Engineered Bacteria and Nanomedicine Platforms: Two Different Approaches for the Common Goal of Removing Tumor Cells

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ABSTRACT

In this paper two different approaches regarding the removal of tumor cells are explained. The first one is taken from the scientific article *Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria*[1]; and the second one stems from the article *A Smart and Versatile Theranostic Nanomedicine Platform Based on Nanoporphyrin*[2].

Whilst the first article follows a synthetic biological standpoint upon modifying E.coli bacteria in order to locate and eliminate tumor cells, the second one is focussed on the use of a polymer-based nanoporphyrin platform, which integrates a broad range of clinically relevant functions.

Author Keywords

E.coli Bacteria, Invasin, Nanoporphyrin, Synthetic Biology, Nanomedicine

INTRODUCTION

Cancer is probably one of the biggest challenges medicine is facing nowadays. Taking into account it cannot be considered a mere disease but an abnormal mutation in the cell reproduction, this fact renders this serious biological anomaly an evasive and extremely difficult-to-resolve nature.

For the last decades the use of radiotherapy and chemotherapy plus surgical interventions has been the optimal tool to eradicate the malign tumors our bodies have wrongly developed. Nonetheless, bearing in mind the aggressiveness of this treatment and the amazing evolution of synthetic biology and nanomedicine, new lines of research have been launched regarding the cancer treatment and cure.

In this article two likely solutions to cope with tumor removal are analyzed: the first one is related to the use of engineered bacteria -a synthetic biological approach; and the second one is based on the use of a polymer-based nanoporphyrin platform -a nanotechnological approach.

Regarding synthetic biology, this is a discipline concerned with the design, construction, and modification of biomolecular systems and organisms to perform specific functions. Synthetic biology tries to apply the engineering paradigm of systems design to biological systems in order to produce predictable and robust systems with novel functionalities that do not exist in Nature. The first use of the term "synthetic biology" was in Stéphane Leduc's publications *Théorie physico-chimique de la vie et générations spontanées* (1910) and *La Biologie Synthétique* (1912). Sixty-four years later, in 1974, the term "synthetic biology" gained its more modern usage when Polish geneticist Wacław Szybalski used it to envision the whole new horizon which was being opened by the progress of the molecular biology.

With respect to nanotechnology, current advances in the domain have been receiving much attention for the past two decades, both from the academia and the industry. A plethora of applications in a wide range of fields are currently available; however, what appears to be amongst the most promising endeavors is the development of nanotechnological constructs targeted for medical use. Nanoparticle-based theranostic agents are emerging as a promising paradigm towards personalized nanomedicine. The integration of imaging and therapeutic functions into a single nano formulation allows precise diagnosis of disease, individualized selection of treatment modality, real-time monitoring of drug distribution/delivery, and assessment of therapeutic outcomes. Several 'soft' organic nanoparticles -for example, paclitaxel (PTX)-loaded polymeric micelles (Genexol-PM), liposomal doxorubicin (Doxil), and PTX-loaded human serum albumin nanoaggregate (Abraxane)- have been approved or are currently in clinical trials for the treatment of human cancers, because of their excellent biocompatibility and drug-loading capacity[2].

To successfully achieve multifunctionality, nanoparticles should be straightforward to prepare and 'intelligent' enough to overcome *in vivo* biological barriers such as interactions with blood proteins, lipoproteins, blood cells, blood vessel walls, and the reticuloendothelial system, and be able to deliver drugs efficiently in a targeted manner to diseased tissues.

THE TWO CONCEPTIONS

A.-The Synthetic Biological Approach

Emerging applications of synthetic biology are amongst others the design of bacteria to produce therapeutic agents and the use of live bacteria as targeted delivery systems. Regarding the latter, bacteria can sense their environment, distinguish between cell types, and transmit proteins to eukaryotic cells[1]. In this delivery situation it is paramount to control the interaction of a bacterium with a mammalian cell and to regulate it in response to environmental stimuli. Bacteria have several systems to interact with and manipulate eukaryotic cells. Redundancies of these systems and their complex regulatory control complicate the engineering of natural bacteria. In contrast and as stated in the paper, the inv gene encoding invasin from Yersinia pseudotuberculosis represents a single-gene output interface for initiating adhesion and invasion of mammalian cells when expressed in E.coli.

Invasin (inv) binds tightly to ß1-integrins present on the surface of many cell lines and induces bacterial uptake. The transfer of inv to E. coli is sufficient to induce the invasion of mammalian cell lines that express ß1-integrins. Moreover, the therapeutic potential of inv^+ E.coli has been explored by constructing strains that can deliver proteins and plasmids into mammalian cells.

