Redox signaling in drug-tolerant persister cells as an emerging therapeutic target

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Summary

Drug-tolerant persister (DTP) cells have attracted significant interest, given their predominant role in treatment failure. In this respect, DTP cells reportedly survive after anticancer drug exposure, and their DNA repair mechanisms are altered to enhance adaptive mutation, accounting for the emergence of drug-resistant mutations. DTP cells resume proliferation upon treatment withdrawal and are responsible for cancer relapse. Current evidence suggests that DTP cells mediate redox signaling-mediated cellular homeostasis by developing various adaptive mechanisms, especially metabolic reprogramming that promotes mitochondrial oxidative respiration and a robust antioxidant process. There is an increasing consensus that disrupting redox homeostasis by intervening with redox signaling is theoretically a promising therapeutic strategy for targeting these sinister cells. In this review, we provide a comprehensive overview of the characteristics of DTP cells and the underlying mechanisms involved in redox signaling, aiming to provide a unique perspective on potential therapeutic applications based on their vulnerabilities to redox regulation.

Keywords: Drug-tolerant persister; Drug resistance; Cancer relapse; Redox signaling; Redox homeostasis

Introduction

Therapeutic resistance represents an intricate conundrum during cancer treatment and can be broadly classified as primary and acquired resistance. Primary resistance refers to the absence of objective response to initial therapy due to preexisting drug resistance genetic mutations within the tumor. However, secondary resistance, i.e., acquired resistance, occurs during cancer relapse despite an initial clinical response attributed to intratumor heterogeneity, including secondary oncogenic mutations, bypass pathway activation, and lineage transformation.[1,2] Although the critical role of genetic alterations in acquired resistance is well-established, recent evidence has demonstrated that nongenetic adaptive mechanisms also participate in this complicated process.[3] The discovery and investigation of these residual drug-tolerant cells have opened a new field that requires innovative strategies.

Drug-tolerant persister (DTP) is a novel concept that embodies nongenetic evolution and reportedly contributes to cancer drug resistance. Cells with a DTP state comprise a rare subpopulation of cancer cells that, when exposed to therapy, exhibit resistance to drug-induced cytotoxicity and ultimately develop drug resistance by enhancing adaptive mutability.[4] In addition, DTP cells underlie minimal residual disease (MRD), accounting for cancer relapse.[5] DTP cells remain quiescent like embryonic diapause and reinitiate proliferation following drug withdrawal.[6] Thus, developing strategies to eradicate DTP cells may lead to promising therapeutic avenues for curative cancer treatment. The main hallmarks of DTP cells have been preliminarily revealed, including slow proliferation, metabolic alteration, phenotypic plasticity and environmental adaptation.[7] However, a key clue is still missing to orchestrate the hallmarks associated with DTP cells.

Reactive oxygen species (ROS) are a class of byproducts of cellular metabolism. An increasing body of literature suggests that excessive intracellular ROS induce oxidative stress, damaging cellular macromolecules and causing mutation-induced malignant transformation.[8,9] In contrast, an appropriate concentration of ROS plays a vital role in physiological activities, such as cell proliferation, biosynthesis, metabolism, aging and differentiation. Therefore, to maintain normal physiological processes, cells have developed antioxidant systems to cope with the overload of ROS and maintain cellular redox homeostasis. Similarly, redox homeostasis in cancer cells is necessary for fueling tumorigenesis

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and development. Interestingly, moderate ROS levels partly facilitate the activation of oncogenic pathways or mutations, including the nuclear factor-kappaB (NF-κB), phosphatidyl-inositol-3-kinase (PI3K)/protein kinase B (AKT), mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinase (ERK) pathways and p53.[10] On the other hand, powerful antioxidant systems and several redox sensors, such as the nuclear factor erythroid 2-related factor 2 (NRF2)/Kelch-like ECH-associated protein 1 (KEAP1) pathways and B-cell lymphoma 2 (BCL2), are well-established to be involved in resistance to oxidative stress induced by various antitumor strategies.[11–13] Unsurprisingly, recent studies have highlighted that redox signaling may also be a master regulator of DTP cells.[3,6] ROS, under the precise regulation of the antioxidant system, seems to run through whole signal networks, facilitating the survival and behavior of DTP cells, such as adaptive metabolic patterns and renewed cell cycles. Accordingly, reasonably targeting redox homeostasis represents an effective strategy to restrict DTP cells. A better understanding of the molecular mechanisms by which DTP cells modulate antioxidant systems to control the available ROS would provide new insights into personalized cancer care.

In this review, we introduce and discuss recent advances focusing on the underlying mechanisms of DTP maintenance, emphasizing redox regulation. Moreover, we propose potential clinical applications targeting DTP cells based on their redox vulnerabilities.

Drug-tolerant persister (DTP): an emerging concept in cancer

DTP cells are considered "invincible in peace, invisible in war" in cancer treatment.[7] DTP was first introduced in microbiology in the 1940s.[14,15] Bigger and colleagues observed that a small proportion of drug-naïve staphylococci could survive in penicillin. The drug-tolerant surviving bacterial cells were identified as persisters, exhibiting resistance based on dormancy-like slow proliferation rather than *de novo* genetic alterations. Of note, upon drug withdrawal, these persister cells reentered a proliferative state and retained sensitivity to penicillin.[16] In 2010, Sharma and colleagues similarly observed a small subpopulation of cancer cells resembling bacterial persisters that remained intact in response to lethal drug exposure. In line with bacterial persisters, the state of these cancer cells was transient and reversible.[17] Further research revealed that the DTP phenotype is mainly mediated by nongenetic mechanisms by triggering insulin-like growth factor 1 receptor (IGF-1R) activity and a distinct chromatin state that requires the induction of lysine-specific demethylase 5A (KDM5A).[17] This finding revealed a novel mechanism underlying drug resistance, challenging the widely recognized opinion that resistance results from sequential genetic mutation. Considering the similarity of nongenetic mechanisms and the consistency of initial feature resumption upon drug removal, the concept of persister cells was introduced to the field of cancer.

