Multiple paths to a drug resistance phenotype: Mutations, translocations, deletions and amplification of coding genes or promoter regions, epigenetic changes and microRNAs

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Abstract

The remarkable responses observed with imatinib (Gleevec®) in the therapy of CML led many scientists to think that drug resistance, long recognized as a problem with “cytotoxic” agents, would soon become a thing of the past. But then reality set in. We learned that imatinib, a wonderful drug by any measure, was also susceptible to the development of resistance, as was gefitinib (Iressa®), and then erlotinib (Tarceva®). This evidence on resistance to “novel agents” together with new data on the complexity of cancer, the rapidly evolving story of microRNAs and their diverse roles, as well as evidence of the importance of epigenetic changes have allowed us to refine our models of drug resistance and how cells acquire these phenotypes. In this overview I will look at examples of how drug resistance develops including older and more recent data on the role of mutations, translocations, deletions, and amplification. The role of epigenetic changes and microRNAs will be discussed, as examples of different mechanisms by which a cell achieves the same end. Recurrent themes that have emerged will be underscored as we seek to understand how drug resistance occurs.

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1. Mutations, tumorigenesis and drug resistance

As targeted therapies missed their mark and fell short of expectations, the difficulty of treating refractory solid tumors was yet again appreciated. Reality hardened as we discovered that even our successes in very refractory cancers prolonged survival for only a few months (sorafenib and sunitinib in renal cell carcinoma) or were substantially active in only a small subset of patients (gefitinib and erlotinib in non-small cell lung cancer). The complexity of the problem and the notion that a drug targeting a single pathway would succeed in eradicating even a majority of solid tumors were concretely addressed when a group of 29 investigators from several institutions published in Science an article

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This paper is the second part of a Debate on genetic and epigenetic causes of drug resistance in cancer.
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harbored an average of 12 (range 4 to 23) mutant CAN-genes while the average number of CAN-genes in colorectal cancers was 9 (range 3 to 18). Even more remarkable, each cancer specimen carried its own distinct CAN-gene mutational signature and no cancer had more than six mutant CAN-genes in common with any other cancer. The authors correctly noted that the number of mutational events is much larger than previously thought. Furthermore, the mutation spectrum was markedly different between breast and colon cancers with all the differences highly significant ($P < 0.0001$). Considering the fact that only point mutations were characterized, and at that, in only about a quarter of all genes, these findings confirmed the complexity of solid tumors.

The authors wrote that “the vast majority of these genes were not known to be genetically altered in tumors and are predicted to affect a wide range of cellular functions, including transcription, adhesion and invasion”. To that we should add drug sensitivity—maybe not a cellular function, but certainly a cellular property. After all why should we believe that the mutations acquired during a tumor’s lifetime affect its biology but not its drug sensitivity? Clinicians have long recognized that “bad cancers” not only behave aggressively but also are invariably drug-resistant. In many cases, these two characteristics are inseparable. Indeed, many of the genes found mutated in the study referred to above have been implicated to affect drug sensitivity. And the recent demonstration of mutations as modulators of sensitivity to imatinib, gefitinib and erlotinib have reinforced for us that drug sensitivity can indeed be modulated by mutations—present before treatment or selected during the course of therapy (Cools et al., 2005).

