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Resistance to Chemotherapy: Short-Term Drug Tolerance and Stem Cell-Like Subpopulations

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Abstract

Personalized medicine in cancer treatment has been a major goal for decades. Recently, the development of several therapies that specifically target key genetic alterations in different malignancies has dramatically improved patient outcome and brought the goal of personalized medicine closer to practicality. Despite the improved specificity of these treatment options, resistance to targeted therapy is common and remains a major obstacle to long-term management of a patient's disease. Often patient relapse is a result of the positive selection of cells with certain genetic alterations that result in a bypass of the therapeutic intervention. Once this occurs, patient relapse is inevitable and further treatment options are limited. The time to relapse is often quite rapid indicating that cancer cells may be primed for adapting to cytotoxic stimuli. Recently, it has been suggested that small subpopulations of cells allow resistance to occur more rapidly. It is thought that these cells are capable of surviving strong apoptotic stimuli until more permanent mechanisms of long-term resistance are developed. In order to decrease the rate of patient relapse, more studies are required in order to identify these subpopulations of cells, understand the mechanisms underlying their drug tolerance, and develop strategies to prevent them from evading treatment.

1. INTRODUCTION

Decades of cancer research have clearly laid a foundation for understanding the common characteristics that are shared between different types of cancer. Generally, these hallmark characteristics include uncontrolled proliferation, apoptotic evasion, and increased invasive potential (Hanahan & Weinberg, 2011). Current research continues to expand our knowledge of these traits in order to increase prevention, detection, and treatment of malignancies. A fundamental step toward this goal is a better understanding of the mechanisms through which a tumor acquires these characteristics. Studies continue to identify abnormalities in signaling pathways that contribute to the malignant phenotype of cancer cells.

Generally, these abnormalities are the result of alterations in key regulatory genes. Alterations of these genes disrupt normal cellular function and are often referred to as driver mutations because they are crucial for the malignant progression of the disease. The ability to identify driving mutations in patient samples and cell lines has been aided by the development of high-throughput assays, such as whole exome sequencing. This may lead to an encyclopedia of cancer mutations that can be used to develop specific targeted therapies. The utilization of targeted therapies that are linked to the mutational makeup of individual patients should lead to more personalized care and improved patient outcome.

Over recent decades, the theoretical possibility of targeted therapy and personalized medicine has become a reality for some malignancies. For example, chronic myelogenous leukemia (CML) is commonly characterized by t(9;22) chromosomal translocation, which results in the formation of the p210 form of the breakpoint cluster region-c-abl oncogene (BCR-ABL) fusion protein. This fusion protein lacks the negative regulatory domain of the ABL protein and, therefore, has constitutive tyrosine kinase activity (Davis et al., 1985). The identification of this fusion protein as a driving mutation of the disease led to the development of imatinib and other second generation inhibitors of this fusion protein. Treatment with these drugs has improved 5-year survival rate of patients with CML to more than 85% (O'Brien et al., 2003).

Mutations are also found in epidermal growth factor receptor (EGFR) family receptors in several different types of cancer. This family of receptors includes EGFR (v-erb-b erythroblastic leukemia viral oncogene homolog—ERBB1), ERBB2/HER2, ERBB3/HER3, and ERBB4. High expression of these proteins is seen in epithelial cancers such as breast and non—small cell lung cancer (NSCLC). Inhibitors such as the small molecule inhibitors, gefitinib and erlotinib, and the monoclonal antibody, herceptin have shown success in treating malignancies dependent on these receptors.

Another key example of targeted therapy has recently been developed for patients with metastatic melanoma. The development of this therapy was driven by the realization that around 50% of melanomas contain mutations in the serine/threonine kinase, v-raf murine sarcoma viral oncogene homolog B1 (B-RAF) (Davies et al., 2002). Less than a decade after this discovery, the mutant B-RAF inhibitor, vemurafenib/PLX4032/zelboraf, was introduced into the clinic. This drug is now the FDA-approved standard of care for patients with mutant V600 B-RAF positive metastatic melanoma and has shown improvement overall and progression-free survival in naïve patients (Chapman et al., 2011).

