Chapter 1

Cancer Drug Resistance: A Brief Overview from a Genetic Viewpoint

José Rueff and António Sebastião Rodrigues

Abstract

Cancer drug resistance leading to therapeutic failure in the treatment of many cancers encompasses various mechanisms and may be intrinsic relying on the patient's genetic makeup or be acquired by tumors that are initially sensitive to cancer drugs. All in all, it may be responsible for treatment failure in over 90 % of patients with metastatic cancer. Cancer drug resistance, in particular acquired resistance, may stem from the micro-clonality/micro-genetic heterogeneity of the tumors whereby, among others, the following mechanisms may entail resistance: altered expression of drug influx/efflux transporters in the tumor cells mediating lower drug uptake and/or greater efflux of the drug; altered role of DNA repair and impairment of apoptosis; role of epigenomics/epistasis by methylation, acetylation, and altered levels of microRNAs leading to alterations in upstream or downstream effectors; mutation of drug targets in targeted therapy and alterations in the cell cycle and checkpoints; and tumor microenvironment that are briefly reviewed.

Key words Intrinsic resistance and pharmacogenetics, Acquired resistance and tumor micro-heterogeneity, Acquired resistance and adaptive compensatory pathways, Uptake and efflux transporters in resistance, DNA repair and resistance, Epigenomics and resistance, Tumor microenvironment and resistance

1 Innate or Intrinsic and Acquired Resistance: Definitions and Mechanisms

Cancer drug resistance classically either stems from host factors (innate or intrinsic resistance) or is an acquired resistance of the tumor cells by means of genetic or epigenetic alterations in the cancer cells [1]. Another way of defining the types of cancer drug resistance is to consider the pharmacokinetic-based resistance and the cell-dependent resistance (for a review *see* ref. 2). For the sake of straightforwardness and from a genetic point of view, let us assume that the mechanisms involved in intrinsic resistance are by and large due to the germinal genetic makeup, and that the mechanisms responsible for acquired resistance rely on mutational or epigenetic phenomena occurring in the tumor cells leading to failure of response to therapeutics. It is well known that tumors

José Rueff and António Sebastião Rodrigues (eds.), *Cancer Drug Resistance: Overviews and Methods*, Methods in Molecular Biology, vol. 1395, DOI 10.1007/978-1-4939-3347-1_1,

exhibit micro-clonality with a high degree of genetic heterogeneity making possible the recruitment of resistant cells to continue growing in spite of the therapy.

This genetic heterogeneity may render the tumor cells particularly versatile in modifying rates of efflux of drugs, up-regulating DNA repair processes, or activating alternative survival signaling pathways [3]. That combination of genomic and epigenetic instability associated with the acquisition of a stem cell-like phenotype is probably an important part of the tumor behavior explaining drug resistance. Such a biological behavior typically characterizes the basis of acquired resistance based on genetic phenomena of tumor cells and quite independent of the constitutional germ-line genome of the patient. A caveat must, however, be alluded to by mentioning that the final therapeutic failure or success also depends on various factors like the competence and effectiveness of the immunological surveillance or the various factors involved in metastasis which are outside the scope of this brief overview, although tumor cell variants may have low immunogenicity due to genetic heterogeneity and become resistant to immune attack [4].

Whatever the mechanisms underlying cancer and its progression, drug resistance is a major problem since it is believed to cause treatment failure in over 90 % of patients with metastatic cancer [5].

2 Intrinsic Resistance

2.1 Intrinsic Resistance, Clinical Expertise, and Clinical Guidelines

In a schematic manner, intrinsic resistance might be defined as a failure of response to the initial drug (or combination of drugs), indicating that before receiving therapy the resistance mechanisms/factors were already present. Intrinsic resistance may result largely, but not only from some major factors: (1) possible pre-existence of resistant cells in the tumor that render the therapy unsuccessful causing or leading anyway to a wrong adequacy of the administered drugs(s) to that particular cancer patient; (2) a different type of unsuccessful treatment may result from the fact that the actual cancer patient has low tolerance to the drug(s) and/or their side effects are unbearable and the dose has to be lowered resulting in putative failure of treatment; or even (3) from factors involved in the ADME (absorption, distribution, metabolism, excretion) such that the drug does not attain its best pharmacokinetic profile to exert its effects on the tumor or is subject to pharmacogenetic patterns which determine different levels of availability of the active metabolite of the drug. Tamoxifen is amongst the examples of the latter.

As far as intrinsic resistance is concerned and even when the prescribed drug(s) belong to the first-line therapy for a specific tumor, there are no easy ways to predict or estimate resistance; neither an easy strategy has been found to overcome resistance, which is based on highly complex and individually variable biological

mechanisms, apart from the few cases where pharmacogenomic patterns can be sought indicating germ-line patterns of low drug efficiency.

In the clinical practice, drug resistance can only be recognized during treatment. Thus, in order to try to prevail over (or tentatively overcome) intrinsic resistance one has to firstly take into consideration the far-reaching clinical competence of the medical staff on the judgement of the adequacy of the treatment and its therapeutic scheme to the particular patient under treatment, as well as the adherence to clinical guidelines, although guidelines are the expression of average patients and many patients are simply not in the average [6]. Understanding the diversity of both genetic and therapeutic factors that can determine innate patient responsiveness to anticancer drugs is thus a multiform endeavor.

2.2 Intrinsic Resistance and Pharmacogenetic Patterns

A main germ-line-determined factor of intrinsic resistance, as pointed out above, encroaching on the ADME variables, is drug metabolism and biotransformation which depends in large part on the activities of cytochromes P450 for phase I and on conjugation reactions for phase II. Cytochrome P450s (CYP) are members of a large superfamily of heme proteins, with a pivotal role in xenobiotic biotransformation, as well as in the endobiotic biosynthesis and catabolism of steroid hormones, bile acid, lipid-soluble vitamins, and fatty acids. At least 57 human microsomal CYPs have been recognized, some 15 of which are involved in drug metabolism [7] whose activities are supported by electron transfer from NADPHcytochrome P450 oxidoreductase (CYPOR) [8]. Interindividual variability in CYP-mediated xenobiotic metabolism is extensive. This type of intrinsic resistance is thus germ-line determined and has as main responsible organs primarily the liver, but also the lungs or the kidneys, among others.