Just a few years ago, references to mutations — usually point mutations — as a cause of drug resistance, meant mutations in genes that for lack of a better term might be said to have global effects on cellular functions. Examples of such genes include p53 and KRAS among others. Both earlier studies as well as more recent investigations provided evidence both in vitro as well as in patients that mutations in these genes could affect drug sensitivity — usually by rendering cells more drug-resistant. For example, in ovarian cancer the p53 mutational status has been shown to be an important determinant of responsiveness to platinum-based chemotherapy, and a strong prognosticator for recurrence-free and overall survival (Concin et al., 2005; Irwin, 2004; Richardson and Kaye, 2005). While in breast cancer treated with an anthracycline, sequence analysis has shown that in most cases the presence of p53 mutations leads to a reduced response rate to chemotherapy (Kandioler-Eckersberger et al., 2000; Anelli et al., 2003). Similarly, K-ras mutations have been shown to confer resistance to therapy in non small cell lung cancer (NSCLC) as well as in metastatic colorectal cancer. In the latter K-ras mutations have an adverse effect on response to the anti-epidermal growth factor receptor (anti-EGFR) antibody, cetuximab, such that the overall survival of patients without K-ras mutation in their tumor is significantly higher compared with those patients with a mutated tumor K-ras (Rosell et al., 2006; Taron et al., 2005; Aviel-Ronen et al., 2006; Lievre et al., 2006). But surprisingly despite the enormous research effort devoted to the analysis of these as well as other proteins, few clinical trials have been able to demonstrate correlations between drug sensitivity and mutations in these genes. Some of this lack of substantiation might be explained by the absence of adequate studies, and by the fact that most current therapies involve combination regimens and not all drugs in a given regimen might have their sensitivity modified by a given protein — for example, microtubule-targeting agents such as paclitaxel are generally regarded to be indifferent to the status of p53. But this lack of clinical substantiation might also be explained in part by the fact that, as the mutational analysis of the breast and colorectal cancers shows, tumors accrue a host of mutations in the course of their development, probably because no single mutation can initiate nor sustain the malignant phenotype — so why should we expect mutations in a single protein to explain drug resistance? So that while robust in vitro data might implicate a given protein as conferring drug resistance, clinical evidence might be lacking or not convincing — in vitro studies that isolate a given protein for analysis might be more informative since they can demonstrate the effect of a single protein, even in a very heterogeneous background.

As our agents have been honed to target specific sites in a protein, a different spectrum of mutations — “acquired” and target specific — have emerged as modulators of drug sensitivity. The most widely documented example has been that of resistance to imatinib. Beginning with a report of “acquired mutations” in patients with imatinib-refractory CML, dozens of studies have now identified over seventy mutations in the BCR-ABL kinase that is the target for imatinib (Gorre et al., 2001; Roche-Lestienne et al., 2002; Shah et al., 2002). Most of the mutations appear to affect the binding of imatinib, either by directly interfering with drug binding to the kinase domain or by affecting the P-loop or the activation loop. An increasing amount of data indicates that the mutations while “acquired” actually pre-exist in the population and are selected during the course of treatment. That this would occur as the mechanism of resistance, especially in patients presenting with the accelerated or blast crisis phases of disease is not surprising, given the large number of cells present at the start of therapy, and the fact that imatinib is not mutagenic, and would thus be unlikely to lead to the acquisition of the mutations. Mutations have also been found in the EGFR. In NSCLC for example, mutations that confer either sensitivity or resistance to gefitinib and erlotinib have been identified (Haber et al., 2005). “Activating mutations” in the EGFR have been shown to render tumors very sensitive to EGFR tyrosine kinase inhibitors so that patients whose tumors harbor these mutations — usually non-smoking oriental women, presenting with metastatic lung adenocarcinomas — have an increased time to progression and survival when treated with EGFR tyrosine kinase inhibitor (Rosell et al., 2006; Taron et al., 2005; Morgillo and Lee, 2005; Aviel-Ronen et al., 2006). And as with imatinib, “acquired mutations” have also been identified in patients whose tumors
become refractory after an initial response (Liu et al., 2006). A recent study looked for “secondary mutations” in patients with NSCLC whose tumors harbored an activating mutation in the epidermal growth factor receptor (EGFR). While the activating mutations rendered these tumors very sensitive to gefitinib, most patients eventually experienced disease progression while still on treatment. In seven of the 14 patients this “acquired resistance” was attributable to a secondary mutation resulting in threonine to methionine transition at codon 790 (T790M) of the EGFR – a mutation that was not found in pretreatment samples from 52 patients including five of the seven tumors with the “acquired mutations” (Kosaka et al., 2006).

While most studies with “targeted therapies” have focused on mutations in the target, it is becoming increasingly clear that these will account for only a fraction of drug resistant cases, and that we will find that mutations in “old targets” also confer resistance to our “targeted therapies”. In the case of imatinib, for example, recent data indicates that inactivation of p53, a frequent occurrence with disease progression in CML, inhibits the in vitro and in vivo response to imatinib without preventing BCR-ABL kinase inhibition (Wendel et al., 2006). P53 is selectively activated by imatinib in BCR-ABL-expressing cells as a result of BCR-ABL kinase inhibition. Concordantly, p53 mutations are associated with progression to imatinib resistance in some human CMLs.

