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Drug persistence – From antibiotics to cancer therapies

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Abstract

Drug-insensitive tumor subpopulations remain a significant barrier to effective cancer treatment. Recent works suggest that within isogenic drug-sensitive cancer populations, subsets of cells can enter a 'persister' state allowing them to survive prolonged drug treatment. Such persisters are well-described in antibiotic-treated bacterial populations. In this review, we compare mechanisms of drug persistence in bacteria and cancer. Both bacterial and cancer persisters are associated with slow-growing phenotypes, are metabolically distinct from non-persisters, and depend on the activation of specific regulatory programs. Moreover, evidence suggests that bacterial and cancer persisters are an important reservoir for the emergence of drug-resistant mutants. The emerging parallels between persistence in bacteria and cancer can guide efforts to untangle mechanistic links between growth, metabolism, and cellular regulation, and reveal exploitable therapeutic vulnerabilities.

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Introduction

The last decades have brought the arrival of an impressive arsenal of therapies for treating cancer. At the same time, countless drug resistance mechanisms have been discovered by which cancer cells avoid and subvert drug treatment. Tumor subpopulations that do not respond to therapeutics are a significant barrier in the treatment of cancer, and cancer remains a major global killer [1].

Recently, it has become clear that even within otherwise drug-susceptible isogenic cancer populations, a subset of cells can enter a persister state, in which they survive prolonged drug exposure [2] (see Table 1 for a list of cancer persister models). While this persister state has only recently started to draw attention in mammalian cells, bacterial persisters were described in literature as early as 70 years ago [3]. The past decade has seen a surge in studies elucidating the mechanisms underlying bacterial antibiotic persistence — as recently summarized in a string of excellent reviews [4–8]. In this review, we compare and contrast persistence in bacteria and cancer cells, and highlight surprising parallels in the underlying persistence mechanisms.

Defining persistence – a persistent challenge

Before delving into persistence mechanisms, we must first define what drug persistence is, and how it differs from other mechanisms of drug insensitivity (Figure 1A).

Bacterial insensitivity to antibiotics is classified phenomenologically into three broad categories that can be distinguished experimentally (compared to a reference sensitive population; Figure 1B), as summarized in Refs. [4,5]. The first category, **drug tolerance,** is the ability of cell populations to withstand transient lethal antibiotic concentrations, while remaining genetically susceptible [4,5]. Experimentally, tolerance manifests as a decreased rate of killing during drug exposure compared to a sensitive reference population (Figure 1B). The second category, **drug resistance**, is the genetically inherited ability of cells to grow at normally lethal antibiotic concentrations [4,5]. Drug-resistant populations show a characteristic increase in minimal inhibitory concentration (the lowest drug concentration needed to prevent bacterial growth); this increase is absent in drug-tolerant populations. In contrast to these two categories, which are defined at the population level, **drug persistence** describes scenarios in which only a subpopulation of cells within a clonal cell population survives prolonged antibiotic treatment, while remaining genetically susceptible to reapplication of the drug [5,9]. An important feature of bacterial drug persistence is its phenotypic reversibility. After drug treatment is stopped, the remaining persister cells will eventually re-establish a population showing the same heterogeneous response when re-treated with the same drug

Table 1

***In vitro* persister model systems in cancer (Sorted by cancer origin and cell line name). Corresponding references are included in brackets.**

