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Commentary

Molecular aspects of cancer cell resistance to chemotherapy

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ABSTRACT

Cancer cell resistance to chemotherapy is still a heavy burden that impairs treatment of cancer patients. Both intrinsic and acquired resistance results from the numerous genetic and epigenetic changes occurring in cancer cells. Most of the hallmarks of cancer cells provide general mechanisms to sustain stresses such as the ones induced by chemotherapeutic drugs. Moreover, specific changes in the target bring resistance to specific drugs like modification in nucleotide synthesis enzymes upon anti-metabolite exposure, in microtubule composition upon spindle poison treatment, in topoisomerase activity upon topoisomerase inhibitor incubation or in intracellular signaling pathways when targeting tyrosine kinase receptors.

Finally, the stemness properties of a few cancer cells as well as components of the tumor stroma, like fibroblasts and tumor-associated macrophages but also hypoxia, also help tumor to resist to anticancer agents. These processes provide an additional level of complexity to the understanding of the tumor resistance phenomenon.

This review aims to describe the different general mechanisms as well as some examples of specific on target modifications inducing cancer cell resistance to chemotherapy at the molecular level. Perspectives to develop more efficient treatment, using genomic signature or more specific biomarkers to characterize putative resistance mechanisms in patients before choosing the more appropriate treatment, will also be discussed.

1. Introduction

In its "Focus on Cancer" March 2011, Nature Medicine has defined four lines of research that still need enormous research efforts in order to ameliorate our understanding of the cancer pathology but also to develop new more efficient therapeutic strategies [1]. Amongst them, research on resistance mechanisms ("insights into treatment failure") remains a key challenge in the fight against cancer.

The first cause of therapeutic failure results from genetic alterations existing before treatment. This is the primary or intrinsic resistance. The second one is induced by drug treatment and is called secondary or acquired resistance. Both are due to mutations in the genome of cancer cells and/or to epigenetic changes. Unfortunately, resistance appears not only to conventional chemotherapy but also to targeted therapies, the so-called "smart drugs" such as kinase inhibitors and tamoxifen that binds to the estrogen receptor [2].

As reviewed by Hanahan and Weinberg [3], cancer cells result from a sequence of mutations in a particular subset of genes (tumor suppressor genes or oncogenes) that triggers unregulated proliferation but that also permits the acquisition of "hallmarks of cancer" that are observed in most cancers. Moreover, enabling characteristics, among which is genome instability, further accelerate tumor progression. Hence, cancer cells contain hundreds to thousands mutations as well as complex chromosome rearrangements [4,5]. Furthermore, each patient harbors a different cancer regarding which genes are mutated, regarding the nature of each mutation, i.e. different mutations for the same gene have been detected in several patients [6], and regarding the sequence of apparition of these mutations. Finally, tumors are very heterogeneous because of the clonal evolution of tumor cell populations driven by genomic instability [7]. These observations partly explain why different patients harboring the "same" cancer may respond differently to a same treatment regimen.

The purpose of this review is to give insight into the molecular mechanisms responsible for resistance of tumors to anticancer agents. They include the mechanisms inducing lower sensitivity to a large panel of drugs as well as the ones responsible for augmenting resistance to a more specific subfamily of therapeutic molecules. It will not overview pharmacological and physiological factors that impair drug delivery, enhance drug metabolism or favor drug elimination.

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2. Mechanisms common to several drugs (Fig. 1)

2.1. Activating mutation of oncogenes or inactivating mutation of tumor suppressor genes renders cancer cells resistant to cell death per se

Dysregulated proliferation signaling pathways is the most described cause of cell transformation. Overexpression of growth factors enabling autocrine mitotic signal, mutation of growth factor receptors as well as mutation/overexpression of signal transduction proteins lead to sustained proliferative signaling and aberrant proliferation [8]. Less known is that proliferation circuits and viability circuits are intimately connected: indeed, proliferative signals do also simultaneously provide survival signals. These survival signals not only prevent cancer cell death per se but also promote cell viability when exposed to stresses, such as the ones generated by anticancer drugs.

One of the most well described examples is "gain-of-function" gene alterations in the PI3K/Akt/mTOR pathway. Phosphatidylinositol 3-kinases (PI3K) are lipid kinases activated downstream of growth factor receptors. These enzymes generated hyperphosphorylated phosphatidylinositol molecules that serve as anchoring platforms for two kinases, PDK and Akt, leading to Akt and mTOR activation. Both enzymes then phosphorylate different substrates involved in regulating cell cycle entry but also anti-apoptotic proteins [9]. Numerous activating mutations into PI3KCA as well as activation of Akt by genetic mutations, genome amplification or by mutations in upstream signaling components have been reported in human tumors [10]. Among Akt anti-apoptotic substrates are Bad, a BH3-only Bcl2 family member which is sequestered in the cytosol, hence maintained inactive, upon phosphorylation; caspase 9 whose phosphorylation is inactivating; FOXO1, FOXO3A and FOXO4 that are forkhead transcription factors which unphosphorylated, localize in the nucleus and induce the transcription of a wide array of target genes involved in the cell cycle and apoptosis such as CDN1B (p27Kip1) and CDN1A (p21Cip1), Fas-L (TNFL6) and BIM. Phosphorylation leads to FOXO sequestration in the cytosol; and ASK1 (apoptosis signal-regulating kinase 1) which, when phosphorylated, activates c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases, hence inducing apoptosis [11]. mTOR is also a major regulator of autophagy (see below).

