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Epistasis and evolutionary dependencies in human cancers

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Cancer evolution is driven by the concerted action of multiple molecular alterations, which emerge and are selected during tumor progression. An alteration is selected when it provides an advantage to the tumor cell. However, the advantage provided by a specific alteration depends on the tumor lineage, cell epigenetic state, and presence of additional alterations. In this case, we say that an *evolutionary dependency* exists between an alteration and what influences its selection. Epistatic interactions between altered genes lead to evolutionary dependencies (EDs), by favoring or vetoing specific combinations of events. Large-scale cancer genomics studies have discovered examples of such dependencies, and showed that they influence tumor progression, disease phenotypes, and therapeutic response. In the past decade, several algorithmic approaches have been proposed to infer EDs from large-scale genomics datasets. These methods adopt diverse strategies to address common challenges and shed new light on cancer evolutionary trajectories. Here, we review these efforts starting from a simple conceptualization of the problem, presenting the tackled and still unmet needs in the field, and discussing the implications of EDs in cancer biology and precision oncology.

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Introduction

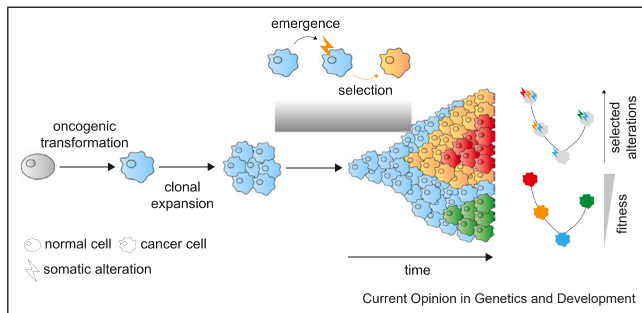
Since the 1950s, evidence indicated that cancer progresses from a single neoplastic cell through the acquisition of advantageous modifications [1–3]. This evidence led to the renowned model of cancer evolution proposed by Peter C Nowell [4••], subsequently corroborated by experimental, technological, and computational advances [5,6]. Whereas, additional evolutionary mechanisms such as adaptive cell plasticity and interactions among tumor, immune, and stromal cell types [7–11] coexist and cooperate in determining tumor phenotypes, here, we will refer to the evolutionary model first proposed by Nowell to explore how epistasis among emerging alterations influences their selection and evolutionary trajectories.

In accordance with Darwinian principles of species evolution, atomic steps of this model are the *emergence* and *selection* of molecular alterations enabling cancer cells to acquire oncogenic phenotypes [12,13]. Here, the term 'alteration' indicates a broad class of genetic and epigenetic modifications that emerge and are selected for during tumor progression. The emergence of these alterations via endogenous and exogenous mutational processes, whereas their selection is associated with their impact on gene functions and the advantage that such consequences confer to the cancer cell. Advantageous alterations give rise to fitter clonal populations, expanding and outcompeting existing clones (Figure 1). Multiple iterations of alteration emergence and selection continuously diversify the tumor-cell population and increase its fitness (Figure 1), which can be "measured" in terms of specific phenotypes [9••,14].

Cancer phenotypes involve the deregulation of multiple cellular pathways, requiring multiple alterations. Indeed, cancer is neither driven by a single alteration, nor the result of the independent action of a random assembly of alterations. *Cancer is determined by the concerted action of specific combinations of alterations*. A prototypical example is the multistage model of colorectal cancer development [15–17••], which is characterized by the sequential acquisition of alterations activating Wnt signaling, for example, loss-of-function mutations of *APC* (~70%[1]) or *RNF43* (~9%), and ERK signaling, for exampple, Kirsten rat sarcoma viral oncogene homolog (*KRAS)* (~41%) or *BRAF* (~12%) gain-of-function mutations. Intriguingly,

[1] Alteration frequencies are estimated based on data from the TCGA colorectal cancer cohort [18] as reported in the cBioPortal [19]. Bookmark Query: https://bit.ly/3xlVq6n.