Nonetheless, no consensus has been reached on how to define DTP cells. A recent report summarized the underlying mechanism of DTP cells into four non-mutually exclusive characteristics: slowing cell proliferation, adapting cell metabolism, changing cell identity, and hijacking the microenvironment. These characteristics, mediated by mechanisms including epigenetic, transcriptional and translational reprogramming, and intercellular crosstalk, account for the phenotype of DTP cells. Accordingly, four criteria which can be assessed experimentally have been proposed to distinguish DTP from other cellular states, such as cancer stem cells (CSCs) and dormant cells: *i*, low proliferation rate of residual cells, *ii*, reduced drug sensitivity, *iii*, reversibility of the cellular phenotype (such as proliferation rate and drug sensitivity), and *iv*, confirming that surviving cells under sustained treatment stress can acquire genetic resistance, i.e. irreversible drug resistance.[7] It has been established that the DTP state is a dynamic and transient situation between sensitive and resistant phenotypes driven by epigenetic inheritance.[18] The temporary and dynamic characteristics of DTP cells are often heterogenous, suggesting that DTP-related phenotypes can be observed at any stage of the disease and dynamically transform to any other subpopulation of cells depending on the context. Importantly, DTP cells account for treatment failure associated with drug resistance and cancer recurrence to a certain extent.

Redox signaling in the features of DTP cells

Increased oxidative stress levels followed by a corresponding enhancement in antioxidant capacity have been documented in tumor cells, especially DTP cells. Given the vital role of DTP cells in the pathogenesis of cancer drug resistance and recurrence, elucidating the redox status and associated signaling pathways of this subpopulation of cells provides a crucial theoretical basis for the developing of effective antitumor strategies.

Slow cell proliferation

Under normal circumstances, antitumor treatment induces intense oxidative stress in rapidly proliferating cancer cells, leading to apoptosis. However, these cells enter a slow to a nonproliferative state under therapeutic stress and thus survive. Recent clinical and experimental studies suggest that epigenetic regulation, including histone modification and DNA methylation, is involved in cellular deceleration and is potentially controlled by redox signaling networks.[7,19–21] Liau et al. found that glioblastomas exhibited a slow-cycling state after treatment with the receptor tyrosine kinase inhibitor dasatinib, exhibiting a Notch signaling-dependent signature

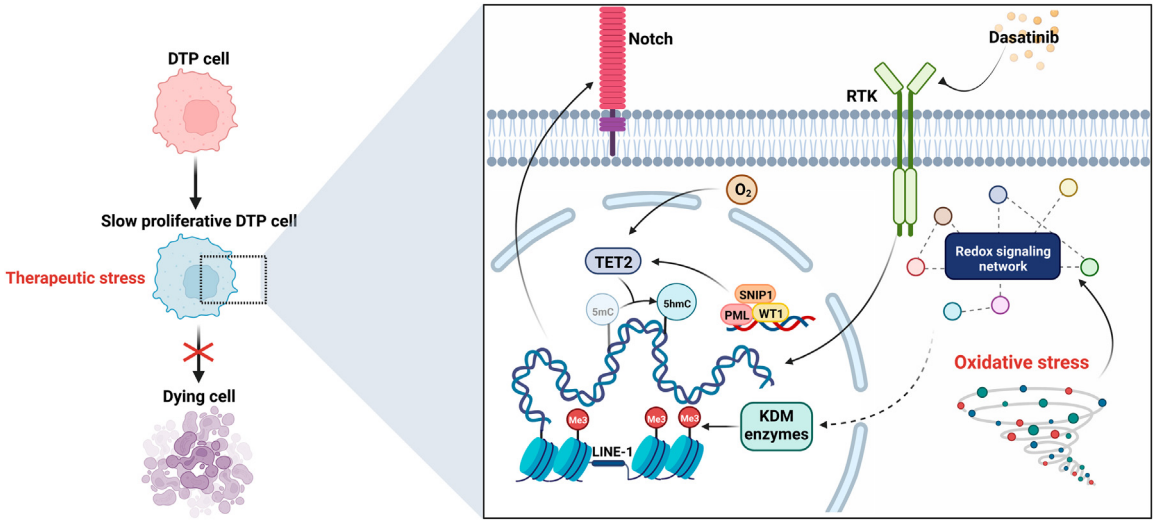

***Fig. 1:* Mechanisms of redox signaling in the slow proliferation of DTP cells.** Under therapeutic stress, cancer cells in the DTP state usually enter cell cycle arrest and exhibit slow or no proliferation. Redox signaling potentially regulates this process in a sophisticated manner by activating epigenetic reprogramming. In particular, histone lysine demethylases (KDMs) and ten-eleven translocation (TET) enzymes under redox modulation mediate chromatin remodelling by modulating histone modification and DNA methylation, respectively. Thus, aberrant epigenomes are involved in the dysregulation of various signaling pathways, such as the Notch pathway, leading to cellular deceleration.