Finally we should note that how often we tabulate that mutations affect drug sensitivity depends to some extent on how one defines “mutations”. The number of examples where mutations affect drug sensitivity is even greater if we consider polymorphisms and protein isoforms as mutations. For example in squamous cancers of the head and neck, a polymorphism encoding either arginine (72R) or proline (72P) at codon 72 of p53 influences the inhibition of the closely related p53 family member, p73, by a variety of p53 mutants. In turn, this polymorphism influences the clinical response to cisplatin-based chemoradiotherapy such that cancers expressing 72R mutants have poorer response rates and shorter survival than those expressing 72P mutants (Bergamaschi et al., 2003; Soussi, 2003). In the case of NSCLC and ovarian cancer on the other hand, low levels of class III beta-tubulin are associated with a better response rate, longer progression-free survival, and improved overall survival when regimens containing paclitaxel are employed (Seve et al., 2005; Ferrandina et al., 2006).

Thus the evidence is unequivocal that mutations – in most cases point mutations – can confer drug resistance. However, with few exceptions such as some mutant p53 proteins that are found at higher levels as a result of reduced mdm-2 transactivation, most mutations do not markedly affect the levels of the protein harboring the mutation. Cells usually rely on other mechanisms to accomplish this, including gene amplification and chromosomal translocations as well as altered expression of microRNAs and epigenetic changes. Higher levels of expression in turn confer resistance.

2. Gene amplification and drug resistance

Gene amplification was one of the earliest mechanisms of resistance to be studied. Its importance was first suggested by insightful observations that recognized gene amplification as the explanation for homogeneously staining regions (HSRs) and by elegant pioneering work describing amplification of the dihydrofolate reductase (DHFR) gene in methotrexate resistant cells (Biedler and Spengler, 1976; Schimke et al., 1978). Other examples of gene amplification and drug resistance followed, including in vitro studies that found amplification of the MDR-1/P-glycoprotein gene in numerous multidrug resistant cell lines (Roninson et al., 1984; Fojo et al., 1985; Van der Bliek et al., 1986). But even as evidence from the basic sciences was accumulating, investigators were looking to patient samples for evidence this might be important. Clinically, an early example of gene amplification as a mechanism of resistance was described in a patient receiving methotrexate therapy found to have increased copy number of the dihydrofolate reductase gene, although this could have been simply a “gene dosage” effect secondary to aneuploidy (Curt et al., 1983); while in a patient with acute myeloid leukemia (AML) resistance to methotrexate was ascribed to gene amplification and overproduction of DHFR (Carman et al., 1984). Other clinical examples followed including a correlation of gene copy with drug sensitivity that emerged as investigators attempted to better identify women with breast cancer whose tumors might respond to the HER2 targeting agent, Herceptin. Originally a correlation was reported between expression of the HER2 protein as determined by immunohistochemistry and Herceptin sensitivity. However, over time, this was refined and correlations were observed between drug sensitivity and the extent of gene amplification (Carlson et al., 2006). So now there is clear evidence of the importance of the copy number in predicting Herceptin sensitivity. Indeed one might argue this was the original “addiction model”. More recently, turning again to the experience with imatinib, gene amplification leading to over-expression of the BCR-ABL protein as a mechanism of acquired resistance has been described, albeit not as frequently as point mutations (Campbell et al., 2002). In colon cancer amplification of the EGFR gene, although not as frequent as initially reported, is also associated with response to the anti-epidermal growth factor receptor (anti-EGFR) antibody, cetuximab (Lievre et al., 2006). And as is the case with point mutations, some of the genes amplified in the course of tumorigenesis may also be important in mediating drug resistance. For example, in the case of neuroblastoma, amplification of N-myc has been correlated with poor outcomes possibly through modulation of the multidrug-resistance gene MRP-1 (Norris et al., 1996; Schleiermacher et al., 2003). Finally in the case of imatinib, and for that matter tumorigenesis in general, point mutations occur more frequently than amplification. This likely reflects the fact that it is more difficult for a cell to acquire and sustain an amplified gene than a point mutation.
3. Gene rearrangements and drug resistance

