

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Adaptive Evolution: How Bacteria and Cancer Cells Survive Stressful Conditions and Drug Treatment

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1792716> since 2023-01-31T15:51:09Z

Published version:

DOI:10.1158/2159-8290.CD-20-1588

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

Adaptive evolution: how bacteria and cancer cells survive stressful conditions and drug treatment

Mariangela Russo^{1,2,*}, Alberto Sogari^{1,2} & Alberto Bardelli^{1,2,*}

¹ Department of Oncology, University of Torino, Str. Prov.le 142, km. 3,95, 10060 Candiolo (TO); ²Candiolo Cancer Institute, FPO – IRCCS, Str. Prov.le 142, km. 3,95, Candiolo (TO) 10060, Italy.

*Corresponding authors.

Email: alberto.bardelli@unito.it; mariangela.russo@unito.it

The authors declare no potential conflicts of interest.

Summary

Cancer is characterized by loss of the regulatory mechanisms that preserve homeostasis in multicellular organisms, such as controlled proliferation, cell-cell adhesion, and tissue differentiation. The breakdown of multicellularity rules is accompanied by activation of “selfish”, unicellular-like life features, which are linked to the increased adaptability to environmental changes displayed by cancer cells. Mechanisms of stress response, resembling those observed in unicellular organisms, are actively exploited by mammalian cancer cells to boost genetic diversity and increase chances of survival under unfavorable conditions, such as lack of oxygen/nutrients or exposure to drugs. Unicellular organisms under stressful conditions (e.g. antibiotic treatment) stop replicating or slowly divide and transiently increase their mutation rates to foster diversity, a process known as adaptive mutability. Analogously, tumor cells exposed to drugs enter a persister phenotype and can reduce DNA replication fidelity, which in turn fosters genetic diversity. The implications of adaptive evolution are of relevance to understand resistance to anticancer therapies.

Main text

Complex multicellular organisms, including mammals, exploit sophisticated and tightly regulated networks and genetic constraints that subject the fitness of individual cells to the fitness of the whole organism. The evolution of multicellularity features underscores selection for cooperation between unicellular entities. Cells must proliferate only when required to fulfill their function, and cell death by apoptosis is sometimes essential to maintain tissue homeostasis (1). However, harmful events, such as somatic mutations or viral infections, may subvert multicellularity constraints and the hallmarks of cancer (the common traits of human tumors) include many features reminiscent of a “selfish”, unicellular-like life (2).

During tumorigenesis, cancer cells forgo the cellular cooperation and the regulatory mechanisms that preserve homeostasis in multicellular organisms (3). Cancer cells display sustained proliferation, evade signals of growth suppression, resist programmed cell death and redirect allocation of resources by promoting neoangiogenesis. Moreover, tumor progression is also characterized by progressive cellular de-differentiation (2) (Figure 1).

By dysregulating cellular processes associated with the transition from uni- to multicellular life, cancer cells activate survival strategies, including rapid proliferation and adaptability to stressful environments, that had been perfected by autonomous organisms such as bacteria (4-7). In other words, cancer, a disease of multicellular organisms, shares biological features with prokaryotic cells (Figure 1).

Keeping in mind the remarkable evolutionary distance between bacteria and mammalian cells, here we discuss the scientific and therapeutic implications of considering cancer cells as selfish forms of life, which subvert multicellularity laws and behave as single competing units much like bacteria (7,8).

The hallmarks of cancer resemble features of unicellular organisms

Cancer can be considered as an atavistic form of life, characterized by competition among individual cells for access to nutrients, survival and proliferation, alike what occurs in bacterial populations (1,7). As a consequence, several hallmarks and acquired capabilities of cancer cells are strikingly similar to properties displayed by microorganisms(2,4). For instance, from a metabolic point of view, cancer cells mainly rely on anaerobic glycolysis (the so-called Warburg effect), which is the prevalent energy source among bacteria (9).

In natural environments, microorganisms face a constant battle for nutrients and have evolved several mechanisms to compete with other individuals for resources, including rapid growth, increased matrix production to secure favorable environments and production of anti-microbial toxins (10,11). Similarly, human cancers display high levels of molecular heterogeneity, and within a single lesion distinct subclones can compete with each other (12). This can be observed especially under treatment with anti-cancer agents, when previously unfit drug-resistant subclones outcompete the dominant drug-sensitive cells (13,14).

Intriguingly, bacteria can also thrive as complex communities regulated by cooperation or competition, either between individuals from the same species or between distinct bacterial strains (4). For example, especially (but not exclusively) when facing hostile environments, bacteria can form biofilms, multicellular aggregates embedded in a proteinaceous extracellular matrix (15). The formation of a biofilm promotes survival of the population, as these complex structures have been shown to protect bacteria from antibiotics, toxins and solvents (16).

Analogously, the interactions between cancer cells and their stroma can be compared to a special form of biofilm, whereby cancer cells orchestrate neoangiogenesis, recruitment of cancer-associated fibroblasts and immune cells to promote the survival of the entire community (2,17,18). Cooperation through paracrine signaling can also promote increased resistance to targeted treatment: for example, we previously showed that in colorectal cancer treatment with anti-EGFR targeted therapy promotes increased secretion of TGF α and amphiregulin from resistant cells, which in turn protect surrounding sensitive cells in the population from EGFR blockade (19).

Both bacteria and cancer can deploy complex responses to hostile environments to protect the population from eradication. Some of the bacteria inside a biofilm are able to switch to planktonic state and colonize distant sites (3,20). Moreover, under stressful conditions some individuals can undergo sporulation, generating highly resistant spores which germinate once favorable growth conditions are restored (15,21). Similarly, some cells within a tumor (possibly in response to challenging circumstances) acquire metastatic competence, i.e. the ability to separate from the cancer of origin and colonize distant organs (22). At the site of colonization, disseminated tumor cells encounter an unfavorable environment, in which they enter a state of dormancy (just like spores) and slowly condition the surrounding stroma until a supportive niche is formed (23).

Notably, competition can lead to selection of variants that are better suited to colonize the environment. The evolution of tumors cells and asexual bacteria is governed by the interplay of genetic drift, heritable variation and Darwinian selection (24). Experimental and computational evidences show that constitutive mutators are present at low frequency in a bacterial population, since they result in the overproduction of lethal mutations. However, in stressful conditions (such as antibiotic treatment or limited access to nutrients) hypermutators are positively selected and can become fixed in the majority of the population (25-29). Similarly, genome instability is a hallmark of cancer, and is favorably selected in tumor cells for its role in fostering the emergence of progressively fitter subclones and supporting tumor evolution (2).

The atavistic hypothesis: cancer as a throwback towards ancestral traits

The atavistic hypothesis states that tumorigenesis involves the reactivation of survival programs dating back to the evolution of unicellular organisms, and the concomitant disruption of complex features evolved to support the intercellular communications required for multicellular organisms' development and physiology (7,30).

Transcriptional analysis of more than 3000 tumor samples across seven different tumor types unveiled that cancers rely on unicellular processes for survival. Dysregulation of genes and cellular processes unique to multicellularity was detected across multiple tumor types, together with strong upregulation of genes conserved in unicellular organisms, including those involved in proliferative signaling, cell death escape, and genomic instability (5,31). Expression of highly conserved genes is linked to drug resistance in cancer cells (32); and tumors often activate transcriptional programs associated with dedifferentiation (33,34).

Analysis of the evolutionary age of genes involved in human cancer revealed increased mutational processes in genes younger than 500 millions of years, suggesting selection for somatic mutations occurring in younger genes preferentially linked to multicellularity (35).

Goode and colleagues used a computational approach to analyze different types of tumors from over 9,000 patients, to unveil accumulation of point mutations and copy number variations (CNV) in genes dating back to metazoan ancestors' genes. Their analysis showed that point mutations disrupt key regulators of multicellularity networks, and that CNVs dysregulate downstream effectors of these pathways (36).