associated with primitive neurodevelopment. Further studies revealed that the upregulation of the Notch pathway was mediated by the redistribution of H3K27me3, which depended on the histone demethylases KDM6A/B.[22] Furthermore, the critical role of histone modification-mediated chromatin remodelling in the induction of a slow-cycling state has recently been reconfirmed in melanoma-derived DTP cells.[23] Interestingly, the redox regulation of chromatin remodelling has been extensively discussed.[24] Additionally, there is direct evidence that oxidative stress can induce histone remodelling accompanied by reactivation of the long-interspersed nuclear element-1 (LINE-1).[25] Thus, although not yet directly investigated, it is highly conceivable that redox signaling networks are involved in the slow proliferative state of DTP cells by regulating the activity of epigenetically relevant enzymes or other components under oxidative stress induced by cancer treatment. However, further studies are needed before conclusions about the detailed molecular mechanisms can be drawn.

In addition to histones, oxidative stress levels can directly affect DNA methylation by modulating ten-eleven translocation (TET) enzymes.[26,27] Mechanistically, TET enzymes oxidize methylcytosine to hydroxymethylcytosine (hmC). It has been reported that TET2 catalyzes the demethylation of 5-methylcytosine (5-mC) by oxidizing 5 mC to 5-hmC, associated with slow-cycling cancer cells.[27–29] Puig and colleagues found that TET2 is required for the survival and drug chemoresistance of slow-cycling cancer cells in multiple tumor types, including melanoma, glioblastoma and colorectal cancer. 5-hmC has been documented to be significantly enriched in chemoresistant cancer cells, suggesting the crucial role of TET2 in determining the unique properties of slow-cycling cancer cells.[30] Consistently, an increasing body of evidence suggests that TET2 recruitment by specific factors, such as promyelocytic leukemia protein (PML), Smad nuclear interacting protein 1 (SNIP1) or Wilms' tumor 1 (WT1), regulated modification and subsequent cell proliferation and therapeutic response.[31–33] Interestingly, the maintenance of TET enzymatic activity relies on oxygen as a cosubstrate.[34,35] Kang et al. found that 5-fluorouracil-resistant colon cancer cells possess lower 5-mC levels and higher 5-hmC levels than parental cells, as well as higher oxidative stress and antioxidant systems.[36] Collectively, the above findings suggest that redox signaling is involved in cell cycle determination via epigenetic regulation in DTP cells (Fig. 1).

Metabolism adaptation

Although cancer metabolism is highly dependent on anaerobic glycolysis, cancer cells entering the DTP state possess metabolic properties similar to resting normal cells and are more dependent on oxidative phosphorylation (OXPHOS) or generate a hybrid metabolic phenotype dependent on both OXPHOS and the glycolytic pentose phosphate pathway. As expected, their redox signaling regulatory networks differ significantly. This phenotype of DTP cells has been observed in various malignancies, including pancreatic ductal

adenocarcinoma, melanoma and myeloid leukemia.[37–39] For example, Viale and colleagues reported that DTP cells from *KRAS*^{G12D} pancreatic ductal adenocarcinoma showed active OXPHOS and impaired glycolysis after inhibiting oncogenic pathways genetically or pharmacologically. DTP cells exhibited a 4-fold increase in the oxygen consumption rate (OCR) compared to cells expressing Kirsten rat sarcoma viral oncogene homolog (KRAS). Further transcriptomic and metabolic analyses revealed that several mitochondrial markers, including peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) and mitochondrial marker voltage-dependent anion channel 1 (VDAC1), were involved in these processes.[37] These results suggest that cells entering the DTP state greatly activate mitochondrial function and that ROS, a byproduct of this metabolic pattern, is bound to increase significantly to orchestrate redox signaling pathways for these DTP cells to survive. Similarly, a subpopulation of slow-cycling melanoma cells was identified to promote the upregulation of OXPHOS by regulating the H3K4 demethylase KDM5B. Notably, DTP cells with high KDM5B exhibited a slow-cycling phenotype accompanied by increased oxygen consumption and H_2O_2 levels.[39] Indeed, this phenomenon has been widely observed and investigated in leukemia persister cells. Farge et al. demonstrated that chemoresistant leukemic cells displayed a high OXPHOS status in response to cytarabine treatment, characterized by high levels of ROS and upregulation of mitochondrial mass and polarization,[38] consistent with findings reported by Larrue et al., which substantiated that enhanced mitochondrial OXPHOS led to drug tolerance or resistance in myeloid leukemia. Mechanistically, calcitonin receptor-like receptors (CALCRLs) were upregulated in cytarabine-treated cells. The adrenomedullin-CALCRL axis contributed to the maintenance of relapse-initiating cells by driving E2F transcription factor 1 (E2F1)- and BCL2-dependent mitochondrial OXPHOS function.[40] Similar findings have been observed in imatinib-treated primitive chronic myeloid leukemia cells.[41]