Gene amplification is by no means the only mechanism used by cancer cells to increase the expression of genes. Rearrangements that lead to the over-expression of critical proteins have been recognized as a mechanism for cellular adaptation and drug resistance for more than two decades. For example, in B-cell lymphomas, non-random translocations in Burkitt’s lymphoma cells were found to consistently juxtapose the c-myc proto-oncogene downstream of a transcriptionally active immunoglobulin gene locus with resultant myc gene deregulation; while in other B-cell tumors, translocations brought BCL-2, at the time a gene of unknown function, into a similar association with the immunoglobulin heavy-chain locus (Pegoraro et al., 1984; Tsujimoto et al., 1984; Tsujimoto et al., 1985; Nowell and Croce, 1990).

Over time it became apparent that the over-expression of Bcl-2 was not only important in tumorigenesis, but also in the drug resistant phenotype so often observed with indolent lymphomas (Miyashita and Reed, 1992; Chao and Korsmeyer, 1998; Cory and Adams, 2002; Mashima and Tsuruo, 2005). And while in a majority of cases, the rearrangements that confer resistance can be classified as endogenous, that is, existing before the start drug therapy, the availability of increasingly sensitive methodologies is allowing us to discriminate between endogenous rearrangements and those acquired after drug exposure. With cytotoxic agents, acquired changes have been found even with agents not generally regarded as mutagenic demonstrating the adaptability of cancer cells. Examples of this include rearrangements involving the MDR-1 gene both in cell culture models and in patients with drug refractory leukemia (Mickley et al., 1997; Huff et al., 2006).

And while BCL-2 and MDR-1 can be regarded as drug resistance genes there is evidence – as noted for point mutations and amplification – that rearrangement of genes, regarded as involved in tumorigenesis or as having more global consequences, can impact drug sensitivity. Thus for example, in childhood acute leukemia drug sensitivity is correlated to the type of rearrangement and the “partner genes” that might be activated as a result of the rearrangement as well as cell lineage (Ramakers-van Woerden et al., 2004; Palle et al., 2005). While the “partner genes” activated by the rearrangements may not directly affect drug sensitivity, it is expected that further research will identify genes whose expression is modulated and that directly affect drug sensitivity. This will clarify how the “partner gene” involved in the translocation indirectly affects drug sensitivity and validate the oncologist’s heuristic – that biologically “bad cancers” are also drug resistant. Evidence for such an indirect effect has already been presented in acute lymphoblastic and myeloblastic leukemias with ALL-1 rearrangements where over-expression of several genes has been found (Rozovskaia et al., 2003). These include (i) CD44, a gene associated with aggressive B-CLL that has been reported to confer resistance to several drugs; (ii) dihydrofolate reductase (DHFR), which as noted above confers resistance to methotrexate; (iii) bleomycin hydrolase (BLMH); and (iv) catalase (CAT), which protects from oxidative stress.

4. Epigenetic changes and drug resistance

Conclusive evidence now exists that aberrant methylation of CpG islands located at or near gene promoters is a mechanism whereby cells can modulate gene expression (Glasspool et al., 2006). While mutations occur with high frequency in cancer, it appears that epigenetic changes such as methylation can occur at an even much higher frequency and have an impact on tumorigenesis as well as drug sensitivity. Advocates of aberrant methylation as a mechanism to alter drug sensitivity argue that while mutation/amplification/rearrangement of a single gene may have an impact, aberrant methylation can simultaneously alter the expression of a large number of genes and that this may have a greater impact on chemosensitivity. According to this view, non-random aberrant CpG island methylation provides a mechanism to affect the expression of multiple genes simultaneously resulting in “polygenic drug resistance” (Glasspool et al., 2006). Changes in methylation of CpG islands and epigenetic regulation following drug selection have been reported in multiple models (Wei et al., 2003). Indeed, as with other “genetic changes” described herein, epigenetic changes modulate drug sensitivity overall, resulting in both increased and decreased drug sensitivity. For example methylation with epigenetic silencing of pro-apoptotic genes (hMLH1 and APAF1) may lead to resistance with inactivation of DNA repair, as is thought to occur following methylation of DNA repair genes (MGMT and FANCF), may result in greater chemosensitivity (Esteller et al., 2000; Soengas et al., 2001; Taniguchi et al., 2003; Teodoridis et al., 2004). At the present time this field remains a “work in progress” that will no doubt elucidate mechanisms of drug resistance. It also remains to be seen whether it affects drug resistance through a different set of genes than those that are “genetically altered”.