However, despite an increased ability to target mutant gene products, most patients still experience tumor relapse over time. Various mechanisms of resistance have been identified in these patients that enable their tumors to bypass targeted therapy. These mechanisms usually result in reactivation of the original pathway targeted by the therapy or activation of new pathways that compensate for the inhibition of the targeted pathway (Fig. 10.1).

The high frequency of patient relapse with targeted therapies suggests that most tumors have inherent plasticity that allows for adaptation to cytotoxic stimuli. One possible explanation for this underlying plasticity is the presence of small subpopulations of cells within a tumor.

These highly adaptable subpopulations of cells are often referred to as stem cell-like or cancer stem cells (CSCs) due to the presence of certain cell surface molecules and the increased expression of stem cell factors in these cells. Studies examining the stem cell subpopulations of tumors have found that they have tumor initiating capabilities and are often resistant to chemotherapeutic treatment. The rate of resistance to targeted therapy suggests that the plasticity of tumor cells has been underestimated and requires more study. Current treatments are still not capable of coping with the evolution of most tumors. This chapter summarizes the latest findings about resistance to targeted therapies with a bias toward studies performed in melanoma.

2. LONG-TERM MECHANISMS OF RESISTANCE TO TARGETED THERAPY

Although treatments targeting various mutations in different cancer types have been developed over the past 10–15 years, a permanent cure for these malignancies still remains elusive for the majority of patients. This is due to emergence of resistance mechanisms in the tumor cells. Once resistance develops, cells undergo positive selection pressure and rapidly expand in order to reestablish the tumor cell population. As this occurs, patients relapse and are no longer responsive to the initial targeted therapy. Subsequent treatment options for such patients are limited.

2.1. Mechanisms of Pathway Reactivation/Therapeutic Bypass

As described earlier, therapeutic resistance is usually associated with either reactivation of the originally targeted pathway or activation of alternative pathways that compensate for the loss of the targeted pathway. One type of resistance mechanism that can lead to pathway reactivation and therapeutic bypass is the development of mutations in the gene that is targeted by the therapeutic intervention. Often, these mutations occur at what are known as “gatekeeper” residues. Mutations at these residues have been shown to interfere with the action of ATP-pocket-binding drugs and, thus, decrease drug efficacy. One study found that mutations in BCR-ABL were present in 29 of 32 patients who relapsed following imatinib treatment (Shah et al., 2002). Of these 29 patients, 10 had developed a mutation at the gatekeeper residue (T315I) (Shah et al., 2002). In NSCLC patients, who were resistant to EGFR inhibitors such as gefitinib, 50% had gatekeeper mutations (T790M) (Oxnard et al., 2011). *In vitro* data also suggest that mutation of the gatekeeper residue of B-RAF^{V600E} (T529) can confer resistance to RAF-inhibitors (Whittaker et al., 2010); however, mutations in the gatekeeper residue of B-RAF have yet to be found in vemurafenib-relapse samples taken from patients (Nazarian et al., 2010).

Additionally, genetic alteration of the targeted gene can occur at areas outside of the gatekeeper residue. For example, one mechanism found to lead to resistance to vemurafenib is the development of a splice variant in B-RAF. Because this splice variant lacks the N-terminal region of B-RAF, it is rendered constitutively active via elevated homodimerization of spliced B-RAF and is insensitive to vemurafenib (Poulikakos et al., 2011). This mechanistic explanation is supported by the fact that expression of a dimerization-deficient mutant form of the splice B-RAF variant displays sensitivity to vemurafenib treatment (Poulikakos et al., 2011).