Relatedly, genes with regulatory function in the communication between genes of unicellular and multicellular origin represent vulnerabilities that, when compromised, promote the emergence of tumorigenesis. For example, *TP53*, *NF1* and *PI3KCA* genes, which are frequently mutated across different tumor types, act as regulatory hubs of multiple processes fundamental for the maintenance of the integrity of genomic networks (31,36).

Whether atavism plays a role on cancer initiation and progression is still debated, as acquisition of cancer's biological features during tumorigenesis can be explained by stochastic genetic alterations and progressive strong environmental selection rather than re-activation of a conserved survival program. However, as discussed below, the two theories are not necessarily mutually exclusive and can coexist (37). According to basic laws of evolution, an enriched fraction of mutants in a population would increase chances of survival of a few individuals under selective conditions. Indeed, unicellular organisms deploy strategies of adaptive mutability to foster evolution in strongly selective environments (see below). Intriguingly, we and others recently observed that cancer adaptation to stress is at least initially fostered by activation of similar "atavistic" survival programs observed in bacteria under stress, thereby promoting mutagenesis and genetic diversity; selection and expansion of phenotypes capable of replicating under stressful environments then occurs (38,39) (see details below).

Bacteria deploy complex responses to survive stressful environments

When facing stressful environments, bacteria deploy rapid and reversible survival programs that foster genetic diversity and facilitate adaptation to changes in the environment (5,40,41). Effective strategies to evade a stressful condition include relocating to a new environment through swimming and the formation of biofilms, as discussed above (3,4).

An additional fascinating strategy exploited by unicellular cells to evade stress-induced death relies on temporary phenotypic switches. Indeed, in bacteria a fraction of cells named "persisters",

evades antibiotic killing by entering a physiologically dormant state displaying transient phenotypic stress-tolerance, without undergoing genetic changes (42,43). Persister cells, differently from permanent genetically resistant cells, reinitiate growth and regenerate a drug-sensitive population upon termination of drug treatment. Importantly, development of resistance eventually occurs upon prolonged drug treatment of persister cells, representing a major cause of antibiotic failure, which leads to recurrent bacterial infections (43).

Many of these stress-adaptations are tailored to the specific stress. For example, the *SOS DNA damage response* induces activation of error-prone DNA polymerases that can bypass the damage and terminate replication; while in case of high temperatures, induction of heat-shock chaperones allows the resolution of misfolded or aggregated proteins (44).

The *SOS response* is triggered by DNA damage caused by cell malfunctioning, stalled DNA replication forks, or conditions such as oxidative stress and antibiotic treatment. In *E. coli* and related bacteria, the coating of RecA protein to single-strand DNA (ssDNA), generated either as a result of DNA damage itself, or following stalled DNA replication forks, or during the DNA repair process, induces the autoproteolytic cleavage of LexA, a transcriptional repressor, leading to upregulation of SOS regulon genes (45,46). The SOS regulon includes genes involved in multiple functions, such as DNA recombination and repair, nucleotide excision repair, DNA synthesis past damaged bases, control of cell-division (45,46). Among these, the activation of specialized low-fidelity DNA polymerases, which temporarily replace canonical replicative polymerases, is responsible for a mutagenic form of DNA replication associated with increased rate of base substitutions and indels (47-49).

However, in addition to stress-specific responses, many bacteria deploy a strong *general stress response* to react to a variety of growth-limiting conditions and stresses, such as nutrient deprivation, stationary phase growth, DNA damage and extreme temperatures or pH (44,50). In *E. coli* and related bacteria, the master regulator of the *general stress response* is the sigma factor RpoS. Different mechanisms regulate the increase of RpoS levels and the induction of the multiple RpoS-dependent effectors; indeed, some are dependent only on RpoS, while others require certain conditions and additional inputs (44,50).

Both *RpoS* and *SOS responses* seem to play a role in persisters formation. In particular the SOS-dependent DNA repair functions are central for persisters formation and recovery. Indeed, emergence of surviving persisters in response to DNA damaging agents is impaired in SOS-deficient mutants (43).

Stress responses in human cells and cancer

Cancer cells are constantly exposed to intracellular and extracellular stressful stimuli, such as hypoxia, reduced nutrient availability, glucose deprivation and DNA damage. In order to foster adaptation and survival, cells deploy a variety of stress-responses to deal with adverse environments. Indeed, cancer cells activate multiple survival strategies, alter gene expression, reprogram metabolic pathways and trigger stress-response mechanisms that can promote mutability and genetic diversity (5).

Cancer cells, like bacteria in response to antibiotics, can enter a reversible drug-tolerant persister state when exposed to targeted therapies (51,52). Through non-genetic mechanisms of drug-tolerance, slowly replicating cancer persister cells escape death and avoid tumor eradication. Of clinical relevance, extended treatment of persister cells inevitably leads to development of resistance and consequent treatment failure (52,53).

In mammals, the kinase mammalian target of rapamycin (mTOR) has been proposed as a possible analog of the bacterial RpoS general stress regulator, which controls the switch to mutagenic break repair in *E. coli* (5). mTOR has a central role in sensing environmental conditions, including lack of growth factors, nutrient deprivation, low oxygen and DNA damage, and regulates many fundamental cell processes, such as protein synthesis, metabolism, cell growth, autophagy and aging (54). Dysregulation of mTOR regulatory signaling is implicated in cancer progression, and mTOR inhibitors have been developed as anticancer agents (54).

Cells are constantly exposed to damage from internal and external sources and have therefore evolved an intricate network of signaling pathways and repair mechanisms to cope with DNA lesions and protect genome stability. Interestingly, several error-prone DNA polymerases have been described also in eukaryotic cells, including yeasts and human cells (49).

Why are error-prone DNA polymerases conserved in highly developed organisms, whereby programmed cell death allows the elimination of cells with damaged genomes?

In human cells exposed to non-stressful conditions, when the replication fork stalls upon encountering a site of DNA damage, canonical replicative DNA polymerases are replaced by specialized polymerases characterized by poor accuracy in nucleotide incorporation and increased rates of base substitutions and frameshifts, ranging from 10^{-3} to 10^{-1} errors per base pair (49,55); propensity to incorporate nucleotides using aberrant DNA primer ends; absence of proof reading 3'→5' exonuclease activity; and moderate-to-low processivity (49,56).

The expression of error-prone DNA polymerases is tightly regulated. In physiological conditions they are involved in multiple biological functions, such as non-homologous end joining (NHEJ) and translesion synthesis (TLS) DNA repair processes (57); somatic hypermutation of immunoglobulin genes (58); protection against UV-induced genetic instability and skin cancer, through the prevention of replication fork collapse and double-strand breaks (DSBs) accumulation (59).

However, according to mathematical models, in growth-limiting hostile environments the evolutionary costs of high fidelity DNA replication may exceed the costs of error-prone replication, becoming therefore a counterproductive strategy, while the proliferation and survival of cells with limited DNA repair capacity is favored, both in microbes and in cancer cells (60).

This suggests that in some instances, also in multicellular organisms, cell survival is prioritized over genome perfection, and occurs by downregulation/repression of DNA repair mechanisms, which is preferred over stalling of DNA replication.

Indeed, DNA damage tolerance (DDT) response, evolved side by side with DNA repair mechanisms, functions in circumstances where the possibility to make errors has a selective advantage. Through the involvement of specialized polymerases able to bypass DNA damage, DDT allows the replication of a damaged template, and postpone the repair at later timepoints, thus lowering the

overall risk of replication fork collapse, genome instability, genetic rearrangements and cell death (57).