5. A potential role for microRNAs (miRNAs) in drug resistance

If one examines the reports describing point mutations, gene amplification and rearrangements as a mechanism of drug resistance, one finds that the genes implicated to confer drug tolerance are often the same or similar. This is the one silver lining in the problem of drug resistance – the number of putative genes appears to be finite – and they are identified as the culprits again and again. Thus it appears that different cells achieve the same end result by modulating a finite number of genes using different mechanisms, a concept underscored by the identification of microRNAs as modulators of drug expression. As noted above, the B cell
lymphoma 2 gene (BCL2), identified originally in patients with follicular lymphomas, was subsequently recognized to have a central role in cell death and survival (Miyashita and Reed, 1992). In subsequent studies, Bcl2 was advocated as an important mediator of drug resistance by favoring survival and inhibiting cell death (Chao and Korsmeyer, 1998; Cory and Adams, 2002). While the ability of Bcl-2 over-expression alone to confer clinical resistance has never been fully proven, numerous studies have reported a correlation between Bcl-2 expression and drug resistance, indicating that at a minimum Bcl-2 expression contributes to the drug resistance phenotype (Wilson et al., 1997). Bcl2 over-expression has been reported in many types of human cancers, including leukemias, lymphomas, and carcinomas (Sanchez-Beato et al., 2003). As noted above, in follicular lymphomas where the Bcl-2 oncoprotein was originally identified and in a small fraction of diffuse B-cell lymphomas the mechanism of BCL2 activation has been shown to involve a translocation of chromosomes 14 and 18 (t(14,18)(q32;q21). This translocation places the BCL2 gene under the control of immunoglobulin (Ig) heavy chain enhancers, resulting in deregulated over-expression (Pegoraro et al., 1984; Tsujimoto et al., 1984; Tsujimoto et al., 1985; Nowell and Croce, 1990). In this case the mechanism responsible for the over-expression is clearly double stranded DNA damage and the subsequent rearrangement. However, in B cell chronic lymphocytic leukemia (CLL) where the malignant, mostly non-dividing, B cells over-express Bcl-2, the BCL-2 gene is juxtaposed to Ig loci in ≤5% of cases and until the recent identification of microRNAs, the mechanism(s) responsible for BCL2 deregulation in B-cell CLL had eluded scientists (Adachi et al., 1990; Kitada et al., 1998).

MicroRNAs (miRNAs) have emerged as a class of genes involved in human tumorigenesis and will likely be identified as mediators of drug resistance – indeed, it can be argued that they already have (McManus, 2003; Gregory and Shiekhattar, 2005). As the name implies, miRNAs are small RNAs usually 19 to 23 bp in length or shorter, that are produced in all mammalian cells (O’Driscoll, 2006; Calin and Croce, 2006). Lacking the ability to encode a protein, these single-stranded miRNAs bind mainly to the 3’ UTR of protein encoding mRNAs through sequences that are imperfectly complementary. The consequences of miRNA binding are that either the bound mRNA is silenced or degraded, resulting in reduced levels of the protein encoded by the mRNA (Bartel, 2004; Lim et al., 2005). Recent evidence has shown that two miRNAs, miR-15a and miR-16-1 are deleted or down-regulated in the majority of CLLs and that miR-15a and miR-16-1 expression is inversely correlated to Bcl-2 expression in CLL (Calin et al., 2002; Calin et al., 2004; Cimmino et al., 2005). This important finding established a mechanism whereby the expression of Bcl-2 could be modulated in B-cell CLL and provided a molecular mechanism for its over-expression. However, the evidence indicates that while the levels of Bcl-2 can be modulated by miRNAs, again the molecular mechanisms appear to be diverse. For example, deletions and translocations leading to down-regulation of two miRNAs, miR-15a and miR-16-1, located in a cluster at 13q14.3, has been reported in approximately 65% of B cell CLL patients (Calin et al., 2002). While a germ-line mutation in the 3’ end of miR-16-1 has been reported to cause low levels of mRNA expression in vitro and in vivo and has also been associated with deletion of the normal allele (Calin et al., 2006).

