Cancer Cells Acquire Resistance to Anticancer Drugs: An Update

Hsing-Pang Lu, Chuck C.K. Chao

The efficacy of cancer chemotherapy is often affected by the emergence of resistant cancer cells. While biochemical and pharmacological mechanisms have been proposed to explain chemoresistance, the genes involved in this process have not been fully identified. We previously used genomic DNA microarrays and quantitative RT-PCR to identify the genes associated with resistance to chemotherapeutic drugs, particularly to the genotoxic agent cisplatin. Notably, knockdown of the cisplatin resistance (CPR) genes that we identified was shown to reduce chemoresistance and to suppress the growth of tumor xenographs in cisplatin-treated mice, indicating that the newly identified CPR genes may represent potential therapy candidates to limit chemoresistance and to improve the efficacy of anticancer drugs. In addition to genetic mutations, researchers have found that epigenetic changes and alternative splicing of specific genes may also allow cancer cells to become resistant to chemotherapeutic drugs. In this article, the authors present an overview of the latest findings in this field, including genetic changes, epigenetic changes and alternative splicing. *(Biomed J 2012;35:464-72)*

Prof. Chuck C.K. Chao

Key words: alternative splicing, cisplatin, chemoresistance, DNA microarray, epigenetic modifications

When cancer cells are first discovered in a patient, many drugs can help to get rid of them. But over time, cancer cells begin to withstand those first-line drugs and continue to grow and spread. Accumulating evidences have shown that the heterogeneity of the cancer cell population influences the cellular response to chemotherapeutic drugs. Several cellular alterations have been proposed to explain resistance to anticancer drugs. The conditions of cisplatin treatment also influence the response of cancer cells. For instance, chronic and long-term exposure to increasing concentrations of cisplatin appears to permanently increase the levels of the nucleophile and detoxifying molecules glutathione and metallothionein which are associated with chemoresistance. Acute exposure to cisplatin on a monthly basis leads to defects on the surface of the cell membrane and reduces drug accumulation inside the cells. On the other hand, weekly pulsed-exposure to cisplatin leads to changes in folate metabolism and oncogene expression. In addition, a single lethal concentration of cisplatin may cause DNA adducts in the treated cells, and in turn cell cycle arrest at the G2 phase and apoptosis. The finding that cisplatin-DNA adducts bind to several cellular proteins, termed cisplatin-damaged-DNA recognition proteins, has attracted considerable attention in this field.[1,2] These proteins include signals that enhance cell survival by mediating DNA repair while others induce cell death by conferring sensitivity to the drug. Over the past decade, numerous genes involved in these pathways have been described;[3] for further information, please referr to the website of the Pharmacogenetics Knowledge Base (PharmGKB; http://www.pharmgkb.org/). It is important to keep in mind that differences may exist between *in vitro* studies and the patients since the environment of a tumor is remarkably different from that of cultured cells in terms of nutrients, growth factors, hormones, pH, intercellular communication, and oxygenation state. In addition, the various oncogene and protein kinase signaling pathways are likely to be differentially regulated in these two environments.

Multiple pathways of anti-cancer drug resistance

Cisplatin resistance (CPR) is the most studied example in anticancer resistance. Herein, its pharmacokinetic views

From the Department of Biochemistry and Molecular Biology, and Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan.

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Correspondence to: Prof. Chuck C.K. Chao, Department of Biochemistry and Molecular Biology, and Institute of Biomedical Sciences, College of Medicine, Chang Gung University. 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan (R.O.C.). Tel: 886-3-2118136; Fax: 886-3-2118700; E-mail: cckchao@mail.cgu.edu.tw