As mentioned briefly above, a representative case of a pharmacogenetic CYP-dependent pattern is the pharmacokinetics of tamoxifen, a drug in clinical use for treatment and prevention of estrogen-dependent breast cancer. Tamoxifen is, however, but a pro-drug, being transformed among other metabolites to endoxifen. Endoxifen is the metabolite with the higher potent antiestrogen effect since it has a much higher affinity for the estrogen receptor and attains higher plasma levels. However, single-nucleotide polymorphisms in the CYP2D6 gene, particularly the presence of two null alleles, predict for reduced tamoxifen metabolism and possibly poorer outcome than expected in patients with a wild-type genotype due to lower biotransformation to endoxifen. However, studies evaluating the impact of genetic polymorphisms resulting in CYP2D6 with reduced or no activity on long-term outcome of breast cancer do not still allow, by and large, a recommendation for typing of CYP2D6 polymorphisms as indicators for predictive outcome of treatment of estrogen-dependent breast cancer. It is expected that the future may bring about predictable

tests to evaluate germ-line-determined pharmacogenetic phenotypes that may help in designing more effective treatments with lower relapse rates. Of course, tumor-associated factors stemming from acquired mutations and/or epimutations should also be considered on their role in the fate of the drug(s) [9].

3 Acquired Resistance

Various mechanisms may bring about acquired resistance. Some of those can be categorized according to the functions which appear modified in the tumor rendering the tumor cell more competitive for growth and metastasis and better resisting cancer drugs.

Categories of acquired drug resistance are seemingly due to secondary genetic alterations (both mutations and epimutations, the latter defined as an abnormal up-regulation of otherwise normally repressed genes, or downregulation of genes active in normal cells, or still by copy number changes), and they encompass namely (1) increased rates of drug efflux of drugs or decreased rates of drug influx into the tumor cells, mediated by transmembrane transporters of drug uptake and/or efflux (e.g., SLCs, ATP-binding cassettes (ABCs)); (2) biotransformation and drug metabolism mainly due to CYPs in the tumor; (3) altered role of DNA repair and impairment of apoptosis; (4) role of epigenomics/epistasis by methylation, acetylation, and altered levels of microRNAs leading to alterations in upstream or downstream effectors; (5) mutation of drug targets in targeted therapy and alterations in the cell cycle and checkpoints; and (6) tumor microenvironment [1].

3.1 Acquired Resistance and Tumor Micro-heterogeneity

In order to tackle the categories of acquired drug resistance, one should thus take into account the recognizable genetic heterogeneity that is present in many tumors (if not all). Indeed, cancer cells within one tumor of a patient at any given moment in time may display overwhelming heterogeneity for various traits related to tumorigenesis, such as those that may modify or modulate all the above categories of acquired resistance leading to angiogenic, invasive, and metastatic potential [10–12].

Tumors, besides turning the organism of the patient into a genetic mosaic, themselves display genetic mosaicism. Tumors are, indeed, composed of subclones, subpopulations of genetically identical cells that can be distinguished from other subclones by the mutations they harbor. Such subclones compete for biological dominance during cancer progression, and drug treatment can lead to formerly minor tumor subclones becoming dominant if they are resistant to treatment. These subclones are indeed positively selected to outgrowth and resistance to apoptosis and although representing a smaller cell population they are endowed with a rapidly growing capacity [13]. There is a crucial need to

understand the mechanisms driving genomic instability so that therapeutic approaches to limit cancer diversity, adaptation, and drug resistance can be developed [14].

Besides inter-tumor heterogeneity which different patients, indeed different genotypic tumors, bear, although probably histologically classified as of the same type, intra-tumor heterogeneity is claiming nowadays our attention [15], since many if not most somatic mutations detected by exome sequencing may not be detected across every tumor region. As pointed out by Castano et al.: "The tumour 'onco-genotype', which defines the collection of disease-related mutations and that evolves over time due to inherent genomic instability, differs obviously among patients so that nearly every tumour cell population is unique, thus adding to the clinical challenges" [16]. Or, as appropriately referred to by Sharma and Settleman, "Cancer is … actually a hundred diseases masquerading as one" [17].

Intra-tumor heterogeneity may have conspicuous consequences in therapeutic failure or cancer drug resistance. The tumor "onco-genotype," which defines the collection of disease-related mutations often occurring mainly as "driver mutations" evolves over time due to inherent genomic instability, not only accumulating various "passenger mutations," but also accumulating mutations, genome rearrangements, and polisomy, involving critical genes for the tumor progression and its resistance to drugs that had previously been found to be effective in the refraining of tumor growth and metastasis. Driver and passenger mutations may change places as the tumor evolves. As such, resistance appears to select for subclones bearing mutations in the genes or pathways targeted by the drug.

But genomic instability and thus heterogeneity leading to resistance may show the way to a rising strategy to overcome this problem through the use of combinations of targeted therapies with the goal of defeating several drivers.

This may, nonetheless, involve insurmountable costs per patient. This is a central problem that should not be neglected since it raises important financial, political, and even ethical questions concerning the access and availability of those drugs that may provide, if not the cure, at least some extra time of life [18].

The very point of using targeted therapies to specific cellular oncoproteins should be traced back to the inspiring concept of "oncogene addiction" coined in 2000 by Weinstein [19–22] whereby despite the multiple genetic and epigenetic abnormalities of the cancer cells, their growth control can often be impaired by the inactivation of a single oncogene, i.e., the "Achilles heel" of the cancer cell that could reasonably be thought to be blocked/inactivated therapeutically.

This innovating cutting-edge concept was based on the assumption that a given oncogene may play a key role on the cell circuitry of signaling pathways of the cells so that they lose cell

cycle control and apoptosis mechanisms leading to sustained proliferation and survival. The examples are many and some brought in the basis for the "oncogene addiction" concept, namely *MYC* (the first in supporting the concept), *RAS* genes, and the most representative activated tyrosine kinases, like the *BCR-ABL* or the ErbB receptor tyrosine kinase family [17]. Unfortunately this key concept and the profound therapeutic basis it helped to create soon uncovered new mechanism of cancer drug resistance, namely by the adaptive compensatory pathways or oncogenic bypass, as discussed below [3, 23].