Although bypass of targeted therapy often results from mutational events arising after treatment initiation, the original genetic makeup of a tumor may also lead to heightened pathway activation and resistance. Specifically, this occurs in melanomas that harbor mutations in neuroblastoma RAS viral oncogene homolog (N-RAS) but have wild-type B-RAF. Despite strong activation of downstream RAF-mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK)1/2 via mutant N-RAS, melanomas with this type of mutational status showed paradoxical hyper-activation of the pathway in response to treatment with vemurafenib (Halaban et al., 2010; Heidorn et al., 2010; Kaplan et al., 2011; Poulikakos et al., 2010). This is thought to be due to increased membrane recruitment and C-RAF heterodimerization of the inhibited form of wild-type B-RAF (Heidorn et al., 2010). For this reason, vemurafenib is not approved for metastatic melanoma patients with wild-type B-RAF.

Patients who have relapsed while on targeted therapies also show pathway reactivation via amplification of the targeted gene. One study has shown that transformed hematopoietic cells cultured continuously in imatinib develop resistance characterized by increased BCR-ABL mRNA and a nearly 10-fold increase in BCR-ABL protein (Weisberg & Griffin, 2000). Additionally, resistance of colorectal cancer cell lines to RAF and MEK inhibition has also been characterized by amplification of B-RAF (Corcoran et al., 2010).

Alterations in other components of the targets downstream pathway may also account for pathway reactivation and resistance to therapy. Several studies have shown that in ERBB2-overexpressing breast cancer cell lines, resistance to EGRF inhibitors can be mediated by upregulation of ERBB3, leading to activation of the same downstream targets originally affected by ERBB2 overexpression (Grovdal et al., 2012). Pathway reactivation has also been shown as a key resistance mechanism to vemurafenib both *in vitro* and in the clinic. For example, a subset of patients who relapsed while on vemurafenib showed mutations in N-RAS, which can activate the MEK-ERK1/2 pathway via other RAF isoforms (Nazarian et al., 2010). Elevation of v-raf-1 murine leukemia viral oncogene homolog 1 (C-RAF) has also been proposed as a potential mechanism of pathway reactivation that would bypass B-RAF inhibition (Montagut et al., 2008), but the data supportive of this notion are *in vitro* based. Additionally, downstream activation of ERK1/2 signaling pathway has been shown to occur in one resistant patient sample via a mutation in MEK1 (Wagle et al., 2011).

2.2. Compensatory Pathway Activation

Although many cases of patient relapse can be attributed to mechanisms that reactivate the targeted pathway, resistance to therapy can also result from increased activity of other oncogenic pathways that lie outside of the influence of the initial target. Patients that develop this type of resistance may still display inhibition of the targeted pathway. However, their disease has evolved in such a way that the tumor cells are no longer dependent on the action of the original driving mutation. Cells that lack this dependency are then capable of reestablishing the tumor population despite the continued action of the targeted therapy.

Some patients that develop resistance to vemurafenib show this type of compensatory mechanism. Elevated expression of platelet-derived growth factor receptor, beta (PDGFR β) was detected in 4 out of 11 samples obtained from patients that experienced tumor relapse

during treatment (Nazarian et al., 2010). Increased expression and activity of this receptor has been shown to enhance growth and survival in cell lines that have developed resistance to RAF inhibitors. Additionally, one study has shown that melanoma cell lines can also develop resistance to RAF inhibition through increased phosphorylation of insulin-like growth factor 1 receptor (IGF-1R) (Villanueva et al., 2010). The study also demonstrated enhanced activation of IGF-1R and/or downstream signaling to v-akt murine thymoma viral oncogene homolog 1 (AKT) in two of five patient samples analyzed.

The plethora of mechanisms resulting in resistance to targeted therapy is a startling realization. Although the identification of aberrations leading to bypass of the target and progression of the disease is important, further investigation into the inherent ability of cancer cells to evade targeted therapy is needed in order to improve the clinical outcome of chemotherapeutic treatments.

3. RESISTANCE OF STEM CELL-LIKE SUBPOPULATIONS

Many investigators have now realized that there are inherent properties of malignant tumors that provide resistance to targeted therapy. This type of resistance is based on the idea that these tumors contain populations of cells with readily available mechanisms in place that prevent the cell from succumbing to strong apoptotic signaling. If the majority of cells in a tumor have mechanisms that provide inherent resistance to a chemotherapeutic treatment, a patient may not show a dramatic response. This has been shown in the clinical trials of vemurafenib where approximately 50% of patients that have mutant B-RAF positive melanomas show low levels of tumor regression or, in some cases, progressive disease following treatment (Chapman et al., 2011). Patients who fail to show significant response to targeted therapy may have tumors with multiple driving mutations that promote proliferation and survival. Therefore, targeting only one of the driving mutations is not sufficient to promote tumor regression.

