Intrinsic and Acquired Chemotherapy Resistance

Subjects: Pharmacology & Pharmacy

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Drug resistance is a commonly unavoidable consequence of cancer treatment that results in therapy failure and disease relapse. Intrinsic (pre-existing) or acquired resistance mechanisms can be drug-specific or be applicable to multiple drugs, resulting in multidrug resistance.

chemotherapy resistance cancer and drug vulnerabilities

1. Introduction

The administration of single anti-cancer drugs sooner or later selects for the occurrence and outgrowth of drug-resistant cancer cell populations, with therapy failure and disease relapse being the ultimate consequences. Focusing on chemotherapeutics, this led to the development of multidrug treatment protocols in which agents with different modes of actions are combined with the aim to suppress the occurrence of drug resistance. In hematological disorders, such as Hodgkin's lymphoma and acute lymphoblastic leukemia, multidrug regimens, such as ABVD (doxorubicin-Adriamycin, bleomycin, vinblastine, and dacarbazine) or CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine sulfate-oncovin and prednisone), when provided to patients with early-stage tumors, can result in 5-year progression-free survival of 80–98%, with many patients being cured. However, for most, if not all other solid and non-solid malignancies, therapy success with multidrug regimens remains to be the exception.

Resistance can be restricted to a specific drug, or affect different drugs with independent modes of action, named multidrug resistance (MDR). However, even in non-solid tumors, chemotherapeutic multidrug regimes result in the appearance of drug-resistant cell populations, containing pre-existing (intrinsic) and newly acquired resistance mechanisms that can be mechanistically separated, as summarized in Figure 1.