Tumor micro-heterogeneity may also be linked to the epithelial–mesenchymal transition (EMT). The epithelial phenotype of cells can undergo transition to a mesenchymal phenotype, a process driven by various transcription factors that is associated with increased motility and invasive capacity as well as increased cancer drug resistance. Signaling pathways activated in EMT seem to include, in some cancers, Wnt/β catenin, Notch, PI3K/AKT, among others, leading to increased resistance to drug treatment, both chemotherapy and targeted therapy, namely resistance to EGFR inhibitors [3, 24]. Nonetheless, EMT may not occur in all tumors, like melanomas, albeit displaying phenotypes with either the expression of high MITF-M and E-cadherin with more differentiated noninvasive behavior, or expressing high N-cadherin, Slug, and Axl and with a more invasive behavior [25].

3.2 Acquired Resistance and Adaptive Compensatory Pathways

Some of the secondary genetic and epigenetic alterations occurring in tumors which may determine cellular diversity with the subsequent occurrence of subclonal heterogeneity may also coexist with adaptive nonhereditary mechanisms, in particular adaptive responses or fluctuation in protein levels downstream to the receptors to targeted therapies, leading to activation of alternative compensatory signaling pathways [3].

This type of bypass to the main pathway by which the drug is exerting its therapeutic effect is what can be called compensatory adaptation or oncogenic bypass [3, 23]. Compensation thus does not affect drug-target interaction but adapts the signaling circuitry of the tumor cell, thus escaping the growth-blocking activity of the drug.

This bypass thus lowers the dependence for tumor growth of the signal transduction pathway whose triggering receptor is being blocked by the drug through the activation of a parallel pathway which results in failure of growth control by the drug being administered. This type of transactivation by other receptor partners thus results in resistance to the target-directed first drug administered and can only be overcome by the use of combinations of targeted therapies, as mentioned above.

Most targeted chemotherapeutic drugs, indeed, block only a single cellular pathway and as consequence cancers frequently acquire resistance by up-regulating alternative compensatory pathways.

But besides multi-targeted therapies, fortunate situations exist, and probably new ones will come to be uncovered whereby some key product genes of the cell circuitry may control more than one signaling pathways. Steroid receptor coactivator-3 (SRC-3), also known as AIB1 (*a*mplified *i*n *b*reast cancer 1), is probably such an example. It is a member of the p160 steroid receptor coactivator family composed of SRC-1 (NCOA1), SRC-2 (TIF2/GRIP1/NCOA2), and SRC-3. SRC-3 coordinates multiple signaling networks, suggesting that SRC-3 inhibition offers a promising therapeutic strategy [26, 27].

4 Uptake and Efflux of Drugs Mediated by Transporters: Role in Resistance

The rates of abnormal efflux or influx of drugs to the cancer cell, as well as their abnormal biotransformation to inactive metabolites, are among the main mediator mechanisms leading to pharmacokinetics-mediated resistance, whereas proficient DNA repair, or lack of an abnormal epigenetic-controlled expression of a key gene product controlling cell cycle regulation, determines a pharmacodynamic resistance.

Multifunctional efflux transporters from the ABC gene family have been known for more than two decades to play a role in multidrug resistance (MDR) of tumor cells conferring resistance to various anticancer drugs. The human genome encodes 48 ABC transporters, organized into seven distinct subfamilies (ABCA–ABCG), and at least 15 of these members are associated with MDR [28].

ABC proteins are involved in the ATP-dependent efflux of substrates such as phospholipids, sterols, bile salts, and amphipathic drugs. While various ABC transporters have been observed to export chemotherapy drugs using in vitro experimental systems, the ones having the major involvement of drug transport seem to be ABCB1, ABCC1, and ABCG2 [1].

Tumors originating from tissues with naturally high levels of ABC transporters' expression may be intrinsically drug resistant (e.g., colon, kidney, pancreas, and liver carcinoma), whereas tumors from tissues with low expression may display an increase only after chemotherapy, acquiring resistance through up-regulation of gene expression. In both cases though, the evolving nature of the initial cancer clone will dictate whether influx/efflux membrane transporters may have a role in cancer drug resistance. Either because in the first case they may not be so much expressed in the genetically altered cancer cell, or because in the latter case cancer cells expressing high levels of resistance due to drug efflux may be selected to proliferate.

In many solid tumors over-expression of ABC transporters and drug resistance is unequivocal. Over-expression of ABCG2, in particular, is associated with resistance to a wide range of different

anticancer agents including mitoxantrone, camptothecins, anthracyclines, flavopiridol, and antifolates [29], but a wider range of cancer drugs are substrates of various ABC transporters [30].

The attempts to use inhibitors of ABC transporters to circumvent ABC-mediated MDR in vivo faced, however, high toxicity observed in vivo in clinical trials, and also because clinical efficacy can only be reached with the inhibition of various transporters.

In the case of breast cancer resistance, the major efflux transporter protein is the breast cancer-resistant protein, a member of the ABCG family (BCRP/ABCG2). It is noteworthy that the c-MET downstream phosphoinositide 3-kinase (PI3K)/AKT signaling activates over-expression to BCRP/ABCG2 in a doxorubicin-resistant ovarian cancer line, thus apparently linking the cell signaling circuitry controlling the cell cycle and proliferation to the levels of expression of a drug efflux transporter showing how intertwined is the network of cancer pathways and mechanisms that more often than not render cancer drug resistance a burdensome phenomenon [28, 31].

In leukemia, it was shown that the expression and functionality of ABCB1 hampers complete remission and survival [32–34]. Acute myeloid leukemia (AML) patients who had joint expression of ABCB1 and ABCG2 had the poorest prognosis [35]. However, the role of ABCG2 as a cause of MDR in acute lymphoid leukemia (ALL) is a matter of debate [36]. In pediatric patients with ALL, ABCB1 does not seem to have a prognostic significance [37].

In chronic myeloid leukaemia (CML) patients ABCG2 gene expression levels correlated with ABCB1 and ABCC1, and interestingly there seems to exist a correlation between efflux genes and the influx gene SLC22A1 which supports the hypothesis that absolute bioavailability may also be influenced by the balance between efflux and influx transport and most of these transporters were also found over-expressed in the majority of resistant CML cell lines [38, 39].

