polyanionic nature [12]. By contrast, free single or double stranded oligonucleotides of the same sequence usually require the use of lipid or cationic transfection agents in order to overcome the Coulombic repulsive forces and enter the cell. Moreover it

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was shown that SNAs possess superior intracellular stability with increased resistance against degradation by nucleases. Additionally, their inherent property to bind complementary nucleic acids inside cells has led to the development of live cell mRNA detection agents [13–15]. Different research groups have shown the possibility of detecting, at real time, cell-to-cell variations in mRNA expressions using SNAs [14,16,17].

Creating ‘smart’ nano drugs by design

Ongoing work in our group is focused on tailoring the design of oligonucleotide-modified AuNPs to develop a new class of drug carriers, which can release their payload only in the presence of specific mRNA cellular signatures [2].

Preliminary experiments were designed to specifically detect and interfere with epithelial to mesenchymal transition, a process heavily involved in tumor metastasis. During epithelial to mesenchymal transition, naturally stationary epithelial cells undergo several biochemical changes to adopt mesenchymal-like characteristics, which include increased motility and elasticity, as well as invasive behavior. Some of these biochemical changes are the loss of epithelial markers (e.g., desmocollin and keratin-8) and the development of mesenchymal markers such as vimentin [18].

The nanoprobe designed by our group were biocompatible and could successfully and selectively detect mRNAs encoding for vimentin, desmocollin or keratin-8 in epithelial and mesenchymal cells in real time. The design of the nanoprobe was further advanced to create a drug delivery system that would only release its payload in the presence of a specific mRNA target. An anticancer drug (doxorubicin) was loaded onto our gold-DNA nanoprobe by means of intercalation between the DNA duplex [19]. Live cell experiments demonstrated that these nanoprobe

released the intercalated DOX only in the presence of specific mRNA targets. For example, if a vimentin-detecting probe was loaded with doxorubicin (DOX), both cell types would uptake the drug-loaded probes, yet the payload would only be released in the vimentin mRNA expressing cells and result in cell death.

This development represents a significant breakthrough toward the elimination of nontargeted drug release and opens up new directions in the treatment of several diseases offering a choice to selectively attack and kill only the cells of interest leaving healthy cells intact.

Future perspective

The design of advanced nanoparticulate systems that can perform more than one task within a complex biological environment is of high importance for the development of a new generation of nanotherapeutic agents. The ability to perform each of these tasks should be controlled by independent stimuli within the complex biological environment. Practically, the future design of nanoparticles should contain a set of key locks each of them releasing an action upon the presence of the appropriate key. Advancement of nanoparticles on this level will allow us to tackle critical issues related to delivery, specificity and efficiency of drugs.

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