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and genetic changes will be used for discussion of the mechanism of anticancer drug resistance. For cisplatin, the mechanisms involved in this process include decreased drug influx, increased drug efflux, activation of detoxification systems, alteration of the drug targets, increased DNA repair, impaired apoptosis, and altered oncogene expression.[4-7] Changes triggered by cisplatin selection in the resistant cells involve a secondary layer of complexity that may include alterations in growth factors and hormone responsiveness, ion transport, nutrient transport and utilization, thymidilate metabolism, oncogene and protein kinase signaling pathways, chromosome structure, and gene expression. Over the past decade, a few specific genes involved in these pathways have been described.The gene products that play crucial roles in the regulation of cells in response to cisplatin have been summarized [Figure 1].[8] For example, the influx of cisplatin into the cells is regulated by SLC31A1 (CTR1), while the efflux out of the cell is controlled by ABCC2 (MRP2), ATP7A, and ATP7B. Once cisplatin is inside the cell, the primary anticancer mechanism is the formation of Pt-DNA adducts, which leads to cell-cycle arrest and apoptosis. Genes involved in DNA repair (e.g. MSH6 and MLH1 for mismatch repair; XRCC1, ERCC1, ERCC2, and XPA for nucleotide excision repair; HMGB1 for recognition of Pt-DNA adducts) decrease the sensitivity of the cells to cisplatin. Several detoxification-related gen es (e.g. myeloperoxidase (MPO), superoxide dismutase 1 (SOD1), glutathione S-transferases M1 (GSTM1), NAD (P) H: quinone oxidoreductase 1 (NQO1), glutathione S-transferases P1 (GSTP1), and metallothioneins (MT)) are also responsible for lowering the intracellular concentration of cisplatin, and therefore play an important role in cellular resistance to the drug. In addition, the DNA polymerase variants POLH and POLB, which are normally involved in translesional DNA replication, have been shown to provide tolerance to cisplatin-based drugs, and therefore represent important determinants of the cellular responses to cisplatin.[8] The model depicted in Figure 1 can also be applied to other Pt-containing drugs as well as some genotoxic and chemotherapeutic compounds.

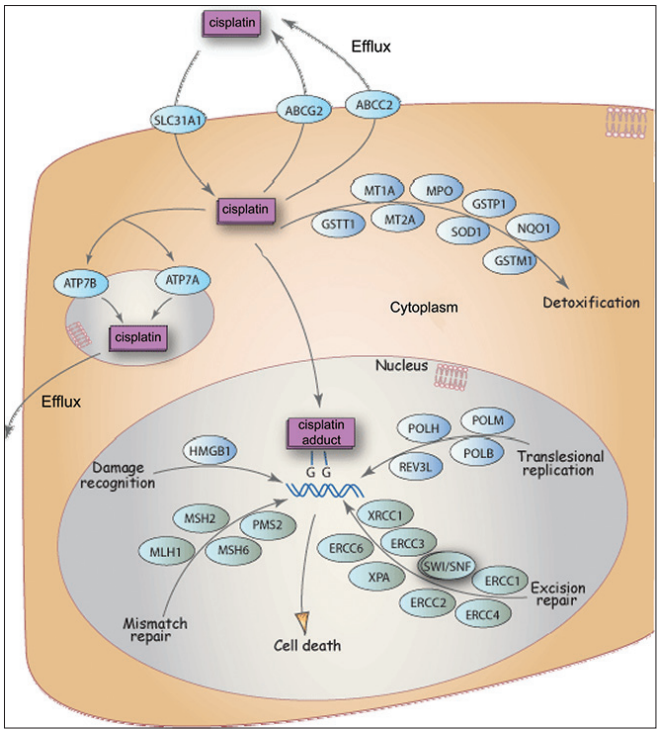

Figure 1: Pharmacokinetic pathways of cisplatin resistance. Several cellular alterations have been proposed to explain drug resistance, including decreased drug influx, increased drug efflux, activation of detoxification systems, alteration of the targets of the drug, increased DNA repair, impaired apoptosis, and altered oncogene expression. Thirty gene products involved in separate pathways are indicated. For additional information, one may refer to the website of the Pharmacogenetics Knowledge Base. (PharmGKB; http://www.pharmgkb.org/)

Genetic changes

DNA microarrays have been used to identify genome-wide gene expression patterns that can be used to predict drug responses along with metastasis, disease relapse, and prognosis of cancer patients.[9,10] Cisplatin-resistant cervix carcinoma HeLa cell lines, which were obtained by repeatedly treating parental HeLa cells with increasing concentrations of cisplatin,[11] have been used to identify the genes involved in cisplatin resistance. Of the altered genes we identified, nine were overexpressed at least twofold in the moderately resistant cells, and more than fourfold in highly resistant cells. Using gene knockdown experiments, we confirmed that the genes identified were involved in cisplatin resistance in various degrees. We recently reported that knockdown of CPR genes sensitized tumor cell lines to cisplatin, but not to the mitosis-disrupting agents such as vincristine and taxol. Thus, these observations suggest that CPR genes may be involved in cells resistance to genotoxic drugs.[12] Specifically, we showed that N-ethylmaleimide-sensitive factor attachment protein alpha (NAPA) and Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) (see below) may play a major role in acquired cisplatin resistance and that this process may rely on the tumor suppressor p53.[12-15] Overall, our results confirm the notion that genetic changes are important for acquired resistance during cancer chemotherapy.

