Ionophore Antibiotics as Cancer Stem Cell-Selective Drugs: Open Questions

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Ionophore antibiotics are classical coccidiostats used to prevent microbial-associated disease in populations of animal livestock. In recent years, these compounds have been additionally considered for the treatment of cancer.

Ionophore antibiotics act by generating pores in biological membranes that dramatically alter the ionic household of cells. Salinomycin, for instance, generates ion channel-like structures that exhibit strong selectivity for $\text{K}^+$, but other mono- or divalent cations are also conducted (e.g., Na$^+$ and H$^+$) [1]. Traditionally, the cell-killing activity of ionophore antibiotics is thought to originate from profoundly deregulated osmosis, as well as from direct cytotoxic effects of the altered biochemical landscape.

The impetus for considering ionophore antibiotics as anticancer agents was given by an article in which Gupta et al. used high-throughput screening of ~16,000 compounds to find that ionophore antibiotics are remarkably potent in killing epithelial (breast) cancer cells [2]. Both salinomycin and the structurally related nigericin bear selective toxicity to cancer stem cells (CSCs), which is approximately 100 times more potent compared with the reference cytotoxic paclitaxel. In the following years, this landmark study stimulated plenty of further research that was retrospectively pursuing two objectives: (a) to confirm the results in independent tumor models including other carcinomas and (b) to test whether ionophore antibiotics are also active in nonepithelial tumor entities such as leukemia, sarcoma, and glioma. Indeed, we now know that ionophore antibiotics target various types of cancer cells virtually irrespective of their tissue origin (e.g., colon/rectum [3], prostate [4], endometrium [5], blood [6], brain [7], and bone [8]). In addition, these drugs share many properties that are useful—and even desirable—for anticancer treatment. For instance, ionophore antibiotics can overcome apoptosis resistance [9], inhibit cell migration/metastasis [10, 11], induce oxidative and endoplasmic reticulum stress [4, 12, 13], synergize with other compounds in combination regimens (e.g., doxorubicin, docetaxel, or vinblastine) [14–16], and interfere with the small GTPase K-ras [17], a so far “undruggable” component of the mitogen-activated protein

kinase/ extracellular signal-regulated kinase pathway acting downstream of the clinically frequently targeted epidermal growth factor receptor. Of note, the drugs are active at nanomolar concentration in cancer cell cultures [18], indicating high potency and/or particular susceptibility of transformed cells to this substance class; conversely, untransformed cells, such as normal peripheral blood lymphocytes, remain unaffected [6]. Thus, the general suitability of ionophore antibiotics as anticancer drugs is beyond dispute, and their testing in clinical studies is being increasingly considered, for instance, with the proprietary salinomycin formulation VS-507 (Verastem Inc., Needham, Massachusetts, http://www.verastem.com) [19].

The question remains as to whether ionophore antibiotics could add value to current treatment protocols for the benefit of patients. In preclinical studies, salinomycin was shown to have toxicity for neuronal [20] and mucosal [21] cell types, the former of which can be prevented by using blocking of mitochondrial cation exchange [22]. More importantly, case reports from clinical pilot studies showed that salinomycin, administered to cancer patients intravenously at a dose of 200–250 $\mu$g/kg every second day for up to 4 weeks, was generally well-tolerated, provoking only minor acute side effects (i.e., tachycardia and mild rapidly resolving tremor), but not inducing severe or long-term side effects (e.g., myelosuppression, neurotoxicity, or nausea/vomiting) [19]. Of note, salinomycin treatment was effective in these patients, leading to partial responses or stable disease in four patients with metastatic breast cancer, one patient with metastatic head and neck cancer, and two patients with a metastasized gynecological cancer. However, even though these early clinical data are encouraging, the critical issue will be whether and to what extent ionophore antibiotics are indeed able to target CSCs. This is because primary therapies are already highly efficient and commonly achieve states of remission, yet they fail to protect against CSC-mediated disease recurrence in the long term.