The association of DDT with cancer is double-faced. On the one hand, defects in DDT are associated with cancer susceptibility and aging (57). On the other, uncontrolled overexpression of error-prone polymerases has been associated with accumulation of mutation and tumorigenesis in lung tumors (61); poorer prognosis in glioma patients (62); and drug resistance (63). Notably, the involvement of DDT and error-prone DNA polymerases in stress-responses affects the mutational profile, leading to a temporary increase of genetic diversity, which can favor adaptation to an hostile environment and foster evolution (38,39,48).

Stress-induced adaptive mutability in *selfish* unicellular entities

Mutations represent the primary source of genetic variation and, together with environmental selection, an important evolutive driving force, both in cancer cells and bacteria (64).

Growing evidence shows that multiple microorganisms, such as bacteria and yeast, adaptively combine stress responses that transiently increase their genomic instability, even during replicative quiescent state, in response to different environmental stresses (5,41,65). The resulting stress-induced adaptive mutability promotes stochastic emergence of fitter mutants, leading to higher rates of adaptive evolution (41,45,65).

Bacteria enter a dormant state when life is unsustainable and recover when it is beneficial to do so. Acquisition of mutations during the stationary phase indicates that cells exposed to stress respond by promoting mutability; a subset of the newly acquired mutations fosters adaptation to the stressful environment.

Indeed, in a constant environment, well adapted populations evolve with a constant low mutation rate; while, when organisms are maladapted to their environments (e.g. in conditions of starvation or when exposed to hypoxia or antibiotic treatment) hypermutator phenotypes can emerge (65,66). These phenotypes transiently provide elevated mutation rates when populations experience bursts of stresses. However, when a new (adapted) population emerges, the mutator phenotype is no longer an advantage and is counter-selected, and eventually the genome of the expanding adapted mutants returns to low mutation rates (45,65).

In *E.coli* stress-induced mutagenesis via mutagenic DNA break repair involves three components: (i) repair of double-strand breaks (DSBs); (ii) activation of the *SOS damage response*, which upregulates error-prone DNA Pol IV; (iii) activation of the RpoS-mediated *general stress response* which allows the accumulation of Pol IV errors in acts of DSB repair (5,45,65). This stress-induced adaptive mutability appears to induce mutagenesis in proximity of DSBs, creating clusters that reduce in density with distance from the DSBs (67). Stress-induced mutagenicity generates distinct forms of genetic changes, including single nucleotide variants, deletions and insertions, copy number variations and chromosomal rearrangements (45,65). Induction of error-prone DNA polymerases and other genes of the SOS regulon is required for most point mutagenesis.

Permanent loss of highly conserved mismatch repair (MMR) proteins, responsible for recognition and repair of mispaired bases and 1-base insertion/deletion, generates constitutive mutators (68). Interestingly, the function of the MMR system is temporarily disabled during adaptive mutability in response to stress. Indeed, overexpression of one or more MMR proteins reduces stress-induced mutations (65).

Downregulation of DNA repair capacity, associated with overexpression of error-prone DNA polymerases, increases the number of errors that persist and become mutations, therefore translating into more mutagenic replication of DNA and genomic instability (69).

Notably, it has been shown that even sublethal concentrations of antibiotics induce downregulation of the MMR system and upregulation of error-prone DNA polymerases which, in turn, promote increased genetic instability (69). This temporary increase in mutation rate promotes the generation of mutants and ultimately the acquisition of resistance to a wide spectrum of drugs, including compounds unrelated to the antibiotic applied (70).

While it is easy to conceive that adaptive mutability is essential for the survival of unicellular organisms exposed to stress, multicellular organisms evolved multiple constraints to ensure genetic integrity and homeostasis, such as the tightly controlled recruitment of error-prone DNA polymerases in the replication of damaged DNA templates described above (5).

However, it has been shown that, when exposed to stressful conditions, tumor cells exploit survival strategies resembling bacterial stress-induced mutagenesis (38,39). For example, constitutive activation of the TGF- β axis causes downregulation of HR-mediated DSBs repair, increased genetic instability and clonal diversity, thus boosting the ability of cancer cells to adapt to drug treatment and the development of chemoresistance (71). Analogously, cancer cells exposed to hypoxia activate a transcriptional regulatory response that downmodulates DNA repair mechanisms such as mismatch repair (MMR) and homologous recombination (HR) (72). Moreover, hypoxia promotes upregulation of error-prone DNA repair pathways, such as NHEJ, and the use of TLS DNA polymerases (72). This switch from highly accurate to error-prone DNA repair creates a permissive milieu for genomic instability; in fact, hypoxic cancer cells display increased mutability and accumulation of frameshift mutations at repetitive microsatellite sequences in reporter plasmids (73).

The remarkable capacity of tumor cells to adaptively modulate their mutagenicity has been observed also in response to targeted agents, similarly to bacteria in response to antibiotics.

Indeed, we recently showed that colorectal cancer (CRC) cells, under stress imposed by targeted therapy, activate a response recapitulating key element of bacterial stress-induced adaptive mutability. Treatment with the anti-EGFR monoclonal antibody cetuximab, alone or in combination with the BRAF inhibitor dabrafenib, induced transient deficiency of both MMR and HR-mediated DNA repair proficiency, and a concomitant switch from high- to a low-fidelity DNA polymerase (38). Notably, treatment with these non-directly genotoxic drugs caused increased DNA damage and the observed response, in turn, translated into increased genetic instability. The stress-induced adaptive mutability phenotype in cancer cells, alike in bacteria, was transitory and confined to maladapted cell populations. When the stress was terminated, either by drug withdrawal or emergence of permanently resistant cells, the MMR and HR levels reverted back (38).

We propose that during tumor development, when breakdown of multicellularity occurs, a stress response aimed at promoting survival over functional and genetic integrity is expediently restored.

Adaptive mutability in response to stress appears to be an evolutionarily conserved process rather than a phenotype restricted to colorectal cancers. Indeed, Cipponi and colleagues observed induction of DNA damage, and a switch to mutagenic DNA replication process in melanoma, prostate, breast and pancreatic cancers exposed to non-genotoxic drugs. This in turn resulted in genetic diversity and development of secondary resistance (39).

Notably, both in melanoma and CRC cells the modulation in DNA repair machinery was not simply a consequence of DNA damage or cell cycle arrest, but was exquisitely linked to the inhibition of the specific oncogene addiction across tumor types, and was abolished when targeted treatment was applied to resistant cells (38,63).

Although the role of stress-induced adaptive mutability in the acquisition of resistance to targeted therapies in cancer requires further elucidation, several indications point in this direction. We observed treatment-induced loss of MMR proteins expression in clinical specimens obtained at maximal tumor response (*nadir*) in MMR proficient CRC patients treated with targeted therapies (38). Moreover, increased mutability at microsatellite regions was detected in preclinical models of acquired resistance (38).

Similarly, treatment of NSCLC cell lines with EGFR inhibitors gefitinib and erlotinib induced increased DNA damage and temporary deficiency in base-excision repair (BER). The resulting increase in genetic instability promoted the acquisition of the EGFR p.T790M mutation, which drives acquired resistance to anti-EGFR treatment (74). In addition, DNA barcoding and mathematical modeling of triple-negative breast cancer cells in response to BET and CDK4/6 inhibitors, showed a higher rate of *de novo* mutagenesis under drug pressure (75).

This growing body of literature suggests that the features of the stress response displayed by microbial organisms have counterparts in cancer cells, and supports the possibility that tumors are a community of individual entities with remarkable survival and evolutionary capabilities (5).

Controllers of adaptive mutability in bacteria and cancer

In *E. coli*, both RpoS-mediated *general stress response* and *SOS DNA damage response* need to be activated to initiate stress-induced adaptive mutability (45,65). The SOS-mediated activation of error-prone DNA polymerases makes the repair of DSBs a source of mutagenesis and increased genetic variations (48). However, RpoS is required to permit the use of error-prone polymerases during stress (47). In addition, although the mechanisms of MMR downregulation during adaptive mutability are not well characterized, RpoS seems to be implicated also in this temporary impairment of DNA repair (76,77). Importantly RpoS, and not error-prone polymerases, appears to contribute to genetic amplification, suggesting a central role for RpoS in the induction of genome instability under stress (77).