Gene upregulation in genotoxic drug resistance

The genes (NAPA, CITED2, CABIN1, ADM, HIST-1H1A, EHD1, MARK2, PTPN21, and MVD), here referred to as cisplatin resistance (CPR) genes [Table 1], were initially reported by our group for their ability to modify the response of non-tumor, but viral transformed, human

Table 1: Levels of upregulated CPR Genes in cisplatin-resistant HeLa cells assessed by DNA microarray analysis

Gene Symbol (RefSeq ID)	Function	R1/HeLa	R3/HeLa
NAPA (NM_003827)	Intracellular transport	2.02±0.33*	7.18±3.11*
CITED2 (NM_006079)	Regulation of transcription	2.58±0.11*	4.38±0.53*
CABIN1 (NM_012295)	Calcineurin binding protein	3.75±0.24*	4.66±0.27*
ADM (NM_001124)	C21-steroid hormone biosynthesis	4.81±0.02*	4.16±0.06*
HIST1H1A (NM_005325)	Histone cluster	4.57±2.93*	7.21±6.26*
EHD1 (NM_006795)	EH-domain containing	2.25±0.13*	4.48±0.87†
MARK2 (NM_004945)	Kinase phosphorylation	2.27±0.52*	4.02±1.28*
PTPN21 (NM_007039)	Tyrosine phosphatase	2.32±0.01*	4.07±1.06*
MVD (NM_002461)	Cholesterol metabolism	2.72±0.21*	4.20±0.84†

Abbreviations: *: $p<0.05$; †: $p<0.01$; R1: Moderate resistance; R3: High resistance.

embryonic kidney HEK293 cells to cisplatin.[12] By using short-hairpin RNA (shRNA) to knockdown the CPR genes individually or in combination, we were able to sensitize HEK293 cells to genotoxic drugs including cisplatin. Among the treatments performed, shRNA knockdown of NAPA — which interacts with its receptor ER-SNARE (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptor) found in the endoplasmic reticulum (ER) and implicated in protein trafficking — was the most efficient treatment able to sensitize cells to cisplatin. Furthermore, shRNA knockdown of a single CPR gene was sufficient to partially reverse acquired cisplatin resistance in HeLa cells. Sensitization to cisplatin following knockdown of CPR genes was also observed in tumorigenic cell lines, such as ovarian cancer cells Sk-ov-3, lung cancer cells H1155, and nasopharyngeal cancer cells CG-1.[12] In addition, cells with low CPR genes levels were protected against cisplatin-induced apoptosis. Based on these results, we propose that the CPR genes identified may represent potential candidates for novel target therapies aimed at preventing resistance to cisplatin during chemotherapy.

Among the CPR candidates we identified, only ADM is an extracellular protein, while other five gene products (EHD1, MARK2, MVD, NAPA and PTPN21) are cytoplasmic, and three (CABIN1, CITED2 and HIST1H1A) are nuclear proteins. The CPR proteins have not been described in the canonical pathways of the MetaCore software (Version 6.3; Build 25485; GeneGo pathway analysis software for systems biology, St. Joseph, MI). ADM has been found to represent an anti-apoptotic factor in different cell lines as well as *in vivo*.[16,17] Although the mechanism of ADM in preventing apoptosis remains unclear, the impairment of apoptosis may still explain acquired cisplatin resistance. An "extracellular region" of ADM is directly upregulated through canonical pathways of transcription factors (SRF and ESR1) in the nucleus. ADM may negatively regulate some transcription factors (e.g. GATA-4) in the nucleus via cytoplasmic GRK2, and may dysregulate c-Src which normally passes signal to p300 in the cytoplasm. Therefore, p300 which regulates many transcription factors, including GATA-4, may be downregulated by ADM. Although GATA-4 is a downstream target of p300, it is probably not part of the pathway of ADM-mediated drug resistance since GATA-4 has been shown to provide a protective role against oxidative stress in the heart.[18] These results suggest that downregulation of p300 by ADM may be a therapeutic target to counteract cisplatin resistance. Our results also indicate that, NAPA and CITED2 may play a major role in acquired cisplatin resistance as seen in the HeLa cell culture and mouse models studied.[12-13] Interestingly, the regulation of cisplatin resistance by both proteins also involves p53 (see below).

