Published in final edited form as:

Trends Pharmacol Sci. 2017 May ; 38(5): 427–437. doi:10.1016/j.tips.2017.02.001.

A New View of Pathway-Driven Drug Resistance in Tumor Proliferation

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Abstract

Defeating drug resistance in proliferation is challenging. Here we propose unifying signaling in cell proliferation with two core pathways, each embodying multiple alternative pathways. We consider drug resistance through an alternative proliferation pathway – within the same or within the other core pathway. Most drug combinations target only one core pathway; blocking both can restrain proliferation. We define core pathways as independent and acting similarly in cell cycle control, which can explain why their products (e.g. ERK and YAP1) can substitute each other in resistance. Core pathways can forecast possible resistance because acquired resistance frequently occurs through alternative proliferation pathways. This concept may help predicting the efficacy of drug combinations. The selection of distinct combinations for specific mutated pathways would be guided by clinical diagnosis.

Keywords

KRAS; K-Ras; RAS; MAPK; PI3K; signaling pathways

The Hallmarks of Cancer and the General Scheme of Drug Resistance

The National Cancer Institute lists not only cancer drugs but also drug combinations (<https://www.cancer.gov/about-cancer/treatment/drugs>). These are also discussed in numerous publications and pharma releases; yet, despite the almost routine deployment of drug combinations in chemotherapeutic regimens, the critical underlying question of *how to select the combinations of pathways to target – among the many possible* – has been largely overlooked.

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In classical publications, Hanahan and Weinberg [1] outlined the eight hallmarks underlying the organizing principle of cancer: sustaining proliferative signaling, evading growth suppressors, reprogramming of energy metabolism, inducing angiogenesis, enabling replicative immortality, resisting cell death, evading immune destruction and activating invasion and metastasis. All hallmarks are required for tumor growth and development; and the first five are directly or indirectly involved in cell proliferation. Drugs successfully striking the hallmarks – even one of them – with lasting effects and acceptable toxicity may treat cancer. Blocking angiogenesis, a requirement for solid tumor progression, has proven challenging [2]. An inherent bypass or parallel pathway might be responsible for its drug resistance. The *general scheme of drug resistance* (outlined in Box 1) formulates mechanisms through which treatments can fail. Within this *drug resistance* framework, Figure 1 enumerates resistance mechanisms related to tumor proliferation – which is our focus – and the corresponding therapeutic strategies. Thus, here, drug resistance is restricted primarily to the root of the cell proliferation hallmarks. *A priori* blocking drug resistance pathways by drug combinations may forestall resistance [3]. The critical question facing cancer treatment today is how to prioritize combinations with clinical relevance.

Box 1

The General Scheme of Drug Resistance

Drug resistance mechanisms (Figure I) that relate to prevention of tumor cell death and tumor proliferation include increased rates of drug efflux, DNA damage repair, alterations in the tumor microenvironment and in drug metabolism, emergence of cancer stem cells, mutations of drug targets, and cell death inhibition [81–83]. Drug efflux [83, 84], a fundamental drug resistance mechanism that works by preventing drug action on its target node, can be promoted by several membrane transporter proteins. Among these, the energy-dependent multidrug efflux pump P-glycoprotein (P-gp) is a major player [84]. In DNA damage repair [85], DNA damage by chemotherapy can be direct (e.g. by platinum-based drugs) or indirect (e.g. topoisomerase inhibitors), eventually leading to cell death or senescence. DNA damage repair and warding off cell death induce cell cycle arrest, which allows repairing the damage. In cancer, cell cycle arrest is disrupted by gain/loss-of-function mutations such as in p53. At least one DNA damage repair pathway is often deregulated. Tumor microenvironment [86] and drug metabolism [82] can be involved via oxidative stress. Resistance may also be metabolic and stromal phenomena [87]. Stem cells display higher levels of drug efflux, DNA damage repair, anti-apoptotic, and pro-survival proteins [88–90]. Mutations [82, 88–90] can take place downstream or upstream of the drug target in the same pathway or in parallel pathways, resulting in activation of proliferative signaling [32], and cell death inhibition [81–83]. Of note, drug resistance mechanisms through signaling includes pathway reactivation which can take place through mechanisms such as alternative signaling, pathway rewiring or crosstalk, with these not necessarily restricted to a certain hallmark of cancer.