Cancer	Cell Line	Target	Drug	Susceptibility
Breast	BT474	HER2	Labatanib, Trastuzumab	BAD/BCL-XL [73]
Breast	BT474	HER2	Lapatanib, Carboplatin + Paclitaxel	GPX4 [11]
Breast	EVSA-T	PI3K	PI3 kinase inhibitor	KDM5 [46]
Breast	SKBR3	HER2	Lapatanib	KDM5 [46]
Colon	Colo205	BRAF	Vemurafenib	KDM5 [46]
Gastric	GTL-16	MET	Crizotinib, Etoposide	ALDH1A1 [36]
Gastric	MKN-4	MET	Crizotinib	ALDH1A1 [36]
Lung	HCC827	EGFR	Erlotinib	BCL-2/BCL-XL, pSTAT3 [74], SOX2 [75]
Lung	HCC827	EGFR	Gefitinib	OCT4 [76], HIF1a, IGF1R [77]
Lung	PC9	EGFR	Erlotinib	BCL-2/BCL-XL, pSTAT3 [74], KDM5 [46], GPX4 [11]
Lung	PC9	EGFR	Gefitinib	IGF-1R, KDM5 [2], OCT4 [76], HIF1a, IGF1R [77]
Ovarian	JCRB		Carboplatin + Paclitaxel	GPX4 [11]
Skin	A375	BRAF	Vemurafenib	GPX4 [11]
Skin	Hs888	BRAF	AZ628	KDM5 [46]
Skin	M14	BRAF	AZ628	KDM5 [46]
T-ALL	DND-41		GSI (Compound E)	BRD4 [78]
T-ALL	KOPT-K1		GSI (Compound E)	BRD4 [78]

(Figure 1B). Experimentally, drug persistence is characterized by a survival curve with two phases — an initial steep decline in cell number followed by a cell number plateau — which is absent in drug-tolerant populations [5,7] (Figure 1B).

Compared with the converging literature view of how to define and distinguish bacterial persisters, terminology is somewhat more diverse in cancer literature. Persistence is sometimes used interchangeably with drug tolerance to describe subpopulations that have an enhanced (and non-genetic) ability to survive drug treatment [2,10,11]. Various other terms have also been used to describe scenarios in which a phenotypically distinct subpopulation survives prolonged drug treatment, including **quiescence** [12], **dormancy** [13] or **cancer stem cells** [14]. *Throughout this review, we will use the term 'persistence' for cases in which a subpopulation survives drug treatment but regains sensitivity after drug removal, and we reserve the term 'tolerance' for cases in which the whole population is more resilient to drug exposure.*

Paths to persistence

How do cells become persisters? We will first briefly discuss mechanisms of bacterial antibiotic persister formation, and then relate these to our current understanding of how cancer drug persisters emerge. In particular, we will focus on the impact of three factors on persistence: cell growth, metabolic activity, and regulatory program.

Arguably the best studied bacterial persistence mechanism are Toxin-Antitoxin (TA) systems [15]. These consist of a stable toxin, which arrests growth by inhibiting vital cellular processes such as transcription or translation thereby inducing the persister state, and a labile antitoxin acting as the antidote [7]. An example is the HipBA module in *Escherichia coli*, which inhibits the glutamyl-tRNA synthetase GltX and thus halts translation [16,17]. Originally identified as a mechanism to prevent plasmid loss, TA systems were shown to induce the stochastic formation of non-growing persisters in exponentially growing cultures [18].

Recent works have identified additional factors that modulate antibiotic persistence. For example, various studies found that the fraction of persisters in different environmental conditions is inversely correlated with the population growth rate, as shown e.g. in Ref. [19] and summarized in Ref. [5]. Additionally, stresses, such as salt-stress, can increase the rate of persister formation [20]. Particularly interesting types of environmental stress are shifts in nutrient availability: bacteria undergoing nutrient shifts, which are typically accompanied by a transient reduction in growth rate, show dramatically elevated persister fractions [21—24]. The examples above evoke a 'tolerance by slow growth' [5] scheme, in which slow-growing bacteria tend to become more resilient against antibiotic treatment, regardless of how exactly the reduction in growth rate came about.

This increase in antibiotic persistence at slow growth could of course simply be the consequence of a reduction in the activity of the antibiotic targets, i.e. the cellular transcription/translation machinery. However, mounting evidence suggests that antibiotic persistence in fact relies on an active cellular program. Various studies have demonstrated that the (p)ppGpp-mediated bacterial starvation program (also termed "stringent response") modulates the rate of persister formation [24—26]. Importantly, mutant strains lacking the stringent response program are readily killed by antibiotic treatment even in starvation conditions [25],