A second signaling pathway that is often overactivated in cancer cells is the Ras/Raf/MAPK pathway. Ras is a small GTP protein also activated downstream of the growth factor tyrosine kinase receptor. It then activates the MAP (mitogen activated protein) kinase cascade of which Raf is the first enzyme. "Gain-of-function" mutations in the three genes encoding Ras, in BRAF, the gene encoding Raf, and in downstream transcription factors lead to unregulated proliferation but also in pro-survival signals.

Another example is addiction of cancer cells to NF-κB activation: constitutive activation of this transcription factor is observed in most cancer cells and inhibition of its activity suppresses the growth of these cells [12]. Several mechanisms have been described that explain this persistent activation both from genomic alterations but also as a consequence of the intratumoral inflammation [13]. NF-κB not only regulates the transcription of inflammatory proteins but also enhances the expression of anti-apoptotic proteins amongst which are BCL-xL and several IAPs.

In addition to the overall induction of positive growth signals, tumor cells also suppress proliferation inhibitors. This is achieved by inactivating mutations in tumor suppressor genes. The RB (retinoblastoma) gene was the first to be discovered as an anti-oncogene. RB "loss-of-function" mutations have been detected in various human tumors [14]. The protein Rb (pRb) regulates cell cycle progression by sequestering the EF2 transcription factor needed for cyclin E and A expression. Disruption of this pathway favors cell cycle entry as well as modulates cancer cell sensitivity to different chemotherapeutic molecules: both elevated and diminished sensitivity has been reported [15,16]. The mechanisms underlying these opposite effects are still unclear but may involve checkpoint bypass as well as regulation of chromosomal stability.

A second well described tumor suppressor is PTEN (phosphatase and tensin homologue deleted from chromosome 10). PTEN is a phosphatase that removes phosphate groups from the hyperphosphorylated phosphatidylinositol molecules generated by PI3K, hence reverting the mitotic signal originating from growth factor binding to their receptor. Inactivating methylation of PTEN promoter and disruptive mutations in PTEN gene result in unregulated activation of the PI3K/Akt pathway, hence as mentioned here above in potent survival signaling [16,17]. More and more reports showed that PTEN plays a role in the response of cancer cells to oncoprotein targeting molecules: loss of PTEN leads to both primary and acquired increased resistance [18].

One exception is p53 mutation, that according to the cancer type, may increase or decrease resistance to drug toxicity. p53, the guardian of the genome, is a transcription factor activated upon stresses amongst which is DNA damage, which increases the expression of genes involved in cell cycle arrest (e.g. p21), DNA repair (e.g. GADD45, PCNA) and, if the damage can not be resolved, in the induction of apoptosis (e.g. Bax, PUMA, NOXA, Fas,...). The gene TP53 is the most frequent target of genetic alterations, being mutated in more than half of human tumors [19]. There is evidence that, in addition to favor genomic instability, p53 mutation is also associated with changes in responses to anti-cancer agents since wild-type p53 induced apoptosis in response to these drugs. Hence, in general, studies in vitro in numerous cancer cell lines as well as in patients demonstrated that cells or tumors harboring mutated p53 are more resistant to drugs compared to wild-type p53 cells when treated with a wide variety of molecules (for a review, see [16]) and is associated with treatment failure [20]. This can be explained by the loss of the upregulation of the p53

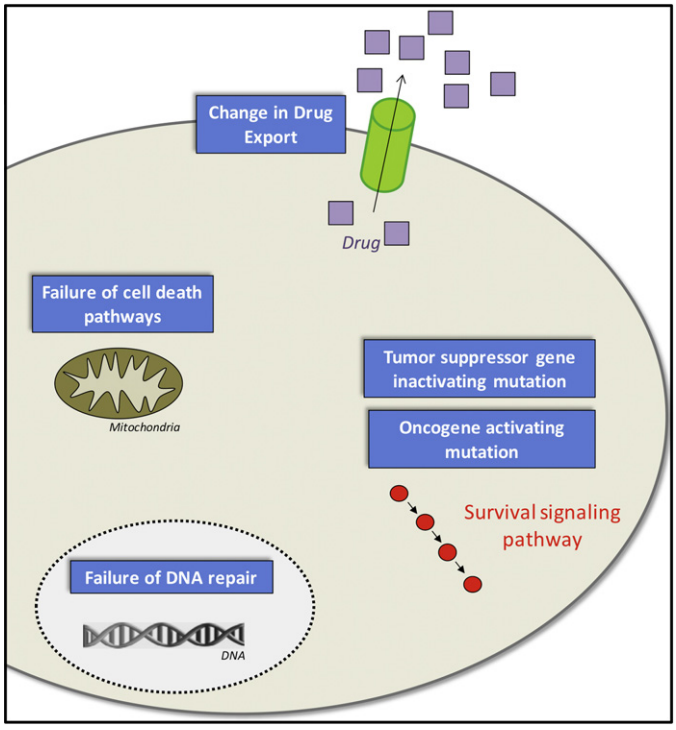

Fig. 1. Overview of drug resistance common mechanisms.