By contrast, here we restrict ourselves to pathways in tumor proliferation. Further, different from earlier pathway-related mechanisms, here we propose that two core pathways converge in cell cycle regulation and that drug resistance can take place either through an alternative pathway within the same core pathway (which is not new), or

within a different core pathway (which is). Each core pathway consists of multiple alternative pathways. To date, drug combinations appear to have largely targeted only alternative pathways within a single core pathway.

Figure 1.

A schematic diagram providing an overview of mechanisms of pathway-driven drug resistance to chemotherapeutics. In principle, cancers result from uncontrolled cell proliferation and dysfunctional regulation of cell death. Three pathway-driven proliferation scenarios (indicated by blue, green, and yellow activation arrows respectively originating from ligand-bound activated receptor to downstream effect of cell growth and proliferation) and two death pathways (intrinsic DNA damage and extrinsic apoptosis, indicated by red arrows) delineate the major drug resistance mechanisms in targeted therapy and in classical chemotherapy. We consider three pathway-driven proliferation scenarios that can emerge in drug resistance: (1) intrinsic (via feedback loop) or acquired (upregulation of a transporter, purple arrows) resistance; (2) whether the resistance takes place within the same pathway or an alternative parallel pathway; and (3) whether the first (early G1 cell cycle phase) or the second (late G1 cell cycle phase) *core* cancer-driven proliferation pathways is involved. Both are required for the transition G1→S in the cell cycle. The two core pathways are shown to initiate transcription. Red ovals denote ligands, and blue hexagons represent drugs; cytoplasmic protein nodes participating in the pathways are in red, transcription factors in blue. Purple color designates drug resistance.

So how to select the combinations of pathways to target among the many possible? Drug combinations often aim at “redundant pathways” for preventing or delaying the development of resistance to treatment. We propose that to be effective, the definition of “redundant pathways” needs to consider the hallmarks of the organizing principle of cancer, and of drug

resistance to restore these hallmarks in therapeutics (Box 1). It should also be guided by the clinics: among the many possible mutated receptors, such as receptor tyrosine kinases (RTKs), only clinical observations can pinpoint *which specific* protein or pathway is involved. Our concept is based on literature reports, which together lead to a powerful model of signaling-driven tumor proliferation (e.g. [4–20]). The landscape of drug resistance is extremely complex, the hurdles immense and much is not understood. Still, like Vogelstein et al. [21], we believe that already now, our knowledge of cancer genomes and signaling in cancer cells can guide and inspire the development of more effective approaches for reducing mortality. Below, even though we overview drug resistance mechanisms (Box 1), our aim is not a broad coverage of drug resistance and therapeutics from the experimental standpoint, such as delivery and dosage. Instead, we aim to take steps toward understanding the underlying principles of why certain drug combinations work – whereas others do not.

The Concept: Restraining Proliferative Growth, the First Hallmark of Cancer

Different than other approaches, we view pathways in terms of their actions at the critical restriction point-of-no-return in the cell cycle [22–24]: the passage from the G1 (Gap 1) to the S (Synthesis) phase. Some pathways act at the early stage of G1; others at the late. Passing through both stages is required for cell growth and division. We reason, and provide observations in support of, that drug resistance stems from a constitutively activated parallel pathway, enabling passage through both stages. We propose that there are two – independent and corresponding – cancer-promoting *core pathways* that accomplish this passage. We define a *core pathway* as encompassing two signaling pathways, one acting in the early G1 stage, the other in the late. Medication platforms typically target and block ‘driver’ genes (genes with mutations that drive cancer); we suggest selecting those inhibiting a *core pathway* and thereby proliferation. Data confirm that resistance can take place through pathways executing similar cell cycle actions (e.g. [4–20]). Thus, *a priori* inhibiting pathways acting at the early or late G1 stage can derail cell cycle progression. Unlike irradiation or chemotherapy, halting pathway-driven proliferation might not always induce cancer cell death, but will restrain tumor size. Based on the oncogenic addiction paradigm [25], once proliferation has been stopped, cells can enter senescence [26] or quiescence (G0 phase in the cell cycle). Unless resumed, the likely (though not necessarily) outcome is apoptosis [26]; i.e. cell death due to suppressed expression or activity of survival factors. Figure 2 portrays the concept emphasizing proliferation as a fundamental hallmark of cancer linking it to G1 cell cycle stages.