Recently, mTOR has been proposed as the master regulator umpiring adaptive mutability in response to stress across multiple cancer types (5,39,63). Using genome-wide functional screens in response to different type of targeted therapies, Cipponi and colleagues identified two classes of

genes involved in development of secondary resistance to targeted therapy: (i) the “solution” genes, directly responsible for conferring resistance to the specific anti-cancer agent used (e.g. *TP53* alterations in response to the MDM2 inhibitor nutlin); ii) “facilitator genes”, capable to indirectly facilitate the process of adaptation to drug-imposed hostile environment. mTOR was identified among the top-ranked facilitator genes, and its pharmacologic inhibition was shown to selectively impair HR and canonical high-fidelity DNA polymerases genes (39).

Moreover, Tempirne and colleagues recently showed that treatment of melanoma cell lines with the BRAF inhibitor dabrafenib induced upregulation of error-prone polymerase κ (Pol κ) and this was central to the emergence of drug-tolerance (63); furthermore, inhibition of mTOR recapitulated the phenotype (63). Polymerase κ is the mammalian ortholog of *E. coli* Pol IV, the error-prone DNA polymerase responsible for base substitutions and indels during mutagenic DNA break repair in *E. coli* (48). Altogether these results suggest a key role for mTOR in fostering genetic diversity and promoting adaptive evolution in cancer cells during drug treatment.

Therapeutic Significance

Overcoming drug resistance remains a major challenge in oncology. While it is generally accepted that the intrinsic molecular heterogeneity which characterizes most tumors plays a major role in the emergence of drug resistance, it remains unclear whether yet to be discovered biological features of cancer cells play a role in therapeutic failures.

The analogies between drug-resistance mechanisms arising in cancer and infectious diseases, such as mutations in the drug-target or activation/amplification of parallel compensatory pathways, suggest that they can provide insights for the development of novel anticancer treatments (78). For instance, the successful experience of combinatorial strategy at treatment initiation in case of HIV and tuberculosis infections highlights the relevance of using drug combinations in order to achieve a stronger tumor shrinkage, while preventing the outgrowth of resistant clones (78).

Here, we propose that further insights on how tumor cells survive adverse environments, and specifically stress generated by therapeutic treatment, can be obtained observing the evolutionary strategies used by unicellular microorganisms in response to drug treatments and stressful environments (79), and could be translated into novel concepts for therapeutic interventions.

In detail, if stress response strategies exploited by unicellular organisms represent phylogenetically conserved mechanisms of survival, then conceptual interpretation of cancer as a community of unicellular organisms can be inspirational for developing new anticancer therapies to curb or restrict the emergence of drug resistance (79,80).

Modern therapeutic regimens often have focused on targeting cells hyper-proliferation features (e.g. targeting aberrant and constitutive activation of oncogenes). Though initially effective, activation of parallel pathways and/or compensatory feedback loops usually leads to treatment failure (81,82), highlighting that cellular proliferation is a redundant, multilayer (and therefore seemingly unbeatable) process in cancer cells. However, by considering at least some features of cancer cells as a phylogenetic throwback, new vulnerabilities emerge. As a matter of fact, drugs targeting cellular processes shared with unicellular organisms are already used in the clinical

practice, including inhibitors of purine and pyrimidine synthesis, proteasome inhibitors, and inhibitors of the mitotic spindle (7).

Notably, alike bacteria in response to antibiotics, cancer cells in quiescent/slowly replicating state may contribute to survival under stress, and represent a reservoir from which mechanisms of drug resistance could eventually emerge (53). Although both tumor progression and response to anticancer drugs are fueled by mutations, the evidence that patients can relapse after a long disease stabilization, or that tumors can be successfully re-challenged with the same drug, cannot be explained by genetic resistance. Dormant drug-tolerant tumor cells might represent a major source of relapse in these instances. The importance of targeting persister cells both in cancer and infectious disease in order to prevent relapse has been previously highlighted (78,83), however little is known about persistence mechanisms.

We and others recently unveiled that cancer persister cells activate a stress-induced adaptive mutability response as an ancestral survival program that leads to increased mutagenicity thus fueling selection of newly generated resistant variants (38,39). Though adaptive mutability increases the chances of survival in stressful environments, this strategy comes at a cost and stress-induced modulation of DNA repair pathways might unveil cell vulnerabilities exploitable for innovative therapeutic strategies (79).

We propose here some therapeutic approaches aiming at preventing the development of drug resistance, targeting cancer cells features resembling the unicellular ones.

As discussed above, in bacteria and cancer cells undergoing adaptive mutability in response to stress, the MMR and HR DNA repair machineries are repressed, and DNA damage is tolerated, thus promoting mutagenesis and improving survival. Interfering with these mechanisms may undermine cellular survival and promote DNA damage-induced cytotoxicity. Indeed, increasing levels of DNA damage to a point which cancer cells cannot tolerate is already exploited in clinical care. Tumors under therapeutic stress display increased endogenous DNA damage, together with delayed repair of radiation- and chemically induced DNA lesions, as pointed out by several works in the literature (see Figure 2) (84-91). Indeed, targeted therapies are already successfully combined with radiotherapy or chemotherapy to increase chances of tumor control.

Multiple evidences indicate modulation in DNA repair systems upon treatment with targeted therapies across different tissues (Figure 2)(38,39,63,74,86,88,90-99). For example, blockade of the RAF/MEK/ERK pathway induces a BRCAness phenotype that sensitizes cells to PARP inhibitors (PARPi), both *in vitro* and *in vivo* (Figure 2). Sun et al showed that targeted therapy inhibiting the MAPK pathway restores sensitivity to PARPi in otherwise PARPi-resistant ovarian cancer cell lines (99). Relatedly, Maertens and colleagues found that cotreatment of melanoma cells with vemurafenib and histone deacetylases (HDAC) inhibitors leads to concomitant downregulation of both HR and NHEJ, an ineffective repair of DSBs and ultimately in cell catastrophe (98) (Figure 2). Based on this, synthetic lethality approaches capitalizing on the transient deficiency of DNA repair systems in stressed cancer cells might be exploited to prevent cancer adaptation and evolution.

Analogously, inhibition of the transcriptional networks controlling induction of adaptive mutability in cancer cells might be successful in preventing development of resistance through *de novo* mutagenesis. While mTOR has emerged as a possible master regulator of adaptive stress-response

in multiple tumors, it has been shown that mTOR inhibition recapitulates the adaptive mutability phenotype rather than preventing it (39). Therefore, the applicability of clinically available mTOR inhibitors for this purpose remains uncertain.

On the contrary, inhibition of hyper-activated error-prone DNA polymerases might eventually interfere with enhanced mutagenic capacity. For example, overexpression of Pol κ , which plays a central role in the adaptive response by regulating genes associated with drug resistance and immune surveillance through non-canonical mechanisms, further increases resistance to treatment, suggesting it as a putative target to curb drug-tolerance (63).

Following the lesson learned from infectious disease, in order to optimize the potential success of these strategies in preventing cancer adaptation and curbing the tumor evolution, targeted therapies and drugs targeting the stress-response phenotype should be administered in combination from the initiation of the treatment (i.e., before development of resistance).

We previously reported the preclinical and clinical potential of concomitant inhibition of multiple nodes along the same pathway in terms of prevention of secondary resistance (81,100) and overall survival in colorectal cancer patients (101,102).