It has been established that DTP cells develop a robust antioxidant response to counter oxidative stress from exogenous cancer treatment or endogenous mitochondrial OXPHOS. Some redox-related transcription factors, such as NRF2, are reportedly involved in the DTP state.[42–44] Takahashi et al. indicated that NRF2 directly upregulates the expression of transient receptor potential cation channel subfamily A (TRPA1), leading to tolerance to oxidative stress generated by chemotherapeutic agents in breast and lung cancer cells.[42] Furthermore, the role of NRF2 in oxidative stress defense has been documented in breast cancer cells with DTP characteristics. In this respect, Fox and colleagues found that HER2 inhibition disrupted cellular redox homeostasis, as evidenced by alterations in reduced glutathione (GSH):oxidized GSH (GSSG) and NADP:NADPH ratios, which in turn led to ROS-dependent cell death. However, for cells that survived this process, activated NRF2 initiated an antioxidant transcriptional program and regulated the synthesis of new nucleotides to reestablish redox homeostasis in a glutathione metabolism-dependent manner. In addition, NRF2 activation facilitated the recurrence of dormant breast cancer cells.[45] In addition to classical redox-related proteins, some stemness-like markers are also involved in oxidative stress tolerance. For example, a drug-tolerant subpopulation was documented to be predominantly enriched with aldehyde dehydrogenase $(ALDH)^{high}$ cells, and inhibition of ALDH resulted in the accumulation of ROS, therefore causing DNA damage and cell death.[46] ALDH contains $NAD(P)^+$-dependent enzymes that attenuate oxidative stress by metabolizing aldehydes, which are toxic products of lipid peroxidation. A subsequent study showed that ALDH conferred the ability to detoxify cancer cells from oxidative damage, thus facilitating resistance to various chemotherapeutic agents.[47] Moreover, the cluster of differentiation-44 variant (CD44v) has been previously reported to defend against ROS, rendering cancer cells resistant to chemotherapy and radiotherapy. Mechanistically, CD44v interacted with and stabilized the glutamate-cystine transporter (xCT) at the cell membrane, resulting in increased intracellular GSH synthesis.[48] Recently, Mashima et al. identified CD44v as a key supporter of DTP cells from gastric cancer,[49] implying the potential existence of CD44v-mediated redox regulation. Furthermore, DTP cells with high CD44 expression in breast cancer exhibited a hybrid metabolic phenotype characterized by increased activity of both the mitochondrial oxidative and glycolytic pathways. ROS and AKT enhanced the flux of glucose into the pentose phosphate pathway, which subsequently countered oxidative stress by increasing the biosynthesis of GSH.[50,51]

Growing evidence suggests that DTP cells also generate alternative metabolic pathways,[7] such as the activation of autophagy, a pathway involved in cellular metabolism by degrading intracellular macromolecules under stress, including oxidative stress.[52] For example, unc-51-like autophagy-activated kinase 1 (ULK1), a key mediator of autophagy, has been associated with tyrosine kinase inhibitor treatment-induced minimal residual disease, one of the main consequences associated with DTP status. Inhibition of ULK1 triggered the upregulation of ROS levels while promoting cell proliferation and sensitivity to targeted cancer therapy.[53] In line with this study, Rehman and colleagues confirmed the important role of autophagy in sustaining the survival of diapause-like DTP cells,[54] which carefully regulated biochemical pathway transitions and maintained redox homeostasis by balancing the production and consumption of metabolites.[55] In addition, several studies have revealed that DTP cells maintain their

metabolic activity by inducing fatty acid β-oxidation (FAO) for redox balance. Shen et al. found increased fatty acid depletion and peroxisomal lipid metabolism in persistent melanoma cells after v-Raf murine sarcoma viral oncogene homolog B (BRAF)/mitogen-activated protein kinase (MEK) treatment. Further mechanistic studies demonstrated that acyl-CoA oxidase 1 (ACOX1)-mediated FAO was induced by the peroxisome proliferator-activated receptor alpha (PPARα)-PGC1α axis, thus protecting DTP cells from lipotoxicity.[56] Consistently, FAO has also been reported to be activated by PPARα-mediated induction of carnitine palmitoyltransferase 1A (CPT1A) in DTP melanoma cells.[57] It is widely thought that FAO may be associated with autophagy in DTP cells. In this regard, You et al. demonstrated that paclitaxel-tolerant persister cancer cells switched their metabolic pattern in FAO, which rendered them vulnerable to xCT inhibitors, i.e., induction of ferroptosis by progesterone receptor membrane component 1 (PGRMC1)-dependent lipophagy.[58] Overall, DTP cells orchestrate various metabolic pathways to adapt to the cellular stress caused by tumor treatment, a process that is partially regulated by redox signaling networks and contributes to maintaining redox homeostasis (Fig. 2).

Tumor cell plasticity

Tumor cell plasticity refers to the ability of cells to drive phenotypic transitions to adapt to environmental diversity. Emerging evidence suggests that cell plasticity is an important mechanism in cancer treatment evasion, as it confers reversible transformation of tumor cells to switch to a target-escapable tolerant phenotypic state in response to drug treatment pressure. The epithelial-mesenchymal transition (EMT) process is an important manifestation of cell plasticity. Interestingly, during this dynamic and reversible process, cancer cells transition from an epithelial to a mesenchymal-like phenotype.[59] The EMT process is precisely orchestrated by redox signaling. ROS are commonly involved in extracellular matrix remodelling, cytoskeletal remodelling, and activation of cell migration-related pathways.[60] It is well-established that EMT renders cancer cells more resistant to therapeutic assault and cell death, observed in a range of epithelial tumors with a DTP-like state, such as colorectal, gastric and breast cancers.[61–63] Furthermore, Weng et al. demonstrated the involvement of EMT in the resistance of non-small cell lung cancer (NSCLC) to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), as evidenced by reduced E-cadherin expression and increased vimentin levels after gefitinib treatment. After vimentin was knocked

