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Perspective

Genetic Interactions in Cancer Progression and Treatment

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As cancer cell genomes are unveiled at a breathtaking pace, the genetic principles at play in cancer are emerging in all their complexity, prompting the assessment of classical genetic interaction models. Here, we discuss the implications of these findings for cancer progression and heterogeneity and for the development of new therapeutic approaches.

Over the past 30 years, intensive research has led to a reasonable understanding of many cellular functions at the molecular level and how these processes go awry in cancer. However, the genetic principles that underlie the evolution of cancer genomes and how combinations of mutations contribute to cancer phenotypes remain poorly defined. These underlying principles are likely to be similar to those governing genetic variation in healthy cells and evolutionary processes in organisms, but this assumption remains untested. Here, we discuss emerging ideas on the genetic interactions in cancer and how they may be used to design new therapeutic approaches.

Cancer Genes and Cancer Genomes

As a cell progresses from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in the cell's behavior. These series of attributes, which are most succinctly described as the "Hallmarks of Cancer" (Hanahan and Weinberg, 2000; Luo et al., 2009b; Hanahan and Weinberg, 2011), are required for the development of a malignant tumor. However, numerous questions remain about how mutations in DNA lead to the acquisition of these traits. First, many "cancer genes" still await discovery, but even when these genes are known, it is mostly unclear how they connect to transformative phenotypes. Moreover, for specific tumor phenotypes, it is not yet known whether transformative mutations are selected from a smorgasbord of functionally equivalent changes in different genes. Or, is cancer progression an ordered procession of mutation acquisition, with each mutation individually and incrementally increasing tumorigenicity, and is the order of acquisition itself important or is it simply the slate of genes that is relevant?

Although the number of cancer genomes currently available for scrutiny is relatively small, this is set to rise dramatically over the next year. Nevertheless, it is already apparent that the landscapes of cancer genomes can be incredibly complex. Tumor cells may possess a panoply (10^4–10^5) of genetic changes compared to germline DNA, which include highly complex structural rearrangements in combination with base changes, insertions and deletions (i.e., indels), and copy-number changes (i.e., amplifications and deletions). These genetic changes likely arise from endogenous chemical reactions with DNA and physiological processes, such as replication, but also from the activity of exogenous mutagenic agents, such as ultraviolet light, chemicals in tobacco smoke, and even chemotherapy. The differential activity of DNA repair processes may considerably influence whether these insults become fixed as heritable mutations. Theoretical considerations have led to the suggestion that only a limited number of genetic alterations (less than 10) may be absolutely required for tumor formation; these are usually referred to as *driver* mutations. If this number is reasonably accurate, it suggests that most of the genetic change in a tumor cell is collateral damage due to either mutagenic exposure and/or defects in DNA repair processes; these changes are called *passenger* mutations.

Mutational changes in genes can be classified biologically by their functional effect on the encoded protein. In cancer research, a long-standing distinction is made between "oncogenes" that incur dominant gain-of-function mutations (i.e., *neomorphic* mutations) and "tumor suppressor" genes that develop recessive loss-of-function mutations (*hypomorphic* mutations). Together these two types of mutations drive disease progression. Mutations can also have dominant-negative (*antimorphic*) effects; when only one allele is lost, this leads to dosage-dependent *haploinsufficiency* (Santarosa and Ashworth, 2004), which has also been recognized as contributing to cancer. A functional subdivision of tumor suppressors into *caretaker*, *gatekeeper*, and *landscaper* genes has also been proposed (Kinzler and Vogelstein, 1997; Michor et al., 2004). Caretaker genes, when mutated, lead to genomic instability and enhanced mutation acquisition. In contrast, gatekeeper genes encode proteins that restrain cell growth, and disrupting their function allows enhanced cell proliferation. Finally, defective landscaper genes foster a microenvironment conducive to tumor cell survival. Doubtless other categories of genes will also have a significant role in cancer.