According to the scientific article, inv^+ E.coli can invade a broad range of tumor cells including epithelial, hepatocarcinoma, and osteosarcoma lines. The bacterial internalization can be synthetically linked to cell density, hypoxia, and inducible inputs (*Appendix*, *Fig. 1*). Under conditions of low cell-density or normal aerobic growth, engineered bacteria are non-invasive. Above a critical cell density or in a hypoxic environment, sensors are activated resulting in the synthesis of invasin from Y. pseudotuberculosis and the invasion of tumoral cells.

Invasin is a long rigid protein that is anchored in the outer membrane and extends 18 nm from the bacterial cell surface. Binding of ß1-integrins does not require additional bacterial proteins to confer invasion since latex beads coated with invasin are taken up by mammalian cells.

Although the efficiency varied, the experiments performed by the North American researchers demonstrated that inv^+ E.coli were capable of invading cancer cell lines of diverse origin. Nevertheless, not all mammalian cells are susceptible

[1] Eukaryotic cells are the type of living cells that form the organisms of all of the life kingdoms except monera. Protista, fungi, plants, and animals are all composed of eukaryotic cells. These cells have evolved ways to partition off different functions to various locations in the cell. In fact, specialized compartments called organelles exist within eukaryotic cells for this purpose.

to invasion: inv+ E.coli will only invade cells actively expressing ß1-integrins, such as those at the leading edge of an epithelial sheet.

As afore-mentioned one potential application of inv+ E.coli is therapeutic bacteria for the treatment of cancer. By restricting the expression of inv to tumor sites, invasion could be confined to malignant cells. The hypoxic environment could provide a cue for detection of tumors and the induction of cancer cell invasion.

It was noticed a remarkable range of bacterial species, including non-pathogens, localized to tumors after intravenous injection -such as E.coli, Vibrio cholerae, Clostridium, Bifidobacterium, Salmonella, and Listeria monocytogenes. This occurred for a wide variety of solid tumors, including bladder, brain, and breast cancers. These microbes imparted their selectivity for tumors by exploiting the hypoxic microenvironment, poor immune surveillance, and the increased availability of nutrients. For instance, after a Salmonella IV injection in mice, the concentration of bacteria in tumors was approximately 10^9 Colony forming units (cfu)/g tissue, whereas in liver it was 10^6 cfu/g and in muscle it was 10^3 cfu/g.

Summing up, invasin presents a scaffold upon which protein engineering could be used to alter its function or enable it to bind new targets. However, engineering the necessary artificial fusions between sensory promoters and output genes is not always easy owing to mismatches in rates of transcription and translation, especially as the complexity of the system increases.

Specific invasion of tumor cells is only one component of an anti-cancer bacterium. Once inside target cells, a cytotoxic or immunostimulatory response must instigate destruction of the tumor. For this effect and as explained in their paper, various bacteria were engineered including Salmonella that metabolized a chemotherapeutic prodrug at tumor sites, strains of Clostridium that secreted TNFa, and strains of BCG and Salmonella that secreted a repertoire of mammalian cytokines.

B.- The Nanomedicine Approach

Multifunctional nanoparticles with combined diagnostic and therapeutic functions show great promise towards personalized nanomedicine. However, attaining consistently high performance of these functions *in vivo* in one single nanoconstruct remains extremely challenging.

In the analyzed paper a robust, smart and highly versatile "all-in-one" porphyrinbased organic nanoconstruct (named nanoporphyrin, or NP) was put forward. This NP platform integrated a variety of imaging and therapeutic functions that included imaging (near-infrared fluorescent imaging (NIRFI), positron emission tomography (PET), magnetic resonance tomography (MRI), dual modal PET-MRI, PTT, photodynamic therapy (PDT), as well as targeted drug delivery. The NPs were formed by the self-assembly of a novel class of hybrid amphiphilic polymers (called telodendrimers) comprising linear polyethylene glycol (PEG), and dendritic oligomers of pyropheophorbide-a (Por, a porphyrin analogue) and CA (porphyrin/c holic acid) (*Appendix, Fig. 2*).

Blood is the first biological barrier for nanoparticle-based drug delivery systems via intravenous administration. Interaction with blood proteins and lipoproteins may cause the dissociation of nanoparticles and lead to premature drug release. In order to further increase the structural stability of NPs in blood circulation, the Chinese and North American researchers applied reversible disulphide cross-linking strategy to the NP platform (CNPs). These CNPs could also efficiently carry a variety of poorly water-soluble chemotherapeutic drugs and molecularly targeted drugs. For instance, DOX could be efficiently encapsulated inside CNPs with a loading capacity of 2.90 mg ml^{-1}. When formulated at 2.55 mg ml^{-1}, the drug-loading efficiency of DOX-loaded CNPs (CNPDOX) was above 85% with final particle size ~30 nm.