Figure 1



Schematic diagram of cancer evolution through the emergence and selection of genomic alterations. Upon transformation of a normal cell (gray) into a cancer cell (blue), cancer cells clonally expand. Uncontrolled proliferation leads to the emergence and selection of new alterations over time, which alter cancer cell phenotypes (color-coded). On the right, clonal evolutionary trajectories are represented indicating either the alterations within each clone (colored lightning bolts, top), or cell fitness (bottom).

~83% of *KRAS* mutations co-occur with *APC* mutations (log2 odds ratio: 1.4), in contrast to only ~39% of *BRAF* mutations (log2OR: −2.5), which instead frequently co-occur with *RNF43* loss (log2OR: 4). Similarly, subsequent alterations in colorectal cancer are not equally represented in *APC–KRAS* and *RNF43–BRAF* tumors. Overall, recurrent combinations of alterations suggest that these alterations alter the cell phenotype in a nonadditive manner through epistatic interactions.

The term *epistasis* has come to encompass various but related phenomena in evolutionary studies, but at its essence, it indicates interactions among alleles such that the effect of mutating one allele depends on the status of another allele [20]. Experimental studies in microbial organisms and yeast have revealed both specific instances of such genetic interactions [21–24] and global epistatic trends [25–28], indicating, for example, a fitness-saturation effect characterized by 'diminishing-returns' epistasis. In cancer, an epistatic interaction between two alterations exists when the effect of one alteration is modulated by the presence or absence of the second alteration. Here, by 'effect', we specifically refer to the ability to alter cell phenotypes rather than the function of the altered protein. An epistatic interaction between two alterations will influence their selection during tumor evolution [29•,30], that is, the epistatic interaction constitutes an *evolutionary dependency* [31•]. Note that selection is influenced by several factors, such as tumor lineage [32–34], the epigenetic status of the tumor cell [35,36], and, as here discussed, preexisting alterations. Hence, epistasis is one but not the only source of evolutionary dependencies (EDs). Longitudinal sample collections provide direct evidence of epistasis-driven EDs. However, such data are challenging to obtain for human tumors. Alternatively, computational approaches have been proposed to infer EDs from molecular profiles of large patient cohorts (Table 1). The underlying principle is that EDs lead to nonrandom patterns of alterations in large cancer genome datasets, that is, specific alterations are more (co-occurrence (CO)) or less (mutually exclusive) frequently observed in the same tumor than expected by chance. Here, we provide a framework of different types of EDs, highlighting their impact on cancer evolution and patterns of occurrence. Next, we explore the key steps to infer EDs and how these are implemented by existing approaches. We highlight unmet challenges and strategies to validate and follow up on computational predictions. Last, we discuss examples of EDs and their impact on tumor phenotypes and response to therapy.

Epistatic interactions influence cancer evolution

Epistatic interactions have been observed as key determinants of tumor evolutionary trajectories [37–41]. Here, we reduce epistasis among cancer alterations to three scenarios and show how these lead to non-random patterns of alterations across large patient cohorts.

Synergistic alterations

The sequential accumulation of genomic alterations deregulates multiple cellular processes, adding the effect of one to the other. When specific combinations of alterations have nonadditive effects, for example, the effect of two alterations is greater than the sum of the effects of the single alterations, we refer to them as *synergistic alterations* (Figure 2a). Synergistic effects are at the basis of tumor initiation and progression. Indeed, several lines of evidence indicate that often a single alteration has little or no effect on cell phenotypes, unless a second is present to remove compensatory mechanisms or 'safety checks'. Prototypical examples involve *TP53*, the loss of which synergizes with alterations in the cell-cycle pathway to promote tumorigenesis [38,42,43]. From an evolutionary perspective, synergistic alterations are likely to be part of recurrent evolutionary trajectories, that is, the presence of one alteration favors the selection of the other. As a result, in large cancer genome datasets, synergistic alterations frequently co-occur in individual tumors and can be inferred from significantly co-occurring alterations [31•,44•].