p53-dependent ER/Golgi pathways in genotoxic drug resistance

We found that NAPA protects the cell against cisplatin.[13] Accordingly, knockdown of NAPA using lentivirus-encoding shRNA (shNAPA) induced ER stress similar to the effect of cisplatin treatment in HEK293 cells. A low dose of cisplatin also elicited a mild ER-stress response associated with the accumulation of the protective proteins BiP and NAPA. Remarkably, knockdown of NAPA induced apoptosis and enhanced cisplatin-induced apoptosis and growth inhibition, thereby sensitizing cancer cells to cisplatin. On the other hand, overexpression of NAPA increased resistance to cisplatin by reducing cisplatin-induced ER stress and apoptosis as well as growth inhibition. The modulatory effects of shNAPA required the tumor suppressor p53 since the effects of NAPA knockdown were reduced by the p53 inhibitor PFT-α (pifithrin-α) and these effects were also reduced in p53-null lung cancer H1299 cells. A partial reversal of cisplatin resistance was also observed in cisplatin-resistant HeLa cells following knockdown of NAPA. Importantly, a combined cisplatin/shNAPA treatment suppressed the growth of tumor xenographs in nude mice. Taken together, these observations suggest that NAPA represents a target of cisplatin, and that knockdown of NAPA expression may improve cisplatin-based cancer therapy. A working hypothesis of ER damage and the related signal

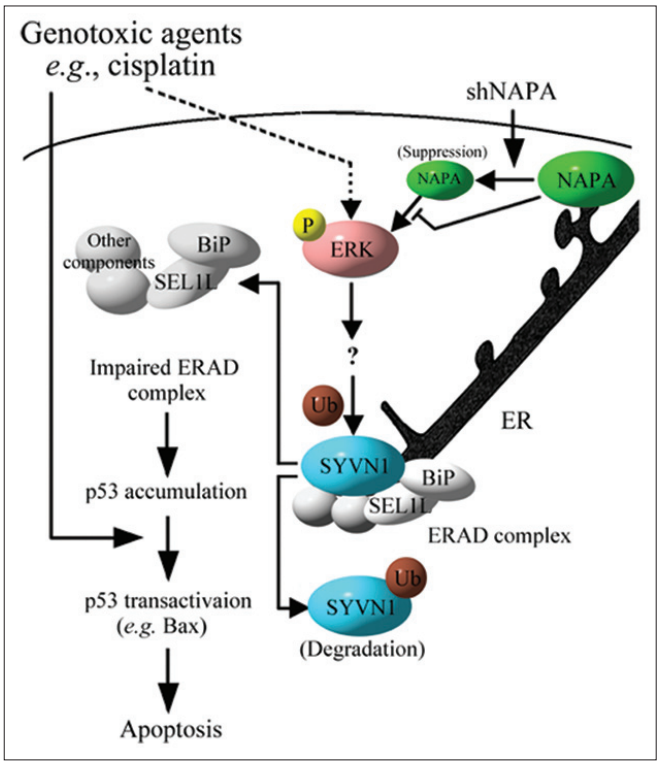

Figure 2: Working model of cisplatin stress and related signal pathways implicated in the regulation of SYVN1 degradation, impaired ERAD complex formation, accumulation of p53, and enhancement of cisplatin-induced apoptosis following NAPA knockdown. [Adapted from ref.[15]].

pathways implicated in cisplatin-induced apoptosis is shown in Figure 2. Severe ER stress induced by high concentration of cisplatin can induce the proapoptotic protein calpain which in turn activates caspase-3 via p53 transactivation of Bax in the nucleus. Among the CPR genes described here, NAPA is especially interesting since it provides a link with the tumor suppressor p53.[15] As a component of the ER-associated degradation (ERAD) complex, SYVN1 (the ER-resident ubiquitin ligase synoviolin) also targets p53 for ubiquitination and proteasomal degradation,[19,20] suggesting a possible crosstalk between ERAD and p53 in regulating apoptosis and ER stress. Together, these observations may be linked to our results that knockdown of NAPA resulted in a p53-dependent sensitization to cisplatin.[15]