However, many models of resistance to targeted therapies have found that inherent resistance to chemotherapy is often a result of small subpopulations of cells that survive treatment and eventually reestablish the initial population. This theory is similar to a recent model of bacterial resistance that involves the presence of “persisting” cells rather than drug-resistant mutants. The presence of these persisting cells in bacterial populations is caused by phenotypic heterogeneity most likely caused by epigenetic mechanisms that increase the probability that some of the individual cells will survive lethal stimuli such as antibiotic treatment (Dhar & McKinney, 2007). It is thought that resistant bacteria colonies develop through these persisting subpopulations. However, only recent data have supported the possibility that this model of resistance could play a role in tumor cells (Sharma et al., 2010).

The idea that a subpopulation of cells is capable of reestablishing an entire tumor population after chemotherapeutic treatment is related to the idea of adaptive resistance that was previously discussed. However, short-term drug tolerance focuses on immediate survival rather than permanent mechanisms that undergo positive selection and ultimately reestablish the ability of the tumor to survive and propagate. For this reason, cancer cells that display

this type of inherent resistance are often called drug-tolerant persisters (Sharma et al., 2010). Although the emergence of the adaptive responses to treatment discussed earlier plays a crucial role in promoting long-term resistance to targeted therapy, the role of short-term adaptations of subpopulations of tumor cells to cytotoxic stimuli remains unclear.

The fact that a tumor is heterogeneous and contains subpopulations of cells is fundamental to cancer biology. This concept suggests that there are different cell types within a tumor that play crucial and specific roles in maintaining malignant properties. For example, evidence suggests that there is a subpopulation of cells in melanoma that are slower cycling and are responsible for maintaining the rapidly proliferating cell population (Roesch et al., 2010). Several studies have found similar results suggesting that a small subpopulation of cancer cells is responsible for establishing the entire tumor cell population. This theory is called the CSC theory and has been the subject of intensive study.

The theory of CSCs assumes that tumors have a similar hierarchy of cellular organization as that seen in normal tissue. Cells are classified as CSCs if they can reestablish a tumor cell population. Subpopulations of CSCs have been identified in many cancer types (Frank et al., 2010) and are usually identified by unique markers that distinguish them from other cancer cells in the population. Some of the molecular phenotypes that have been associated with CSC subpopulations include CD34⁺CD38⁻ for acute myeloid leukemia (AML), CD44⁺CD24⁻ for breast cancer, CD133⁺ for colon cancer and pancreatic cancer, CD90⁺ for liver cancer, CD44⁺CD117⁺ for ovarian cancer, and ABCB5⁺ for melanoma (reviewed in Frank et al., 2010).

One of the most salient features of the CSC theory is that these subpopulations of cells are responsible for the establishment of all other cancer cells within a tumor. This means that the bulk of tumor cells may represent differentiated forms of the original CSCs. These differentiated cancer cells are thought to have a limited life span and an increased sensitivity to immune detection, hypoxia, and chemotherapeutic treatments. However, because CSCs are believed to have unlimited self-renewal, the effects of cytotoxic stimuli on this subpopulation is believed to be mitigated. This is supported by the fact that tumors that are resistant to therapeutic interventions have maintained the presence of the CSC subpopulation (Reya et al., 2001; Scheck et al., 1996). The fact that CSCs are more resistant to cytotoxic stimuli has dramatic implications for cancer treatment.