Pathway-Based Drug Resistance Mechanisms

Acquired (and innate) resistance can take place through pathway reactivation [3, 27–29]. Box 1 describes the four signaling pathways-related mechanisms of drug resistance. Targeted therapy aims to block oncogenic proteins in a cancer-driven pathway. Drugs enter either through passive diffusion or uptake by a transporter; thus, the first drug resistance mechanism can be intrinsic or acquired upregulation of a transporter to increase the efflux rate across the plasma membrane. Because oncogenic proteins constitutively turn on proliferation pathways, the second resistance mechanism involves circumventing drug action either by an acquired second mutation to reduce drug binding or by overexpression of the

target protein to outnumber the drug molecules. Overexpression can take place either via an intrinsic feedback loop or an acquired alternative gene expression pathway. In the third mechanism cell growth and proliferation is reactivated either by an alternative *core proliferation pathway* or by a parallel pathway within the same *core pathway*. The fourth mechanism elicits acquired loss-of-function that triggers programmed cell death, or activation of a survival factor outweighing initiation of cell death or of an inhibitor blocking cell death. Both can be downstream branches of the proliferation pathway.

Therapeutic Scenarios to Counteract Pathway-Based Resistance

Therapeutic efforts often focus on inhibition downstream of the oncogenic protein (Figure 3). This follows the rationale that drug resistance via pathway reactivation is more likely to take place upstream. Examples include MEK and ERK inhibitors to accompany Raf inhibitors in *BRAF*-mutant melanomas [30–32]. Even though downstream pathway inhibition may effectively suppress resistance driven by pathway reactivation, it may also promote resistance driven by alternative pathways. ERK1/2 (particularly in the intestine) and ERK5 provide an example. Deletion of ERK1/2, or inhibition of MEK1/2, leads to enhanced activity of the ERK5 pathway. Targeting both pathways causes a more effective suppression of cell proliferation in colorectal cancer (CRC) cell lines, pointing to ERK5 as a bypass rescuing MAPK signaling [33]. Pathway-activating mutants in the Ras-MAPK, Notch1, PI3K-mTOR, and ER (estrogen receptor) signaling pathways [32], conferred resistance to some clinically relevant therapies through alternative upstream pathways. Activation of the Notch1 pathway promoted resistance to tamoxifen (an ER-targeted therapy), and γ -secretase inhibitor, which inhibits Notch signaling, and restored tamoxifen sensitivity. Likewise, Notch1 pathway activation promoted acquired resistance to MAPK inhibitors in *BRAF*^{V600E} melanoma cells, which suggested that Notch1 signaling may be a target in certain drug-resistant breast cancers and melanomas. Taken together, both downstream and upstream inhibition strategies have merits and shortcomings.

The complex, intrinsic pathways-based drug resistance mechanisms, which vary across cells, hamper the identification of the distinct pathways [34]. Bypass mechanisms can turn on (or off) a downstream effector usually through phosphorylation. Resistance to gefitinib by reactivation of PI3K/Akt signaling through activation of MET or insulin growth factor-1 receptor (IGF-IR) signaling in EGFR mutant non-small cell lung cancer (NSCLC) is one example [29, 35, 36]; resistance to vemurafenib in *BRAF*^{V600E} cells in melanoma where upregulated COT kinase promotes ERK activation is another [28]. Overexpression of RTKs PDGFRA and IGF-IR may also confer resistance to vemurafenib [37, 38]. Additionally, inhibition of a pathway node may relieve feedback control to reactivate the pathway; for example, TOR inhibitors activate PI3K/AKT [39], as do MEK inhibitors [40].

Drug combinations targeting the same protein, pathway or different pathways have been challenging. We suggested “pathway drug cocktails” aimed at redundant pathways [41], a classified repertoire of which could be compiled. However – *how* to determine these has not been clear, nor did they relate to fundamental cancer processes.

Support for Core Pathways-Based Drug Cocktails

Our basic premise is that oncogenic signaling pathways should be perceived in terms of the mechanisms through which they act, including the stage in cell life (e.g. division, growth). A focus on cell cycle is not new. Studies on normal intestinal epithelial cells (IECs) indicated a correlation between ERK1/2 activation and G1/S phase transition, whereas pharmacological or molecular inhibition of ERK1/2 abrogated cell proliferation [24, 42–44]. We classify normal proliferation pathways according to the cell cycle stage that they act in, and suggest that when one pathway is inhibited a corresponding and independent one can take over. This bestows the ability to act prophylactically.