Notably, as we previously demonstrated, interfering with oncogenic addiction causes an increased DNA damage and shifts the dependency of cancer cells towards DNA damage tolerance pathways to cope with increased instability (38). The concomitant inhibition of multiple pathways with agents targeting simultaneously oncogene addiction and the adaptive response activated in cancer cells exposed to targeted therapies might therefore restrain the emergence of resistant variants by denying activation of the salvation mechanisms in cancer cells.

The concept of a paradigmatic shift from re-active to a pro-active strategy is indeed central to the approach of targeting mechanisms of adaptive mutability and resistance. Currently, in most instances medical oncologists wait for resistance to emerge, next the resistant tumor is molecularly profiled and then therapy is modified accordingly. Instead, one can envision to act pre-emptively by interfering with the cellular factors involved in adaptive mutability to restrain the emergence of resistance, rather than passively wait for the tumor to evolve under therapy.

While it remains still unclear whether the stress-induced adaptive response occurs also in normal cells, inhibition of DNA damage response has been previously tested in cancer patients with manageable toxicity (103). Moreover, combinatorial regimens might allow the administration of lower doses of single agents by exploiting synergism exerted by parallel inhibition of multiple survival pathways in cancer cells.

In conclusion, further exploration of the mechanisms through which bacteria and cancer cells survive and evolve under stress could provide new therapeutic options aimed at curbing adaptive mutability unleashed by drug pressures in cancer cells with altered genome functions, while limiting the side effects on normal cells.

Acknowledgments

This work was supported by FONDAZIONE AIRC under 5 per Mille 2018 - ID. 21091 program – P.I. Alberto Bardelli; H2020 grant agreement no. 635342-2 MoTriColor to A.B.; AIRC under IG 2018 - ID. 21923 project – P.I. Alberto Bardelli; AIRC-CRUK-FC AECC Accelerator Award contract 22795 to Alberto Bardelli; Genomic-Based Triage for Target Therapy in Colorectal Cancer Ministero della Salute, Project no. NET 02352137 to Alberto Bardelli.; Ministero Salute, RC 2019 to Alberto Bardelli; Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 per mille 2015 Ministero della Salute to Alberto Bardelli.

Figure legends

Figure 1. The hallmarks of tumor cells and the biological features of unicellular organisms.

Cancer cells display properties that parallel the behavior of unicellular organisms. The biologic, genetic and metabolic features shared between cancer and bacterial cell populations include: competition between clones, glycolytic metabolism, formation of communities by manipulating the environment, stress responses leading to increased genetic instability and adaptation to hostile conditions such as drug treatments.

Figure 2. Effect of targeted therapies-induced stress on the DNA repair machinery in cancer cells.

Treatment of cancer cells with several targeted therapies (indicated on the left with the corresponding molecular targets) results in enhanced DNA damage, accompanied by a promotion of tolerance over DNA damage recognition and repair, and upregulation of error-prone DNA polymerases. The concomitant downmodulation of the DNA repair systems (depicted on the right) results in transient functional DDR deficiency which, in turn, is permissive for increased mutagenesis. While fostering genetic diversity and, therefore, survival, modulation of the DDR unveils possible vulnerabilities that might be exploited to eradicate cancer cells that undergo stress-induced adaptive evolution and foster the onset of resistance to targeted agents. The numbers in brackets indicate the references corresponding to each targeted treatment and associated DNA repair modulation. MMR, mismatch repair. HR, homologous recombination. NHEJ, non-homologous end joining. NER, nucleotide excision repair. BER, base excision repair.

References

1. Aktipis CA, Boddy AM, Jansen G, Hibner U, Hochberg ME, Maley CC, *et al.* Cancer across the tree of life: cooperation and cheating in multicellularity. *Philos Trans R Soc Lond B Biol Sci* **2015**;370(1673) doi 10.1098/rstb.2014.0219.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* **2011**;144(5):646-74 doi 10.1016/j.cell.2011.02.013.

3. Lambert G, Estévez-Salmeron L, Oh S, Liao D, Emerson BM, Tlsty TD, *et al.* An analogy between the evolution of drug resistance in bacterial communities and malignant tissues. *Nat Rev Cancer* **2011**;11(5):375-82 doi 10.1038/nrc3039.
4. Ben-Jacob E, Coffey DS, Levine H. Bacterial survival strategies suggest rethinking cancer cooperativity. *Trends Microbiol* **2012**;20(9):403-10 doi 10.1016/j.tim.2012.06.001.
5. Fitzgerald DM, Hastings PJ, Rosenberg SM. Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance. *Annu Rev Cancer Biol* **2017**;1:119-40 doi 10.1146/annurev-cancerbio-050216-121919.
6. Rosenberg SM, Shee C, Frisch RL, Hastings PJ. Stress-induced mutation via DNA breaks in *Escherichia coli*: a molecular mechanism with implications for evolution and medicine. *Bioessays* **2012**;34(10):885-92 doi 10.1002/bies.201200050.
7. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. How the evolution of multicellularity set the stage for cancer. *Br J Cancer* **2018**;118(2):145-52 doi 10.1038/bjc.2017.398.
8. Vincent M. Cancer: a de-repression of a default survival program common to all cells?: a life-history perspective on the nature of cancer. *Bioessays* **2012**;34(1):72-82 doi 10.1002/bies.201100049.
9. Lineweaver CH, Davies PC, Vincent MD. Targeting cancer's weaknesses (not its strengths): Therapeutic strategies suggested by the atavistic model. *Bioessays* **2014**;36(9):827-35 doi 10.1002/bies.201400070.
10. Hibbing ME, Fuqua C, Parsek MR, Peterson SB. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* **2010**;8(1):15-25 doi 10.1038/nrmicro2259.
11. Nadell CD, Drescher K, Foster KR. Spatial structure, cooperation and competition in biofilms. *Nat Rev Microbiol* **2016**;14(9):589-600 doi 10.1038/nrmicro.2016.84.
12. Sun R, Hu Z, Sottoriva A, Graham TA, Harpak A, Ma Z, *et al.* Between-region genetic divergence reflects the mode and tempo of tumor evolution. *Nat Genet* **2017**;49(7):1015-24 doi 10.1038/ng.3891.
13. Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, *et al.* Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nature Medicine* **2015**;21(7):795-801 doi 10.1038/nm.3870.
14. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* **2017**;168(4):613-28 doi 10.1016/j.cell.2017.01.018.
15. Claessen D, Rozen DE, Kuipers OP, Sjøgaard-Andersen L, van Wezel GP. Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. *Nat Rev Microbiol* **2014**;12(2):115-24 doi 10.1038/nrmicro3178.
16. Monds RD, O'Toole GA. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol* **2009**;17(2):73-87 doi 10.1016/j.tim.2008.11.001.
17. Sahai E, Astsaturou I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, *et al.* A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer* **2020**;20(3):174-86 doi 10.1038/s41568-019-0238-1.
18. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, *et al.* Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* **2018**;24(5):541-50 doi 10.1038/s41591-018-0014-x.
19. Hobor S, Van Emburgh BO, Crowley E, Misale S, Di Nicolantonio F, Bardelli A. TGF α and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. *Clin Cancer Res* **2014**;20(24):6429-38 doi 10.1158/1078-0432.CCR-14-0774.
20. Butler MT, Wang Q, Harshey RM. Cell density and mobility protect swarming bacteria against antibiotics. *Proc Natl Acad Sci U S A* **2010**;107(8):3776-81 doi 10.1073/pnas.0910934107.
21. Branda SS, González-Pastor JE, Ben-Yehuda S, Losick R, Kolter R. Fruiting body formation by *Bacillus subtilis*. *Proc Natl Acad Sci U S A* **2001**;98(20):11621-6 doi 10.1073/pnas.191384198.
22. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell* **2011**;147(2):275-92 doi 10.1016/j.cell.2011.09.024.
23. Hüsemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, *et al.* Systemic spread is an early step in breast cancer. *Cancer Cell* **2008**;13(1):58-68 doi 10.1016/j.ccr.2007.12.003.