We also observed that GS28, a Golgi-SNARE protein, forms a complex with p53 in HEK-293 cells.[21] Given that p53 represents a tumor suppressor that affects the sensitivity of cancer cells to various chemotherapeutic drugs, we examined whether GS28 may influence the level of sensitivity to cisplatin. Indeed, knockdown of GS28 using shRNA induced resistance to cisplatin in these cells. On the other hand, overexpression of GS28 resulted in cells sensitized to cisplatin, whereas no sensitization effect was observed in cells treated with the mitotic spindle-damaging drugs vincristine and taxol. Accordingly, we observed that knockdown of GS28 reduced the accumulation of p53 and its proapoptotic target Bax. Conversely, ectopic expression of GS28 induced the accumulation of p53 and Bax as well as the proapoptotic phosphorylation of p53 on Ser46. Further experiments showed that these cellular responses could be abrogated by the p53 inhibitor PFT-α, indicating that GS28 may affect the stability and activity of p53. However, the modulatory effects of GS28 on cisplatin sensitivity and p53 stability were absent in p53-null H1299 cells. As expected, ectopic expression of p53 in H1299 cells restored the modulatory effects of GS28 on sensitivity to cisplatin. Notably, GS28 was found to form a complex with MDM2 (murine double minute 2), an E3 ligase of p53, in H1299 cells. Furthermore, the ubiquitination of p53 was reduced in GS28-overexpressing cells, confirming that GS28 enhances the stability of the p53 protein.[21] Taken together, these results suggest that GS28 may potentiate cells to genotoxic agents-induced apoptosis by inhibiting the ubiquitination and degradation of p53 [Figure 3].

Epigenetic changes

Phenotypic diversity arises in tumors just as it does in developing organisms, and tumor recurrence frequently manifests due to the selective survival advantage of divergent drug-resistant cells. In addition to genetic mutations, researchers have found that epigenetic changes represent non-mutational mechanisms that are also involved in drug resistance. Unlike the genetic changes that occur in cancer cells, however, epigenetic modifications can occur quickly in response to environmental changes. For example, accumulating evidences suggest that a small population of the so-called "cancer stem cells" are intrinsically refractory to a variety of anticancer drugs, an observation possibly due to enhanced drug efflux.[22] Studies have shown that epigenetic modifications are involved in this process, suggesting that acquired drug resistance does not necessarily require stable heritable genetic alterations.[23]

Histone deacetylases play an important role in epigenetic regulation

Histone deacetylases (HDACs) play an important role in the epigenetic regulation of gene expression by catalyzing the removal of acetyl groups which stimulates chromatin condensation and represses transcription. Given that aberrant epigenetic changes are a hallmark of cancer, HDACs represent promising targets for pharmacological treatments. Inhibition of HDAC can induce cell cycle arrest, promote cell differentiation, and stimulate cell death in tumor cells. These properties have prompted numerous preclinical and clinical investigations to evaluate the efficacy of HDAC inhibitors against various malignancies.

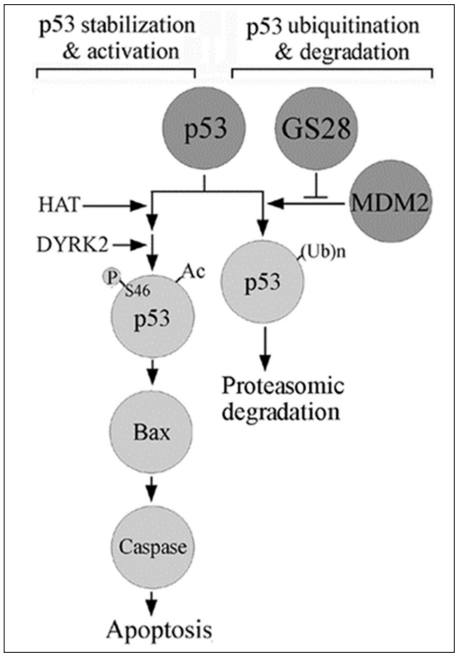

Figure 3: Working model of cisplatin-induced stress and related signal pathways implicated in the positive regulation of cisplatin-induced apoptosis by GS28. Under unstressed conditions, p53, which is usually rapidly degraded through MDM2-mediated ubiquitination, is blocked by GS28 (right part of the model). In response to cisplatin stress, p53 is modified to escape from degradation (such as being acetylated by HAT). When cells are exposed to cytotoxic concentration of cisplatin (causing severe DNA damage), p53 is additionally phosphorylated by DYRK2 at Ser46, for example, which strongly transactivates Bax and induces apoptotic cell death (left part of the model). The involvement of HAT and DYRK is based on previous studies. [Adapted from ref.[21]].