The fluorescence resonance energy transfer (FRET) between the DOX and NPs was used to monitor the realtime drug release in human plasma. The FRET signal was very stable when CNPDOX was incubated

in human plasma for 24h at 37 °C, indicating that the DOX release from CNPs was slow. However, there was dramatic and immediate FRET signal decrease with each light exposure at 24 and 28h, indicating that CNPs could be triggered to release drug via illumination, probably as a result of local heat generation. Those results indicated that DOX release was slow in the presence of human plasma but could be facilitated through illumination or by adding glutathione (GSH).

Intracellular delivery of CNP-DOX was investigated as well in SKOV3 ovarian cancer cells. After 2h of incubation, DOX was released from CNPs and transported into the nucleus (*Appendix*, *Fig. 3*).

In the scientific paper it was demonstrated the excellent delivery of NPs to target tumor cells after intravenous injection. Using NIRF signal technology it was observed that at 1h post injection the overall NIRF signal from CNPs was very low inside the tumor tissue. Significant NIRF signal was observed surrounding the blood vessels at 24h, indicating the accumulation and partial dissociation of CNPs into tumor tissue around blood vessels. At 48h, NIRF signals diffused throughout the entire tumor implying excellent tissue penetration and dissociation of CNPs at the tumor site.

Therefore, CNPs could be used as programmable releasing nanocarriers that minimized the drug release in human plasma but could be triggered to release the drug content when exposed to light and intracellular reducing agents. At the same dose of DOX, the combination treatment with CNPDOX showed the best anti-tumor activity and totally inhibited tumor growth throughout the study.

The NP platform reported possesses several properties that are unique and favorable as theranostic agent, amongst others: excellent efficiency for drugloading, biocompatible, monodisperse and relatively small (20~30 nm), and it can be reversibly stabilized via disulphide bonds. As exposed in the paper, regarding both ovarian cancer xenograft model and murine transgenic breast cancer model, these novel NPs could be used as: (1) amplifiable nanoprobes to increase the sensitivity of multimodal imaging for tumor detection, (2) nanotransducers that can be activated to generate heat and ROS efficiently at tumour sites for PTT/PDT dual therapy via a singlewavelength light and (3) nanocarriers with programmable releasing property that can minimize the premature drug release in blood and allow efficient release upon light irradiation and/or triggered by the endogenous reducing agent present at the tumor site or in cancer cells.

Although NPs can in principle reach tumor sites throughout the body, PTT and PDT, however, they are limited by the region of the body accessible to light irradiation and the depth of NIR laser penetration into tissues. As explained in the article, the improvements in light delivery methods and targeting property of NPs via targeting moieties for specific type of tumors will undoubtedly further increase the therapeutic efficacy and accelerate the translation of such NPs to the clinic. For tumors that are beyond the reach of the light, anti-tumor effects can still be achieved with a chemotherapeutic agent such as PTX and DOX encapsulated inside the NPs.

CONCLUSION

Engineered bacteria or nanoporphyrin platforms?

From my standpoint and according to what has been explained in this paper, it's not necessary to go through that disjunctive stance: we can and should count on both approaches on fighting against cancer.

As afore-explained the North American researchers engineered the interaction between bacteria and cancer cells to depend on heterologous environmental signals. They characterized invasin from Yersinia pseudotuburculosis as an output module that enabled Escherichia coli to invade cancer-derived cells, including HeLa, HepG2, and U2OS lines. This approach could be used to engineer bacteria to sense the microenvironment of a tumor and to respond by invading cancerous cells and releasing a cytotoxic agent.

With respect to NPs, they could be used as a nontoxic nanoplatform for hydrophobic anticancer drug formulation, yielding relatively small and monodisperse nanotherapeutics with high drug-

loading efficiency. As afore-mentioned, to minimize premature drug release in the circulation, NPs could be cross-linked by disulphide bonds. Such CNPs could be triggered to release the drug content by light exposure and intracellular reducing agents.

Both the engineered bacteria and the integrated nanomedicine platform -by far less aggressive than the current medical treatments based on radiotherapy, chemotherapy, and surgical interventions- seem to show promising results and to raise high expectations as extremely versatile procedures against cancer: surely the hardest and toughest challenge ever faced by medicine.

REFERENCES

1. J. Christopher Anderson, Elizabeth J. Clarke, Adam P. Arkin, and Christopher A. Voigt, *Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria*. Howard Hughes Medical Institute, California Institute of Quantitative Biology Department of Bioengineering University of California; Physical Biosciences Division E.O. Lawrence Berkeley National Laboratory: and Department of Pharmaceutical Chemistry, California Institute of Quantitative Biology, The University of California San Francisco.