apoptotic target genes. However, other studies also suggest that mutation of p53 can render cells or tumors more sensitive to treatment [21,22]. One hypothesis to explain this discrepancy is that p53 induces cell cycle arrest in response to DNA damaging agents, thus allowing time to repair. In the absence of functional p53, cells progress through the cell cycle with damaged DNA and fails to divide appropriately [23]. Another possible explanation is that some mutations in the DNA binding domain lead to protein stabilization and "gain-of-function" [24]. Therefore, the influence of p53 mutation on chemosensitivity is very complex and may vary according to the type of mutation, to the cellular context and to the class of chemotherapeutics. It is also to be noted that not only mutations in the TP53 gene have been described to regulate p53-dependent induction of cell death. Mutations in the gene encoding several of its regulators, such as amplification in the Mdm2 gene, which encodes a p53 ubiquitin ligase, or inactivation point mutations in the gene encoding $p14^{ARF}$, a Mdm2 antagonist, have been shown to generate a similar phenotype as p53 mutation.

2.2. Changes in drug export

A major process in which cancer cells exhibit reduced sensitivity to multiple unrelated drugs is multidrug resistance. This phenomenon is mediated by multidrug resistance proteins which belong to the ATP-binding cassette (ABC) transporter family. 48 ABC transporters have been identified in the human genome, which are efflux pumps with broad specificity. Substrates include endogenous molecules but also anticancer drugs. Resistance results because increased drug efflux lowers intracellular drug concentration hence, decreasing their intracellular effects [25]. It has however to be noted that in addition to be a drug efflux pump, Pgp is also a regulatory protein that influences diverse cellular processes, such as the p53 network that also plays a role in mediated chemoresistance [26]. To date, at least 15 of these proteins have been characterized to confer resistance to most of the spectrum of currently used anticancer agents. Amongst them, P-glycoprotein (MDR, Pgp or ABCB1), multidrug resistance protein 1 (MRP1 or ABCC1) and ABCG2 are the most frequently associated with multidrug resistance [27]. High expression of the Pgp can be inherent to specialized tissues from which the cancer cells originate. However, overexpression of Pgp as well as of other ABC proteins can be induced by exposure of the cells to the drugs. The upregulation of Pgp expression is complex and may result from different mutational events but also from epigenetic modifications of its promoter [28]. The impact of the induction of this phenomenon on clinical outcome is one of the major hurdles in the treatment of cancer in patients [29].

2.3. Changes in DNA damage response

Maintenance of the genomic integrity is critical to prevent cancer development as indicated by the cancer-prone phenotype of several DNA damage response (DDR) mutants [30]. Genomic instability has been quoted as an "enabling characteristic" by Hanahan and Weinberg [3] which allows cancer cells to accumulate mutations, chromosomal rearrangements and epigenetic changes that, upon Darwinian selection, drive malignant progression. Given these potential dramatic effects, cells have evolved a complex failsafe network of mechanisms aimed at preventing the transmission of damaged DNA during mitosis. According to the type of DNA lesions, several pathways are switched on: base excision repair (BER) copes with single strand breaks (SSB), homologous recombination (HR) and non-homologous end joining (NHEJ) act on double strand breaks (DSB), nucleotide excision repair (NER) takes care of adducts and mismatch repair processes base mismatches, insertions and deletions [31]. DSB are the most lethal lesions that can be repaired in a conservative way by homologous recombination acting only during S and G2 phases while they can be repaired throughout the whole cell cycle by NHEJ in an error-prone manner. It has to be noted that in normal cells, detection of DNA damage evokes a process aimed at inhibiting cell cycle in order to allow DNA repair systems to work but if they fail, apoptosis is activated to eliminate damaged cells in order to prevent precancerous cells to survive.