The observations that HDAC inhibitors show preferential toxicity toward transformed cells and that these drugs synergistically enhance the anticancer activity of many other chemotherapeutic agents have further generated interest for this novel drug class. The different mechanisms of apoptosis induced by HDAC inhibitors and the use of these drugs in combination with other anticancer agents have been extensively reviewed [Table 2].[24] A hallmark of HDAC inhibitors, for example, is their ability to induce p21 and subsequently to cause cell cycle arrest, primarily at the G1 phase. As shown in Figure 1, inhibition of HDAC activity also induces apoptosis via both the extrinsic (death receptor) and intrinsic (mitochondrial) pathway. The action of HDAC inhibitors is associated with increased expression of pro-apoptotic genes and decreased expression of anti-apoptotic genes, thus shifting the balance toward cell death. Due to this effect, HDAC inhibitors enhance the efficacy of many conventional proapoptotic anticancer agents. Furthermore, HDAC inhibitors have been reported to inhibit tumor angiogenesis and to induce autophagy. Another significant event associated with HDAC inhibitor-mediated cell death is the generation of reactive oxygen species (ROS) which appear to play an important role in activating the intrinsic apoptosis pathway.[24]

Emergence of drug-resistant cancer cells requires lysine (K)-specific demethylase 5A (KDM5A)

While modeling the acute response to various anticancer agents in drug-sensitive human tumor cell lines, we consistently detected a small population of reversibly "drug-tolerant" cells. These cells demonstrated a more than 100-fold reduction in drug sensitivity and maintained viability via engagement of insulin-like growth factor 1 receptor (IGF1R) signaling and via altered chromatin states that required the HDAC lysine-specific demethylase 5A (KDM5A/RBP2/Jarid1A). This drug-tolerant phenotype was transiently acquired at low frequency by individual cells within the population. Notably, the drug-tolerant cancer cell subpopulation could be selectively ablated by the treatment with IGF1R inhibitors or by chromatin-modifying agents. Together, these findings suggest that populations of cancer cells may employ dynamic survival strategies in which individual cells transiently assume a reversible drug-tolerant state to protect the population from eradication by potentially lethal drugs.[25,26]

Epigenetic modulation for overcoming resistance to anticancer hormonal therapy

It has been found that epigenetic changes are responsible for the resistance that many breast cancers acquire against the estrogen-blocking drug tamoxifen following 18 months of treatment. Surprisingly, while no mutations that correlated with resistance were detected in the treated breast cancer cells, resistant cancer cells expressed the survival gene Akt at much higher levels than susceptible cells. Apparently, cancer cells used histone tags, or chemical markers on chromatin, to expose the Akt gene and increase its transcription. The higher signal of Akt allowed cancer cells to stay alive even in the presence of tamoxifen and this by stimulating growth and proliferation and by preventing cell death.[27] Therefore, tumor cells may tag genes responsible for chemoresistance in order to pass them to the next generations, thus giving daughter cells an advantage in the presence of the drug.

Nuclear pore complex architecture regulates resistance to anticancer drugs independently of genetic mutations

Cancer cells may also use a trick from viruses in order to switch between resistance and susceptibility. Kohtz and colleagues explored the reasons why ovarian carcinomas sometimes become resistant to a drug and why cells become

Table 2: Characteristics of histone deacetylases (HDACs), their inhibitors and target genes

			Class I	**Localization**	**Selected Targets**
SAHA, TSA, LBH589, PXD101	Valproic Acid, Butyrate	MS-275 / Depsipeptide	HDAC1	Nucleus	Androgen Receptor
					P53
					MyoD
					E2F-1
					SHP
					Stat3
			HDAC2	Nucleus	Glucocorticoid Receptor
					Bcl-6
					YY-1
					Stat3
			HDAC3	Nucleus	RelA
					YY-1
					GATA-1
					SHP
					Stat3
			HDAC8	Nucleus	
			Class IIa		
			HDAC4	Nucleus/Cytoplasm	GATA-1
					GCMa
					HP-1
			HDAC5	Nucleus/Cytoplasm	Smad7
					GCMa
					HP-1
			HDAC7	Nucleus/Cytoplasm	Plag1/Plag2
			HDAC9	Nucleus/Cytoplasm	
		Tubacin	**Class IIb**		
			HDAC6	Mostly Cytoplasm	α-tubulin
					Hsp90
					SMP
					Smad7
			HDAC10	Mostly Cytoplasm	
			Class IV		
			HDAC11	Nucleus/Cytoplasm	
			Class III		
Nicotinamide	Cambinol	EX-527	Sirt1	Nucleus	P53
					FOXO
					NF-kB
					PGC-1a
			Sirt2	Cytoplasm	Histone H4
					α-tubulin
			Sirt3	Nucleus/Mitochondria	Acetyl-CoA synthetases
			Sirt4	Mitochondria	Glutamate dehydrogenase
			Sirt5	Mitochondria	
			Sirt6	Nucleus	DNA polymerase B
			Sirt7	Nucleus	RNA polymerase I