The Nature of Genetic Interactions in Cancer

Clearly, the complexity of cellular systems does not arise from the independent action of a large number of different genes but is rather the result of extensive genetic interactions among

Box 1. Glossary

This glossary presents the consensus definitions of terms used in the text. Definitions of many terms vary, and many of the concepts and terms overlap considerably in meaning.

Mutations and Alleles

- ***Driver mutation*:** Genetic alteration that provides an advantage to cells facilitating tumor formation and survival. Driver mutations may not necessarily be required at all points in the natural history of the disease.
- ***Passenger mutation*:** Tumor-specific genetic alteration that is not essential for tumor formation.
- ***Gain-of-function mutation*:** Mutation that increases a gene product's activity or results in a new function. Subclassifications include *hypermorphs*, for which the mutation increases normal gene function; *neomorphs,* for which the dominant gain of function is distinct from normal function; and *antimorphs*, for which the dominant mutations act to oppose normal gene function.
- ***Loss-of-function mutation*:** Mutation that decreases or destroys the gene product's function. Subclassifications include *amorphs*, where the mutation completely ablates normal function (also known as a *null mutation*); and hypomorphs, where the mutation partially disrupts normal function.
- ***Haploinsufficiency*:** An allele dosage effect in which diploid cells carry a single active copy of a particular gene and that copy is insufficient to support the function of two wild-type alleles.
- ***Caretaker, gatekeeper, and landscaper genes*:** A classification of genes involved in cancer. Caretaker genes encode products that engage in maintaining the stability of the genome, such as the genes involved in DNA repair. Gatekeeper genes encode proteins that restrain cell growth, and their loss of function allows enhanced cell proliferation and the transmission of mutations. Landscaper genes encode products that, when mutated, contribute to the abnormal, neoplastic growth of cells by fostering a microenvironment conducive to unregulated cell proliferation.
- ***Gradualism*:** The gradual and stepwise accumulation of tumorigenic mutations over time.
- ***Chromothripsis*:** The nearly simultaneous acquisition of multiple mutations in a tumor cell via the catastrophic shattering and then reassembly of chromosomes.

Gene Interactions

- ***Epistasis or gene interaction*:** When the effects of one gene are modified by one or several other genes, which are often called *modifier genes*. In contrast to dominant/recessive effects that describe the interaction between alleles of the same gene, epistatic effects describe the interaction between genes at different loci. When the phenotype of multiple genes is expressed within the context of a gene interaction, these genes are defined as *epistatic*; when the genes' phenotype is repressed within the context of the gene interaction, these mutations are defined as *hypostatic*. In functional terms, these gene interactions can also be defined as *genetic suppression* or *enhancement effects*; that is, the combination of gene effects on a phenotype is less or more profound than the expected combination of single-gene effects, respectively.
- ***Synthetic lethality*:** A gene interaction in which single-gene defects are compatible with cell viability, but the combination of gene effects results in cell death (Figure 1). The term synthetic lethality is from the Greek, *συνδυάζω* or "syndiazo," which means "two or more features combined."
- ***Synthetic sickness*:** A gene interaction in which a combination of changes in different genes generates a greater deleterious effect on the fitness of a cell than would be expected given the individual phenotypes of individual mutations (Figure 1B).