Faulty DDR mutants not only predispose cells to become transformed cells but also affect chemosensitivity. Most anticancer agents induce DNA damage in order to kill cancer cells. Some DNA damage signaling deficiencies lead to chemoresistance since cell cycle arrest is not triggered upon DNA damage and cells go through the cell cycle unrepaired. This is for example the case for regulators of the ATM pathway [32]. In other cases, increased sensitivity is observed: the cells go through the cell cycle unrepaired and fail to undergo proper chromosome segregation during the mitosis phase and die [33]. This can be mimicked upon specific inhibition of components of the DDR machinery used in combination with DNA damaging agents in order to improve treatment efficacy [34]. However, genetic reversion of DDR defects has been observed in several types of cancer like acute myeloid leukemia of in BRCA-associated ovarian and pancreatic cancers: secondary mutations leading to restoration of protein function and drug resistance do occur [35]. Pathway rewiring may also alter responses of DNA repair-deficient cells to DSB-inducing agents. Suppression of the error-prone NHEJ that results in detrimental mutations in parallel to stimulation of homologous recombination, for example through 53BP1 overexpression or XRCC4 suppression, reverse BRCA1 deficiency-induced chemosensitivity [36]. On the other hand, the dependence on BRCA1-deficient cells on the NHEJ pathway makes them exquisitely sensitive to PARP (poly-ADP ribose polymerase) inhibition. The action of PARP1, the most studied of all the PARP family protein, is essential for repair of SSB, predominantly through the BER mechanism. This is the concept of synthetic lethality, in which the inhibition of two proteins leads to cell death while blockage of one of them has no effect [37]. Targeting the DNA damage response in BRCA1, BRCA2 or ATM deficient cells is thus promising and reached phase 3 clinical trials [38].

2.4. Changes in the apoptosis pathway

Resisting cell death is yet another hallmark of cancer. Numerous studies have shown that programmed cell death serves as an obstacle to cancer development by eliminating damaged cells. Apoptosis can be initiated by the extrinsic pathway mediated by death receptors on the cell surface or by the intrinsic pathway whereby the BH3 members of the Bcl2 protein family function as damage sensors that transmit pro-apoptotic signals to the mitochondria. These signals lead to increased mitochondrial permeability, release of cytochrome c and caspase activation. Caspases cleave cellular substrates leading to the typical morphological and biochemical changes observed in apoptotic cells. Tumor cells have developed a vast array of strategies to limit or circumvent programmed cell death. Two non exclusive approaches have been described: overexpression of anti-apoptotic proteins like Bcl-2, Mcl-1, FLIP, IAPs,... and inactivation of pro-apoptotic genes such as mutations in genes encoding caspases [39], in pro-apoptotic Bcl-2 members, e.g. Bax or alterations in the p53 pathway (reviewed in [40]).

These defects non only favor tumor growth but also render cancer cells resistant to therapy. Indeed, most of the anti-cancer therapies, either using drugs or radiations, kill cancer cells mainly by inducing apoptosis. The knowledge of the mechanisms that cancer cells have evolved to evade apoptosis is now used to design

strategies to overcome apoptosis resistance. The challenge will be to translate this into efficient but non toxic clinical applications [41]. Examples of such strategies are the use of BH3 mimetics aimed to antagonizing the anti-apoptotic Bcl-2 proteins [42], reactivation of the p53 pathway either with molecules interacting directly with p53 or by inhibiting Mdm2 [43], or via TRAIL death receptor activation [44]. Moreover, another mechanism involving polymorphism of the host has been recently uncovered: a germline deletion of the second intron of BCL2L11, encoding the BH3-only protein Bim, which impairs the generation of the protein [45]. Deficiency in Bim expression following targeted therapy was already known to lead to lack of sensitivity (reviewed in [46]).

Over the last years, the concept of programmed cell death has been broadened to other types of cell death including necroptosis, senescence and autophagy. Autophagy is a self-degradative pathway that enables cells to cope with stresses. It delivers portion of cytosol, possibly including organelle(s), to the lysosome via its inclusion in a double membrane vesicle. It is primarily a pro-survival pathway but it can also sustain or counteract apoptosis and vice versa [47]. Alterations in the autophagy pathway have been shown to exert tumor suppressive or tumor promoting function according to the tumor development stage. Furthermore, recent data showed that autophagy is also triggered by chemotherapeutic drugs, mostly participating to cell resistance rather than to cell death [48]. Therapeutic interventions using autophagy inhibitors, but also autophagy activators, as a way to improve the cell killing efficacy of chemotherapy have emerged. However, more work is still needed to use autophagy manipulation in cancer therapy in regard to this apparent contradictory role of autophagy [49].

3. Mechanisms specific to one (one class) of drug(s) (Fig. 2)

3.1. Specific mechanisms of resistance to anti-metabolites

An antimetabolite molecule is a chemical that inhibits the use of an endogenous metabolite. Such substances are often similar in structure to the metabolite that they interfere with, such as the antifolates, that block the metabolism of folic acid, or 5-fluorouracil that stops thymidine synthesis by inhibiting thymidylate synthase, thus selectively inhibiting DNA over RNA synthesis. These molecules interfere with DNA production and therefore cell division and the growth of tumors are halted.

Folate as well as anti-folate molecules like methotrexate or pemetrexed, are selectively imported into cells via specific transporters, predominantly the proton-coupled folate transporter (PCFT/SLC46A1). This influx is facilitated in cancer cells since the tumor microenvironment is often acidic. However, qualitative, i.e. inactivating mutations, or quantitative, i.e. allele loss, decreased expression or silencing due to epigenetic changes or loss of function of transcription factors, alterations in this transport but also in the other proteins involved in folate influx or efflux lead to antifolate resistance. Increased expression of specific metabolizing enzymes like gamma-glutamyl hydrolase (γ-GH) as well as overexpression of dehydrofolate reductase or thymidylate synthase and mutations that decrease their affinity toward antifolates also result into decreased sensitivity toward these molecules. These processes are thoroughly reviewed in Gonen and Assaraf [50].