It is worth noting that namely hematopoietic stem cells express higher levels of ABCB1 than their matured counterparts, which contributed to the concept that cancer stem cells may represent a small subset of cancer cells within a cancer that have the ability to self-renew, thus constituting a reservoir of self-sustaining cells [2], which does not set aside the concept that genetic diversification and clonal selection by the cancer drugs may simultaneously occur with a reiterative process of clonal expansion from stem-like cells in some tumors [40].

Efflux pumps of the ABC transporters' family are subject to microRNA-mediated gene regulation. As a matter of fact, it appears that ABC transporters are entrenched in a concerted microRNA-guided network of concurrently regulated proteins that mediate altered drug transport and cell survival upon defy by cancer drugs or adverse survival conditions due to exposure to environmental detrimental compounds. There is increasing evidence that

microRNAs are crucially involved in coordinating and fine-tuning this complex network of proteins mediating increased drug efflux and cell survival. microRNA-93, for example, activates c-Met/PI3K/Akt pathway which in turn activates over-expression to BCRP/ABCG2, as mentioned above [41].

microRNAs play, therefore, an important epigenetic role in controlling the levels of expression of ABC transporters' genes, being thus connected with drug distribution as well as with drug resistance [42].

5 DNA Repair and Cancer Drug Resistance

Mutations in genes involved in the DNA damage response (DDR) can increase the risk of developing cancer which is plentifully illustrated by the various cancer syndromes involving mutations in genes coding for repair enzymes, from ataxia telangiectasia (ATM), or Fanconi anemia (FANC genes) to breast and ovary cancers (BRAC1/BRAC2).

It is also well established that, besides rare Mendelian gene defects of high penetrance, common variations (e.g., SNPs) in DNA repair genes of low penetrance may alter protein function and the individual's capacity to repair damaged DNA, hence increasing cancer susceptibility. However, those SNPs occurring in DNA repair genes may also possibly have an important role in cancer drug resistance [43–50].

Many cancer drugs exert their effects by and large by causing DNA damage, like epirubicin, doxorubicin, 5-fluorouracil, or cisplatin which find their place as first-line drugs for some cancers. DNA damage entails the triggering of the DDR which is a key mechanism enabling cancer cells to survive through repair of the induced DNA lesions and thereby developing resistance [51]. But lack of DDR proficiency can also definitely contribute to cancer drug resistance.

BRCA genes are involved in repairing DNA through homologous recombination following DNA strand breaks (DSB). The second hit is acquired in the tumor genome, rendering these tumors susceptible to DNA-damaging agents once they have defects in their DNA repair machinery. Moreover, some 60–80 % of breast tumors from BRCA1 mutation carriers display a triple-negative phenotype (TNBC) [52]. BRCA gene products are nonfunctional in a subset of sporadic triple-negative breast tumors (TNBCs), generally through promoter hypermethylation or other epigenetic pathways, and also by mutations occurring concurrently with tumors' progression heterogeneity. This has been termed the "BRACness" of sporadic TNBCs. BRCAness leads to a better response upon intensive exposure to alkylating agents as adjuvant chemotherapy and to hypersensitivity to DSB-inducing agents such as bifunctional alkylators and platinum salts, but not doxorubicin and docetaxel. Also the clinical responses are lower with

taxane- and/or anthracycline-based neoadjuvant chemotherapy (NAC) in the case of tumors bearing BRACness [53]. Thus, up- or downregulation of DDR genes may provide tumor cells with escape mechanisms to cancer drugs and induce chemotherapy resistance.

Disabling alterations in DNA repair pathways are frequently observed in cancer. These DNA repair defects may either be mutations or epimutations and are specific to cancer cells. It is thought that these molecular defects produce a "mutator phenotype," which allows cancer cells to accumulate additional cancer-promoting mutations.

The molecular understanding of DNA repair mechanisms, namely DSB repair, has led to the development of targeted therapies to selectively trigger cancer cells that display defects in homologous recombination-mediated DNA DSB repair. These pharmacological approaches for the treatment of homologous recombination-defective tumors predominantly aim at repressing the activity of PARP1, which is crucial for base excision repair, or inhibit the nonhomologous end joining kinase DNA-PKcs (DNA-dependent protein kinase, catalytic subunit). Whereas normal cells can bypass PARP1 (poly ADP-ribose polymerase 1) inhibitor- or DNA-PKcs inhibitor-induced genotoxic lesions via homologous recombination, homologous recombination-defective cancer cells are unable to properly repair DNA DSBs, in the presence of PARP1 or DNA-PKcs inhibitors, ultimately leading to apoptotic cancer cell death [54].

The identification of genes associated with the DNA repair activity and related with individual response to chemotherapeutic agents is therefore crucial since it may allow the development of customized strategies for cancer treatment. The recent approval by the US FDA of olaparib, a (PARP) inhibitor, is a relevant move towards the class of personalized cancer drugs targeted to the blocking of DNA repair functions ultimately triggering cell death.

Indeed, the search for targeted therapies has also focused on DNA repair pathways [51], besides the ones developed for molecular players having a key role on the cell circuitry of signaling pathways of the cells so as to avoid that they lose cell cycle control and apoptosis mechanisms, as mentioned above. Efforts are now also focused in targets of DNA integrity, or, stated otherwise, not only targeted to "gatekeepers" as the genes that should be inactivated for a cell to become cancerous, but also targeted to "caretakers," the genes involved in maintaining genetic stability [55].

The biological significance of DNA repair mechanisms is highlighted by the fact that their deregulation can contribute to the initiation and progression of cancer, but on the other hand, DNA repair can confer resistance to front-line cancer treatments, might there be cancer drugs or radiotherapy which relies on the generation of DNA damage to kill cancer cells. The way cancer cells (or cancer stem cells) recognize DNA damage and undertake DNA repair is therefore a key mechanism for therapeutic resistance or recurrence [56].