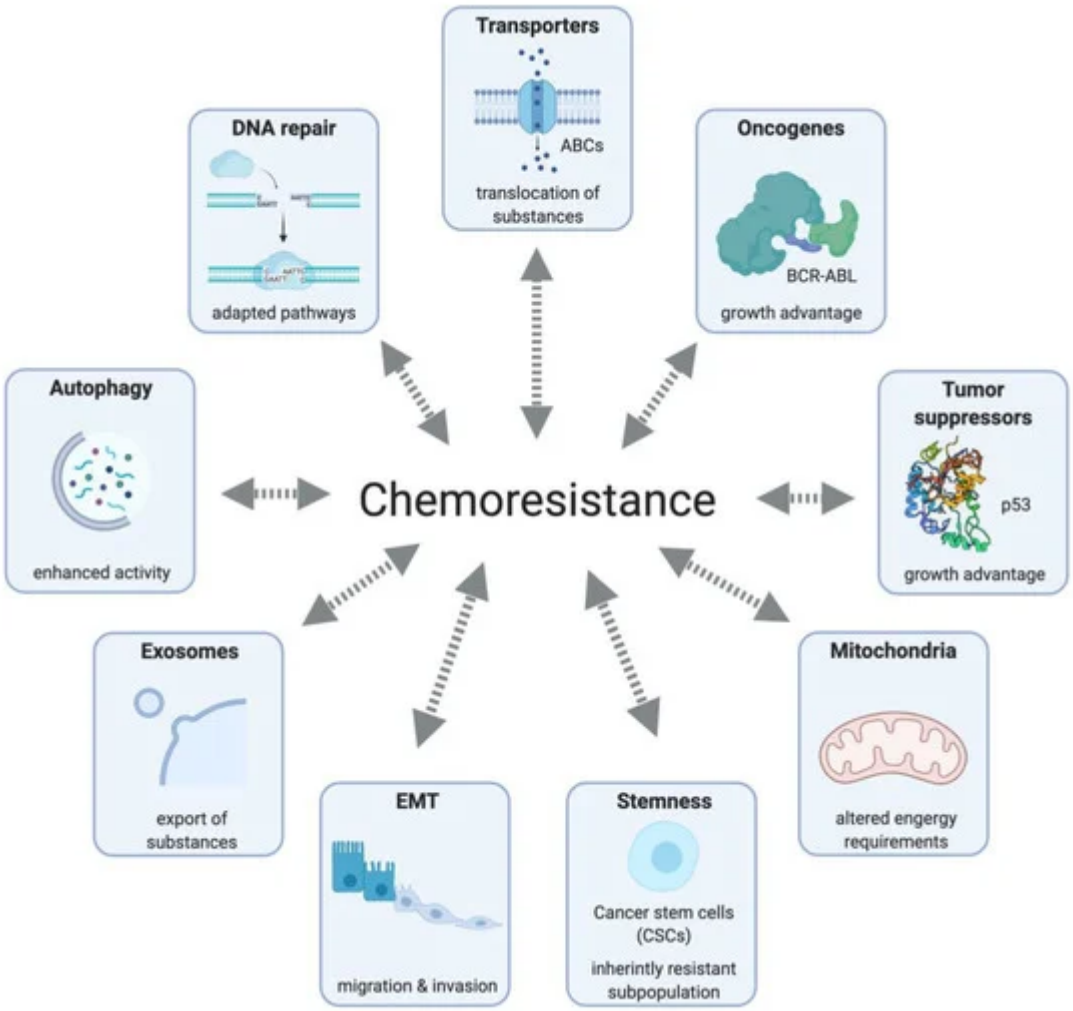

Figure 1. Mechanisms contributing to chemoresistance include molecule transporters that increase the drug efflux, reducing their intracellular concentrations; higher proliferation induced by oncogene activation or mutations in tumor suppressor genes; deregulation of apoptosis and metabolic reprogramming due to mitochondrial alteration; invasive phenotypes caused by overexpression of stem cell markers; existence of inherently resistant cell subpopulations which present a certain degree of quiescence and a high expression of stem cell markers, as well as drug efflux and anti-apoptotic proteins; elevated secretion of exosomes by tumor cells, which mediate the transfer of cargos that can promote resistance by several mechanisms (e.g., growth advantage, drug efflux); pro-survival function, mediated by increased autophagic activity; the activation of alternative DNA repair pathways.

Intrinsic resistance may be defined as the pre-existence of resistance mechanisms before therapy is initiated. The reasons are heterogeneous and include (1) the pre-existence of therapy-resistant cell populations; (2) low therapy tolerance of the patient or the occurrence of unbearable side-effects; (3) an inability of the therapy to reach its needed pharmacokinetic profile by means of altered absorption, distribution, metabolism, or excretion. In contrast to intrinsic mechanisms, acquired resistance may be defined by the appearance of drug-resistant cell populations containing secondary genetic modifications acquired during the course of therapy, ultimately, as with intrinsic resistance, leading to therapy failure. Acquired resistance mechanisms include, but are not limited to (1) increased rates of drug efflux or decreased rates of drug influx into the tumor cells, mediated by transmembrane transporters

of drug uptake and/or efflux; (2) biotransformation and drug metabolism, mainly due to CYPs (Cytochromes P450s) in the tumor; (3) altered role of DNA repair and impairment of apoptosis; (4) role of epigenetics/epistasis by methylation, acetylation, and altered levels of microRNAs leading to alterations in upstream or downstream effectors; (5) mutation of drug targets in targeted therapy and alterations in the cell cycle and its checkpoints; (6) the tumor microenvironment. Importantly, cancers can become chemotherapy resistant by combinations of these mechanisms. For instance, the action of methotrexate depends on its active transport into cells through the reduced-folate transporter 1 (RFT-1), its conversion to a long-lived intra-cellular polyglutamate, and its binding to the dihydrofolate reductase (DHFR), which leads to the inhibition of the synthesis of thymidylate and purines and the induction of apoptosis. Cellular defects in any of these steps can lead to drug resistance. Mutations in RFT-1, amplification or mutation of DHFR, loss of polyglutamation, and defects in the apoptotic pathway have all been shown to lead to the loss of efficacy of methotrexate [1][2].

Anti-cancer drug resistance mechanisms, however, can be accompanied by the emergence of new and therapy-restricted vulnerabilities. For example, resistance can arise as a compensation for the effects of treatment due to the “addiction” of cancer cells to a specific oncogene. Functional genetic screens have been used to identify such acquired vulnerabilities in several cancer cell lines [3][4]. These dependencies, or collateral sensitivities, should be clinically exploited, as was demonstrated for drug-resistant melanoma. Through a “one-two-punch” strategy, treatment with vemurafenib (B-Raf inhibitor) leads to increased levels of reactive oxygen species (ROS) in resistant cells, rendering them particularly sensitive to the treatment with the histone deacetylase inhibitor vorinostat [5]. This exemplifies how comprehensive genetic mapping of drug resistance can be applied to synchronize a cancer cell population, enabling the collateral targeting of the resistance-associated vulnerability. In this review, we discuss targeted and unbiased gene perturbation efforts in elucidating the mechanisms behind chemoresistance and, whenever available, highlight associated vulnerabilities for a potential clinical exploration.

2. Translating Chemoresistant Vulnerabilities—Conceptual and Technological Strategies

The majority of the studies presented in this review harness the concept of collateral sensitivity as a type of synthetic lethality that could be potentially exploited in cancer therapy. The idea that resistance towards a drug must inevitably come accompanied by an increased sensitivity to other compounds has led to the search of these acquired vulnerabilities in cancer cell lines resistant to chemotherapeutic agents, as well as to targeted drugs. Collateral sensitivities have been reported with cancer chemotherapeutics, including an increased sensitivity to microtubule inhibitors, cisplatin or cytarabine in paclitaxel-resistant CHO (Chinese hamster ovary) cells [6]. For example, Jensen et al. screened a panel of SCLC cell lines resistant to different chemotherapy agents and tested their sensitivity to 20 additional drugs [7]. With this approach, they identified cross-resistance as well as collateral sensitivities. Interestingly, vulnerabilities were found for all the resistant cell lines tested, one of them being the previously reported higher sensitivity to cisplatin in paclitaxel-resistant cells. MDR is often caused by the overexpression of drug efflux pumps, such as the ABC transporter MRP1, and this overexpression could itself be exploited as a collateral sensitivity that can be associated with the depletion of critical intracellular compounds such