Box 1. *Continued*

- ***Synthetic viability*:** The reverse of synthetic lethality. One gene alteration causes lethality, but in the presence of a second alteration in another gene, the cell is viable (Figure 1).
- ***Hard and soft synthetic lethalities:*** Describes the extent to which synthetic lethal interactions between two genes are modulated by changes in additional genes. Hard synthetic lethalities are relatively unaffected by changes in additional genes, whereas soft synthetic lethalities can be readily rescued by alterations in other genes. Ideally, the preferred targets of cancer therapy are hard synthetic lethalities.
- ***Non-cell-autonomous synthetic lethality*:** Synthetic lethality that occurs between two or more genes in different cells.
- ***Functional buffering*:** The ability of complex molecular systems to buffer against the tendency of new alleles to impair cell fitness or viability. This term is also known as *genetic canalization*.
- ***Gene addiction*:** The ability of a cell to become completely dependent upon the activity of a gene. In cancer, tumor cells often become addicted to the activity of oncogenes. A prime example of gene addiction is the dependency of chronic myelogenous leukemia cells on the constitutive activity of ABL kinase.
- ***Functional redundancy*:** When two or more genes, proteins, or pathways perform similar, interchangeable activities. Synthetic lethality can occur between genes with functional redundancy.
- ***Induced essentiality*:** A refined model of the synthetic lethality concept; when the functional buffering that occurs in response to a cancer gene mutation results in a dependency or addiction to another gene.
- ***Genetic capacitor*:** A protein or molecular system that restricts the ability of genetic variation to change phenotype. Heat shock proteins are regarded as an example of a genetic capacitor because they restrict the deleterious effects of a highly mutated cancer proteome.
- ***Network compatibility*:** When the acquisition of successive mutations in the natural history of a tumor is defined by their compatibility with pre-existing mutations and the functional buffering that has occurred alongside existing mutations.

them. Although definitions for "genetic interactions" vary, they can often be described as *epistasis* and then further subclassified as either *enhancement* or *suppressive* effects. For example, considering the effect of mutating two genes, *A* and *B*, on a phenotype such as cell fitness (Figure 1), these genes have an *enhancement* interaction when a combination of mutations in both genes has a far greater effect on cell fitness than would be predicted by a simple combination of individual gene effects. Conversely, a *genetic suppression* interaction produces a less profound effect when both genes are mutated than would be expected from the combination of individual gene effects. These gene interactions can be extreme and result in cell death or at least a significant reduction in the fitness of cells. For example, *synthetic lethality* and *synthetic sickness* (Dobzhansky, 1946; Lucchesi, 1968) describe the scenario where single-gene defects are compatible with cell viability but the synthesis or combination of gene effects results in cell death or a significant impairment of fitness. At the other extreme is a combination of gene effects that rescues the lethal effects of a single gene change; we term this effect *synthetic viability*, given its inverse relationship to synthetic lethality.

So how do such concepts impact upon our understanding of the genetic interactions in cancer? Cooperative interactions