2. Yuanpei Li, Tzuyin Lin, Yan Luo, Qiangqiang Liu, Wenwu Xiao, Wenchang Guo, Diana Lac, Hongyong Zhang, Caihong Feng, Sebastian WachsmannHogiu, Jeffrey H. Walton, Simon R. Cherry, Douglas J. Rowland, David Kukis, Chongxian Pan & Kit S. Lam. *A Smart and Versatile Theranostic Nanomedicine Platform Based on Nanoporphyrin.* Department of Biochemistry and Molecular Medicine, UC Davis Comprehensive Cancer Center, University of California Davis, Sacramento; Division of Hematology/Oncology, Department of Internal Medicine, University of California Davis, Sacramento; Department of Oncology, PLA Cancer Research Institute of the Second Affiliated Hospital, Third Military Medical University, Chongqing; National Chengdu Center for Safety Evaluation of Drugs, West China Hospital, Sichuan University, Chengdu; Beijing Institute of Technology; NSF Center for Biophotonics Science and Technology, University of California Davis, Sacramento; Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento; UC Davis NMR Facility, Davis, Sacramento; Department of Biomedical Engineering, Center for Molecular and Genomic Imaging, University of California Davis, Davis, California; VA Northern California Health Care System, Mather, California

3. Marina Ntika, Petros Kefalas, and Ioanna Stamatopoulou, *Formal Modelling and Simulation of a Multi-agent Nano-Robotic Drug Delivery System,* South-East European Research Centre (SEERC), Research Centre of the International Faculty of Sheffield, and The University of Sheffield International Faculty.

APPENDIX - FIGURES

Figure 1.- Design for Induction-Dependent Invasion of a Cancer Cell.

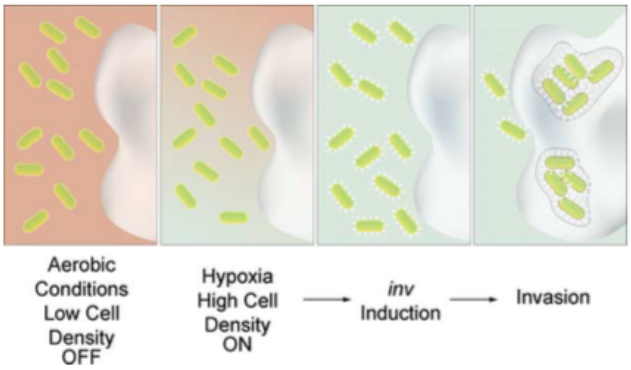

Figure 2.- Design, Synthesis, and Characterizations of NPs

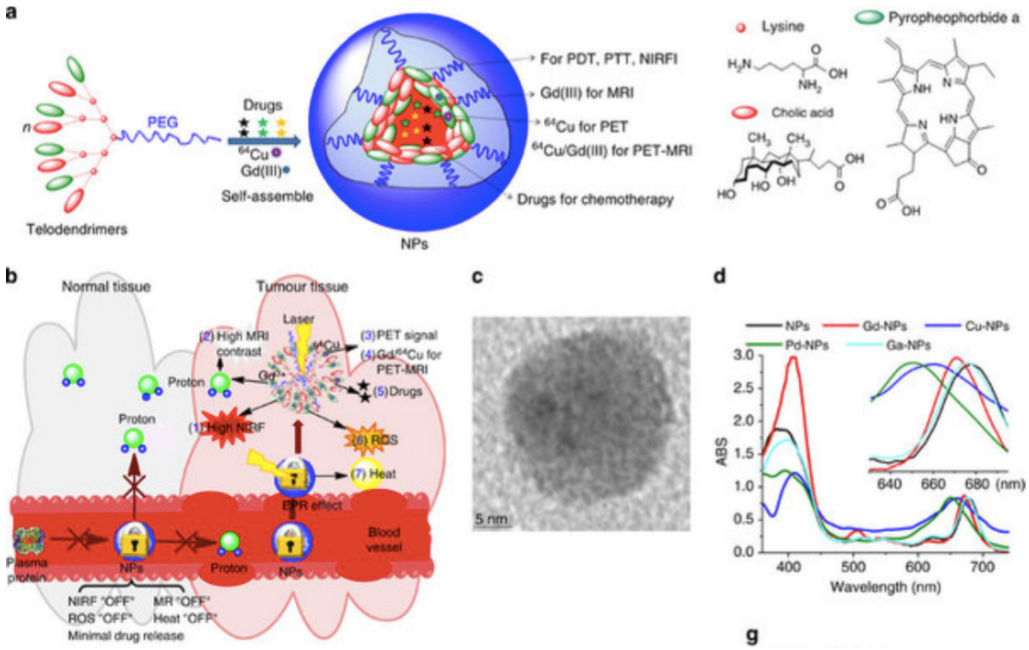

Figure Figure 3.- Intracellular Delivery and Photocytotoxicity of NPs against cancer cells