Dual functionality nanobioconjugates: a new tool for intracellular bacterial targeting in cancer cells?

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“It is time that therapy spectrum be shifted from ‘one drug for all cell types’ to a personalized one using modular multifunctional nanocargos effectively targeting the specific cancer tissue and bacteria within.”

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Historical perspective

The mammalian–bacterial relationship has existed as a long commensal affair since the time human evolution began. The gut flora is full of innumerable bacteria that not only metabolize certain carbohydrates that are unprocessable by the human body, but also act as a shield against foreign pathogens by providing a favourable milieu [1,2]. The significance of this symbiotic relationship took a paradigm shift in the late 19th century when the German scientist, Wilhelm Busch, reported for the first time the presence of *Streptococcus* bacteria in two patients with sarcoma. Since then, multiple reports have been published that portray this cellular cooperative alliance in a different light. It is now evident that bacteria can behave both as cancer causative or cancer prevailing agents, and can even remit cancer in certain situations [3]. In the majority of these cases, the outcomes of association are however, sadly negative.

Bacteria gain entry into the tumor through the leaky vasculature (resulting from disorganized angiogenesis) via chemotaxis toward purines, lactic acid, ribose sugars and other such chemoattractants present in the tumor niche. Once inside, the immigrant anaerobic or facultative bacteria can find refuge in nutrient-rich, hypoxic regions of the tumor tissues while remaining dormant for years, evading immune clearance by hiding in the body's own cancer cells, and can become pathogenic as they find an immunologically favorable environment. Cancer patients are especially at risk of developing bacterial infections due to prolonged neutropenia, lymphocyte dysfunction and use of invasive devices [4]. It is now believed that long-term presence of opportunistic bacteria can even trigger cancer in healthy cells, although the exact mechanisms for this still remain elusive.

The Nobel prize winning research of Warren and Marshall on *Helicobacter pylori* role in gastric cancer is a great example of this major breakthrough in molecular biology. Strong links have also been reported for *Salmonella typhi* causing gall bladder cancer, *Mycoplasma* resulting in ovarian and lung cancer and many more [3]. A recent article in the journal *Science* demonstrated that the presence of bacteria inside tumor have also the potential to create resistance against cancer therapy due to their inherent capability of cleaving chemotherapeutics into inactive metabolites [5]. In this way, specific links between infectious pathogens and cancer can greatly impact the design of therapy.

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Current scenario

So far, more than 60 cancer therapeutic drugs have been launched in the market while many more are under clinical trials. The conventional ones include alkylating agents (e.g., cisplatin), antimicrotubule mediators (e.g., paclitaxel), cytotoxic inhibitors (e.g., doxorubicin) and antibody therapies (e.g., herceptin), etc. Free forms of these drugs have

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poor solubility, fast clearance rates and improper distribution during systematic delivery due to which their efficacy is significantly reduced inside the body. This leads to their higher drug dose administration and cytotoxic side effects. One way to address this issue is through the use of nanoformulations that allow packaging drugs into hydrophobic/hydrophilic compartments to increase their solubility and retention time [6]. Further PEGylation and functionalization of the nanoformulated surface helps to improve their circulation period [7]. A great example of such a nanoformulation that became the first US FDA-approved nanodrug more than 20 years ago is Doxil [8,9]. It functions by entering the cancer site passively via the enhanced permeability retention effect owing to the complex intratumoral capillary fenestration and *de novo* lymphangiogenesis [10,11].

In addition to cancer therapeutics, the immunosuppressed cancer patients are routinely given antibiotics to avoid secondary infections [12]. These antibiotics also suffer poor bioavailability in the same way as cancer drugs but are continued to be given orally, intravenously or via infusion in the free form without any particular target in mind. This rampant, undirected use of antibiotics causes many side effects including antimicrobial resistance, which is one of the biggest health challenges of this century [13,14]. Any discrimination that does take place, happens more through the principle of exclusion, as drugs do not penetrate all tissues equally well. The drug's preferential accumulation at the site of action is thus a result of a number of different parameters like molecular structure, electrical charge and hydrophobicity, etc. Needless to say, no antibiotic can be effective unless it interacts with the bacteria. This is a major challenge for tackling intracellularized pathogenic infections as the drugs are simply not designed to act on the surface markers present on mammalian cells.