6 Epigenomics and Resistance: The Role of Methylation, Acetylation, and microRNAs

Whatever the mutations involved in the initiation of a cancer, not all may end up as a clinically diagnosed cancer in all individuals. One possible important reason for this is that the outcome of a mutation can depend upon other genetic variants in the genome. This can broadly define epistatic interactions, which may increase the effects of the hypostatic gene or, conversely, alleviate its effects [57]. They can occur between different variants within the same gene or between variants in different genes. The latter might be important to consider in cancer since the wealth of "passenger mutations" in a cancer may modulate the effect of the "driver mutation," acting as putative modifier genes. For example mutations in *ERS1*, the gene coding for the estrogen receptor (ER), have been linked to treatment failure and shown to be recurrent in metastatic clinical samples playing an important role in acquired endocrine therapy resistance [58].

But epimutations may also play an important role, by up- or downregulating the expression of receptors, might them be hormonal receptors or receptors used by targeted therapies.

In estrogen-dependent breast cancer, approximately some 20 % of ER-positive tumors lose its expression during tamoxifen treatment. This loss of expression may be the result of epigenetic silencing of ER expression.

Several mechanisms have been proposed to explain the absence of ER expression. These mechanisms involve epigenetic changes such as aberrant methylation of CpG islands of the ER promoter and histone deacetylation. This fact has been used as a predictor of poor outcome and tamoxifen resistance. Other mechanisms proposed in the loss of ER expression are hypoxia, over-expression of EGFR or HER2, and MAPKs hyperactivation. Also, PI3K pathway activation confers anti-estrogen resistance. Also, altered expression of specific microRNAs has been implicated in tamoxifen resistance development predicting the outcome and therapeutic response in breast cancer [59].

Steroid receptor coactivator-3 (SRC-3) promotes numerous aspects of cancer, through its capacity as a coactivator for nuclear hormone receptors and other transcription factors, and via its ability to control multiple growth pathways simultaneously. Gene amplification and protein over-expression of Sarc3 are well established. In fact, SRC-3 is over-expressed in 60 % of breast cancers which may be implicated in tamoxifen resistance [27], namely through potentiating of E2F1 activity (a target of pRb-mediated repression). Binding of SRC to transcription factors will further recruit other chromatin modification factors, such as acetyltransferases and methyltransferases that modify the chromatin structure and alter the transcription levels of their target genes. Thus, it is

conceivable that these changes may affect the expression levels of many genes. In tamoxifen-treated breast cancer patients, SRC-3 over-expression is associated with high levels of HER-2/*neu*, tamoxifen resistance, and poor disease-free survival [60].

Mechanisms involved in epigenetic-driven drug resistance encompass epigenetic changes resulting in gene transcription of drug transporters (ABCB1), pro-apoptotic genes (*DAPK*, *APAF-1*), DNA repair proteins (MLH1, MGMT, FANCF), and histone modifiers (KDM5A). Fortunately, treatment of drug-resistant tumor cell populations bearing epimutations with cytotoxic or targeted drugs in combination with epigenetic drugs, such as inhibitors of histone deacetylases (e.g., vorinostat, trichostatin A), DNA methyl transferases, and histone methyltransferases, may reverse a drug-resistant epigenome into a drug-sensitive epigenome, thereby rendering tumor cells sensitive to the cytotoxic or targeted drug. Indeed, the large variability in drug resistance of individual cells is to be found, maybe not primarily in cancer cells' mutations due to genetic instability of the tumor, but also and most decisively in the different transcriptional network states produced by epigenetic mechanisms in the same cancer genome [61].

Epigenetic regulation, particularly by microRNAs, besides DNA methylation or histone acetylation, plays an important role in carcinogenesis and oncotherapy. The approximately 2000 different human microRNA species identified form a intertwined network of concurrently regulated proteins that mediate cell survival upon a challenge by cancer drugs, and as already mentioned above they may control the levels of expression of ABC transporters' genes, being thus connected with drug resistance [42]. Also long noncoding RNAs (lncRNAs) are also able to regulate mRNAs' levels of expression correlated with cancer drug resistance. The fine-tuning of the ncRNA system is on the other hand also regulated by hypermethylation making the whole of the epigenetic machinery a self-regulated system whose overall implications in cancer drug resistance are yet to be fully uncovered.

7 Tumor Microenvironment and Resistance

The tumor microenvironment (TME) consists of vascular cells, fibroblasts, infiltrating immune cells, the extracellular matrix (ECM), and the signaling molecules bound to it [62]. TME has many roles in tumor progression and metastasis, including the creation of a hypoxic environment, increased angiogenesis, and invasion and changes in expression of noncoding RNAs. There is a molecular cross talk between the tumor and its microenvironment that determines tumor progression [63].

The microenvironment could be a major niche where some mechanisms of drug resistance may take place through the reduction

of drug distribution throughout the tumor, therefore protecting high proportions of cells from damage induced by the drug [64]. The dissection of interactions between tumors and their microenvironment can reveal important mechanisms underlying drug resistance [64, 65].

It is noteworthy that landscaper genes seem to facilitate the growth of neoplastic lesions by creating a microenvironment that aids in unregulated cellular proliferation. Loss of components of the extracellular matrix (ECM) may lead to a microenvironment which can stimulate unregulated growth, clonal proliferation, and ultimately neoplastic lesions [66].

There are also interesting data suggesting that, at least in patients with *BRCA1/2*-related breast cancers, genomic alterations in the stroma coexist equally with alterations in the epithelium, and, thus, the genetically unstable stroma might provide for a microenvironment that functions as a landscaper that positively selects for genomic instability in the epithelium [67]. However, this might not be the case in other situations whereby the suggestion of epithelial:mesenchymal interactions remains but a possibility in the causality of malignant development [68].