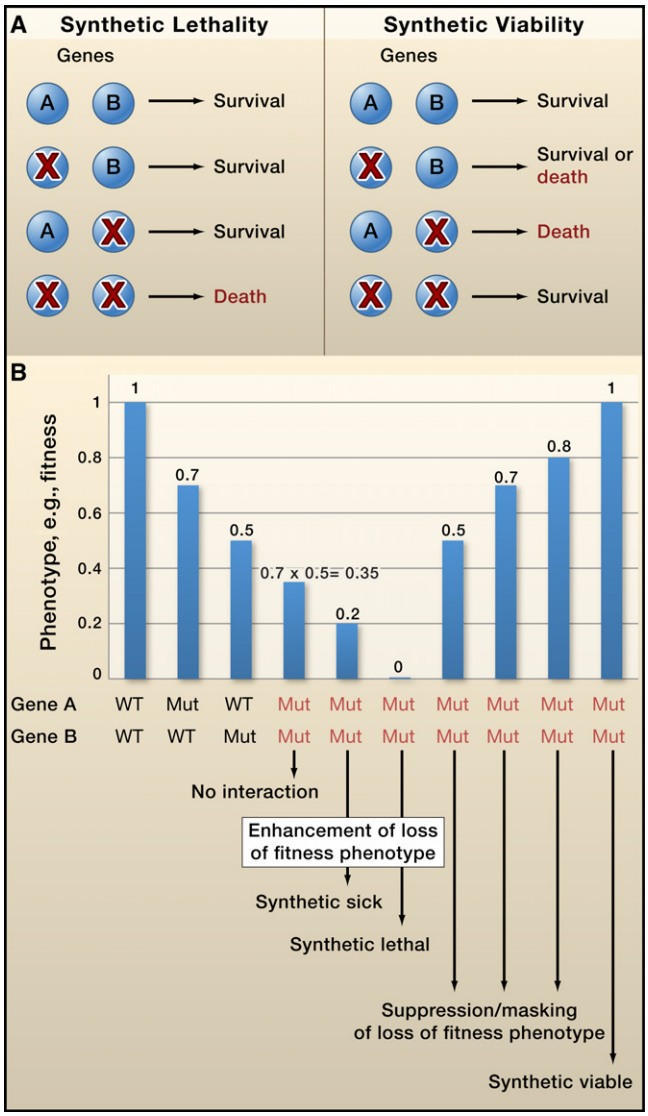


Figure 1. Gene Interactions in Cancer

(A) Extreme forms of genetic interaction are defined by synthetic lethality (in which a combination or synthesis of gene mutations causes cell death) and the reverse scenario, synthetic viability (in which a combination of gene effects rescues the lethal effects of a single gene change).

(B) Different modes of genetic interaction defined by quantitative effects on a phenotype, such as cell fitness. Here the value 1 represents the maximal fitness of cells, and the individual effects of changes in genes A (0.7) or B (0.5) are shown. When no interaction between genes A and B exists, the simple combination of effects (shown here as 0.7 × 0.5 = 0.35) is expected; any deviation from this value suggests an interaction between genes A and B.

between oncogenes, such as the transcription factor gene *MYC* and the GTPase gene *RAS*, have long been recognized as contributing to the processes of transformation and immortalization (Land et al., 1983). This type of interaction was originally viewed as a genetic enhancement effect. However, and perhaps counterintuitively, some cancer drivers, such as activated mutant *RAS*, while imparting a selective advantage to tumor cells can also cause deleterious effects, and gene interactions can act to mitigate these. RAS activation causes oncogene-induced senescence, a process driven by the excessive firing of multiple replication forks, the activation of ATM, and a DNA damage response (Bartkova et al., 2006; Di Micco et al., 2006). Inactivation of ATM suppresses the effect of RAS on oncogene-induced senescence and therefore represents an example of synthetic viability.

Similarly, loss of either *BRCA1* or *BRCA2* tumor suppressor gene function in cells triggers a cell-cycle arrest at the G_2/M checkpoint that can be suppressed by the inactivation of *P53* (Connor et al., 1997; Liu et al., 2007). This situation may also be viewed as a relationship between gatekeeper and caretaker mutations, in which caretakers such as *BRCA1* and *BRCA2* protect the genome against the potentially mutagenic and tumorigenic effects of DNA damage, whereas gatekeepers such as P53 induce cell death or cell-cycle arrest of cells that have lost caretaker function. Similarly, loss of VHL (Von Hippel-Lindau tumor suppressor) function normally causes cellular senescence, but inactivation of a second tumor suppressor, RB (Retinoblastoma), can suppress this process (Young et al., 2008).

As a final example of genetic suppression, the reliance of many tumor cells on molecular chaperones, such as HSP90, may be viewed as reflective of an entire series of gene interaction effects. Molecular chaperones facilitate protein folding and aggregation in normal cells, and in tumor cells HSP90 probably suppresses some of the deleterious effects of a highly mutated proteome, acting as a form of *genetic capacitor* (Whitesell and Lindquist, 2005).

The simple gene interactions described above almost certainly reflect the nature of biological systems: complex networks of interactions that maintain cellular homeostasis, fitness, and survival in the face of environmental and/or genetic change. In essence, these gene interactions provide *functional buffering* (Hartman et al., 2001), sometimes described as *genetic canalization*—that is, the buffering of pathways against the tendency of new alleles to make nonoptimal phenotypes (Gibson and Wagner, 2000; Waddington, 1959). For example, loss of cell-cycle checkpoints functionally buffers and reduces the deleterious effects of BRCA dysfunction or VHL loss, allowing the cell to survive, albeit with a modified phenotype.