Analysis of published reports [22, 23] reveals that there are two *core pathways* that act similarly and independently in cell cycle control; together, they additively drive tumor initiation. The first is MAPK (Raf/MEK/ERK) and PI3K/Akt/mTOR; this *core pathway* acts mainly via phosphorylation. The second *core pathway* encompasses Hippo (YAP1) and WNT (β -catenin, or other pathways that lead to the expression (or activation) of Myc such as Notch, Hedgehog, etc.). This *core pathway* acts through direct transcription regulation. Both *core pathways* act at the G1→S restriction point; ERK in the first *core pathway* and YAP1 in the second act in the early, cell cycle reentry stage from the quiescent (G0) state to G1. PI3K in the first *core pathway* and β -catenin (or Notch, Hedgehog, or eukaryotic translation initiation factor 4E (eIF4E), etc.) in the second operate in the late stage of G1. The actions in the early + late stages prompt the G1→S progression (Figure 2). This correspondence and independence in the *core pathways* suggest that blocking ERK can be compensated by overexpressed/mutated YAP1; and blocking YAP1 can be overcome by overexpressed/mutated ERK. Similarly, inhibition of PI3K can be overcome by oncogenic β -catenin and *vice versa*; both act via the protein Myc. Inspection of the literature vindicates this notion: drug resistance to ERK is executed by overexpressed YAP1; drug resistance to PI3K can involve β -catenin (Notch, Hedgehog, eIF4E). Drug resistance to K-Ras – which involves both MAPK and PI3K – can bear mutations in both YAP1 and Myc pathways. Together, these can result in more aggressive cancers [45]. In line with this, YAP1 can rescue B-Raf inhibition (both execute the early G1 reentry step), whereas β -catenin can rescue PI3K (both act in the late stage to progress into the S phase) but not B-Raf, which functions at the G1 reentry stage. Thus, our thesis is that to exit quiescence and pass through the G1→S restriction point a *core pathway* must have both early and late actions. For the two *core pathways* there are four such combinations (Figure 2).

Hence, signaling cues may vary: hormone/growth factor-stimulated receptors (in receptor tyrosine kinase (RTK)-Ras), or mechanical cell-cell contact (Hippo/YAP1 and WNT/ β -catenin). However – irrespective of the cue and specific *core pathway* – cell cycle reentry and progression into the S phase will take place with the consequences unchanged, which can explain why YAP1 and β -catenin are observed in analyses of drug resistance in K-Ras4B cancers [8, 9, 20, 22, 23]. Recent studies of colorectal cancer support the independent and corresponding roles of PI3K and β -catenin in the cell cycle G1 phase. Overexpressed β -catenin can defeat PI3K and Akt inhibition [46]. Inhibition of MAPK substrate tankyrase (TNKS) in resistant CRC together with PI3K/Akt inhibitors quells tumor growth [47]. However, even though in WNT and *KRAS*-driven CRC cells inhibition of β -catenin

collaborates with K-Ras inhibition, it is not the case in cells carrying *BRAF* mutations [48], in line with *BRAF* mutations corresponding to YAP1. In another example [49], genetic screening identified YAP1 as the reason for resistance in Raf inhibitor vemurafenib-treated B-RAF^{V600E} cancer cells. At the same time, suppressing YAP1 promoted sensitivity to Raf and MEK inhibitors in diverse tumors, including lung, melanoma, colon, thyroid and pancreas. Inhibiting both YAP1 and Raf (or MEK) is effective in *BRAF*- and *RAS*-mutant tumors [20]. PI3K/mTOR inhibitor treatment activates MSK1 (mitogen- and stress-activated protein kinase 1). MSK1 phosphorylates β -catenin at Ser552 and regulates its nuclear translocation and transcriptional activity. The inhibition of MSK1 and PI3K/mTOR decreased β -catenin phosphorylation [50]. Lung carcinoid and somatostatinoma were treated with either the pan-PI3K inhibitor, BKM120, the dual PI3K-mTOR inhibitor, BEZ235, or one of these in combination with the MEK inhibitor, PD0325901. BKM120 and BEZ235 decreased proliferation and increased apoptosis; combination with PD0325901 significantly enhanced the effects of either alone, without systemic toxicity [51], all in line with the two *core pathways* model. Expression and transcriptional activity of β -catenin increased in tamoxifen-resistant cells and were inhibited by β -catenin small-molecule inhibitor, suggesting that β -catenin plays a role in tamoxifen-resistant breast cancer [52]. Similarly, oncogenic K-Ras signaling promotes the WNT/ β -catenin pathway in colorectal cancer through LRP6 (low-density lipoprotein receptor-related protein 6), a component of the WNT-Fzd-LRP5-LRP6 complex that triggers β -catenin signaling [42].