24. Sprouffske K, Merlo LM, Gerrish PJ, Maley CC, Sniegowski PD. Cancer in light of experimental evolution. *Curr Biol* **2012**;22(17):R762-71 doi 10.1016/j.cub.2012.06.065.
25. Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, *et al.* Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* **1997**;277(5333):1833-4 doi 10.1126/science.277.5333.1833.
26. Mao EF, Lane L, Lee J, Miller JH. Proliferation of mutators in *A* cell population. *J Bacteriol* **1997**;179(2):417-22 doi 10.1128/jb.179.2.417-422.1997.
27. Taddei F, Radman M, Maynard-Smith J, Toupance B, Gouyon PH, Godelle B. Role of mutator alleles in adaptive evolution. *Nature* **1997**;387(6634):700-2 doi 10.1038/42696.
28. Tenaillon O, Taddei F, Radman M, Matic I. Second-order selection in bacterial evolution: selection acting on mutation and recombination rates in the course of adaptation. *Res Microbiol* **2001**;152(1):11-6 doi 10.1016/s0923-2508(00)01163-3.
29. Tanaka MM, Bergstrom CT, Levin BR. The evolution of mutator genes in bacterial populations: the roles of environmental change and timing. *Genetics* **2003**;164(3):843-54.
30. Davies PC, Lineweaver CH. Cancer tumors as Metazoa 1.0: tapping genes of ancient ancestors. *Phys Biol* **2011**;8(1):015001 doi 10.1088/1478-3975/8/1/015001.
31. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. Altered interactions between unicellular and multicellular genes drive hallmarks of transformation in a diverse range of solid tumors. *Proc Natl Acad Sci U S A* **2017**;114(24):6406-11 doi 10.1073/pnas.1617743114.
32. Wu A, Zhang Q, Lambert G, Khin Z, Gatenby RA, Kim HJ, *et al.* Ancient hot and cold genes and chemotherapy resistance emergence. *Proc Natl Acad Sci U S A* **2015**;112(33):10467-72 doi 10.1073/pnas.1512396112.
33. Chen H, He X. The Convergent Cancer Evolution toward a Single Cellular Destination. *Mol Biol Evol* **2016**;33(1):4-12 doi 10.1093/molbev/msv212.
34. Chen H, Lin F, Xing K, He X. The reverse evolution from multicellularity to unicellularity during carcinogenesis. *Nat Commun* **2015**;6:6367 doi 10.1038/ncomms7367.
35. Cisneros L, Bussey KJ, Orr AJ, Miočević M, Lineweaver CH, Davies P. Ancient genes establish stress-induced mutation as a hallmark of cancer. *PLoS One* **2017**;12(4):e0176258 doi 10.1371/journal.pone.0176258.
36. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. Somatic mutations in early metazoan genes disrupt regulatory links between unicellular and multicellular genes in cancer. *Elife* **2019**;8 doi 10.7554/eLife.40947.
37. Thomas F, Ujvari B, Renaud F, Vincent M. Cancer adaptations: Atavism, de novo selection, or something in between? *Bioessays* **2017**;39(8) doi 10.1002/bies.201700039.
38. Russo M, Crisafulli G, Sogari A, Reilly NM, Arena S, Lamba S, *et al.* Adaptive mutability of colorectal cancers in response to targeted therapies. *Science* **2019**;366(6472):1473-80 doi 10.1126/science.aav4474.
39. Cipponi A, Goode DL, Bedo J, McCabe MJ, Pajic M, Croucher DR, *et al.* mTOR signaling orchestrates stress-induced mutagenesis, facilitating adaptive evolution in cancer. *Science* **2020**;368(6495):1127-31 doi 10.1126/science.aau8768.
40. Rosenberg SM. Evolving responsively: adaptive mutation. *Nat Rev Genet* **2001**;2(7):504-15 doi 10.1038/35080556.
41. Foster PL. Adaptive mutation: implications for evolution. *Bioessays* **2000**;22(12):1067-74 doi 10.1002/1521-1878(200012)22:12<1067::AID-BIES4>3.0.CO;2-Q.
42. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. *Science* **2004**;305(5690):1622-5 doi 10.1126/science.1099390.
43. Harms A, Maisonneuve E, Gerdes K. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science* **2016**;354(6318) doi 10.1126/science.aaf4268.
44. Gottesman S. Trouble is coming: Signaling pathways that regulate general stress responses in bacteria. *J Biol Chem* **2019**;294(31):11685-700 doi 10.1074/jbc.REV119.005593.
45. Foster PL. Stress-induced mutagenesis in bacteria. *Crit Rev Biochem Mol Biol* **2007**;42(5):373-97 doi 10.1080/10409230701648494.

46. Sutton MD, Smith BT, Godoy VG, Walker GC. The SOS response: recent insights into umuDC-dependent mutagenesis and DNA damage tolerance. *Annu Rev Genet* **2000**;34:479-97 doi 10.1146/annurev.genet.34.1.479.
47. Ponder RG, Fonville NC, Rosenberg SM. A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol Cell* **2005**;19(6):791-804 doi 10.1016/j.molcel.2005.07.025.
48. McKenzie GJ, Lee PL, Lombardo MJ, Hastings PJ, Rosenberg SM. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol Cell* **2001**;7(3):571-9 doi 10.1016/s1097-2765(01)00204-0.
49. Goodman MF. Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem* **2002**;71:17-50 doi 10.1146/annurev.biochem.71.083101.124707.
50. Battesti A, Majdalani N, Gottesman S. The RpoS-mediated general stress response in *Escherichia coli*. *Annu Rev Microbiol* **2011**;65:189-213 doi 10.1146/annurev-micro-090110-102946.
51. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **2010**;141(1):69-80 doi 10.1016/j.cell.2010.02.027.
52. Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, *et al.* Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nature Medicine* **2016**;22(3):262-9 doi 10.1038/nm.4040.
53. Ramirez M, Rajaram S, Steininger RJ, Osipchuk D, Roth MA, Morinishi LS, *et al.* Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. *Nat Commun* **2016**;7:10690 doi 10.1038/ncomms10690.
54. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **2017**;168(6):960-76 doi 10.1016/j.cell.2017.02.004.
55. Tissier A, McDonald JP, Frank EG, Woodgate R. poliota, a remarkably error-prone human DNA polymerase. *Genes Dev* **2000**;14(13):1642-50.
56. Rattray AJ, Strathern JN. Error-prone DNA polymerases: when making a mistake is the only way to get ahead. *Annu Rev Genet* **2003**;37:31-66 doi 10.1146/annurev.genet.37.042203.132748.
57. Ghosal G, Chen J. DNA damage tolerance: a double-edged sword guarding the genome. *Transl Cancer Res* **2013**;2(3):107-29 doi 10.3978/j.issn.2218-676X.2013.04.01.
58. Seki M, Gearhart PJ, Wood RD. DNA polymerases and somatic hypermutation of immunoglobulin genes. *EMBO Rep* **2005**;6(12):1143-8 doi 10.1038/sj.embor.7400582.
59. Yoon JH, McArthur MJ, Park J, Basu D, Wakamiya M, Prakash L, *et al.* Error-Prone Replication through UV Lesions by DNA Polymerase θ Protects against Skin Cancers. *Cell* **2019**;176(6):1295-309.e15 doi 10.1016/j.cell.2019.01.023.
60. Breivik J, Gaudernack G. Resolving the evolutionary paradox of genetic instability: a cost-benefit analysis of DNA repair in changing environments. *FEBS Lett* **2004**;563(1-3):7-12 doi 10.1016/S0014-5793(04)00282-0.
61. O-Wang J, Kawamura K, Tada Y, Ohmori H, Kimura H, Sakiyama S, *et al.* DNA polymerase kappa, implicated in spontaneous and DNA damage-induced mutagenesis, is overexpressed in lung cancer. *Cancer Res* **2001**;61(14):5366-9.
62. Wang H, Wu W, Wang HW, Wang S, Chen Y, Zhang X, *et al.* Analysis of specialized DNA polymerases expression in human gliomas: association with prognostic significance. *Neuro Oncol* **2010**;12(7):679-86 doi 10.1093/neuonc/nop074.
63. Temprine K, Campbell NR, Huang R, Langdon EM, Simon-Vermot T, Mehta K, *et al.* Regulation of the error-prone DNA polymerase Polk by oncogenic signaling and its contribution to drug resistance. *Science Signaling* **2020**;13(629):eaau1453 doi 10.1126/scisignal.aau1453.
64. Lipinski KA, Barber LJ, Davies MN, Ashenden M, Sottoriva A, Gerlinger M. Cancer Evolution and the Limits of Predictability in Precision Cancer Medicine. *Trends Cancer* **2016**;2(1):49-63 doi 10.1016/j.trecan.2015.11.003.
65. Galhardo RS, Hastings PJ, Rosenberg SM. Mutation as a stress response and the regulation of evolvability. *Crit Rev Biochem Mol Biol* **2007**;42(5):399-435 doi 10.1080/10409230701648502.