Dual targeting: a new outlook

The above insights lead us to ask a pertinent question: “*Why have anticancer and antibacterial therapies been studied as two independent frameworks for this long despite sufficient evidence that the presence of one cell type provides an opportunity for the other to flourish?*” Perhaps it is time to cast a fresh look at the present drug delivery approaches and re-engineer them to allow facile dual-targeting of both cell types simultaneously. A recent study by Mitragotri's group at University of California, Berkeley (CA, USA) showed an enhanced synergistic combinatorial therapy for tumor targeting by co-packaging two anticancer drugs in the same liposome carrier [15]. Such incorporation and synergistic use of more than one drug to enhance the efficacy of standard therapies is indeed the latest trend in targeted deliveries. Rationale designing of drug delivery systems can further help in reducing toxicity by enhancing targeting abilities and reducing nonspecific distribution.

One way to improve the targeting efficiency and uptake of nanocarriers is to append them with ligand moieties (e.g., folic acid, antisense oligonucleotides, aptamers and monoclonal antibodies) that are specific and selective to the receptors expressed (or overexpressed) on the cancer cell surface [16,17]. Once inside, the drug can be remotely offloaded via the endosomal pathway by hydrolytic cleavage or rapid degradation of the drug carrier triggered by an acidic environment [18,19]. Our group is one of the first to demonstrate the successful targeting of cancer cell-internalized pathogenic bacteria. This was done using gold nanoparticles co-functionalized with antibacterial peptides and folic acid [20]. The nanobioconjugates showed enhanced antimicrobial efficacy as compared with the free drug on intracellularized bacteria without affecting the mammalian cells. The next logical step for this study is to combine anticancer and antibacterial therapy into a single vehicle for co-targeting cancer and bacterial cells, an activity we have already started in our group.

“Why have anticancer and antibacterial therapies been studied as two independent frameworks for this long?”

Future perspective

In spite of the inspiring progress made so far, multiple challenges are still foreseen in the clinical translation of cancer–bacterial dual-targeting, single-delivery platforms as the next-generation nanodrugs. The first challenge relates to the location of the bacteria inside the tumor site. While some bacteria naturally reside intracellularly within the cells, others prefer to hang out in the extracellular matrix of the cancer tissue [21,22]. It may also so happen that in some cases, the bacteria co-exist in both locations [23,24]. These situations demand the development of nanoscaffolds with spatio-temporal tunability and multiscale drug release profiles. Another important design criterion relates to the sequence of drug release to deal with bacteria that have the tendency to escape into blood or translocate to other favorable environments (e.g., local lymph nodes) for survival [25]. At last, in spite of the best efforts, efficient tumor targeting still remains a major challenge in the field. Commonly targeted surface receptors

such as transferrin (TfR1) and folate (FR- α) that are overexpressed in many tumors are also present in normal tissues albeit in low amounts resulting in marginal benefits.

The most recent systems paving way for an exciting new era in nanomedicine are biologically engineered nanocargos. They utilize the natural colonizing properties of bacteria and viruses to deliver drug to the diseased sites. A 2016 study by Din *et al.* showed how a clinically relevant bacterium could be engineered using tools of synthetic biology to lyse synchronously at a threshold population density and release genetically encoded anticancer cargo on demand in the required vicinity [26]. Although, natural carriers like bacteria and viruses are superior over synthetic analogs due to their inherent colonizing tendency into tumor cells, combating their immune response inside the body poses a big challenge. PEGylation and synthetic peptide coatings can to some extent minimize this immune response but no guidelines clearly favor the possibility of their clinical acceptance in the near term.

“... the most recent systems paving way for an exciting new era in nanomedicine are biologically engineered nanocargos.”

Conclusion

In summary, the field of drug delivery is constantly evolving and opening new avenues of research as our understanding of cancer biology and its various nuances deepens. The realization that tumor cells have a symbiotic liaison with their microenvironment and the microorganisms that inhabit this space creates new opportunities for scientists to study the role of the human microbiome in the initiation, progression and termination of cancer. It is time that therapy spectrum be shifted from ‘one drug for all cell types’ to a personalized one using modular multifunctional nanocargos effectively targeting the specific cancer tissue and bacteria within. The ultimate success of these initiatives, however, critically depends on the research funding received from the federal government and the active collaboration between investigators across various disciplines.

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