8 Concluding Remarks

Substantial scientific advances over the last years have allowed us to understand the genomic landscapes and portraits of individual tumors [69, 70]. Numerous genetic alterations have been identified in individual tumors, but the number of cancer-promoting genes is considered relatively small, in the order of 100–150 [69]. Two to eight driver gene mutations can be found in tumors, but the vast majority will be passenger mutations. These driver genes can be grouped into well-known signaling pathways, the fittingly called hallmarks of cancer [71]; tumor-promoting mutations are seemingly involved in three major biological processes, cell fate, cell survival, and genome maintenance [69]. Hence, their identification has led to the concept of tailored mechanism-based targeted therapies aimed at inhibiting some of the specific oncogenic pathways mentioned above. This strategy has the advantage of only targeting tumor cells while doing little or no harm to normal tissues. The vast information garnered by the latest genome-wide sequencing studies has not yet been fully translated into the clinic, but several instances of targeted therapies have emerged from the identification of specific alterations in driver genes (i.e., those that confer a growth and survival advantage), such as protein kinases and development of small-molecule inhibitors. As discussed above, targeted therapies include epidermal growth factor receptor (EGFR) inhibitors, human epidermal growth factor receptor 2 (HER2), or breakpoint cluster region-Abl

proto-oncogene 1 (BCR-ABL) inhibitors with some success [72]. Nevertheless, outcomes of these targeted therapies have revealed to be suboptimal, particularly as their usage becomes more widespread, and clinical responses are generally short-lived. Unfortunately, in most patients with solid tumors, the cancer evolves to become resistant within a few months [73]. Drug resistance to these targeted therapies arrives sooner or later. In some cases initial drug resistance can be attributed to misexpression of a number of genes, frequently occurring in refractory tumors, and responsible for cellular drug extrusion, as discussed [74]. However, in most cases drug resistance is due to multiple factors, including under- or overexpression of specific targets, mutations in target genes, and epigenetic alterations in DNA [2, 75].

To understand the reasons for the apparent inevitability of cancer drug resistance, one must focus on the knowns and the unknowns of cancer development and progression. First and foremost, the clinical detection of a tumor occurs many years, perhaps decades, after the initial oncogenic triggering event. The average time it takes for a tumor to reach detection size varies with several factors, including tissue affected, rate of tissue self-renewal, and exposure to mutagens and carcinogens. For example, it has been estimated that colorectal cancer requires about 17 years for a large benign tumor to evolve into an advanced cancer [76]. When comparing different tumors with different progression periods, the number of accumulated mutations and genomic alterations will necessarily be different. As a consequence, the response of different tumors towards chemotherapy will depend on these mutational landscapes. It follows that different tumor sensitivities will arise, and cellular adaptation to chemotherapy will necessarily be more or less effective and rapid. It also follows that resistance to chemotherapy will depend on the number of cells with sufficiently wide mutational landscapes that could allow escape from cell death. Recent studies have indicated that at the onset, tumors already possess mutated cells that could be responsible for resistance. According to Tomasetti et al. [77] more than half of somatic mutations in self-renewing tissues are already present before the onset of neoplasia, and the number of mutations correlates with the age of the patient. It is plausible that some of these mutations could drive drug resistance. Indeed, several experimental and theoretical studies have reached the conclusion that a small number of cells resistant to any targeted agent are always present in large solid tumors at the start of therapy and that these cells clonally expand once therapy is administered [78]. If this is the case, then treatment with multiple therapy could be more effective in delaying the onset of resistance. In accordance, recent mathematical modeling suggests that dual therapy results in higher long-term disease control for most patients, whereas for some patients with larger disease burdens triple therapy would be more effective [79, 80]. Nevertheless, one constraint on this approach is the expectable higher systemic

toxicity with multiple drug regimens. Hence, ideally one should detect a tumor at the earliest stage possible and comparison of tumors should be performed with similar mutational landscapes, more likely correlated with age. Current efforts are beginning to address this issue, notably in the potential use of circulating tumor cells (CTCs) to detect tumors earlier [81].

Hence, it would be desirable to use a combination of genomics, proteomics, and functional assays to evaluate the mechanisms underlying drug resistance. Unfortunately, the difficulty in accessing tumors and the low amount of biological material available from high-grade tumor specimens preclude this approach. Thus, the usage of drug-resistant cell lines in vitro has been invaluable in elucidating specific resistance pathways, and shall continue to be so.

Finally, we should take into account the dynamic nature of resistance mechanisms. One of the reasons for failure to eradicate tumor cells could well lie in the successive alternation of one resistance mechanism with another, as cells proliferate in vivo and adapt to drug regimens. This would mean that current strategies to circumvent drug resistance would have to depend on continuous monitoring of patients and prescription of a cocktail of chemotherapeutic drugs, each targeting one or more of known drug resistance pathways. The feasibility of such an approach, especially in what regards toxicity and efficacy, is not predictable. Ideally, the earliest that the tumor is detected the lower the heterogeneity of tumor cells, and the more successful the therapy should be. However, in the long term, drug resistance is unfortunately probably inevitable [2].

References

1. Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med 53:615–627
2. Rodrigues AS, Dinis J, Gromicho M, Martins M, Laires A, Rueff J (2012) Genomics and cancer drug resistance. Curr Pharm Biotechnol 13:651–673
3. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2013) Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 13: 714–726
4. Kim R, Emi M, Tanabe K (2007) Cancer immunoediting from immune surveillance to immune escape. Immunology 121:1–14
5. Longley DB, Johnston PG (2005) Molecular mechanisms of drug resistance. J Pathol 205:275–292
6. Cooper RA, Straus DJ (2012) Clinical guidelines, the politics of value, and the practice of medicine: physicians at the crossroads. J Oncol Pract 8:233–235
7. Guengerich FP (2006) Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J 8:E101–E111
8. Palma BB, Silva ESM, Urban P, Rueff J, Kranendonk M (2013) Functional characterization of eight human CYP1A2 variants: the role of cytochrome b5. Pharmacogenet Genomics 23:41–52
9. Higgins MJ, Rae JM, Flockhart DA, Hayes DF, Stearns V (2009) Pharmacogenetics of tamoxifen: who should undergo CYP2D6 genetic testing? J Natl Compr Canc Netw 7:203–213
10. Marusyk A, Polyak K (2010) Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 1805:105–117
11. Polyak K (2011) Heterogeneity in breast cancer. J Clin Invest 121:3786–3788
12. Salk JJ, Fox EJ, Loeb LA (2010) Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol 5:51–75