66. Gonzalez C, Hadany L, Ponder RG, Price M, Hastings PJ, Rosenberg SM. Mutability and importance of a hypermutable cell subpopulation that produces stress-induced mutants in *Escherichia coli*. *PLoS Genet* **2008**;4(10):e1000208 doi 10.1371/journal.pgen.1000208.
67. Shee C, Gibson JL, Rosenberg SM. Two mechanisms produce mutation hotspots at DNA breaks in *Escherichia coli*. *Cell Rep* **2012**;2(4):714-21 doi 10.1016/j.celrep.2012.08.033.
68. Roberts SA, Gordenin DA. Hypermutation in human cancer genomes: footprints and mechanisms. *Nat Rev Cancer* **2014**;14(12):786-800 doi 10.1038/nrc3816.
69. Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, *et al.* β -Lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. *Nat Commun* **2013**;4:1610 doi 10.1038/ncomms2607.
70. Kohanski MA, DePristo MA, Collins JJ. Sublethal Antibiotic Treatment Leads to Multidrug Resistance via Radical-Induced Mutagenesis. *Molecular Cell* **2010**;37(3):311-20 doi 10.1016/j.molcel.2010.01.003.
71. Pal D, Pertot A, Shirole NH, Yao Z, Anaparthi N, Garvin T, *et al.* TGF- β reduces DNA ds-break repair mechanisms to heighten genetic diversity and adaptability of CD44+/CD24- cancer cells. *Elife* **2017**;6 doi 10.7554/eLife.21615.
72. Scanlon SE, Glazer PM. Multifaceted control of DNA repair pathways by the hypoxic tumor microenvironment. *DNA Repair* **2015**;32:180-9 doi <https://doi.org/10.1016/j.dnarep.2015.04.030>.
73. Mihaylova VT, Bindra RS, Yuan J, Campisi D, Narayanan L, Jensen R, *et al.* Decreased Expression of the DNA Mismatch Repair Gene *MLH1* under Hypoxic Stress in Mammalian Cells. *Molecular and Cellular Biology* **2003**;23(9):3265 doi 10.1128/MCB.23.9.3265-3273.2003.
74. Cao X, Zhou Y, Sun H, Xu M, Bi X, Zhao Z, *et al.* EGFR-TKI-induced HSP70 degradation and BER suppression facilitate the occurrence of the EGFR T790 M resistant mutation in lung cancer cells. *Cancer Letters* **2018**;424:84-96 doi <https://doi.org/10.1016/j.canlet.2018.03.004>.
75. Ge JY, Shu S, Kwon M, Jovanović B, Murphy K, Gulvady A, *et al.* Acquired resistance to combined BET and CDK4/6 inhibition in triple-negative breast cancer. *Nat Commun* **2020**;11(1):2350 doi 10.1038/s41467-020-16170-3.
76. McKenzie GJ, Harris RS, Lee PL, Rosenberg SM. The SOS response regulates adaptive mutation. *Proc Natl Acad Sci U S A* **2000**;97(12):6646-51 doi 10.1073/pnas.120161797.
77. McKenzie GJ, Rosenberg SM. Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. *Curr Opin Microbiol* **2001**;4(5):586-94.
78. Glickman MS, Sawyers CL. Converting cancer therapies into cures: lessons from infectious diseases. *Cell* **2012**;148(6):1089-98 doi 10.1016/j.cell.2012.02.015.
79. Rosenberg SM, Queitsch C. Medicine. Combating evolution to fight disease. *Science* **2014**;343(6175):1088-9 doi 10.1126/science.1247472.
80. Al Mamun AA, Lombardo MJ, Shee C, Lisewski AM, Gonzalez C, Lin D, *et al.* Identity and function of a large gene network underlying mutagenic repair of DNA breaks. *Science* **2012**;338(6112):1344-8 doi 10.1126/science.1226683.
81. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **2012**;483(7387):100-3 doi 10.1038/nature10868.
82. Amodio V, Yaeger R, Arcella P, Cancelliere C, Lamba S, Lorenzato A, *et al.* EGFR blockade reverts resistance to KRAS G12C inhibition in colorectal cancer. *Cancer Discov* **2020** doi 10.1158/2159-8290.CD-20-0187.
83. Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, *et al.* Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* **2017**;551(7679):247-50 doi 10.1038/nature24297.
84. Chinnaiyan P, Huang S, Vallabhaneni G, Armstrong E, Varambally S, Tomlins SA, *et al.* Mechanisms of Enhanced Radiation Response following Epidermal Growth Factor Receptor Signaling Inhibition by Erlotinib (Tarceva). *Cancer Research* **2005**;65(8):3328 doi 10.1158/0008-5472.CAN-04-3547.