Thymidylate synthase is also a target for pyrimidine analogs like 5-fluorouracil or gemcitabine. Since the basis of the action of these anti-cancer agents is thymidylate synthase inhibition, the expression level of this enzyme is very important for setting the level of sensitivity. Hence tumors with low levels of thymidylate synthase are generally more sensitive. However, it should be noted that it is not always the case [51]. The presence of mutations in the coding sequence of this enzyme may also alter the degree of inhibition reached by similar levels of 5-fluorouracil [52]. Finally, since 5-fluorouracil is metabolized by dihydropyrimidine dehydrogenase (DPD), a reduction in its efficiency is also observed in tumors with high level of this enzyme [53].

3.2. Specific mechanisms of resistance to platinium derivatives

Cisplatin and its derivatives are made of a heavy metal complex containing platinium that form adducts with macromolecules, mainly DNA. DNA-induced damages prevent DNA synthesis and RNA transcription, finally leading to the induction of cell death mainly through apoptosis. Cytoplasmic targets also participate to the induction of cells through ROS production and depletion in reducing equivalents. The cytotoxic potential of cisplatin may be dampened by pre-target processes including reduced accumulation of the molecule and an increased sequestration by GSH. The first mechanism is associated with increased expression of efflux pumps but also with reduced uptake via downregulation or internalization of the plasma membrane copper transporter CTR1 while the latter results from acquired elevated GSH concentration via the induction of enzymes that catalyze GSH synthesis.

Two non-exclusive strategies have been evidenced in cancer cells that explain on-target resistance. The first one is an increased DNA adduct repair capacity: overexpression of XPA or ERCC1 (increased NER proficiency) or of BRCA1 (increased HR efficacy) has been detected in cisplatin resistant cancer cells. On the other hand, the second mechanism is ascribed to the loss of propagation of the DNA damage signal to the apoptotic machinery leading to replicative bypass, DNA damage tolerance and enhanced cell survival. This is well illustrated by the hyperresistance of cells harboring defects in mismatch repair genes such as MLH1 or MSH2. These mechanisms are described in details in the reviews from Galluzzi et al. [54] and Siddik [55].

3.3. Specific mechanisms of resistance to spindle poisons

Spindle poisons can be classified into two categories: the ones that stabilize microtubules like taxanes and epothilones and the others that destabilize microtubules such as vincristine and vinblastine. Binding of these molecules to β-tubulin thus disturbs microtubule dynamics needed to disassemble cytoskeleton upon cell division and to form the mitotic spindle that insures correct chromosome segregation. They exert their anti-cancer activities through activation of spindle assembly checkpoint thereby arresting cells in mitosis. Mitotic arrest then activates apoptosis pathway via the degradation of Mcl1, an anti-apoptotic member of the Bcl2 family, mediated by its JNK-dependent phosphorylation in a timely dependent fashion [56], as well as via the inhibition of Bcl2 which is also mediated by JNK phosphorylation. Cancer cells may however evade tubulin-binding agents by several mechanisms. The expression of the different β-tubulin isoforms is dysregulated, for example through the overexpression of βIII-tubulin that displays less affinity for the drugs. Mutations in the predominant β1-tubulin gene have also been evidenced in vitro and recent evidence show that they may also occur in patients [57]. Alterations in proteins that regulate microtubules such as stathmin, Tau and MCAK (mitotic centrosome associated kinesin) are also implicated in drug resistance [58,59]. Dysfunctional regulation of proteins involved in the spindle assembly checkpoint, such as high expression of the kinases Aurora, as well as in apoptotic signaling have also emerged as putative mechanisms of resistance to tubulin-binding agents [60].

Besides alterations in microtubule physiology, the HER2 signaling cascade is also a mechanism for escape from spindle poison cytotoxicity: enhanced survival, decreased apoptosis, drug