[adapted from ref.[24]]

susceptible again after the drug is no longer taken.[28] Such a change is unlikely to be caused by genetic mutations, because these are usually not reversed so quickly. Prior studies have shown that nuclear pores, which help transport proteins from the nucleus to the cytoplasm and vice versa, could influence gene expression by interacting with chromatin at the periphery of the nucleus. Specifically, nuclear pores can activate transcription by shielding DNA from repressors, or by hindering transcription because repressor proteins lurk in the region near the nuclear boundary, suggesting that nuclear pores might be altered in different cancer cell types. Using electron microscopy to visualize the nuclei of cisplatin-resistant cancer cells, researchers observed that nuclear pores looked abnormal: the pore complexes appeared to be hollow and disassembled and to contain a gatekeeper protein in the center, a finding similar to the pores affected by viruses that co-opt cellular transport machinery for their own purposes. Partial knockdown of nucleoporin p62 (NUP62) by small-interfering RNA (siRNA) conferred resistance to cisplatin in cultured high-grade ovarian carcinoma cells. Treatments with NUP62 siRNA and cisplatin left resistant cells in a state of dormancy and some dormant cells could be induced to proliferate by transient induction of NUP62 expression from an ectopic expression construct.[28] *In vivo*, the dormant cells may be located at metastatic sites or even at the site of the original tumor until a factor induces their malignancy and makes them start growing again. These results suggest that functional links exist between nuclear pore complex architecture, chromatin regulation, and cancer cell survival.

We also found that the anti-apoptotic function of CITED2 during cisplatin treatment is also p53-dependent.[14] A recent study showed that knockdown of CITED2 using siRNA in Pt complex-resistant ovarian cancer cells improved the sensitivity of these cells to Pt compounds.[29] DNA damage-induced phosphorylation of p53 enhances the association of this protein with the CBP/p300 transcriptional coactivators which results in increased acetylation and stability of p53.[30-32] Yet, these gene products are unlikely to interact in cancer cells since NAPA is found in the ER[33] whereas CITED2 transcriptional regulatory proteins are found in the nucleus.[34] Furthermore, CITED2 modifies acetyltransferase like p300 that may modulates chromatin proteins, leading to chemoresistance-associated gene expression in a epigenetic manner. In this case, CITED2, like NAPA, may represent a target of cisplatin and may regulate drug sensitivity by influencing the stability of p53. This model could represent a new molecular mechanism to explain cisplatin resistance in cancer cells.

Alternative splicing

Yet another way that cancer cells use to acquire drug resistance is associated with protein processing. In normal cells, signal-activated RAS recruits BRAF (or v-Raf murine sarcoma viral oncogene homolog B1) to the cell membrane and activates its kinase domain through dimerization. Activated BRAF, in turn, triggers MEK and ERK protein kinases, through phosphorylation, and promotes cell proliferation and survival. In contrast, the mutant BRAFV600E continuously sends signals to MEK and ERK even in the absence of activation by RAS. Notably, BRAFV600E is highly sensitive to inhibition by vemurafenib. Between 40 and 80% of melanoma patients have a mutated BRAF (BRAFV600E) which turns on cellular growth and division signaling pathways. Vemurafenib exploits the fact that BRAF proteins in healthy cells pair up with other BRAF proteins to form a multiprotein complex, while the mutated BRAF protein acts as a single compound. This single structure can be hundreds of times more effective in activating cell division than the normal paired BRAF complexes. Vemurafenib targets tumor cells by only inhibiting the stand-alone mutant version, while allowing the twinned version in healthy cells to act unimpaired [Figure 4]. Unfortunately, many patients develop resistance to vemurafenib within 18 months, and their tumors progress. Some of the resistant tumor cells generate a variant form of BRAF that is shorter. However, the shorter BRAF protein is not due to a mutation in the protein-coding region of DNA. Instead, deletions of exons in the gene lead to alternative splicing that generate the shorter version, which can bind to itself, rendering the protein undetectable under

