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Diagnosis of cancer multidrug resistance by bacterium-mediated imaging

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ABSTRACT

Multidrug resistance (MDR) is a phenomenon expressed by many tumors affecting the chemotherapy efficacy, treatment decision, and the disease prognosis. Considering its great implication, non-invasive approaches are needed to identify this phenomenon in early stages of the disease. This article discusses the potential of the emerging non-invasive bacterium-mediated imaging of cancer in diagnosis of MDR. This potential is derived from the effect of cancer MDR on the pharmacokinetics of certain antibiotics, which are substrates of the MDR proteins. Since MDR proteins actively pump their substrates outside the resistant cancer cells, the elimination of the employed reporter bacteria, proliferating within MDR cancer cells, would require a larger dose of these antibiotics compared to those inside non-MDR cancer cells. These bacteria bear reporter genes that produce specific signals such as bioluminescent, fluorescent, magnetic, or radioactive signals that can be detected by non-invasive imaging modalities. Therefore, the presence, degree, and mechanism of MDR can be estimated by comparing the concentration of the employed antibiotic, required to cease these signals (reflecting the elimination of the bacteria), to a pre-determined reference. The real time imaging of MDR cancer and the early diagnosis of MDR, offered by this approach, would provide a better tool for preclinical studies of MDR, and allow a prompt choice of the most appropriate therapy.

Introduction

Multidrug resistance

Multidrug resistance (MDR) is a phenomenon expressed by many tumors, representing the main cause of chemotherapy failure. MDR is defined as the intrinsic or acquired resistance of cancer cells to structurally and functionally unrelated drugs [1]. Many mechanisms are involved in MDR including Adenosine triphosphate Binding Cassette (ABC) transporters up-regulation [2–4] and limited drug penetration in solid tumors [5].

ABC transporters up-regulation and the significance of multidrug resistance

ABC transporters, especially P-glycoprotein (Pgp) and Multi Drug Resistance associated protein (MRP), are major contributors to MDR. They work by an efflux mechanism maintaining a very low concentration of their substrates (such as chemotherapeutic agents), insufficient to carry out their effect [3,6]. These proteins are usually over-expressed in patients refractory to chemotherapy; this overexpression is generally associated with poor prognosis [2,4,7]. Thus, MDR phenotype can affect the treatment decision of cancer. For instance, if the tumor is MDR, then one of three strategies should be considered during therapy: avoiding the MDR pumps, using drugs that are not substrate of the pumps; reversing the resistance, using MDR pump inhibitors [4,8]; or even exploiting it, using protector drugs substrate to the MDR pumps [6,8].

Cancer multidrug resistance and antibacterial chemotherapy

Cancer MDR implication goes beyond cancer to affect antibacterial chemotherapy. Since MDR reduces the intracellular accumulation of some antibiotics (substrate of the MDR pumps), it reduces their activity against intracellular bacteria: increasing their minimum inhibitory concentration (MIC) [6,9]. These antibiotics are diverse including and belong to various classes: macrolides, azalides, ciprofloxacin, ofloxacin, clindamycin, rifampicin, chloramphenicol, doxycycline, and trimethoprim [9,10]. Again, MRP and Pgp play an important role in this reduced

Abbreviations: MDR, multidrug resistance; ABC, Adenosine triphosphate Binding Cassette; Pgp, P-glycoprotein; MRP, multidrug resistance associated protein; MIC, minimum inhibitory concentration; [^{99m}Tc] MIBI, technetium labeled sestamibi; MRI, magnetic resonance imaging; PET, positron emission tomography; BLI, bioluminescent imaging; FLI, fluorescent imaging.

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intracellular activity, where their selective inhibitors reverse this reduced activity [10].

Diagnosis of cancer multidrug resistance: from invasive to non-invasive approaches

So far, the methods developed for MDR diagnosis are mostly invasive. They involve testing the tolerance of cancer cells (isolated from primary tissues) to chemotherapeutic drugs. This can be indicated by the change in the half inhibitory concentration, cell growth curve, cell proliferation index, resistance index, or apoptosis index. They can also assess cytotoxic drugs (such as doxorubicin) pump out rate or detect multidrug resistance genes [1].

Radio-labeled substrates of MDR proteins have been investigated as non-invasive alternatives for functional imaging of MDR. Technetium (^{99m}Tc) labeled sestamibi ([^{99m}Tc] MIBI) is the first and most studied of these radio-labeled substrates. It is a ^{99m}Tc-labeled lipophilic cation originally introduced for imaging myocardial perfusion [11,12]. [^{99m}Tc] MIBI passively diffuses into the cell, accumulating in the mitochondria; however, in MDR cells, it is expelled by the MDR proteins i.e. its retention in tumors correlates inversely with the degree of MDR proteins expression [11]. As it diffuses passively into the cells, we should question its ability to differentiate between resistances due to up-regulation of MDR proteins, and limited drug penetration (due to poor distribution caused by distended distance between cancer cells and vasculatures in tumors), which in turn can affect the therapy decision. However, this approach is not clinically employed yet, and in vivo approaches are still needed to clinically evaluate MDR in early stages of the disease [1,11].