13. Baguley BC (2011) The paradox of cancer cell apoptosis. Front Biosci (Landmark Ed) 16: 1759–1767
14. Burrell RA, McGranahan N, Bartek J, Swanton C (2013) The causes and consequences of genetic heterogeneity in cancer evolution. Nature 501:338–345
15. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366:883–892
16. Castano Z, Fillmore CM, Kim CF, McAllister SS (2012) The bed and the bugs: interactions between the tumor microenvironment and cancer stem cells. Semin Cancer Biol 22:462–470
17. Sharma SV, Settleman J (2007) Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes Dev 21:3214–3231
18. Fleck LM (2013) "Just caring": can we afford the ethical and economic costs of circumventing cancer drug resistance? J Pers Med 3:124–143
19. Weinstein IB (2000) Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. Carcinogenesis 21:857–864
20. Weinstein IB, Joe A (2008) Oncogene addiction. Cancer Res 68:3077–3080, discussion 3080
21. Weinstein IB, Joe AK (2006) Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. Nat Clin Pract Oncol 3:448–457
22. Weinstein IB (2002) Cancer. Addiction to oncogenes—the Achilles heal of cancer. Science 297:63–64
23. Borst P (2012) Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? Open Biol 2:120066
24. da Silva SD, Hier M, Mlynarek A, Kowalski LP, Alaoui-Jamali MA (2012) Recurrent oral cancer: current and emerging therapeutic approaches. Front Pharmacol 3:149
25. Kim JE, Leung E, Baguley BC, Finlay GJ (2013) Heterogeneity of expression of epithelial-mesenchymal transition markers in melanocytes and melanoma cell lines. Front Genet 4:97
26. Tien JC, Xu J (2012) Steroid receptor coactivator-3 as a potential molecular target for cancer therapy. Expert Opin Ther Targets 16:1085–1096
27. Ma G, Ren Y, Wang K, He J (2011) SRC-3 has a role in cancer other than as a nuclear receptor coactivator. Int J Biol Sci 7:664–672
28. Vadlapatla RK, Vadlapudi AD, Pal D, Mitra AK (2013) Mechanisms of drug resistance in cancer chemotherapy: coordinated role and regulation of efflux transporters and metabolizing enzymes. Curr Pharm Des 19:7126–7140
29. Robey RW, Polgar O, Deeken J, To KW, Bates SE (2007) ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev 26:39–57
30. Lemos C, Jansen G, Peters GJ (2008) Drug transporters: recent advances concerning BCRP and tyrosine kinase inhibitors. Br J Cancer 98:857–862
31. Jung KA, Choi BH, Kwak MK (2015) The c-MET/PI3K signaling is associated with cancer resistance to doxorubicin and photodynamic therapy by elevating BCRP/ABCG2 expression. Mol Pharmacol 87:465–476
32. Marie JP, Zittoun R, Sikic BI (1991) Multidrug resistance (mdr1) gene expression in adult acute leukemias: correlations with treatment outcome and in vitro drug sensitivity. Blood 78:586–592
33. Legrand O, Simonin G, Perrot JY, Zittoun R, Marie JP (1998) Pgp and MRP activities using calcein-AM are prognostic factors in adult acute myeloid leukemia patients. Blood 91:4480–4488
34. Wuchter C, Leonid K, Ruppert V, Schrappe M, Buchner T, Schoch C, Haferlach T, Harbott J, Ratei R, Dorken B, Ludwig WD (2000) Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. Haematologica 85:711–721
35. Benderra Z, Faussat AM, Sayada L, Perrot JY, Chaoui D, Marie JP, Legrand O (2004) Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. Clin Cancer Res 10:7896–7902
36. Steinbach D, Legrand O (2007) ABC transporters and drug resistance in leukemia: was P-gp nothing but the first head of the Hydra? Leukemia 21:1172–1176
37. Steinbach D, Furchtbar S, Sell W, Lengemann J, Hermann J, Zintl F, Sauerbrey A (2003) Contrary to adult patients, expression of the

multidrug resistance gene (MDR1) fails to define a poor prognostic group in childhood AML. Leukemia 17:470–471

38. Gromicho M, Magalhaes M, Torres F, Dinis J, Fernandes AR, Rendeiro P, Tavares P, Laires A, Rueff J, Sebastiao RA (2013) Instability of mRNA expression signatures of drug transporters in chronic myeloid leukemia patients resistant to imatinib. Oncol Rep 29:741–750
39. Gromicho M, Dinis J, Magalhães M, Fernandes A, Tavares P, Laires A, Rueff J, Rodrigues A (2011) Development of imatinib and dasatinib resistance: dynamics of the drug transporters expression ABCB1, ABCC1, ABCG2, MVP and SLC22A1. Leuk Lymphoma 52:1980–1990
40. Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481:306–313
41. Ohta K, Hoshino H, Wang J, Ono S, Iida Y, Hata K, Huang SK, Colquhoun S, Hoon DS (2015) MicroRNA-93 activates c-Met/PI3K/Akt pathway activity in hepatocellular carcinoma by directly inhibiting PTEN and CDKN1A. Oncotarget 6:3211–3224
42. Haenisch S, Werk AN, Cascorbi I (2014) MicroRNAs and their relevance to ABC transporters. Br J Clin Pharmacol 77:587–596
43. Silva S, Moita R, Azevedo A, Gouveia R, Manita I, Pina J, Rueff J, Gaspar J (2007) Menopausal age and XRCC1 gene polymorphisms: role in breast cancer risk. Cancer Detect Prev 31:303–309
44. Bastos HN, Antao MR, Silva SN, Azevedo AP, Manita I, Teixeira V, Pina JE, Gil OM, Ferreira TC, Limbert E, Rueff J, Gaspar JF (2009) Association of polymorphisms in genes of the homologous recombination DNA repair pathway and thyroid cancer risk. Thyroid 19:1067–1075
45. Conde J, Silva S, Azevedo A, Teixeira V, Pina J, Rueff J, Gaspar J (2009) Association of common variants in mismatch repair genes and breast cancer susceptibility: a multigene study. BMC Cancer 9:344
46. Santos LS, Gomes BC, Gouveia R, Silva SN, Azevedo AP, Camacho V, Manita I, Gil OM, Ferreira TC, Limbert E, Rueff J, Gaspar JF (2013) The role of CCNH Val270Ala (rs2230641) and other nucleotide excision repair polymorphisms in individual susceptibility to well-differentiated thyroid cancer. Oncol Rep 30:2458–2466
47. Santos LS, Branco SC, Silva SN, Azevedo AP, Gil OM, Manita I, Ferreira TC, Limbert E, Rueff J, Gaspar JF (2012) Polymorphisms in base excision repair genes and thyroid cancer risk. Oncol Rep 28:1859–1868
48. Gomes BC, Silva SN, Azevedo AP, Manita I, Gil OM, Ferreira TC, Limbert E, Rueff J, Gaspar JF (2010) The role of common variants of non-homologous end-joining repair genes XRCC4, LIG4 and Ku80 in thyroid cancer risk. Oncol Rep 24:1079–1085
49. Silva SN, Tomar M, Paulo C, Gomes BC, Azevedo AP, Teixeira V, Pina JE, Rueff J, Gaspar JF (2010) Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes XRCC2, XRCC3, NBS1 and RAD51. Cancer Epidemiol 34:85–92
50. Silva SN, Gomes BC, Rueff J, Gaspar JF (2011) DNA repair perspectives in thyroid and breast cancer: the role of DNA repair polymorphisms. In: Vengrova S (ed) DNA repair and human health. InTech. doi:10.5772/22944
51. Rodrigues AS, Gomes BC, Martins C, Gromicho M, Oliveira NG, Guerreiro PS, Rueff J (2013) DNA repair and resistance to cancer therapy. In: Chen C (ed) DNA repair and resistance to cancer therapy, new research directions in DNA repair. Intech. doi:10.5772/53952
52. Atchley DP, Albarracin CT, Lopez A, Valero V, Amos CI, Gonzalez-Angulo AM, Hortobagyi GN, Arun BK (2008) Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol 26:4282–4288
53. Akashi-Tanaka S, Watanabe C, Takamaru T, Kuwayama T, Ikeda M, Ohyama H, Mori M, Yoshida R, Hashimoto R, Terumasa S, Enokido K, Hirota Y, Okuyama H, Nakamura S (2015) BRCAness predicts resistance to taxane-containing regimens in triple negative breast cancer during neoadjuvant chemotherapy. Clin Breast Cancer 15:80–85
54. Dietlein F, Reinhardt HC (2014) Molecular pathways: exploiting tumor-specific molecular defects in DNA repair pathways for precision cancer therapy. Clin Cancer Res 20:5882–5887
55. Kinzler KW, Vogelstein B (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386(761):763
56. Dexheimer TS (2013) DNA repair pathways and mechanisms. In: Mathews LA, Cabarcas SM, Hurt EM (eds) DNA repair of cancer stem cells. Springer, Dordrecht, Netherlands, pp 19–32. doi:10.1007/978-94-007-4590-2_2
57. Lehner B (2011) Molecular mechanisms of epistasis within and between genes. Trends Genet 27:323–331
58. Alluri PG, Speers C, Chinnaiyan AM (2014) Estrogen receptor mutations and their role in breast cancer progression. Breast Cancer Res 16:494