Redundant alterations

Simultaneous acquisition of alterations having similar downstream consequences is not expected to increase tumor fitness (Figure 2b), that is, the advantage provided by an alteration is reduced by the presence of another one with the same functional effect. From an evolutionary perspective, *redundant alterations* will not be

Table 1

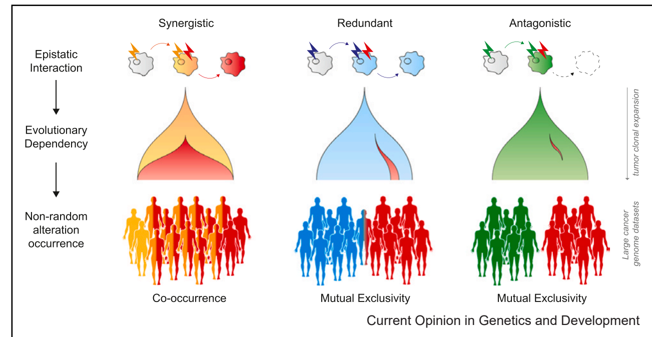
Computational approaches for ED inference. MCMC: Markov chain Monte Carlo, ILP: integer linear programming, BLP: binary linear programming, GA: genetic algorithm, SA: simulated annealing.

Method	Year	Prior knowledge required	ED type	Pairwise only	Covariates	Search strategy	Test strategy
RME [115]	2011	No	ME	No	No	Greedy search of ME alteration sets from a network of ME-enriched pairs.	It compares number of bits required to encode alteration matrix under ME or H_0
MEMo [45••]	2012	Yes	ME	No	No	Search for cliques of recurrent alterations within pathways.	Edge-switching permutation strategy
Dendrix [55]	2012	No	ME	No	No	MCMC search for alteration set maximizing ME and coverage.	Score optimization
MDPFinder [60]	2012	Yes	ME	No	No	BLP and GA searches for alteration sets maximizing ME and coverage. The GA approach integrates mutation and gene- expression data.	BLP provides the exact maximum. GA proceeds through score optimization.
Multi-Dendrix [54]	2013	No	ME	No	No	ILP search for alteration sets maximizing ME and coverage.	Score optimization
muex [66]	2014	No	ME	No	No	Probabilistic generative model accounting for coverage, impurity, and error rates.	Likelihood ratio of the ME model and independence model.
CoMDP [51]	2014	No	ME/CO	No	No	BLP search of CO sets of alterations, where each set has high ME and coverage.	Permutation test.
TiMEx [67]	2015	No	ME	No	No	Probabilistic generative model accounting for alteration emergence and observation time.	Likelihood ratio of the ME model and independence model.
Mutex [53]	2015	Yes	ME	No	No	Search for ME alteration sets converging on downstream node in a pathway network.	Fisher's test-based approach.
GAMToC [56]	2015	No	ME/CO	No	No	SA search for alteration sets maximizing total correlation score.	G-test limits the module size and determines statistical significance.
CoMe [30]	2015	No	ME/CO	Yes	Tumor and mutation type	Exhaustive pairwise test.	Permutation test.
CoMEt [58]	2015	No	ME	No	No	MCMC-based search for ME alteration sets.	Exact statistical test for ME conditioned to alteration frequency
DISCOVER [61]	2016	No	ME/CO	No	Tumor type	Exhaustive pairwise test followed by network-module search on ME graph.	Poisson-binomial distribution. Probabilities of gene/sample-specific mutation estimated from the alteration matrix.
MOCA [57]	2016	No	ME	No	Tumor type	Greedy search based on pairwise Fisher's exact test.	It defines an iterative procedure to extend hypergeometric test to alteration sets.
WExT [65]	2016	No	ME	No	No	Exhaustive search of alteration sets with minimal filtering criteria.	Weighted exact ME test preserving gene-alteration frequency. Weights reflect gene/ sample-specific mutation probability.
MEGSA [116]	2016	Optional	ME	No	No	Multiple-path linearsearch algorithm. Optionally, pathway information can be used.	Likelihood-ratio test of ME for an alteration set.
WeSME (WeSCO) [68]	2016	No	ME/CO	Yes	No	Exhaustive pairwise test.	Weighted sampling of gene alterations based on sample- alteration frequency.
SELECT [31•]	2017	No	ME/CO	No	Tumor and mutation type	Exhaustive pairwise test followed by module search on graph derived from ME/CO pairs.	Blockwise edge-switching permutation strategy to test significant weighted mutual information (pairs).
BeWith [52]	2017	Yes	ME/CO	No	No	ILP search for module sets exhibiting predefined ME/CO patterns.	WeSME and WeSCO are used for statistical significance.
FaME [63]	2021	No	ME	No	No	Exhaustive pairwise test. Optimized matrix-product computation.	Fisher's exact test