Several studies have demonstrated that the CSC subpopulation displays decreased sensitivity to radiation treatment. Radiation therapy causes DNA damage in cells, which leads to mitotic catastrophe and cell death. It is believed that CSCs may be resistant to radiation by increasing DNA damage repair mechanisms. For example, in normal stem cells, the activation of the wingless-type MMTV integration site family, member (Wnt)/ β -catenin pathway plays an important role in normal stem cells and has been shown to be involved in resistance to DNA damage (Eyler & Rich, 2008). Previous studies have also shown that sorted mammary CSCs have increased the activity of the Wnt/ β -catenin pathway following irradiation (Chen et al., 2007; Woodward et al., 2007). Other studies have also implicated the involvement of Chk1/2 and Notch signaling as possible explanations for the decreased radiosensitivity of CSCs (Bao et al., 2006; Phillips et al., 2006).

Evidence also suggests that the CSC subpopulation is resistant to DNA-damaging chemotherapeutic agents. For example, one study has demonstrated that CSCs from gliomas show resistance to temozolomide, carboplatin, VP16, and Taxol (Liu et al., 2006). Because CSCs are less sensitive to DNA damage caused by radiation therapy, it is not surprising that this subpopulation of cells shows little effect upon exposure to these types of agents. However, it is thought that the resistance that CSCs show against DNA-damaging agents may lie outside of their ability to upregulate DNA repair mechanisms. Both normal stem cells and CSCs express high levels of drug pumps such as ATP-binding cassette (ABC) transporters. These channels transport agents out of the cell and diminish the likelihood of having efficacious doses of the chemotherapeutic agent reach crucial areas of the cell. It is thought that these channels play an important role in the ability of CSCs to gain resistance to systemic therapies, for example, amplification of the ABC transporter, ATP-binding cassette, subfamily G, and member 2 (BCRP) reduces efficacy of imatinib (Burger et al., 2004). Other ABC transporters, such as ATP-binding cassette, subfamily B, and member 1 (MDR1), have been shown to remove agents such as paclitaxel (Green et al., 2006).

In addition to the characterization of CSC subpopulations, investigations have also aimed to understand the regulation of stemness factors that may be inherently expressed in various malignancies. For example, POU class 5 homeobox 1 (Oct4) has been shown to be highly upregulated in bladder cancer tissue compared to normal tissue (Atlasi et al., 2007). Oct4 and Nanog expression have also been shown to enhance malignancy and epithelial-mesenchymal transition (EMT) properties of lung adenocarcinomas (Chiou et al., 2010). Additionally, increasing the expression of Oct4 enhances invasiveness and drug resistance in colorectal cancer and lung cancer cell lines (Chen et al., 2008).

A stemness factor that appears to have an important role in melanoma is forkhead box D3 (FOXD3). FOXD3 is a forkhead transcription factor that is crucial for maintaining pluripotency and self-renewal in embryonic stem cells (Hanna et al., 2002; Liu & Labosky, 2008) possibly by regulating other stem cell factors such as Nanog and Oct4 (Pan et al., 2006). Recent studies have demonstrated that FOXD3 is upregulated in response to inhibition of mutant B-RAF in melanoma cells (Abel & Aplin, 2010). This upregulation of FOXD3 has been shown to provide resistance to cell death induced by RAF inhibitors, such as vemurafenib (Basile et al., 2011). However, preliminary data indicate that FOXD3 prevents cell death independent of changes in the expression patterns of many key regulators of apoptosis such as Bim-EL, Bmf, and Mcl-1 (Basile et al., 2011). Instead, it is believed that FOXD3 is influencing other targets that provide compensation for the strong apoptotic stimuli that has previously been shown to be associated with loss of ERK1/2 signaling (Shao & Aplin, 2010).

The fact that FOXD3 is upregulated rapidly after inhibition of RAF/MEK in mutant B-RAF melanoma cells in order to prevent cell death suggests that these cells have an inherent ability to adapt to environmental stresses. Although the role of FOXD3 in melanoma seems to be complex, the increased expression of this stemness factor in response to inhibition of strong pro-survival signaling may be the result of an “emergency response” that the cells use to withstand a sudden change in the equilibrium of their malignant phenotypes. Because this

response prevents cell death, these cells may be capable of going into a state of dormancy until they regain malignant properties through other mechanisms (Fig. 10.2).