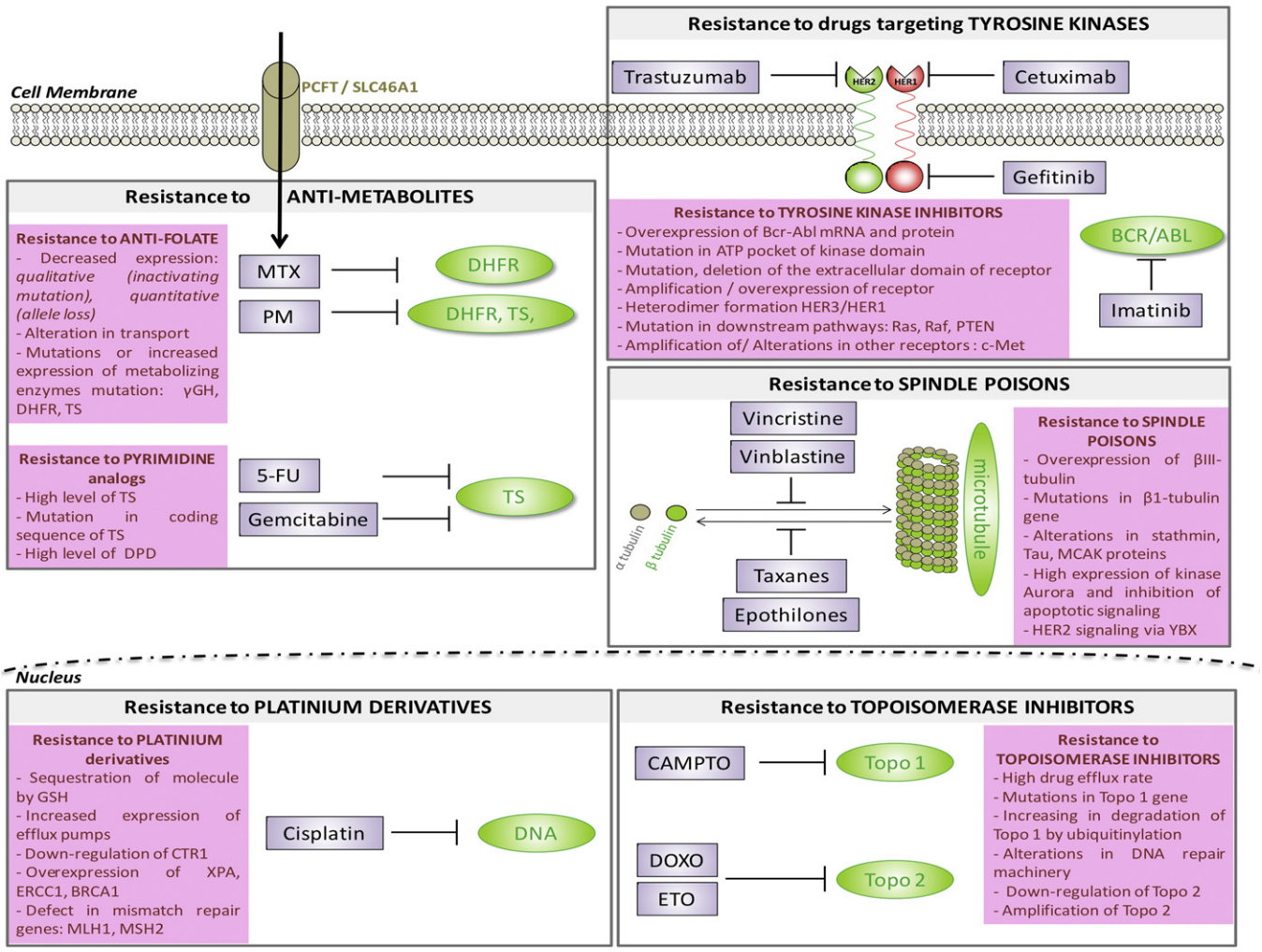

Fig. 2. Intracellular resistance mechanisms to specific class of drug(s). Drugs are symbolized by purple rectangles. Their targets are represented by green ovals. Resistance mechanisms are listed for each class of drugs in pink rectangle. *Abbreviations*: CAMPTO, camptothecin; DPD, dihydropyrimidine dehydrogenase; DHFR, dehydrofolate reductase; DOXO, doxorubicin; ETO, etoposide; γ-GH, γ-glutamyl hydrolase; GSH, glutathione; MTX, methotrexate; PM, pemetrexed; TS, thymidylate synthase; Topo 1/2, topoisomerase 1/2; 5-FU, 5-fluorouracil.

efflux and drug metabolism are contributing to this phenomenon [61]. Furthermore, the transcription factor YBX (Y-box binding protein-1), activated via the signaling pathways initiated at the HER2 receptor, determines a positive feedback loop on the activation of the receptor, hence sustaining the cell survival. This has been observed not only in breast cancer [62] but also in myelomas [63].

3.4. Specific mechanisms of resistance to topoisomerase inhibitors

Topoisomerases are essentially involved in the control of DNA topology. These enzymes can be categorized into two groups according to the fact that they catalyze the breaking and re-ligation of one (type I) or two strands of DNA (type II). Topoisomerase key function is the relaxation of supercoiled DNA ahead of the replication fork and of the RNA polymerase during transcription but also during DNA repair. Topoisomerase 1 inhibitors include camptothecin and its analogs that prevent the re-ligation, hence leading to single strand DNA breaks. Topoisomerase 2 poisons like etoposide and doxorubicin generate increased levels of topo2-DNA covalent complexes, arresting DNA replication and RNA transcription, eventually leading to double strand DNA breaks. Similar to other chemotherapeutic drugs, ineffective cellular uptake and/or high efflux rate could be responsible for drug resistance. Furthermore, distinct mutations in the topoisomerase 1 encoding gene lead to resistance specific to the drugs targeting this enzyme. Enhanced degradation of the topoisomerase 1 via ubiquination in the ternary complex enzyme-DNA-drug has also been evidenced. Finally, alterations in the DNA repair machinery largely influence cancer cell sensitivity to topoisomerase 1 inhibitors (for an extensive review, see [64]). Besides augmented drug efflux, the main modality of resistance to topoisomerase 2 inhibitors is topoisomerase 2 downregulation (reviewed in [65]). Nevertheless, topoisomerase 2 amplification has also been reported, but in many if not all cases, it is associated to ERBB2 amplification. Co-amplification is correlated with enhanced sensitivity to different topoisomerase 2 poisons [66].