Table 1 *(continued)*

Method	Year	Prior knowledge required	ED type	Pairwise only	Covariates	Search strategy	Test strategy
CRSO [117]	2021	No	Mixed	No	No	Search for sets of CO alterations that maximize coverage and minimize a passenger- probability matrix.	Score optimization
FSME [62]	2021	No	ME	Yes	No	Exhaustive pairwise test followed by greedy search of modules of significant pairs.	ME and coverage-based statistic asymptotically following a normal distribution under the null hypothesis.
MeSCAN [59]	2021	No	ME	No	No	MCMC search for alteration sets maximizing a ME statistic accounting or gene and sample mutation rates.	Score optimization and local FDR estimation.
coselens [118]	2022	No	ME/CO	Yes	No	Exhaustive pairwise test.	Difference between number of estimated driver mutations (dNdS) in gene A when gene B is mutated and when it is not.

Figure 2



A model of epistasis-driven EDs in cancer. The emergence of a new alteration (red) has different effects in tumors with different preexisting alterations. Top: Epistatic interactions exist between synergistic (red and yellow), redundant (red and blue), and antagonistic alterations (red and green). Center: Synergistic alterations lead to the emergence of a fitter clone. Redundant alterations lead to the emergence of a new clone with no further selective advantage. Antagonistic alterations lead to the emergence of a new clone with lower fitness, which is likely to disappear. Bottom: In large cancer genome datasets, synergistic alterations are frequently co-occurrent, whereas redundant and antagonistic alterations are frequently mutually exclusive.

selected in the same tumor. As a result, redundant alterations are expected to be mutually exclusive across large datasets. Indeed, multiple studies have shown that mutual exclusivity (ME) is common among genomic alterations impinging on the same pathway. Examples include mutually exclusive alterations of the Rb pathway, such as activation of cyclin-dependent kinases (CDK) and inactivation of the CDK inhibitor p16 or *RB1* itself [18,43]. Hence, significant mutually exclusive alterations could reflect alterations having similar downstream consequences.

Antagonistic alterations

The advantage provided by one alteration might become a disadvantage in the presence of another alteration. When their CO is lethal, their interaction is termed *synthetic lethal*. Whether synthetic lethal or simply disadvantageous, the copresence of *antagonistic alterations* is negatively selected during tumor evolution, leading, in principle, to perfect ME (Figure 2c). A well-known example of synthetic lethal interaction are loss-of-function alterations of the DNA repair genes *BRCA1/2* and *PARP1* [46,47], which have exposed a therapeutically actionable vulnerability. Indeed, PARP inhibitors are well-established treatment options for cancers exhibiting alterations in the *BRCA1* or *BRCA2* genes [48]. The appeal of exploiting synthetic lethal interactions for therapy has fostered the search for mutually exclusive alterations, often incorporating additional functional data to discriminate between redundant and antagonistic interactions [49,50].

Overall, alterations involved in these interactions are expected to be found significantly co-occurring or to be mutually exclusive across large cancer genome datasets, providing an opportunity to search for non-random patterns of occurrence to infer EDs in cancer.

Computational inference of evolutionary dependencies

The problem of inferring EDs from large cancer genome datasets has been tackled by several approaches. In general, each method needs to 1) define the type of non-random pattern to search for, 2) implement a strategy to search for these patterns, and 3) test their significance against an expected distribution.