4. PLASTICITY OF TRANSIENTLY DRUG-TOLERANT SUBPOPULATIONS

In order to eradicate these stem cell-like subpopulations of cancer cells, it is necessary to gain a better understanding of the inherent plasticity of cancer cells. Current research suggests that cancer cells are highly adaptable to a variety of stimuli. These adaptations occur quickly and efficiently as if the cancer cell itself is preprogrammed to respond to continuous environmental changes. Therefore, the root cause of resistance to therapeutic treatments is likely to lie within this adaptability.

Many mechanisms of adaptive resistance such as those discussed earlier are based on the idea that alterations in a single gene or pathway lead to resistance. However, it is unlikely that the majority of relapse cases can be explained this simply. Instead, many now believe that drug resistance is caused by widespread epigenetic changes. These epigenetic changes reflect an inherent plasticity of small subpopulations of cells that can alter complex signaling networks quickly and efficiently in order to survive cytotoxic stimuli.

It has previously been shown that several oncogenic pathways can influence epigenetic changes. For example, loss of adenomatous polyposis coli (APC) is known to result in dysregulation of DNA methyltransferases that promote undifferentiated states similar to stem cell-like populations (Rai et al., 2010). Transformed breast epithelial cells were also shown to have higher levels of spontaneous conversion of non-stem cells to stem-like cells than their nontransformed counterparts (Chaffer et al., 2011). Additionally, oncogenic RAS can alter global histone modification (Pelaez et al., 2010).

Epigenetics has also been implicated in drug-resistant populations of cells. Originally, it was thought that methylation of certain genes would impact resistance to therapy. For example, studies of ovarian cancer have found that hypermethylation of the mismatch repair gene, mutL homolog 1 (MLH1), can confer resistance to DNA-damaging agents (Brown et al., 1997; Strathdee et al., 1999). However, recent evidence suggests that global epigenetic changes may contribute to the ability of cancer cells to develop resistance. This seems to be especially crucial for short-term survival of small subpopulations of persisting cells. Sharma et al. (2010) have found global chromatin alterations in drug-tolerant subpopulations of cancer cells that are associated with increased expression of the histone demethylase, jumonji/AT-rich interactive domain-containing protein 1A (JARID1A). This study found that increased expression of JARID1A was essential for the formation of drug resistance in their experimental systems. Therefore, results from this study would suggest that global epigenetic changes are crucial in the development of resistance to chemotherapeutic treatments.

The presence of stem cell-like subpopulations with global epigenetic modifications offers several key advantages that increase the tumor's ability to gain resistance to therapy. One advantage that this model of drug resistance offers is the efficiency by which global gene expression can be controlled. Increased activity of a few key epigenetic factors can change

the expression patterns of hundreds of genes, whereas mutational changes that alter the same number of genes would take a much greater amount of time. Indeed, a previous study has shown that gene alterations by DNA methylation are far more common than mutations in genetic sequence (Bhattacharyya et al., 1994). Because resistance to therapy in the clinic can occur rapidly, it is likely that drug-tolerant subpopulations of cells may gain resistance using epigenetic mechanisms that can alter genetic expression more quickly than positive selection of permanent genetic mutations.

Another advantage that this model of drug resistance offers is the plasticity and reversibility of these drug-tolerant subpopulations of cells. Because epigenetic modifications can be removed, the changes to gene expression that accompany epigenetic alterations are capable of being transient. This reversibility would not occur with mutations in the genetic sequence, which would remain permanent unless there was a negative selection pressure.

Several studies have demonstrated the plasticity of subpopulations of cancer cells. For example, a study of melanoma cells found that there was a slow-cycling subpopulation of cells that was maintained at a very low frequency in the population (Roesch et al., 2010). This subpopulation had elevated expression of the histone 3 lysine 4 (H3K4) demethylase, JARID1B, which again indicates the use of epigenetic modification (Roesch et al., 2010). Interestingly, this study found that the JARID1B-positive cells were essential for continuous growth of the tumor cell population, and even if this subpopulation was removed, previously JARID1B-negative cells were capable of becoming JARID1B-positive cells (Roesch et al., 2010). This indicates that differing populations of melanoma cells are capable of reversing their JARID1B status, demonstrating a high level of plasticity.