The Two Core Pathways Target Only One Cancer Hallmark

Numerous drug combinations tackle redundant pathways. They inhibit oncogenic processes; however, often their initial potent response fades, unable to prevent an eventual relapse. In principle, one way to improve the effectiveness of a two-drug combination could be to co-target pathways that inhibit multiple hallmarks. For example, to grow – and then divide – cancer cells need to switch their metabolic regulation from catabolic to anabolic. Thus, a superior two-drug combination strategy could target simultaneously two hallmarks, proliferation and metabolism. A second way to improve the outcome could be to harness drug combinations that target just one cancer hallmark; as we discuss here these combinations would simultaneously block the two *core pathways* to shutdown tumor proliferation.

Why Two Core Pathways?

Signaling in stem cells differs from differentiated cells [53]. Conserved pathways in development and maintenance of adult stem cells include Notch, BMP (bone morphogenic protein), hedgehog, FGF (fibroblast growth factor), TGF- β (transforming growth factor β), and WNT signals [54], with WNT a dominant factor in self-renewal [55, 56]. Overactivation of canonical WNT signaling is a hallmark of cancer stem cells, and key regulator of colorectal cancer [57]. Canonical WNT regulates self-renewal of stem cell systems. That Notch and β -catenin pathways appear more frequently in stem than in differentiated cells can be seen in fast-cycling colorectal cancer cells with stem-like properties (colon cancer-initiating cells, CCIC). By contrast, slow-cycling CCIC express markers that are independent of Myc. Stem cells undergo cell division and growth. Myc induces stemness

and blocks differentiation [58]. WNT, Notch, Hedgehog, etc. development-related signaling pathways are all preferred stem cell pathways [59]. YAP1 is also essential for differentiation [60] and early development [61], which involves cell division and growth. On the other hand, MAPK and PI3K appear to be preferred in cell cycle control in differentiated cells. G0→G1 progress needs MAPK; however, in development, the cell may not go through a G0 state altogether. The idea of drugging CSC is not new [62, 63]. Drugs under development indeed target signaling pathways, such as the WNT, the Sonic Hedgehog and the Notch pathways, aiming to either preferentially block CSC renewal or drive the cells into differentiation. However, like chemotherapy, for the most part, to date they were nonspecific.

The Two Core Pathways Can Help Clinical Trials and Computational Modeling

Even though most drug combinations emerged based on clinical experience, ongoing efforts also aim to understand the underlying principles of successful (synergistic) combinations to improve drug response – though not necessarily aiming to overcome resistance.

Computational approaches based in mathematical modeling, stochastic search techniques, context-based methods like gene expression or targeted phosphoproteomics profiling, and network-based methods have been developed to predict and assess features involved in effective synergistic drug response. Innovative rational approaches that predict or prioritize synergistic drug pairs include two dream challenges to predict drug combinations in 2014 [64, 65], and in 2016 (the AZ-Sanger Drug Combo Prediction Challenge). The major strategies, resources and techniques for the prediction of drug sensitivity in cell lines and patient-derived samples have been recently reviewed [66]; more recent works have since also appeared [67, 68]. Some of these specifically focus on targeted drugs, providing more specific treatment options.