Characteristically, CSCs represent a phenotypically and functionally distinct cancer cell subpopulation responsible for
most of the malignant potential of a tumor [23]. For example, cancer initiation and metastatic dissemination during progression are both governed by this dedicated cell pool, making CSCs a prime target for new-generation cancer therapies. However, therapeutic CSC elimination has proven difficult because these cells are slow-cycling, have elaborate systems of detoxification, and cope very well with DNA damage. As a consequence, they carry an intrinsic resistance to both systemic (chemotherapy and targeted therapy) and local (irradiation) treatment modalities, allowing persistence predisposing the patient to relapse.

Compounds targeting and eradicating CSCs are thus urgently needed to allow an increasing rate of long-term cures. After the identification of ionophore antibiotics as breast CSC-targeting drugs, the CSC-selective toxicity of these compounds was corroborated in numerous independent studies so that ionophore antibiotics currently represent lead CSC inhibitors. Moreover, a potential mechanistic basis for their CSC-targeting nature was established when it was shown that they interfere with canonical stem cell pathways such as Wnt/b-Catenin [6] and hedgehog [24] and downstream modulate core factors of the epithelial-to-mesenchymal transition (i.e., ZEB1) [14], a cellular process mechanistically linked to the acquisition of stem cell properties [2]. Nonstem cells, including untransformed cells, exhibit little to no activity of these signaling cascades and therefore manage to evade ionophore antibiotic toxicity, explaining the safety profile of these compounds. Yet it is important to note that the selectivity of ionophore antibiotics for CSC populations is not fully understood to date and subject to current research [17].

Despite these promising data, however, our group recently disclosed a potent CSC-intrinsic mechanism of protection against ionophore antibiotics. Specifically, we found that side population (SP)-defined ovarian CSCs evade salinomycin- or nigericin-induced cell death through transporter-mediated drug extrusion, even though efflux-independent contributions cannot entirely be ruled out [18]. Harnessing the genetic specificity of RNA interference and transporter-selective pharmacological inhibition, we finally uncovered a causal role of ABCB1 (also known as P-glycoprotein, MDR1, and CD243) and ABCG2 (also known as Bcrp1 and CD338) in mediating resistance (Fig. 1). In view of the presumably high frequency of drug transporter expression among CSC populations [25, 26], our principal conclusion was that ionophore antibiotics may be less suited for CSC-directed treatment than initially thought. However, does this mean that ionophore antibiotics are irrelevant for cancer therapy?

From today’s perspective, the suitability of ionophore antibiotics as breast CSC-targeting drugs depends to a great extent on the prevalence of the relevant transporters (i.e., ABCB1 and ABCG2) among different CSC subsets. Moreover, it remains to be determined whether other efflux pumps (e.g., those that belong to the ABCC family) also mediate resistance to this substance class.

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Despite this, it is reasonable to believe that tumor entities in which CSCs are essentially defined by the SP phenotype (e.g., ovarian cancer and melanoma) [27, 28] are generally less susceptible to ionophore antibiotic-based treatment modalities. Conversely, breast and brain tumor stem cells—which are mostly defined by CD44^high/CD24^low and CD133^+ surface phenotypes, respectively [29, 30]—might be
more sensitive to ionophore antibiotic targeting, because drug transporters are not necessarily coexpressed. It is expected that single cell systems biology (e.g., using the recent mass cytometry/ CyTOF platform or RNA sequencing of individual cells) will help to identify those tumor entities (and subtypes thereof) that are most amenable to ionophore antibiotic targeting. In parallel, further development and adjustments in the molecular structure of ionophore antibiotics [31] should be contemplated to potentially resensitize drug transporter-expressing CSCs to this substance class or otherwise enhance their performance.

In summary, ionophore antibiotics are promising compounds with potent anticancer and anti-CSC activity. However, resistance mediated by drug transporters potentially confines their use to CSC populations that do not exhibit appreciable drug efflux capacity. Further research is required to delineate which CSC subsets are likely to be susceptible to ionophore antibiotic cytotoxicity, and it is proposed that profiling the tissue stem cells from which they arise may provide valuable input here. Ultimately, the testing of ionophore antibiotics in large clinical trials, especially salinomycin and nigericin, should involve the selection of eligible tumor entities and patient cohorts by drug transporter status in addition to general and clinical CSC estimates, such as stem cell frequency and likelihood of recurrence. This commentary should thus serve as a gateway for the rational design of clinical trials in the future to foster the precision medicine-compliant use of ionophore antibiotics in cancer patients.

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