Microbial-mediated imaging of cancer

Microbial-mediated imaging is a noninvasive novel approach that is being investigated for imaging of cancer [13–16]. It depends on the natural preference of some bacteria and viruses for the tumor microenvironment. This preference is attributed to the nutrient-rich, hypoxic and immune-hiding niches provided by tumors' microenvironment [17]. Thus, attenuated bacteria and viruses bearing reporter genes are successfully employed to reveal locations of solid tumors and metastases in animal studies. These reporter genes are expressed to produce signals such as: luciferase-catalyzed luminescence and green fluorescent protein fluorescence, which can be imaged by sensitive photon detectors [13,16,18]; magnetic resonance imaging (MRI) contrast enhancers like ferritin, which can be imaged by MRI [19]; and radionuclides, which can be imaged by positron emission tomography (PET) [15]. The viruses and bacteria studied as tumor-colonizing live vector are diverse including vaccinia viruses, *Bifidobacterium breve*, *Escherichia coli*, *Vibrio cholera*, and *Salmonella* species [14,16].

Salmonella typhimurium is one of the most studied bacteria employed in targeting cancer. The wild-type has the ability to infect a wide host range—including human and mice—and can be easily engineered to carry foreign genes [20]. Moreover, *Salmonella* preferentially colonizes tumor xenografts in mice rather than normal tissues achieving tumor implant/normal tissue ratios of 10,000:1 [21]. Being a facultative anaerobe, *Salmonella* can survive in both oxygenated and hypoxic conditions; thus, it could accumulate in small metastasis and solid tumors [13,17,20].

To assure safety and increase tumor-targeting capabilities, the attenuated *S. typhimurium*, *VNP20009*, was developed by msbB (Lipid- A- modified) and purI (purine auxotrophs) mutations, respectively [22,23]. The msbB mutation largely decreased host TNF-α induction, reducing the induction of proinflammatory cytokines, and purI mutation made *Salmonella* auxotrophic for purine, which is abundant in tumors [22]. *S. typhimurium* is also susceptible to a variety of antibiotics including azithromycin and ciprofloxacin [24], which are substrates to the MDR proteins: Pgp and MRP, respectively [10].

Hypothesis

We propose that bacterium-mediated imaging of cancer can diagnose MDR by using antibiotics substrate to the MDR proteins: monitoring the change in the concentration required to eliminate the intracellular bacteria. The reporter bacteria (such as bioluminescent bacteria) proliferating within MDR cancer cells would require a larger dose of antibiotics to be eliminated, than those present in non-MDR cancer cells; this elimination would be reflected in the cease of the signals produced by the reporter bacteria (such as bioluminescent signals) (Fig. 1). Thus, the presence and degree of MDR can be accurately estimated by comparing the MIC of the employed antibiotic, required to cease the signals, to a predetermined reference.

Hypothesis discussion

Significance and potentials

Considering the great implication of MDR on cancer prognosis and treatment options, an early, convenient and cost effective diagnostic method of MDR is needed. Bacterium-mediated imaging of cancer is a noninvasive, safe, efficient, versatile and relatively simple method [14,25] that can satisfy this need. It involves the administration of reporter bacteria intravenously, and its selective proliferation in cancer is detected by a suitable imaging system after a predetermined time [16]. To detect MDR, antibiotics—substrate to the MDR proteins and effective against the bacteria—can be administrated in an increasing dose, and the effective dose that eliminates the bacteria, and thus the signal reported by them, is to be determined. We can evaluate MDR by comparing this dose to a known reference of non-MDR tumor: if the dose required is more than the known reference, then the tumor is MDR. For facultative intracellular bacteria, such as *S. typhimurium*, the extracellular bacteria would be eliminated at a specific dose, independent of MDR. Thus, MDR can be diagnosed by comparing the antibiotic dose required to eliminate the intracellular bacteria to those required to eliminate extracellular bacteria (constant as they are not challenged by the up-regulated ABC transporters on cancer cell membrane) i.e. comparing the first antibiotic doses required to decrease the signals (extracellular bacteria), and those required to completely cease the signals (intracellular bacteria in resistant cells).

Besides the diagnosis of MDR caused by ABC transporters up-regulation, bacterium-mediated imaging of cancer could also diagnose MDR caused by other pharmacokinetic parameters, such as limited drug penetration in solid tumors due to reduced blood supply and diffusion resistance. Moreover, as the bacteria disseminate all over the tumor [26], it can differentiate between both causes of resistance. The reduced vasculature causes reduced drug penetration to the core, thus the signal (produced by the bacteria) will persist for higher antibiotic doses only in the core; while the resistance caused by ABC transporters up-regulation will require higher antibiotic dose all over the tumor (depending on the distribution of the resistant cells), i.e. the pattern, by which the signal ceases, would reflect the mechanism of resistance. Another benefit of this approach