The NCI combination screening has systematically tested the efficacy of all pairwise combinations of over 100 FDA-approved anticancer drugs in the NCI-60 panel of human tumor cell lines. Over 5,000 combinations and 300,000 experiments were screened with these panels. Only one combination has entered clinical trials (<https://deainfo.nci.nih.gov/advisory/ctac/1114/5%20-%20Holbeck.pdf>). On their own, these experiments are not able to explain specific successes and failures. The screening strategies involved ‘rational’ combinations hitting multiple nodes in a pathway, or hitting parallel pathways. Despite the extensive molecular characterization of the NCI-60 panel by gene mutation, DNA copy number, DNA methylation, and expression of mRNA, protein and microRNA [69], the reason for this disparity and why specifically this combination was successful, whereas others failed has been unclear. Recently the NCI retired the NCI-60 panel, launching a rejuvenated repository of cancer models [70]. These approaches, the testing of the NCI-60 panel of human tumor cell lines – and presumably along similar lines the new cells from ‘patient-derived xenografts’ (PDXs) which substitute it, tagged with data regarding the tumor genetic make-up and gene expression patterns, and the donor’s treatment history – involve comprehensive, broad brute-force trials.

Computations and modeling are critically important in prediction; but they too may lack the underlying conceptual framework which would help in understanding why specific predicted combination may – or may not – work. Additionally, the outcome of clinical trials also needs to be understood in terms of the fundamental processes of the cell. To date, these approaches have focused on synergistic drug response, with the drug combinations largely targeting the same core pathway; going forward a two-core pathways framework may help both clinical trials and computational modeling in capturing the principles of proliferative signaling in drug resistance.

Concluding Remarks

How to maximize the benefits of pathway-driven pharmacology in tumor proliferation is a key concern (see Outstanding Questions). New, creative strategies are being developed, such as those aiming to target *RAS*-driven cancers [71–79]. However, even if successful, drug resistance is likely to ensue. To fight resistance, chemotherapy often involves combination regimen; but exactly how to select drug combinations to block proliferating tumor cells is a question that is still in its infancy. The theoretical number of possible combinations is vast; how then to prioritize combinations with clinical relevance? The concern is aggravated by the differential signaling across isoforms and cancer subtypes [72, 80]. To date drug combinations in the clinic have been largely based on experience with the drugs and their availability. The risk of higher toxicity, modes of applications as well as cell type-specific signaling have also been contemplated.

Outstanding Questions Box

- How to maximize the benefits of treatments of mutated proteins in specific tissues? Are certain pathways more likely to acquire resistance than others in different cell types? And related to this, how to classify acquired resistance pathways with respect to the blocked protein and the tumor type?
- How would the clinical identification of the specific oncogenic receptor tyrosine kinase affect the selection of the drug combinations? Is acquired drug resistance to certain drug-blocked receptors more likely to be coupled with a distinct *core pathway*?
- Here we focus on tumor proliferation, the first hallmark of cancer. Within the clinical setting of drug resistance, how to effectively prioritize the selected combination, apart from the considerations above? Which additional factors are at play?
- Finally, how to rationally list drug combinations in the most effective way to help the physician?
- Is there a cross-talk between the two independent core proliferation pathway? If yes, which is what data suggest, how does it complicate drug resistance?

Combination chemotherapy that focuses on alternative pathways controlling the same cell cycle stage contends that no matter what are the stimulations – e.g. a growth hormone or

cell-cell contact – the pathways can substitute for each other and be independent from each other. Our *core pathways* outlook organizes the oncogenic signaling map, clarifies observations of drug resistance and can help modeling to forecast it. To be useful, we envision a detailed map with the pathways originating from the respective cell surface receptors, and propagating to their final cell cycle actions. The parallel and independent *core pathways* – which could be delineated from the map – would mirror the acquired alternative resistance pathways that emerge to restore the proliferative cancer hallmark. Going forward, we believe that combinatorial drug regimens to avert drug resistance would benefit from consideration of the organizing principle of cancer, as well as a mechanistic grasp of alternative signaling in cell proliferation.

Acknowledgments

This project has been funded in whole or in part with Federal funds from the Frederick National Laboratory for Cancer Research, National Institutes of Health, under contract HHSN261200800001E. This research was supported [in part] by the Intramural Research Program of NIH, Frederick National Lab, Center for Cancer Research. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

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Trends Box

- Due to the expected emergence of drug resistance, to maximize survival benefits, combinatorial drug treatments are routinely employed in chemotherapeutics. Aiming to block oncogenic signaling either by targeting the same pathway or a combination of pathways accomplishing the same cellular function are frequently contemplated strategies.
- To counteract pathway-based resistance, efforts focus either downstream of the protein making it harder to achieve pathway reactivation; however, an acquired alternative pathway can take over as in the case of ERK5 pathway bypassing blocked ERK1/2 or MEK1/2. By contrast, upstream blockage can elicit downstream pathway activation.
- Drug combinations to block proliferation pathways are in development, but the fundamental combinatorial principle is still elusive.