Large-scale mutagenesis studies in worms and yeast suggest that these functional buffering networks are relatively pervasive. Most single-gene deletion mutations do not impair viability or fitness, at least under laboratory conditions. This suggests that either the majority of genes are not involved in maintaining cellular viability or cell division or, perhaps more likely, functional buffering against the dysfunction of an individual gene is common (Giaever et al., 2002; Kamath et al., 2003). Supporting this latter hypothesis, analysis of viable yeast strains with deletion mutations indicates that nearly all mutants display perturbed gene expression profiles. This suggests that the loss of almost any nonessential gene may result in a compensatory change in the molecular network (Hughes et al., 2000). Clearly, this is potentially bad news for cancer therapies that aim to block individual pathways. However, systematic functional screens in model organisms have also shown that each nonessential gene typically has between 10 and 30 synthetic lethal partners (Baryshnikova et al., 2010), suggesting that intervening at a "sweet spot" might overcome compensatory network rearrangements.

Concepts of gene interaction and functional buffering are critical for understanding tumor evolution. Most existing models of cancer progression have assumed that in the majority of cases, the acquisition of cancer-promoting mutations occurs cumulatively, perhaps over years to decades (Jones et al., 2008). However, mutagenic "big bangs," such as those driven by telomere attrition (O'Hagan et al., 2002), or *chromothripsis* (the shattering and then reassembly of chromosomes) (Stephens et al., 2011) challenge the universality of this *gradualism* dogma. Regardless of the mechanism, it seems reasonable to assume that cancer progression is partly shaped in some form by both synthetic lethal and synthetic effects. For example, given that BRCA dysfunction induces an acute cell-cycle arrest in the presence of functional P53, it seems reasonable to propose that P53 dysfunction usually precedes BRCA loss of function, generating a permissive state and synthetic viability.

In the example above the order in which mutations occur is likely defined by the genetic interactions involved. In other situations it may be that the nature of the genes mutated is more important than the order (Anderson et al., 2011). Currently, it is unknown which situation is the more prevalent phenomenon, which is due in large part to the paucity of studies examining the order in which oncogenic driver mutations accumulate from an original clonal population. Nevertheless, some general principles may be emerging.

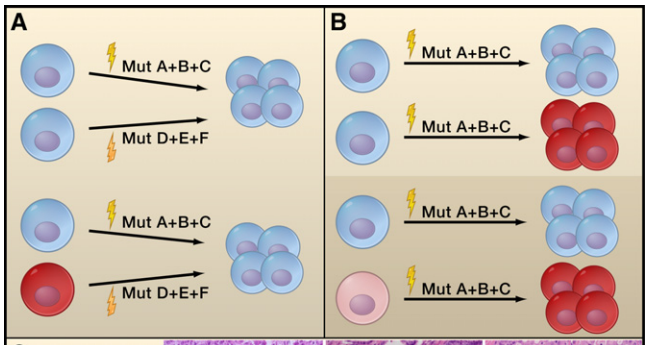
First, for the cell to remain viable when two mutations occur successively in a cell, the second mutation must be compatible with the effect of the first mutation. Moreover, the second mutation must also be compatible with any functional buffering mechanism that mitigates the deleterious effects of the first mutation. In addition, driver mutations cannot be synthetically lethal with mutations that occur earlier in the ultimate tumor cell lineage. Most mutations in the natural history of a tumor that result in a phenotype may need to fit into this schema of *network compatibility.* If this is the case, it could result in genetic canalization. For example, in the face of tumor-associated mutations and the network remodeling that mitigates the potentially deleterious effects of mutation, the spectrum of subsequent mutations that are compatible with these changes could be restrictive. This could ultimately cause an evolutionary progression that is increasingly stereotyped. Moreover, this genetic ratcheting may partially explain the relatively nonrandom histological features of tumor types. It may also explain the recurrence of specific mutations in particular types of tumor; in some cases once an initial driver mutation is in place, the eventual fate of any surviving daughter cell is essentially sealed, or at least partially defined.