59. Garcia-Becerra R, Santos N, Diaz L, Camacho J (2012) Mechanisms of resistance to endocrine therapy in breast cancer: focus on signaling pathways, miRNAs and genetically based resistance. Int J Mol Sci 14:108–145
60. Yan J, Tsai SY, Tsai MJ (2006) SRC-3/AIB1: transcriptional coactivator in oncogenesis. Acta Pharmacol Sin 27:387–394
61. Wilting RH, Dannenberg J-H (2012) Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. Drug Resist Updat 15:21–38
62. Theodora F (2014) Tumor plasticity driven by spatial micro-heterogeneity in the tumor microenvironment contributes to therapy resistance. J Carcinog Mutagen. doi: 10.4172/2157-2518.S8-006
63. Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G (2013) Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol 23:522–532
64. Brown JM, Giaccia AJ (1998) The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Res 58:1408–1416
65. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, Davis A, Mongare MM, Gould J, Frederick DT, Cooper ZA, Chapman PB, Solit DB, Ribas A, Lo RS, Flaherty KT, Ogino S, Wargo JA, Golub TR (2012) Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature 487:500–504
66. Srivastava S, Grizzle WE (2010) Biomarkers and the genetics of early neoplastic lesions. Cancer Biomark 9:41–64
67. Weber F, Shen L, Fukino K, Patocs A, Mutter GL, Caldes T, Eng C (2006) Total-genome analysis of BRCA1/2-related invasive carcinomas of the breast identifies tumor stroma as potential landscaper for neoplastic initiation. Am J Hum Genet 78:961–972
68. Playford RJ (2001) Landscaper seeks remunerative position. Gut 48:594–595
69. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW (2013) Cancer genome landscapes. Science 339:1546–1558
70. Cgar N (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
71. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
72. Haber DA, Gray NS, Baselga J (2011) The evolving war on cancer. Cell 145:19–24
73. Kaiser J (2011) Combining targeted drugs to stop resistant tumors. Science 331:1542–1545
74. Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2:48–58
75. Ramos P, Bentires-Alj M (2015) Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. Oncogene 34:3617–3626
76. Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, Traulsen A, Nowak MA, Siegel C, Velculescu VE, Kinzler KW, Vogelstein B, Willis J, Markowitz SD (2008) Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 105:4283–4288
77. Tomasetti C, Vogelstein B, Parmigiani G (2013) Half or more of the somatic mutations in cancers of self-renewing tissues originate prior to tumor initiation. Proc Natl Acad Sci U S A 110:1999–2004
78. Diaz LA Jr, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA, Kinzler KW, Oliner KS, Vogelstein B (2012) The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486:537–540
79. Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, Moon YS, Yaqubie A, Kelly N, Le DT, Lipson EJ, Chapman PB, Diaz LA Jr, Vogelstein B, Nowak MA (2013) Evolutionary dynamics of cancer in response to targeted combination therapy. eLife 2:e00747
80. Komarova NL, Katouli AA, Wodarz D (2009) Combination of two but not three current targeted drugs can improve therapy of chronic myeloid leukemia. PLoS One 4:e4423
81. Polzer B, Medoro G, Pasch S, Fontana F, Zorzino L, Pestka A, Andergassen U, Meier-Stiegen F, Czyz ZT, Alberter B, Treitschke S, Schamberger T, Sergio M, Bregola G, Doffini A, Gianni S, Calanca A, Signorini G, Bolognesi C, Hartmann A, Fasching PA, Sandri MT, Rack B, Fehm T, Giorgini G, Manaresi N, Klein CA (2014) Molecular profiling of single circulating tumor cells with diagnostic intention. EMBO Mol Med 6(11):1371–1386