Fig. 1.

An outline of the general scheme of drug resistance adopted by tumor cells to sustain the hallmarks of cancer and the pharmacological strategies. The cartoons portray only those resistance mechanisms that relate to tumor initiation. **(A)** These include *DNA damage repair*, which may act by rewiring the cellular network such that repair signaling proceeds through a parallel (alternative) oncogenic pathway; *Proliferation*, the fundamental hallmark of cancer, requires cell growth and division which relates to the cell cycle. Proliferation can – but does not necessarily – take place through inhibition of cell death (in the figure connecting via the *cell death inhibition* node), or through survival pathways, as in case of the MAPK, PI3K, Hippo and WNT pathways. Parallel pathways are at play in this hallmark; *Cell death inhibition*, many pathways prevent cell death and are pro-survival. It can be expressed via parallel or same pathway; *Drug target mutations*, can take place through mutations in an enzyme in the same pathway (often downstream the inhibited target); *Drug efflux*, assuming that the drug has reached its target, like *drug target mutations*, drug efflux can affect the same pathway; *Drug metabolism alteration*, an important hallmark of cancer, may not relate to signaling-driven proliferation thus not considered here. Pro-survival pathways along with inhibition of cell death sustain tumor cell proliferation. **(B)** The hallmarks of cancer proposed by Hanahan and Weinberg [1] as they relate to drug resistance mechanisms in Figure 1A. They characterized neoplastic diseases as sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Relating these to the drug resistance mechanisms, reducing proliferative signaling and enhancing growth suppressors are involved in proliferation; promoting cell death relates to cell death inhibition; preventing replicative immortality corresponds to DNA damage repair; suppressing angiogenesis, which provides nutrition, could relate to stage 2 cancer; finally, inactivating invasion and metastasis does not relate to tumor initiation thus not in Figure 1A.

Fig. 2.

An overview of the cell cycle phases and two examples of independent *core pathways* in the G1 stage. The first *core pathway* involves the MAPK (Raf/MEK/ERK) and PI3K/Akt/mTOR pathways, and the second *core pathway* encompasses Hippo (YAP1) and WNT (β -catenin, or other pathways that lead to the expression (or activation) of Myc such as Notch, Hedgehog, etc.). Both of *core pathways* are parallel. At the G1→S restriction point, ERK and YAP1 regulate the post-mitosis G1 phase (G1-pm), whereas PI3K and β -catenin regulate the pre-synthesis G1 phase (G1-ps). ERK and YAP1, and PI3K and β -catenin, correspond to each other because they act in the *same* cell cycle stage; MAPK and PI3K cell cycle actions are consecutive as are those of YAP1 and β -catenin.

Fig. 3.

A cartoon illustrating two independent parallel pathways in the post-mitosis G1 phase (G1-pm) in cell proliferation: MAPK and the Hippo pathways. This figure depicts the new view proposed in this work. In the first pathway, EGFR signaling activates Ras which turns on the MAPK pathway. This leads to the activation of transcription factors such as Elk1 and c-Jun. In the second parallel pathway, signaling takes place through the Hippo pathway. This results in phosphorylation of YAP1 and thus its degradation. On the left hand-side oncogenic mutant B-Raf^{V600E} is targeted by drug, and thus the MAPK pathway is inhibited. Drug resistance can take place by inactivation of the Hippo pathway. The unphosphorylated YAP1 (right hand-side, bottom) binds to the transcription factor TEAD. The complex binds to the DNA leading to transcription of genes encoding proteins acting in cell growth and division at the same cell cycle stage as the MAPK pathway does. Thus, MAPK and Hippo are independent *core* (parallel) pathways. An alternative strategy that the cell can adopt in drug resistance is activating MEK5 to take over MEK1/2 inaction. Under physiological conditions, MEK5 is activated by an unknown cell surface receptor (depicted by a question mark here). MEK5 bypasses MEK1/2 in the same (MAPK) pathway.