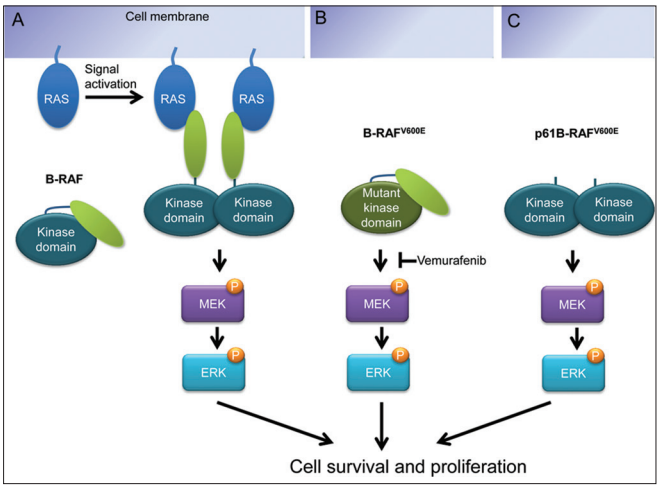

Figure 4: The two faces of oncogenic BRAF. A, In normal cells, signal-activated RAS recruits BRAF to the cell membrane and activates its kinase domain through dimerization. Active BRAF, in turn, triggers MEK and ERK protein kinases, through phosphorylation (denoted with the letter P), to promote cell proliferation and survival. B, Mutant BRAFV600E continuously sends signals to MEK and ERK, even in the absence of activation by RAS. BRAFV600E is highly sensitive to the anticancer drug vemurafenib. Poulikakos et al. reported that BRAFV600E essentially works as a monomer. C, The authors also show that p61BRAFV600E—the truncated variant of BRAFV600E has an increased propensity to form dimers and that this is associated with resistance to vemurafenib. [Adapted from ref.[36]].

vemurafenib treatment, processes which appear to account for vemurafenib resistance.[35]

These findings are fascinating from a mechanistic standpoint, but we may ask whether they are clinically relevant. Solit and colleagues reported that six of 19 samples from patients with drug-resistant melanoma expressed BRAFV600E truncated variants similar to p61BRAFV600E. However, this sample number was too small to assess the prevalence of p61B-RAFV600E in the human population. Nonetheless, the finding is still impressive since it suggests that enhanced BRAFV600E dimerization may significantly contribute to vemurafenib resistance in patients with melanoma. In addition to the need to analyze larger sample sizes, these observations should be corroborated in animal models. This study and others indicate that any event promoting RAF dimerization may lead to resistance to vemurafenib and possibly to other similar RAF inhibitors.[35] How can we get around this problem? Co-administering RAF and MEK inhibitors could be a solution. Indeed, as p61B-RAFV600E-expressing cells have been found to remain sensitive to a MEK inhibitor, shutting down both RAF and MEK activity might prevent the growth of resistant cells.[35]

Conclusions

While cancer cells use an intimidating array of tactics to evade drug therapies, researchers are developing ways to target resistant cells. Given that the mechanisms underlying chemoresistance in cancer therapy are getting clear, targeted therapy or combination therapy can be established in laboratory models and clinical settings. Although the expanding tumor cell population may be successfully targeted, drug-resistant cells may persist and sustain the tumor or enter in a state of dormancy before igniting a future relapse. Researchers are now making more progress into understanding how cancer cells acquire drug resistance, and they are finding that genetic mutations are just one of many strategies cancers use to evade death. In this mini-review, we have presented new mechanisms of anticancer resistance, revealing additional changes in cancer receiving therapy. The findings in this selected literature convincingly explain the loss of several powerful target therapies. In human trials, adding a compound that removes histone tags to tamoxifen regimen can make resistant breast cancers sensitive to the drug again. Similarly, ongoing Phase II trials are testing a combination of vemurafenib and another drug that inhibits a compound in the cellular division pathway in melanoma patients that are resistant to vemurafenib. And for those cancers that have switched between quiescence and active growth, simply treating the patients again with the same medicine later on can sometimes be effective. While there are general principles that apply to cancer resistance, current treatments still require a tailored approach that uses frequent biopsies of tumors to see the nature of the genetic and epigenetic mutations that they have acquired. Unfortunately, with more thorough genetic sequencing, it is also becoming clear that there is no single answer, even for a single patient. Instead, combination approaches usually provide the best results.

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