Studies of drug resistance have also demonstrated increased plasticity of subpopulations of cells. Previously, it was mentioned that a study by Sharma et al. (2010) found that drug-tolerant subpopulations of cells had high expression of the histone demethylase, JARID1A. Additionally, this study found that the drug-tolerant status of this subpopulation was highly reversible. Drug-resistant subpopulations that were grown in the absence of treatment regained sensitivity to the inhibitor within 9–30 passages (Sharma et al., 2010). Spontaneous heterogeneity of this drug-tolerant subpopulation was also detected using CD133 as a marker (Sharma et al., 2010). This means that the drug-resistant state of these cells is transient and indicates high levels of plasticity influenced by the absence or presence of cytotoxic stimuli.

5. POTENTIAL STRATEGIES TO TREAT DRUG-TOLERANT SUBPOPULATIONS

Although original theories suggested that using multitargeted inhibitors may offer the best chance of overcoming resistance to therapy, new evidence suggests that cancer cells have complex levels of adaptability. This adaptability appears to be based on the plasticity of subpopulations of cells within the tumor that are capable of global epigenetic modifications. Therefore, overcoming resistance to targeted therapy may benefit from using chromatin-modifying agents, such as histone deacetylase (HDAC) inhibitors (Fig. 10.3).

HDACs are associated with transcriptional repression. This is accomplished by the removal of key acetyl groups on histone proteins. This removal leads to chromatin condensation, which prevents transcriptional machinery from associating with genomic regions. Most HDACs belong to three main classes. Class I HDACs reside exclusively in the nucleus, while class II HDACs shuttle between the nucleus and the cytoplasm (Monneret, 2007). Class III HDACs are classified based on sequence similarity to the yeast Sir2 homolog. Each HDAC differs in tissue specificity and substrate binding. For example, HDAC1 (Class I) is ubiquitously expressed in various tissue types and has substrates that include E2F1, while HDAC5 (Class II) is expressed in heart, smooth muscle, and brain tissue and has substrates that include SMAD7 (Dokmanovic et al., 2007).

In cancer, it is believed that dysregulation of HDACs might lead to altered function of key tumor suppressor genes, such as tumor protein 53 (p53) (Murphy et al., 1999). Therefore, many researchers have explored the feasibility of using HDAC inhibitors to treat various malignancies. Many HDAC inhibitors have been developed, and several of them have shown efficacy in both *in vitro* and *in vivo* cancer models. It is believed that HDAC inhibitors work through several mechanisms, which are often independent of chromatin structure. This is because HDACs target a variety of nonhistone proteins such as transcription factors and DNA repair enzymes (Khan & La Thangue, 2011). In fact, it has been shown that HDAC inhibitors may affect the mRNA levels of only 5–10% of genes based on microarray studies (Peart et al., 2005). Despite the ambiguities of their mechanism, HDAC inhibitors have shown to be a promising avenue for cancer therapy.

For example, vorinostat/suberoylanilide hydroxamic acid (SAHA) is an FDA-approved HDAC inhibitor used to treat cutaneous T-cell lymphoma (CTCL). Prior to its approval, vorinostat was assessed in phase 1–2 trials. In these trials, vorinostat was well tolerated in patients and gave a response in around 30% of CTCL patients (Duvic et al., 2007). Currently, more clinical trials are investigating other HDAC inhibitors as well as combination treatment of vorinostat and other chemotherapeutic agents for various types of malignancies.

Because resistance to chemotherapy is likely to rely on chromatin modifications, it may be beneficial if certain chemotherapeutic treatments were used in combination with HDAC inhibitors. Combination treatment of HDAC inhibitors such as vorinostat with kinase inhibitors such as erlotinib or sorafenib has shown to be more effective at preventing the establishment of drug-tolerant colonies versus treatment with single-agent therapies (Sharma et al., 2010). The addition of HDAC inhibitors was also able to decrease resistance to nonspecific DNA-damaging agents such as cisplatin (Sharma et al., 2010).