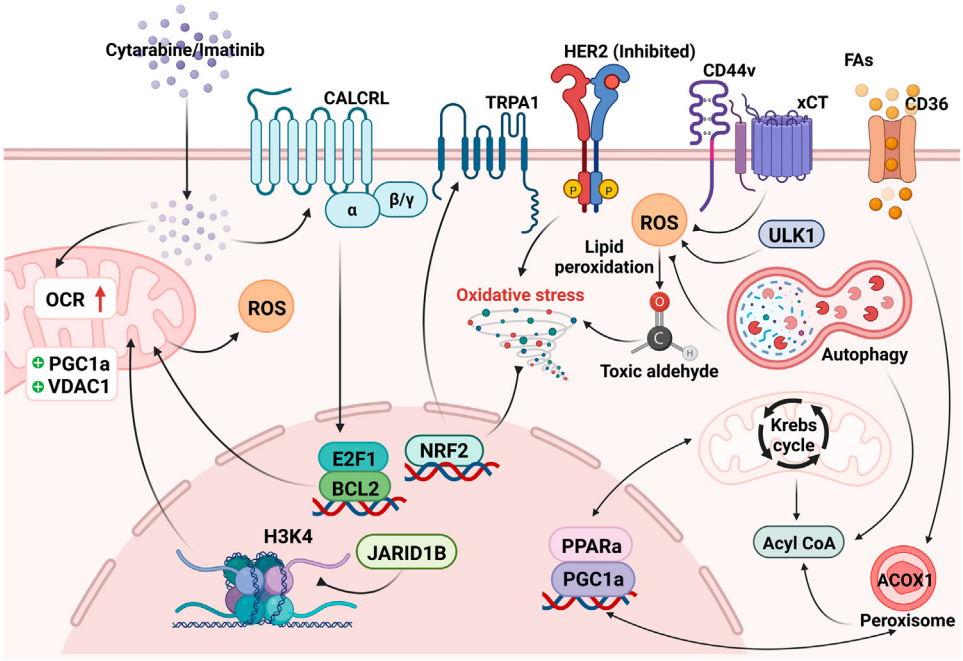

***Fig. 2:* Molecular pathways of DTP cells with metabolic adaptation in redox homeostasis.** DTP cells depend on OXPHOS or a mixed metabolic phenotype by modulating mitochondrial functions and epigenetic reprogramming to develop a robust antioxidant system. For example, the key redox protein NRF2 can initiate the antioxidant transcriptional program in response to disruptions of redox homeostasis induced by metabolic stress or therapeutic stress. In addition, lone or in combination, autophagy and FAO help protect DTP cells from oxidative stress-related lipotoxicity.

down in resistant cells, gefitinib sensitivity was restored, suggesting that mesenchymal features confer drug resistance in DTP-like cancer cells.[64] Consistently, Aldonza et al. found that cancer cells experiencing paclitaxel treatment failure also exhibited resistance to EGFR-TKIs. The persistence of this resistance was dependent on changes in the EMT status of the cells, as shown by the upregulation of N-cadherin, vimentin and Slug.[65] Indeed, these studies do not directly indicate the involvement of redox signaling. However, oxidative stress induced by cancer therapy is ubiquitous. Accordingly, there are solid grounds to believe that ROS regulate the expression of EMT-related proteins in DTP cells to a certain extent, as demonstrated by studies in 5-fluorouracil-resistant human colon cancer cells[66] (Fig. 3a).

Transdifferentiation is another type of cellular plasticity involving irreversible transformation from one mature lineage into another. Transdifferentiation has been implicated in drug tolerance in various malignancies, such as melanoma, lung cancer, and basal cell carcinoma.[67] For example, Sánchez-Danés et al. found that administration of the smoothened inhibitor vismodegib induced a subpopulation of persisting slow-cycling cells characterized by leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) expression and active Wnt signaling. This drug-tolerant conformation was accompanied by tumor differentiation, and vismodegib inhibited oncogene-mediated hair follicle reprogramming and promoted interfollicular epidermis (IFE) and infundibulum-like features.[68] Consistently, Biehs and colleagues revealed that residual basal cell carcinoma cells after vismodegib treatment initiated plasticity with a phenotype similar to stem cells of the IFE and isthmus; in contrast, untreated cells exhibited a similar phenotype to hair follicle bulges. Plastic transformation was mediated by rapid activation of the Wnt pathway and key transcription factors, independent of the activity of the Hedgehog pathway.[69] Coincidentally, a study found that Lgr5^{+} intestinal stem cell regeneration was regulated by Wnt/β-catenin signaling and depended, at least in part, on the activation of NRF2-governed redox signaling.[70] Indeed, several recent reports have linked the engagement of redox signaling with tumor plasticity in DTP cells. ROS has been reported revealed to modulate squamous transdifferentiation from lung adenocarcinoma to squamous cell carcinoma (SCC) and subsequently result in drug resistance in NSCLC.[71] Other research groups consistently reported that redox stress accelerates lung adenocarcinoma (ADC) to SCC transdifferentiation[72] (Fig. 3b).