85. Tanaka T, Munshi A, Brooks C, Liu J, Hobbs ML, Meyn RE. Gefitinib Radiosensitizes Non–Small Cell Lung Cancer Cells by Suppressing Cellular DNA Repair Capacity. *Clinical Cancer Research* **2008**;14(4):1266 doi 10.1158/1078-0432.CCR-07-1606.
86. Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, *et al.* Radiation-induced Epidermal Growth Factor Receptor Nuclear Import Is Linked to Activation of DNA-dependent Protein Kinase. *Journal of Biological Chemistry* **2005**;280(35):31182-9 doi 10.1074/jbc.M506591200.
87. Lim J, Yang K, Taylor-Harding B, Wiedemeyer WR, Buckanovich RJ. VEGFR3 Inhibition Chemosensitizes Ovarian Cancer Stemlike Cells through Down-Regulation of BRCA1 and BRCA2. *Neoplasia* **2014**;16(4):343-53.e2 doi <https://doi.org/10.1016/j.neo.2014.04.003>.
88. Robb R, Yang L, Shen C, Wolfe AR, Webb A, Zhang X, *et al.* Inhibiting BRAF Oncogene-Mediated Radioresistance Effectively Radiosensitizes BRAF. *Clin Cancer Res* **2019**;25(15):4749-60 doi 10.1158/1078-0432.CCR-18-3625.
89. Liu J, Jiang G, Mao P, Zhang J, Zhang L, Liu L, *et al.* Down-regulation of GADD45A enhances chemosensitivity in melanoma. *Scientific Reports* **2018**;8(1):4111 doi 10.1038/s41598-018-22484-6.
90. Li W, Melton DW. Cisplatin regulates the MAPK kinase pathway to induce increased expression of DNA repair gene ERCC1 and increase melanoma chemoresistance. *Oncogene* **2012**;31(19):2412-22 doi 10.1038/onc.2011.426.
91. Estrada-Bernal A, Chatterjee M, Haque SJ, Yang L, Morgan MA, Kotian S, *et al.* MEK inhibitor GSK1120212-mediated radiosensitization of pancreatic cancer cells involves inhibition of DNA double-strand break repair pathways. *Cell Cycle* **2015**;14(23):3713-24 doi 10.1080/15384101.2015.1104437.
92. Newshean S, Bonner JA, LoBuglio AF, Trummell H, Whitley AC, Dobelbower MC, *et al.* Cetuximab Augments Cytotoxicity with Poly (ADP-Ribose) Polymerase Inhibition in Head and Neck Cancer. *PLOS ONE* **2011**;6(8):e24148 doi 10.1371/journal.pone.0024148.
93. Li L, Wang H, Yang ES, Arteaga CL, Xia F. Erlotinib Attenuates Homologous Recombinational Repair of Chromosomal Breaks in Human Breast Cancer Cells. *Cancer Research* **2008**;68(22):9141 doi 10.1158/0008-5472.CAN-08-1127.
94. Friedmann BJ, Caplin M, Savic B, Shah T, Lord CJ, Ashworth A, *et al.* Interaction of the epidermal growth factor receptor and the DNA-dependent protein kinase pathway following gefitinib treatment. *Molecular Cancer Therapeutics* **2006**;5(2):209 doi 10.1158/1535-7163.MCT-05-0239.
95. Ibrahim YH, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, *et al.* PI3K Inhibition Impairs BRCA1/2 Expression and Sensitizes BRCA-Proficient Triple-Negative Breast Cancer to PARP Inhibition. *Cancer Discovery* **2012**;2(11):1036 doi 10.1158/2159-8290.CD-11-0348.
96. Juvekar A, Burga LN, Hu H, Lunsford EP, Ibrahim YH, Balmaña J, *et al.* Combining a PI3K Inhibitor with a PARP Inhibitor Provides an Effective Therapy for BRCA1-Related Breast Cancer. *Cancer Discovery* **2012**;2(11):1048 doi 10.1158/2159-8290.CD-11-0336.
97. Mo W, Liu Q, Lin CC-J, Dai H, Peng Y, Liang Y, *et al.* mTOR Inhibitors Suppress Homologous Recombination Repair and Synergize with PARP Inhibitors via Regulating SUV39H1 in BRCA-Proficient Triple-Negative Breast Cancer. *Clinical Cancer Research* **2016**;22(7):1699 doi 10.1158/1078-0432.CCR-15-1772.
98. Maertens O, Kuzmickas R, Manchester HE, Emerson CE, Gavin AG, Guild CJ, *et al.* MAPK Pathway Suppression Unmasks Latent DNA Repair Defects and Confers a Chemical Synthetic Vulnerability in *BRAF*-, *NRAS*-, and *NF1*-Mutant Melanomas. *Cancer Discovery* **2019**;9(4):526 doi 10.1158/2159-8290.CD-18-0879.
99. Sun C, Fang Y, Yin J, Chen J, Ju Z, Zhang D, *et al.* Rational combination therapy with PARP and MEK inhibitors capitalizes on therapeutic liabilities in. *Sci Transl Med* **2017**;9(392) doi 10.1126/scitranslmed.aal5148.
100. Misale S, Arena S, Lamba S, Siravegna G, Lallo A, Hobor S, *et al.* Blockade of EGFR and MEK intercepts heterogeneous mechanisms of acquired resistance to Anti-EGFR therapies in colorectal cancer. *Science Translational Medicine* **2014**;6(224) doi 10.1126/scitranslmed.3007947.

101. Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, *et al.* Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* **2016**;17(6):738-46 doi 10.1016/S1470-2045(16)00150-9.
102. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, *et al.* Encorafenib, Binimetinib, and Cetuximab in. *N Engl J Med* **2019** doi 10.1056/NEJMoa1908075.
103. Pilié PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nat Rev Clin Oncol* **2019**;16(2):81-104 doi 10.1038/s41571-018-0114-z.

Fig.1

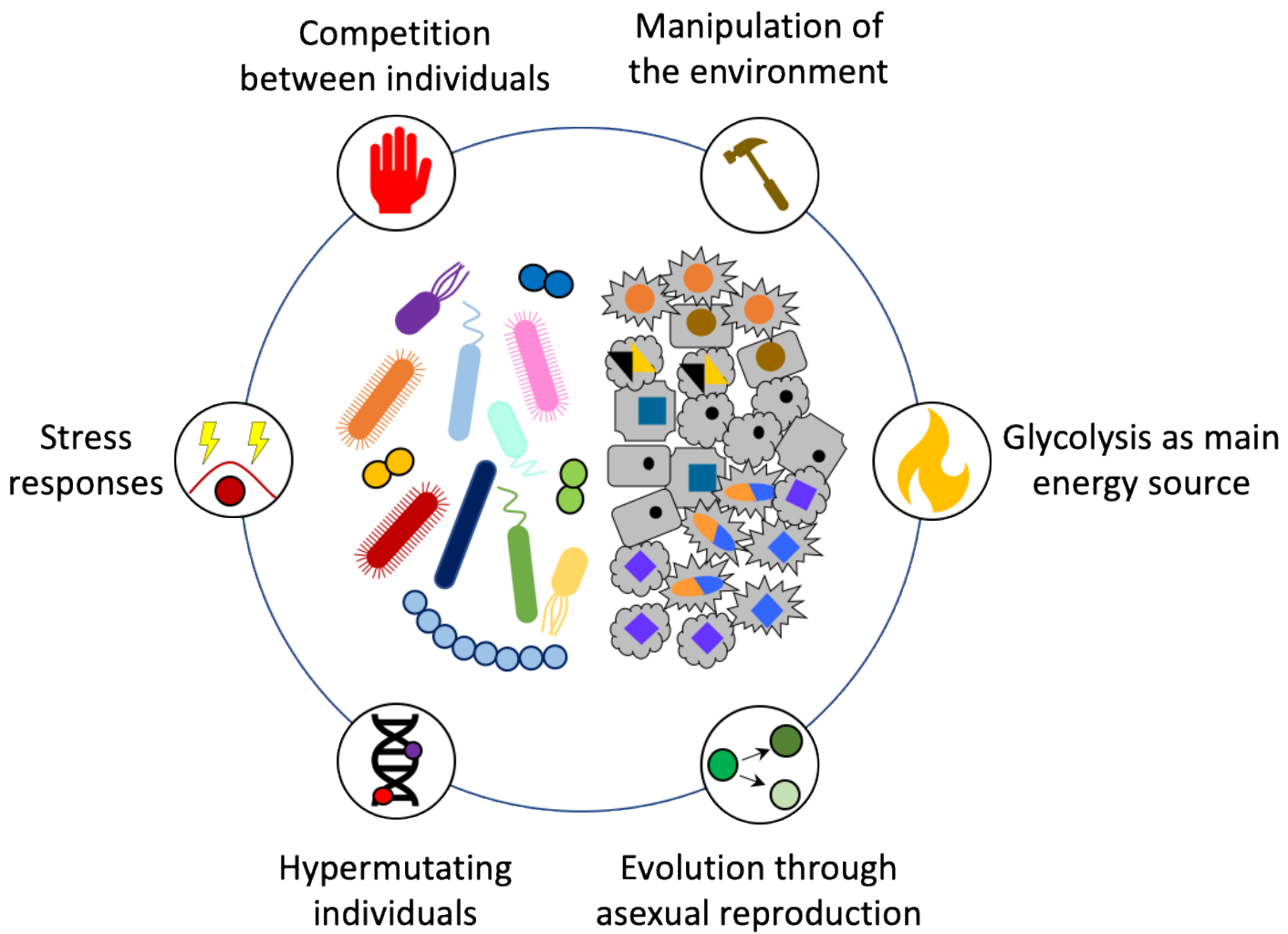


Fig.2