Despite dramatic *in vitro* results, clinical data supporting the efficacy of using HDAC inhibitors in combination with other chemotherapeutic treatments have yet to be shown. A recent clinical trial exploring the efficacy of erlotinib in combination with vorinostat in 16 patients with NSCLC was prematurely terminated due to serious adverse events and a lack of efficacy (Clinical Trial Identifier: [NCT00251589](#)). This suggests that more specific chromatin modifying agents may be needed to increase efficacy and prevent toxicity resulting from combinatorial therapies.

6. CONCLUSION

Despite improved treatment options for many types of cancer, long-term management still remains elusive for the majority of patients. New evidence suggests that resistance to chemotherapy occurs in a step-wise process that begins with the inherent plasticity and stem cell-like phenotype of small subpopulations of cells within the tumor. These drug-tolerant subpopulations persist through strong apoptotic stimuli but remain in a dormant state of growth inhibition. Eventually, more permanent mechanisms of resistance are developed and result in therapeutic bypass and reacquired growth potential of the tumor. At this point, the patient presents with relapse of their original disease despite the presence of their targeted treatment. Further study is needed in order to clarify the mechanisms of inherent resistance shown by these small subpopulations of cells and identify new treatment options that may potentially target this type of resistance.

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ABBREVIATIONS

ABC	ATP-binding cassette
ABL	c-abl oncogene
AKT	v-akt murine thymoma viral oncogene homolog 1
AML	Acute Myeloid Leukemia
APC	adenomatous polyposis coli
BCR	breakpoint cluster region
BCRP	ATP-binding cassette, subfamily G, member 2
B-RAF	v-raf murine sarcoma viral oncogene homolog B1
CML	chronic myelogenous leukemia
C-RAF	v-raf-1 murine leukemia viral oncogene homolog 1
CSC	cancer stem cell
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ERBB	v-erb-b erythroblastic leukemia viral oncogene homolog
ERK	extracellular signal-regulated kinase
FOXO3	forkhead box D3

H3K4	histone 3 lysine 4
HDAC	histone deacetylase
IGF-1R	insulin-like growth factor 1 receptor
JARID	jumonji/AT-rich interactive domain-containing protein
MDR1	ATP-binding cassette, subfamily B, member 1
MEK	mitogen-activated protein kinase
MLH1	mutL homolog 1
N-RAS	neuroblastoma RAS viral oncogene homolog
NSCLC	non-small cell lung carcinoma
OCT4	POU class 5 homeobox 1, p53 tumor protein 53
PDGFRβ	platelet-derived growth factor receptor, beta
SAHA	suberoylanilide hydroxamic acid
WNT	wingless-type MMTV integration site family, member

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Figure 10.1. Canonical model of drug resistance.

Drug resistance is generally thought to occur through permanent adaptations in signaling pathways that promote regrowth of the tumor. Often, this occurs through increased expression of secondary activators or downstream effectors, acquired mutations in pathway components, or increased activity of secondary pathways.

Figure 10.2.

The role of stemness in drug resistance. Resistance to therapy is believed to be a complex, multistep process that begins with the short-term, inherent plasticity of subpopulations of cancer cells. The cytotoxic stimuli resulting from inhibiting a target can result in enhanced expression/activity of stemness factors in persisting subpopulations of cells. Expression of these stemness factors results in genetic plasticity that allows these cells to remain in a dormant, drug-tolerant state.

Figure 10.3. The proposed treatment modalities to overcome drug tolerance.

Using targeted therapies will provide beneficial results by decreasing cell growth and increasing cell death. However, chromatin-modifying agents may be necessary to overcome the drug-tolerant state that results from the increased genetic plasticity of certain subpopulations of cancer cells exposed to cytotoxic stimuli. Therefore, the combination of these two agents may eliminate drug-persisting cells and decrease the rate of patient relapse.