Patterns of alterations

The possible types of non-random patterns increase with the number of alterations simultaneously considered. Approaches can be distinguished in those that only test pairs of alterations (pairwise) and in those that test sets of arbitrary size (geneset). Testing patterns within genesets increases the detection power, especially for low-frequency alterations. However, the number of testable genesets increases exponentially with the number of input alterations. As a result, approaches analyzing genesets use special heuristics to reduce the number of hypotheses (see next section). Concerning the type of pattern, in the pairwise scenario, only two patterns are possible: ME and CO. Interestingly, while several algorithms have been proposed to identify ME, only few support the analysis of CO patterns (Table 1). In the geneset scenario, most algorithms extend the discovery of either only ME or only CO among multiple genes. Three approaches, CoMDP [51], BeWith [52], and SELECT [31•], look for patterns comprising both ME and CO within a geneset. CoMDP searches for modules with within-module ME and between-module CO. BeWith extends this idea, searching for distinct patterns within and between modules and uses prior knowledge to search for modules of functionally related genes. Last, SELECT first identifies pairwise patterns (ME and CO) and then clusters these patterns into genesets enriched for ME and CO alterations. Interestingly, genesets identified by these methods nicely overlap with known biological pathways, supporting the notion that EDs are enriched within cellular pathways.

Searching for candidate patterns to test

The search for nonrandom patterns of alterations is challenging for genesets, where their number grows exponentially with the geneset size. Here, a distinction exists between *de novo* algorithms, and those using *prior knowledge* to filter the search space (Table 1). Algorithms leveraging prior knowledge, prefilter the number of hypotheses to test based on external information, typically a network of gene relationships (e.g. protein-interaction networks or canonical pathways). For example, MEMo [45••] and Mutex [53] search and test ME only among highly connected genes in the network. De novo algorithms adopt heuristics to select candidate geneset of interest. Typically, a score is defined to assess the degree of ME or CO within a geneset and optimization strategies are used to identify modules maximizing such scores. When searching only for either ME or CO, simple scores combine the total number of samples exhibiting more than one alteration in a geneset (overlap), and the total number of samples with at least one alteration in a geneset (coverage) [54,55]. Alternatively, strategies based on information theory have been introduced in GAMToC [56], which computes the total correlation of a geneset, and SELECT, which computes a weighted version of mutual information to score gene pairs. Once a score is defined, optimization algorithms can be used to identify the geneset that maximizes that score. These include greedy algorithms, as in MOCA [57] or Mutex, Markov chain Monte Carlo methods, as in Dendrix [55], CoMEt [58], and MeSCAN [59], simulated annealing, as in GAMToC, or integer or binary linear programming approaches, as in Multi-Dendrix [54], MDPFinder [60], and BeWith [52]. A feature of these approaches is the ability to detect alteration sets comprising more than 2 alterations without requiring each pair within the set to exhibit itself a significant pattern of occurrence. In contrast, SELECT, DISCOVER [61], and FMSE [62] first exhaustively search for pairwise EDs and then identify genesets by clustering these pairwise hits. On the one hand, de novo approaches provide the greatest power to discover unexpected associations, which is limited when restricting the search within cellular pathways. On the other hand, unfiltered searches deal with a rapidly growing number of hypotheses, increasing the risk of false-positive discoveries, and challenging the interpretability of the results.

Determining the statistical significance of a pattern

Once a set of gene pairs or genesets has been identified, the statistical significance of the alteration pattern must be assessed. Most methodologies employ a frequentist statistical framework to estimate the likelihood that a score is observed given a null model assuming independent alterations. Null distributions can be defined analytically, by deriving a mathematical formulation of the distribution, or empirically, by estimating the null distribution through data permutation (Monte Carlo procedure). Analytical approaches need to make assumptions on the family and shape of the null distribution. For example, Mutex, MOCA, and FaME [63] are based on a Fisher's exact test for ME. This test makes the strong assumption that all samples have the same probability of acquiring a given alteration. This is however hardly true in cancer, where tumors can exhibit dramatically different mutation rates [64•], that is, different probability of acquiring a new mutation. Failure