Although clear evidence that miRNAs have a role in drug resistance has yet to be provided, the plethora of mechanisms whereby Bcl-2 levels can be modulated by miRNAs, together with the fact that Bcl-2 is expressed in many tumors, and the evidence that Bcl-2 expression has been shown to correlate with drug resistance means that evidence implicating a role for miRNAs in drug resistance will soon emerge. A recent study suggests that the micro-RNA gene miR125b-1 may be the target of chromosome 11q deletion and relates to increased anthracycline sensitivity of breast cancer (Climent et al., 2007). One can predict that other examples will follow.

6. Conclusion

The micro-RNA-mediated form of drug resistance adds yet another mechanism of drug resistance; yet one could argue that the molecular basis will be similar to those for which we have evidence: mutations, deletions, translocations and amplification. In a way, this will reinforce the thesis that, while diverse mechanisms of drug resistance exist, the molecular basis of these is similar and involves at some level genetic or epigenetic changes that recur over and again, differing only in their manifestations.

The recent data describing the incidence of mutations in breast and colon cancers, the prevalence of rearrangements, the evidence that epigenetic changes occur commonly and the emergence of microRNAs as a mechanism of resistance – and at that one not evaluated in the mutational analysis – provide ample mechanisms by which a cell can acquire a drug resistance phenotype (Table 1). Regardless whether the resistance is mediated by a gene that has global effects on cellular functions such as p53, by a drug transporter or by one of the glutathione transferases, it is clear that a genetic or epigenetic change affecting only one gene can confer a multidrug resistance phenotype whose complexity can be further augmented by similar changes involving other genes. The evidence indicates that such genetic and epigenetic changes can exist even before initial therapy and can contribute to intrinsic resistance or can be acquired as a result of therapy and manifest as an acquired phenotype. Hence, while aneuploidy might also generate progeny tolerant to multiple drugs (Duesberg et al., this issue), the evidence indicates that point mutations, deletions, translocations, amplification, epigenetic changes and altered microRNAs levels can accomplish this as well and most likely in a simpler more direct manner.
Table 1
Genetic and Epigenetic Causes of Drug Resistance