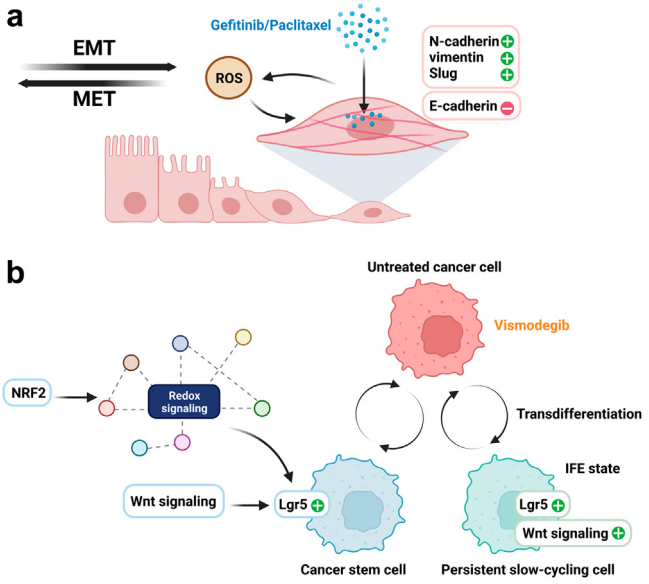

Fig. 3: **Redox-mediated plasticity in DTP cells.** (a) Cancer therapy induces oxidative stress, which activates the EMT process and confers a mesenchymal-like phenotype on DTP cells. Several proteins such as E-cadherin, N-cadherin, vimentin and Slug, are involved in this process. (b) Under therapeutic stress, tumor cells undergo transdifferentiation by modulating redox signaling, thus showing a DTP cell-associated phenotype. Lgr5 and Wnt signaling are key to this process.

Domesticating the microenvironment

The tumor microenvironment (TME) is a complex ecosystem that includes multiple nontumor cell types, such as fibroblasts, immune cells, and endothelial cells and has been associated with tumorigenesis and development. Recently, it has been found that treated residual tumors, i.e., those entering the DTP state, possess a higher percentage of macrophage and fibroblast infiltration and cytokine and collagen expression than untreated tumors. Importantly, oxidative stress is involved in TME remodelling, therefore regulating the drug-tolerant phenotype of tumors (Fig. 4).

Cancer-associated fibroblasts (CAFs) are a recognized cell type associated with tumor drug resistance and mediated by oxidative stress-mediated secretion of soluble factors.[73,74] Wang et al. found that after EGFR-TKI treatment, lung cancer cells recruited CAFs and promoted the production of hepatocyte growth factor (HGF), which subsequently activated MET (specific HGF receptors) in cancer to restore the PI3K/AKT pathway, ultimately leading to tumor resistance.[75] In line with this, Straussman and colleagues proposed that HGF-induced activation of the MET/PI3K/AKT pathway conferred tumor cell resistance to BRAF inhibitors in melanoma, glioblastoma, and colorectal cancer.[76] In addition to interacting with cancer cells through the secretion of growth factors, CAFs can promote the remodelling of the local stromal microenvironment. For example, BRAF inhibitors resulted in drug tolerance in BRAF-mutant melanoma cells by inducing tumor stromal remodelling. Fibronectin axial stiffness increased integrin β1 and focal adhesion kinase (FAK) signaling, which then reactivated the inhibited BRAF-dependent ERK signaling in melanoma cells.[77] Furthermore,

antiangiogenic therapy induced upregulation of β1 integrin/FAK signaling, mediating glioblastoma-microenvironment interactions and subsequent mesenchymal-type drug resistance.[78] Collectively, oxidative stress influences the redox signaling pathway in CAFs, which in turn causes drug resistance. Notably, this process appears to be a common feature of several cancers in response to various therapies.

Tumor-associated macrophages (TAMs) can differentiate into M1 and M2 subtypes. M1 macrophages can produce ROS and proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL23, and tumor necrosis factor-alpha (TNF-α), exerting antitumor effects. In contrast, M2 macrophages are thought to have protumorigenic effects due to the production of pro-oncogenic cytokines such as IL-10, IL-13 and transforming growth factor-beta (TGF-β).[79,80] Of note, TAMs are regulated by oxidative stress, thereby promoting immunosuppression and mediating treatment tolerance.[81] For example, Walens et al. found that macrophage infiltration promoted drug resistance and recurrence of breast cancer when human epidermal growth factor receptor 2 (HER2) was suppressed. Mechanistically, HER2 downregulation triggered a TNF-α/inhibitor kappa B kinase (IKK) pathway-dependent inflammatory gene expression program, triggering the secretion of C–C motif chemokine ligand 5 (CCL5), which subsequently promoted macrophage infiltration and collagen deposition in residual tumors.[82] It is widely thought that local redox levels affect the polarization of macrophages. However, the direction of ROS-induced polarization is often indeterminate and may depend on the ROS concentration or other contexts.[83,84]

Intriguingly, neutrophils have also been found to be involved in tolerance after tumor treatment. Glodde et al. reported that tumors receiving cancer immunotherapy promoted the recruitment of neutrophils through the HGF/c-MET pathway. The recruited neutrophils acquired immunosuppressive properties and suppressed T-cell function.[85] Interestingly, HGF and its receptor MET are target genes of hypoxia-inducible factor 1 (HIF1) affected by redox homeostasis.[86] A recent study reported that programmed cell death 1 (PD-1) blockade therapy could induce a discrete subpopulation of immunotherapy persister cells (IPCs) directly resistant to $CD8^+$ T-cell-mediated killing. Specifically, IPCs expressed Snai1 and stem cell antigen 1 (Sca-1), showing hybrid epithelial-mesenchymal features.[87] It is highly conceivable that redox signaling pathways regulate these IPCs.