to account for these differences will inflate pattern significance. Strategies to account for nonuniform alteration frequencies have been proposed by computing weighted ME and CO tests. Examples include a recursively approximated weighted test in WeXT [65] or by estimating the probability of a gene to be mutated in each sample in DISCOVER. Alternatively, muex [66] and TiMEx [67] proposed a probabilistic generative model accounting for multiple covariates, such as tumor purity, mutation-calling error rates, and time of alteration emergence at observation. Here, the significance is estimated by the likelihood ratio of the ME and independence models. Among permutation-based strategies, WeSME and WeSCO [68] use weighted ME and CO tests by sampling mutated samples based by their alteration frequency. MEMo models alterations as the adjacency matrix of a bipartite graph, having genes and samples as nodes, and randomizes the occurrences by randomly sampling two edges and switching their ends. The edge-switching technique for graph randomization [69] preserves both gene and sample alteration frequencies.

The appeal of permutation procedures is that they provide simple strategies to control for multiple covariates. For instance, tumor types and subtypes are known to be enriched or depleted for specific alterations. When multiple tumor (sub-)types are analyzed together, ignoring this information will invariably overestimate pattern significance [29•]. For example, clear-cell renal carcinoma and invasive bladder cancer are respectively enriched for *VHL* and *KMD6A* mutations, which exhibit high lineage specificity [70,71]. A permutation strategy ignoring tumor types will call *VHL* and *KMD6A* mutations significantly mutually exclusive, erroneously inferring an epistatic interaction between the two alterations. To address these issues, a blockwise permutation strategy defines submatrices, or ‘blocks’, within the alteration matrix based on a set of covariates and permutes alterations within each block, but not between different blocks. Exploiting this strategy, SELECT performs the edge-switching permutation in a blockwise manner.

Open challenges: sample size, alteration frequency, and molecular heterogeneity

Nonrandom patterns of alterations can emerge for several reasons beyond epistasis-driven EDs. Although some of these factors can be accounted for by the null model, some remain difficult to address. Permutation strategies preserving the total number of alterations observed in each gene and sample might still fail to estimate the actual *tumor mutation burden*. Indeed, ED-inference approaches typically retain genes altered above a predetermined frequency threshold or known cancer drivers. The total number of mutations in these genes might not reflect the overall tumor mutation burden in the same dataset. In addition, mutation emergence is driven by different *mutational processes* [72]. For example, tumors exhibiting microsatellite instability harbor a higher fraction of frameshift mutations than other tumor subtypes [73]. ED-inference approaches typically ignore variant classification assuming equally distributed variant types. As a result, they might overestimate CO patterns when enriched for specific variant types. Similarly, the mutation rate is expected to be highly variable across the genome, potentially making the emergence of specific gene mutations more or less likely than others [74]. While most approaches preserve gene-mutation frequency, implicitly accounting for this variability, explicit models might be required to better disentangle functional and neutral mutations. Indeed, a large fraction of observed missense mutations (i.e., amino acid substitutions) are likely functionally neutral [74–77]. Filtering these mutations inevitably reduces statistical power but it is recommended as neutral mutations are not under selection and should not exhibit nonrandom patterns of occurrence [44•]. Moreover, several oncogenes exhibit multiple mutational hotspots [33], not necessarily affecting the protein in the same manner [78••–80]. Different mutations of the same gene might establish different EDs and, thus, these should be separately analyzed. However, such analyses are possible only for a few mutation hotspots where the alteration frequency is sufficiently high to detect statistically significant patterns. In the future, the explosion of target sequencing in the clinic will fill the data gap for these analyses, at least for genes included in clinically adopted panels.

Result interpretation and follow-up

The interpretation and validation of computational prediction pose the greatest challenges. Here, we propose a few strategies to validate and follow up on predicted EDs.