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<th>Preclinical data</th>
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<td><strong>Mutations</strong></td>
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<td>Mutations in p53 confer resistance to most cytotoxic agents. NCI Anticancer Drug Screen: cells with MT p53 less sensitive to cytotoxic agents with exception of microtubule-targeting agents</td>
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<td>Ovarian cancer: p53 status important determinant of responsiveness to platinum chemotherapy, and strong prognosticator for recurrence-free and overall survival – WT better than MT</td>
<td>Concin et al., 2005; Irwin, 2004; Richardson and Kaye, 2005</td>
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<td>Activating k-ras mutations specifically detected in gefitinib-resistant cells, suggesting a correlation with resistance to EGFR antagonists</td>
<td>Janmaat et al., 2006</td>
<td>Breast cancer: In most cases p53 mutations lead to reduced response rate to chemotherapy</td>
<td>Kandioler-Eckersberger et al., 2000; Anelli et al., 2003</td>
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<td>N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen identified almost exclusively BCR-ABL kinase domain mutants although ENU is expected to induce mutations in multiple proteins</td>
<td>Bradeen et al., 2006</td>
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<td>Lievre et al., 2006</td>
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<td>P53 polymorphism encoding arginine (72R) or proline (72P) influences inhibition of family member, p73, by p53 and in turn drug sensitivity</td>
<td>Bergamaschi et al., 2003</td>
<td>Head and neck cancer: 72R/72P polymorphism of p53 influences clinical response to cisplatin-based chemoradiotherapy Cancers expressing 72R mutants have poorer response rates and shorter survival</td>
<td>Bergamaschi et al., 2003; Soussi, 2003</td>
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<td>Treatment with antisense oligonucleotides to class III beta-tubulin sensitized drug-resistant cells to Taxol</td>
<td>Kavallaris et al., 1999</td>
<td>NSCLC and ovarian cancer: Low levels of class III beta-tubulin are associated with improved response rate, progression-free survival, and overall survival when paclitaxel-containing regimens are used</td>
<td>Seve et al., 2005; Ferrandina et al., 2006</td>
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<td><strong>Gene Amplification</strong></td>
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<td>Homogeneously staining regions in drug resistant cells; and amplification of dihydrofolate reductase (DHFR) gene in methotrexate resistant cells correlated with resistance</td>
<td>Birdler and Spengler, 1976; Schimke et al., 1978</td>
<td>Increased copy number of dihydrofolate reductase (DHFR) gene in patients receiving methotrexate</td>
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<td>Breast cancer: Correlation of HER2 gene copy with sensitivity to herceptin</td>
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<td>Up-regulation of the Bcr-Abl protein associated with amplification of the BCR-ABL gene and imatinib resistance</td>
<td>Mahon et al., 2000</td>
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<td>In non-small cell carcinoma (HNSCC) cell lines without “activating EGFR mutations” a trend suggesting association between EGFR gene copy number and drug sensitivity was observed for both gefitinib (P = 0.0498) and cetuximab (P = 0.053)</td>
<td>Erjala et al., 2006</td>
<td>Colon cancer: Amplification of the EGFR gene is associated with response to the anti-epidermal growth factor receptor (anti-EGFR) antibody, cetuximab</td>
<td>Lievre et al., 2006</td>
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<td>Preclinical data</td>
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<td>Data suggesting a common mechanism controls N-myc expression irrespective of copy number, and hence in cell lines with N-myc amplification all copies contribute to expression. In 6/8 neuroblastomas (NB) data consistent with N-myc in circular extra-chromosomal DNA amplification structures: 5/6, double minute chromosomes, 1/6 smaller DNA circles. 2/8 NB with larger (presumably chromosomal) amplification structures</td>
<td>Lutz and Schwab, 1997; VanDevanter et al., 1990</td>
<td>Neuroblastoma: Amplification of N-myc correlated with poor outcomes in neuroblastoma</td>
<td>Norris et al., 1996; Schleiermacher et al., 2003</td>
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### Gene Rearrangements

| Rearrangements involving the MDR-1 gene in cell culture models | Mickley et al., 1997; Huff et al., 2006 | Rearrangements involving the MDR-1 gene in patients with drug refractory leukemia | Mickley et al., 1997; Huff et al., 2006 |

### Epigenetic changes

| Epigenetic silencing of pro-apoptotic genes (hMLH1 and APAF1) results in resistance | Soengas et al., 2001 |
| Methylation of DNA-repair genes (MGMT and FANCF) result in chemosensitivity | Taniguchi et al., 2003; Esteller et al., 2000 |
| Human Gliomas: Methylation of the promoter of the DNA-repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) is a predictor of responsiveness to alkylating agents | Esteller et al., 2000 |

### MicroRNAs

| BCL2 down-regulation by miR-15 and miR-16 has an important functional consequence: the activation of the intrinsic apoptosis pathway. Drug-induced apoptosis in B-CLL cells inversely related to Bcl-2/Bax ratios | Cimmino et al., 2005; Pepper et al., 1997 |
| A cluster of miRNA, including miR-320, miR-200b, miR-21, miR-23a, miR-141, miR-27a, and miR-34a, were expressed in all cell lines. MiR-21, miR-141, and miR-200b were highly over-expressed in malignant cholangiocytes. Inhibition of miR-21 and miR-200b increased sensitivity to gemcitabine | Meng et al., 2006 |
| B cell chronic lymphocytic leukemia (CLL): Expression of two miRNAs, miR-15a and miR-16-1, that are deleted or down-regulated in the majority of CLLs is inversely correlated to Bcl-2 expression | Adachi et al., 1990; Kitada et al., 1998; Calin et al., 2002; Calin et al., 2004 |

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