3.5. Specific mechanisms of resistance to drugs targeting tyrosine kinase receptors or other signaling kinases

Several tyrosine kinase receptors responsible for dysregulated proliferation of cancer cells are direct targets of specific "smart drugs". These therapies include kinase inhibitors like imatinib and gefitinib, monoclonal antibodies specific for the receptor itself, such as cetuximab or trastuzumab, or for the ligand, such as bevacizumab that targets VEGF. Recently, an inhibitor of Raf, an intermediate of the signaling cascade, has also been developed,

vemurafenib. The first therapeutically successful treatment was the use of imatinib in chronic myeloid leukemia patients. Imatinib inhibits the kinase resulting from the fusion of Bcr and Abl genes. The primary cause for relapse in patients is due to the reactivation of the kinase due to point mutation in the ATP binding domain of the kinase that prevents imatinib binding [67]. On the other hand, in vitro, resistance is most often related to Bcr-Abl mRNA and protein overexpression.

Mutations that modify receptor-inhibitor interactions resulting from point mutation, deletion of the extracellular domain or alternative splicing have also been described for other tyrosine kinase receptors (for a review, see [68]). The EGFRvIII variant is such an example: it results from an in-frame deletion of 267 amino acids in the extracellular domain, resulting in a constitutively active protein [69]. Other mutations in the EGFR coding sequence have also been described that all lead to constitutive activation. Amplifications and/or overexpression of the targeted receptor may also occur that contribute to escape inhibitor-induced cytotoxic effects. Contrariwise, downregulation of VEGFR2 upon VEGFR signaling blockers has been observed in tumor endothelial cells, leading to loss of their VEGF dependence [70]. The use of other receptors to transphosphorylate the inhibited tyrosine kinase receptor via the formation of dimers other than HER2/HER2 also stems for resistance, e.g. elevated EGFR/HER3 or EGFR/EGFR dimers is responsible for a loss of trastuzumab sensitivity. The resistance to inhibitors may also be mediated by mutations not in the receptor itself but in downstream effectors like K-Ras, Raf or PTEN, rendering the cells insensitive to the inhibition of the upstream receptor. Combining inhibitors of both the receptor and of the downstream signaling protein may alleviate this resistance. Resistance due to the induction of alternative/compensatory signaling pathways via the upregulation of other receptors like Met, IGF-1R or PDGFRA is also frequently observed in patients [71]. Expression of an abnormal spliced B-Raf lacking exons 4–8 that aberrantly dimerizes and that is resistant to vemurafenib inhibition has been reported [72]. Ras mutations may also render cells resistant to tyrosine kinase receptor inhibition. Finally, tumor cells may also hijack stromal cells to secrete growth factors like HGF that will compensate for their inhibited addiction pathway in a paracrine manner [73,74]. The identification of these complex mechanisms must be pursued in order to be translated into effective new therapies.

4. Role of the tumor environment

4.1. Tumor hypoxia affects chemosensitivity

A compelling body of evidence indicates that most of human solid tumors contain hypoxic areas [75]. Hypoxia is the consequence not only of the chaotic proliferation of cancer cells that place them at distance from the nearest capillary but also of the abnormal structure of the new vasculature network resulting in transient blood flow [76]. Oxygen deficiency triggers the activation of hypoxia-specific transcription factors, the HIFs (hypoxia-inducible factors), that regulate the expression of genes whose products help cells and tissues to cope with this stress. Not only HIFs are part of the cancer cell arsenal aimed to increase their survival but they also mediate drug resistance [77]. Metabolic changes, multidrug resistance protein overexpression, inhibition of apoptosis, induction of autophagy and inhibition of DDR are part of the cellular reprogramming triggered by HIFs that compromises the effectiveness of chemotherapy [78].

Hypoxia is a pivotal driving force of malignant progression [79]. Furthermore, hypoxia-induced resistance and escape compel cancer cell to metastasize by selecting more resistant cells but also through the upregulation of genes involved in each step of the metastasis process. Indeed, HIFs are master regulators of the expression of genes implicated in epithelial-to-mesenchymal transition (repression of β-catenin), in cell migration (increase in MMP2, in uPAR), in homing (increase in CXCR4 and in its ligand, SDF1) and in the establishment of the pre-metastatic niche (increase in LOX) (for a detailed review, see [80]). Since patient survival rate is closed related to the development of distal secondary tumors, association between hypoxia, resistance and the development of metastatic disease represents a significant obstacle to successful treatment.