Therapeutic targeting of DTP cells by interaction with redox signaling

Given that epigenetic adaptations are driving forces for DTP cell survival and function maintenance, targeting these key epigenetic enzymes, such as KDMs, has been shown in preclinical studies to be a potential approach to eradicate DTP cells.[88–96] However, most epigenetic enzyme inhibitors have not been approved for clinical practice due to poor selectivity and organ toxicity.[89,90] In this section, we propose to disrupt the redox homeostasis of DTP cells by modulating redox signaling to ablate these cells and prevent relapse.

Over the past few years, several studies have claimed that the dependence of DTP cells on redox homeostasis makes them vulnerable to ROS-related cell death, such as ferroptosis, an iron-dependent regulation of cell death triggered by toxic lipid peroxides.[97] Glutathione peroxidase 4 (GPX4) is a central regulator of ferroptosis that prevents ROS accumulation and reduces hydroperoxides, rescuing cell membranes from lipid peroxidation damage.[98,99] In recent years, Viswanathan and colleagues have identified GPX4 as an important target for the induction of ferroptosis in DTP cells.[100,101] Cotreatment with GPX4 inhibitors, such as RSL3 and ML210, to induce ferroptosis in cancer cells effectively reduced the residual persister cell pool across many lineages. However, due to the poor pharmacokinetic properties of existing GPX4 inhibitors, several efforts have been made to improve their deficiencies to pave the way for targeting GPX4 in DTP cells. Gao et al. developed a micelle made of an arachidonic acid-bound amphiphilic copolymer. They encapsulated RSL3 in this micelle, which significantly improved the limitations of the drug.[102] Furthermore, significant efforts have been undertaken to develop or validate other suitable therapeutic

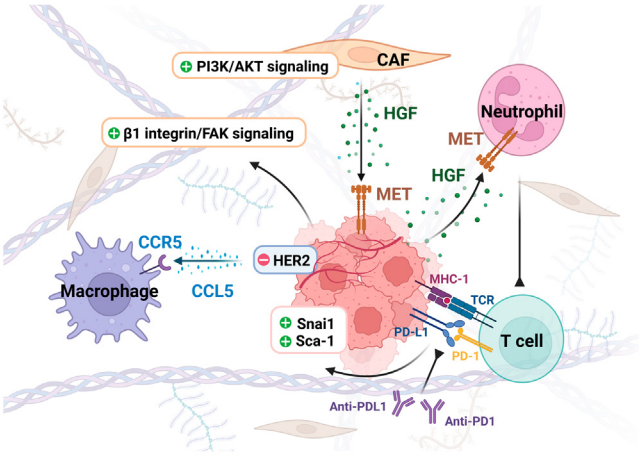

Fig. 4: **Mechanisms of redox signaling-regulated DTP cells domesticating the tumor microenvironment.** After treatment, DTP cells activate redox signaling and subsequently recruit a variety of nontumor cells in the tumour microenvironment, which help tumors against therapeutic toxicity and contribute to tumor relapse on an adaptive occasion. Specifically, CAFs are involved in HGF-MET axis-mediated activation of oncogenic pathways, including the PI3K/AKT pathway and β1 integrin/FAK signaling. CCR5-expressing TAMs are recruited into residual tumours in response to CCL5 to facilitate recurrence. Neutrophils are mobilized by HGF/MET signaling against T cell-mediated anticancer immune responses. Additionally, DTP cells expressing Snai1 and Sca-1 can directly resist $CD8^+$ T cell-mediated killing.

compounds. For instance, an itaconate derivative, 4-octyl itaconate, was reported to induce ferritinophagy-dependent ferroptosis and eliminate carboplatin-tolerant cancer cells in retinoblastoma.[103] Antoszczak and colleagues synthesized a series of small molecule chimeras of salinomycin derivatives and iron-reactive dihydroartemisinin. Then, they identified the activities of these agents in inducing ferroptosis in DTP cells in pancreatic cancer.[104] Some agents targeting GPX4 and GSH, such as altretamine, NOV-002 and sodium selenite, have entered clinical trials (NCT00002936, NCT00499122 and NCT02184533). Among these, NOV-002, combined with neoadjuvant chemotherapy, yielded good results in patients with HER-2 negative breast cancer, and is more effective in patients with hormone receptor-positive breast cancer, suggesting that further clinical trials are highly warranted.[105] In addition to GPX4, other targets of ferroptosis,[106–109] such as apoptosis-inducing factor mitochondria-associated 2 (AIFM2)/ferroptosis suppressor protein 1 (FSP1),[106] have also been identified, providing novel therapeutic ideas for eliminating DTP cells.