as glutathione, ATP and metals. MRP1-overexpressing cells usually show reduced levels of glutathione (GSH) [8][9][10], which can be additionally decreased using other therapeutic agents, such as verapamil [11][12], that further increase GSH efflux, leading to oxidative stress and apoptosis [13][14]. Oxidative stress induced by high levels of ROS is characteristic of chemotherapy-treated cells [15][16][17], and several redox-regulating enzymes could be potentially targeted, including mitochondrial electron transporters and GSH metabolism-related enzymes (reviewed in [18]). Another approach to exploit this acquired vulnerability is the “one-two-punch” strategy, elaborated by the Bernards’ group [5]. Similar to chemotherapy-resistant cells, melanoma cells resistant to the BRAF inhibitor vemurafenib have increased levels of ROS, which can be additionally increased by using the histone deacetylase inhibitor vorinostat. This sequential treatment leads to much higher levels of ROS in the resistant cells, causing severe DNA damage and apoptosis. The clinical efficacy of the “one-two-punch” hypothesis is currently evaluated with vorinostat treatment in patients with resistant BRAF V600E mutated advanced melanoma [19], and the results will hopefully prove that the concept is clinically relevant.

These discussed studies also demonstrate the usefulness of genetic perturbation screens in the investigation of acquired vulnerabilities in chemotherapy-resistant cancer cells. Many of the hits found to act synthetic lethal with the drugs in the resistant cells pertain to cell proliferation, DNA damage repair or the apoptosis pathways, which makes them potentially targetable with inhibitors of these signaling pathways. For instance, CHEK1 is a commonly found hit in screens that search for genes that could render cells more susceptible to gemcitabine treatment when downregulated. In fact, it has been shown that CHEK1 can be targeted with the inhibitor AZD7762 to sensitize pancreatic cancer cell lines to gemcitabine both in vitro and in vivo [20][21]. Apoptotic pathway alteration is another commonly found feature in chemoresistant cells, usually evidenced by an increased expression of the anti-apoptotic protein Bcl-2 [22][23][24]. Under chemotherapy selective pressure, tumor cells can become “addicted” to Bcl-2 expression for survival, so an obvious strategy to overcome this resistance mechanism may be the use of Bcl-2 inhibitors (reviewed in [25][26]). Pearce et al. showed that the increased expression of Bcl-2 in paclitaxel-resistant lung cancer cells could be exploited by a different approach [27]. Bcl-2 can be converted from an anti-apoptotic protein to a pro-apoptotic one through interaction with Nur77 in the mitochondria. The authors demonstrated that Bcl-2 can be pharmacologically targeted with a peptide that mimics Nur77, preferentially inducing apoptosis in Bcl-2 overexpressing paclitaxel-resistant cells and inhibiting tumor growth in a zebrafish xenograft model.

Most unbiased gene perturbation efforts investigate what would resemble intrinsic resistance mechanisms in the sense that the genetic perturbation is introduced prior to short-term exposure to a given drug. However, searching for targetable collateral sensitivities that emerge as a result of long-term drug exposure might be more beneficial, as these model system likely more accurately mimic the heterogenous chemoresistance profiles found in patients. It is also of great importance to be able to prioritize cancer therapeutic targets, and this should be done on the basis of context-penetrating mechanisms of resistance and/or vulnerabilities. In an effort to link drug response to genomic alterations, Garnett et al. performed a systematic screen in a panel of 639 cancer cell lines with 130 targeted agents and chemotherapeutic drugs [28]. As expected, strong associations were found for oncogenes that are targets of their specific drug, but they also found marked sensitizing genotypes in small subsets of cell lines, highlighting the need for large panels of cell lines to uncover potentially relevant genomic features. To address the

above-mentioned aspects, a larger number of drug-resistant cell lines pertaining to different cancer entities should be investigated systematically, with the “The Resistant Cancer Cell Line (RCCL) collection” representing a valuable resource that awaits its comprehensive genetic characterization (https://www.wass-michaelislab.org/rccl.php). Another important aspect is that resistance rarely emerges from a single mechanism. More often, several co-occurring gain-of-function and/or loss-of-function mechanisms synergistically contribute to a patient’s chemoresistance profile. In this context, technologies for the robust performance of genetic multiplexing approaches for the simultaneous perturbation of multiple loci are needed [29][30]. While these combinatorial approaches gain momentum in the identification of synthetic gene interactions, their general application and analysis are far from standardized. However, these approaches hold great potential to uncover druggable vulnerabilities otherwise missed in monogenic screens due to functional buffering effects [31].

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