4.2. Influence of stromal cells

The tumor is not only constituted of cancer cells but also of different types of stromal cells and many investigations support the notion that tumor stromal cells play important roles in tumor initiation, progression, metastasis as well as resistance to treatments. Cancer-associated fibroblasts (CAFs) make the bulk of the cancer stroma [81]. Furthermore, the induction of angiogenesis brings in not only endothelial cells but also immune cells. Amongst them, tumor-associated macrophages (TAMs) and the related inflammation represent the 7th hallmark of cancer [82]. Indeed, TAMs contribute to malignant cell survival and proliferation, to angiogenesis as well as to metastasis [83]. Adaptive and reciprocal signaling dialog between tumor cells and their surrounding environment also contributes to drug resistance [84]. Cytokines and growth factors secreted by TAMs, CAFs or tumor vasculature endothelial cells participate to this phenomenon. This has been demonstrated in vitro but also in vivo in patients for TAMs [85] and CAFs [86]. Cell adhesion-mediated resistance is also arising from the attachment of cancer cells to stromal cells or the components of the extracellular matrix [87]. Multiple interaction mechanisms are also evoked to explain escape from targeted therapies not only from the tumor cells but also from the endothelial cells upon treatment with angiogenesis inhibitors [70].

5. Role of cancer stem cells

Cancer stem cells (CSCs) are defined by their capacity to self-renew, differentiate as well as regenerate and propagate a malignant cell population when injected in vivo [88]. CSCs have been originally discovered in leukemia but now they have also been isolated from most human solid cancers. A level of hierarchy is maintained, and similar to normal stem cells, CSCs give rise to transit-amplifying cells that are more differentiated and do not have the ability to regenerate a tumor, to finally produce the bulk of cancer cells. If the tumors develop from CSCs, these cells are the cells that need to be killed upon treatment to eradicate the tumor. If some, even a few, are left intact, they will be responsible for tumor relapse. Not only all of them must be eliminated but it seems that CSCs are equipped with specialized defenses against anticancer agents, that might explain drug-resistant residual disease [89]. CSCs, like normal stem cells, express high level of ABC transporters, such as Pgp and ABCG2 [90]. Moreover, relative quiescent, active DNA-repair capacity and resistance to apoptosis are other stemness-related properties that confer drug resistance [91]. This is particular true since two recent reports demonstrated that stemness property is dynamically regulated and reactivated in "apparently" non CSCs [92] and that more than one fourth of melanoma cells can generate tumors in severely immunocompromised mice [93]. This suggests that CSC population may be renewable.

In addition to the inherent insensitivity of CSCs to chemotherapy, interactions of CSCs with their microenvironment including cellular and extracellular matrix components of this so-called

niche, may also contribute to drug resistance [94]. A better understanding of these CSC-niche interactions may help to device new strategies to kill CSCs.

6. Conclusion and perspectives

Cancer cells develop multiple and complex mechanisms to evade drug induced cytotoxicity. Furthermore, the interaction of cancer cells with their microenvironment also influences treatment outcome. This complexity was well exemplified by Beier et al. [95] for glioblastomas. Chemoresistance therefore represents a significant impediment to successful cancer therapy. A better understanding of these mechanisms is thus a medical need that requires to be met. The development of pharmacologic inhibitors impinging on these pathways indeed opens novel opportunities for the design of new and more efficient therapeutic strategies for patients. However, the example of targeting ABC transporters has failed until now due to high toxicity [96]. Combinations of several drugs targeting different pathways still represents the new highway since it may avoid secondary resistance and/or compensatory rewiring and increase efficacy. The crucial importance to target CSCs, that are more refractory to treatment, to achieve cure is to be underlined, for example by taking the opportunity that developmental signaling pathways, like Wnt, Notch and Hedgehog, are reactivated in these cells [97].

Two recent works established a huge set of data available to search for new mechanisms of chemoresistance and for drug discovery. On one hand, the Cancer Cell Line Encyclopedia compiled extensive data on 947 cancer cell lines regarding gene expression and sequencing data completed with data of their drug-sensitive profiles to more than twenty different anticancer drugs [98]. On the other hand, Garnett et al. [99] screened several hundred cancer cell lines for their sensitivity to 130 drugs, in parallel with their expression profile and genetic abnormalities Complementarily, in order to overcome the limitation of cancer cell lines, genomic and cDNA sequencing of cancer specimens is undertaken [100]. Another line of future investigations is the exploitation of the knowledge that host factors intervene as determinants of efficacy and resistance. New immunotherapies taking into account these factors may prove useful, particularly for metastatic patients.

All these efforts aimed in the same direction: personalized medicine. For this, identification of predictive biomarkers and/or gene expression profiling (“signature”) will help to stratify patients who would benefit from one or another drug. The battle with cancer cells is not yet won but extensive research must go on to achieve the ultimate goal, i.e. to cure the patients.

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