Distilling computational predictions with orthogonal evidence

ED inference was facilitated by large-scale cancer genome profiling projects, such as The Cancer Genome Atlas (TCGA) [81]. These datasets allow the analysis of matched genetic, epigenetic, transcriptional, and proteomic profiles and, thus, assessing the impact of EDs on tumor phenotypes. Given a predicted ED between two alterations, samples can be stratified as those exhibiting either only one of the two alterations, or both, or none and the resulting groups can be compared in terms of transcriptional, epigenetic, and clinical features. Information about the functional role of the genes involved in a specific ED can be used to test more specific hypotheses, for example, downstream pathway activation inferred by transcriptional signatures or protein phosphorylation. These datasets represent a gold standard not only for ED inference, but also for ED interpretation. Since epistatic interactions among altered genes are likely to emerge within cellular pathways [31•], verifying that EDs identified by a given tool are enriched within

pathways provides evidence that results are driven by epistatic interactions rather than other confounding factors. Moreover, focusing on ED sets that are enriched within one or a few pathways could facilitate the biological interpretation of the results, the design of follow-up analyses, and formulation of hypotheses to experimentally validate. The downside of this approach is that of limiting the space of discovery to potentially already well-studied targets.

An orthogonal strategy for ED validation uses data from high-throughput perturbation screenings on cancer cell lines [82–85••]. These experiments record the effect of gene inhibition across hundreds of cell lines with comprehensively annotated genomic profiles [86]. A recommended resource is the cancer Dependency Map (DepMap — https://depmap.org/portal/). As before, cell lines can be categorized as those exhibiting either only one or both alterations, and response to inhibition of one of the two altered genes can be compared among these groups. Recently, this strategy was adopted to show that, for most ME EDs, cell lines harboring both alterations were resistant to inhibition of one of the two altered genes, in contrast to cell lines that were only altered in the gene that was inhibited [44•]. These experiments indicate that cells exhibiting two redundant alterations compensate for the loss of one of the two altered genes (typically an activated oncogene). Cell lines harboring both alterations exhibited a stronger sensitivity to inhibition of one of the two genes than cell lines only harboring one alteration, that is, tumors exhibited increased dependence on synergistic alterations. Gene essentiality screenings have also been used to discover EDs potentially associated with synthetic lethal interactions [86,87]. These strategies can benefit from increasingly available datasets beyond gene essentiality, such as single-drug and drug-combination screenings [88,89] and screenings adopting patient-derived xenografts [90,91] or tumor organoids [92]. Two major limiting factors should be noted: first, the sample size is often insufficient to study many EDs, second, the tested phenotype is limited to cell growth and viability, hence, EDs affecting other phenotypes such as immune evasion or cell migration cannot be tested. Increasing the number of screened models and exploring alternative screening modalities, for example, coculture of tumor and immune cells, will be critical to address these challenges.

From evolutionary dependencies to precision oncology

Distilled and possibly functionally validated EDs could be used in follow-up analyses to study cancer evolutionary trajectories and response to therapy. Evolutionary trajectories reconstruct the history of a tumor, from its early driving events to the late appearance of lesions promoting metastatic progression or resistance to therapy [93–95]. Identifying recurrent trajectories across patients [96••] could predict which tumors are more likely to progress and/or respond to a given therapy [97,98]. Such approaches could use the notion of ED as a prior to promote trajectories comprising co-occurrent alterations and penalizing trajectories comprising mutually exclusive alterations. In addition, evolutionary trajectories could provide insight on the temporal order and tumor clonality of EDs. Indeed, co-occurrent alterations might not necessarily co-occur in the same cell and subclonal heterogeneity might confound mutual exclusivity estimation. With growing availability of multiregion samples and single-cell datasets, both these problems warrant further investigations.

ED clinical implications are probably more direct in precision oncology. With the advent of targeted sequencing in the clinic, therapeutic choices can be tailored to the tumor mutational profile [99–101]. However, response to targeted therapies remains highly heterogeneous. Response variability can in part be explained by the presence of additional alterations, modulating or even ablating treatment efficacy [102,103]. For example, official treatment guidelines from the National Comprehensive Cancer Network (NCCN) for non-small cell lung cancer report lack of therapeutic efficacy of epidermal growth factor receptor (EGFR)-selective inhibitors in the presence of KRAS-activating mutations (NCCN Guidelines Version 3.2022[2]). Here, EDs could represent promising hypotheses to test the association between therapeutic response and combinations of alterations (rather than single alterations). In addition, results from gene essentiality screenings could effectively translate into clinical insight: synergistic alterations increasing tumor dependencies could be used to select patient cohorts more likely to respond to a specific treatment, whereas redundant alterations could pinpoint mechanisms of resistance to therapy. Preclinical studies and cohorts with matching mutational and treatment data will provide an opportunity to test these hypotheses.