With a better understanding of the redox signaling network in DTP cells, other underlying mechanisms and potential targets have gradually been revealed. For example, DTP cells are strongly dependent on OXPHOS and the corresponding antioxidant system. Therefore, targeting mitochondrial OXPHOS represents a promising strategy to eradicate DTP cells based on the disruption of cellular redox homeostasis. Indeed, Kuntz et al. used tigecycline (an antibiotic that inhibits mitochondrial protein translation) to disrupt OXPHOS in chronic myeloid leukemia (CML), reducing the probability of MRD following conventional therapy; thus, imatinib in combination with tigecycline could eradicate leukemic stem cells.[41] In line with this, oligomycin (a Fo-ATPase inhibitor of complex V) has been reported to reduce mitochondrial respiration, impacting the spherogenic and tumorigenic potential of KRAS ablation-resistant pancreatic cancer cells.[37] Moreover, ALDH is widely believed to be a promising target for killing DTP cells via a ROS-plus-ROS strategy. Overwhelming evidence substantiates that pharmacologic inhibition of ALDH activity leads to cell death in the drug-tolerant subpopulation via ROS accumulation.[46] Recently, our team identified a key role for Niemann-Pick C1-like 1 (NPC1L1) in maintaining redox homeostasis in multidrug-resistant (MDR) persister cells. Mechanistically, NPC1L1 prevented lipid peroxide accumulation-mediated oxidative stress primarily by promoting the uptake of its substrates, vitamin E and cholesterol. Fortunately, NPC1L1 is the direct target of ezetimibe, an approved antihyperlipidemic drug. We used ezetimibe in combination with a chemotherapeutic agent and MDR inhibitor to induce oxidative stress-dependent cell death in MDR cancer cells, thereby eliminating DTP cells and avoiding tumor recurrence.[6]

Perspectives and conclusions

The discovery of DTP cells offers a novel perspective on treatment failure due to drug resistance and cancer recurrence. Targeting DTP cells has made curative treatment for cancer possible. Over the past few years, various potential mechanisms, including slow nonproliferation, adaptive energy depletion, phenotypic plasticity, and microenvironmental adjustments, have been revealed to mediate the survival of DTP cells. Given that almost all mechanisms of DTP cells are associated with redox signaling, manipulating the redox homeostasis of cells represents a promising therapeutic avenue for eradicating DTP cells. At least two therapeutic strategies exist to target the redox state of DTP cells: one involves the induction of oxidative stress by increasing intracellular ROS levels while using pro-oxidants or disrupting the cellular antioxidant system, and the approach is to induce reductive stress by removing ROS from cells with antioxidants. However, compromised ROS signaling may impair ROS-mediated antitumor properties during therapy, especially classical chemotherapy and radiation. Therefore, the most attractive strategy is to promote ROS accumulation by inducing exogenous ROS or interfering with the endogenous antioxidant system. Indeed, several redox modulators have been reported to be effective in feasible preclinical models and clinical trials.[110,111]

It must be acknowledged that the detailed biology of DTP remains elusive, which is an obstacle to the establishment of an integrated redox-DTP interaction network and the discovery of targeted therapeutic strategies. More advanced research strategies must be established to examine unexplored issues concerning DTP cells. Efforts to develop affordable and reliable methods to elucidate DTP remains an area of urgent need. Moreover, current strategies to manipulate the redox state are almost all based on macroscopic regulation and lack precise schemes, which means that ROS-based DTP-targeted drugs require more investigation to ensure their safety and efficacy.

As a largely underexplored and relatively novel concept, DTP has attracted increasing attention as a potential target for curative cancer therapy. Interestingly, the earlier discovery of MRD as a reservoir of drug resistance and cancer recurrence can be considered a clinical DTP state. There is a rich literature available substantiating the critical role of redox signaling in DTP cells. Corresponding therapeutic strategies, such as eradicating persistent cells by inducing ferroptosis, have been repeatedly confirmed to be effective by many research groups. We advocate that a deeper understanding of the crosstalk mechanisms between DTP cells and ROS provides strong theoretical support for future cancer therapy. However, there is still a long way to go before clinical translation.

Search strategy and selection criteria

The references from our review were retrieved from PubMed and Google Scholar databases using the keywords "drug tolerance persistence" or "nongenetic mechanisms" or "cancer" or "reactive oxygen species" or "redox signaling". In addition, the references in relevant articles were used as important literature sources. Priority was given to searching for articles published between 2015 and 2022. Some well-known concepts in this review were cited regardless of their publication date.

Outstanding questions

Given the extensive involvement of redox signaling in regulating DTP cell biological functions, targeting the redox signaling network of DTP cells to bring it under control represents an extremely promising therapeutic strategy. However, it remains unclear which therapeutic strategies are effective before clinical translation.

First, since the harm of DTP cells mainly comes from their re-entry into a proliferative state after therapeutic withdrawal, keeping them in a sleepy state may be an effective strategy. Importantly, existing cell cycle inhibitors as well as inhibitors of ROS-dependent proliferation signaling pathways seem to have great potential to ameliorate the adverse consequences caused by DTP cells by maintaining their nonproliferative state.

In addition, the DTP state is reversible; cancer cells can resume proliferation and drug sensitivity upon drug withdrawal, implying that awakening these cells to exit a slow or nonproliferative state represents an effective strategy for controlling this subset of malignant cells.

We advocate the direct ablation of DTP cells by disrupting the redox balance based on the modulation of redox signaling. However, as a new concept in the field of cancer, our understanding of DTP cells is very limited. The discovery of lethal targets depends on advances in high-throughput sequencing and bioanalytical methods, and better application of novel preclinical models.

Contributors

XWW and CHH designed the conception. ZZ wrote and edited the manuscript. ZZ and YHT drew the figures. All authors read and approved the final manuscript.

Declaration of interests

The authors declare that they have no competing interests.

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