Discussion

A systemic understanding of cancer genomic alterations is necessary to decipher how and why specific combinations of alterations are preferentially selected during tumor evolution [104].

In lung adenocarcinoma (LUAD), frequent oncogenic mutations target the *KRAS* and *EGFR* oncogenes in a mutually exclusive manner [105]. Transgenic mouse models and cancer-cell lines showed that only cells expressing one of the two oncogenes formed tumors, while forced expression of the second was deleterious [41],

[2] https://www.nccn.org/guidelines/category_1.

driving overactivation of ERK signaling [106]. A similar mechanism was shown for concurrent *EGFR* overexpression and oncogenic *BRAF* mutations in melanoma: *EGFR* induction was deleterious in *BRAF*-mutant tumors but became beneficial upon treatment with a selective *BRAF* inhibitor, rescuing cell proliferation [40]. In colorectal cancer, *KRAS* and *EGFR* mutations were shown to coexist in the same tumor but not in the same cell, giving rise to separate tumor clones [107]. Interestingly, the *EGFR*-driven clone was dominant in treatment-naive tumors, EGFR therapeutic inhibition led the *KRAS*-driven clone to take over, but this phenotype was reverted upon treatment withdrawal. Interestingly, *EGFR*-driven and *KRAS*-driven tumors develop following different evolutionary trajectories [108]. For example, distinct LUAD subtypes are defined by the copresence of *KRAS* and *STK11* (a.k.a. LKB1) or *KRAS* and *TP53* mutations [39]. Concurrence of *KRAS* and *STK11* mutations leads to worse prognosis, reduced expression of immune markers, and lack of response to PD-1 blockade [109••]. Frequently co-occuring mutations often lead to more aggressive phenotypes when combined. Beyond *KRAS* and *STK11*, other examples include mutations of *NPM1*, *DNMT3A*, and *FLT3* in acute myeloid leukemia [110], and *KDM6A* (a.k.a. UTX) and *FGFR3* in bladder cancer [111]. Notably, CO can be asymmetric when the advantage provided by a specific mutation emerges only in the context of another one. For example, *CCNE1* copy-number amplifications almost always co-occur with *TP53* mutations [31•], but not vice versa. *CCNE1* amplifications enhance *CCNE1* expression, leading to *RB1* inhibition and G1-to-S transition through the CCNE1–CDK2 complex. However, overactivation of *CCNE1* triggers p21 in a p53-dependent manner, which limits *CDK2* abundance [38,112]. Hence, p53 effectively limits the oncogenic potential of *CCNE1* and *CCNE1* overactivation provides a proliferative advantage only in *TP53*-negative tumors. A similar asymmetric dependence was shown for *SMAD4* loss, which is advantageous to pancreatic tumors harboring *KRAS* oncogenic mutations [113]. These and many more examples have demonstrated the relevance of studying cancer genomic alterations not as independent but as cooperating events to understand the diversity of tumor phenotypes and therapeutic responses.

Finally, although most approaches have investigated epistatic interactions among somatic mutations and copy number alterations, the landscape of cancer alterations extends well beyond these genetic lesions. EDs among epigenetic alterations have rarely been investigated [114]. Similarly, aberrant transcription, gene fusions, catastrophic events (e.g. whole-genome doubling or chromotripsis events), cell–cell interactions, inflammation states, and so on, all contribute to cancer evolutionary trajectories. Future work should integrate these multiple data types. Overall, multiple factors cooperate to influence tumor evolution. Any effort to anticipate and possibly steer this process ultimately needs to learn which combinations of these factors are possible, which are favorable, and which can be used to disrupt the progression of the disease.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

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