Heterogeneity in Cancer: Context Dependence of the Phenotypic Effects of Mutations

Multiple mechanisms have been proposed to explain the phenotypic heterogeneity of cancer, including differences in the cells of origin, heterotypic interactions between cancer cells and their microenvironment, and the plasticity of so-called cancer "stem cells" (i.e., cells that have an indefinite potential for self-renewal and that drive tumorigenesis) (Vargo-Gogola and Rosen, 2007; Visvader, 2011). In addition, the interplay among the functional consequences of genetic and heritable epigenetic aberrations, synthetic lethal and viable interactions, and genetic canalization likely play major roles in generating the diversity of cancer phenotypes.

The availability of cancer genomes at base-pair resolution is allowing a detailed analysis of the relationships between cancer genotypes and phenotypes (Figure 2). In particular, the genomes of "rare" types of cancers are providing useful information. Rare types of cancer, which often have specific histological appearance, may be driven by pathognomonic genetic aberrations (i.e., ones that are distinctively characteristic of a particular disease) (Figure 2C). An example of this comes from sequencing studies in two rare and histologically distinct types of ovarian cancer, granulosa cell tumors and clear cell carcinomas. Mutations in the transcription factor *FOXL2* (Shah et al., 2009a) are common in granulosa cell tumors, whereas ovarian clear cell carcinomas have recurrent genetic changes affecting a few driver genes, particularly *ARID1A* (*AT Rich-interactive domain 1A* [*SWI-like*]) and *PPP2R1A (Protein Phosphatase, Subunit A, R1-alpha isoform)* (Jones et al., 2010; Wiegand et al., 2010). Some rare cancer types also have similar phenotypes and driver events but can occur in different anatomic sites. For example, adenoid cystic carcinomas of both breast and salivary glands are characterized by almost identical histological features, and both tumors carry the oncogenic *MYB-NFIB* fusion gene (Persson et al., 2009) (Figure 2A). These examples suggest that sequencing the phenotypic extremes represented by the "rare" cancer types may constitute a way to reduce the heterogeneity of cancers a priori and thus facilitate the identification of recurrent driver mutations.

Adding an increasing level of complexity to our understanding of genotype-phenotype relationships in cancer, a number of tumors are now known to share identical driver mutations even though the tumors arise in distinct anatomical sites and exhibit different histological features and clinical behavior. These observations suggest that some mutations may generate divergent phenotypes in different environments and cell types (Figure 2B). Activating *KIT* (*v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog*) mutations drive not only gastrointestinal stromal tumors but also mucosal and acral (i.e., palmoplantar and subungual) melanomas and mast cell disorders (Davies et al., 2006; Davies and Samuels, 2010). In addition, the *ETV6-NTRK3 (ETS variant 6-neurotrophic tyrosine kinase, receptor, type 3*) fusion gene can drive tumors from distinct anatomical sites, with different differentiation lineages and dissimilar clinical behaviors (Lannon and Sorensen, 2005). Although it is not completely clear why this occurs, the interplay between cell of origin and the specific mutation undoubtedly provides some explanation for convergent and divergent phenotypes (Figures 2A and 2B). One possible mechanism underlying this phenomenon might be that a driver mutation has an overt impact on the ability of cells to differentiate, so that these aberrations cause convergence toward the same phenotype regardless of the cell of origin. Nevertheless, in other cases, the cell origin does influence the phenotype. For example, mutations in *Ctnnb1* (*β-catenin*) have little impact on progenitor cell populations in the cerebellum, but they may drive medulloblastoma development when present in embryonic dorsal brainstem cells (Gibson et al., 2010).



Characteristic	Common epithelial malignancies	Special histological types of epithelial malignancies	Rare epithelial malignancies
Phenotypic diversity	High	Medium	Rare
Genome	Instable	Low level of instability	Stable
Mutations, copy number changes rearrangements	Multiple, nonrecurrent	Few, affacting multiple genes in few pathways	Tumor-type specific genetic aberrations
Cases to identify passengers, drivers	Hudreds-to-thousands	Fewer cases	Small numbers
Pathognomonic mutations	No	Unlikely	Yes
Example	High grade invasive ductal carcinoma	Tubular carcinoma	Secretory carcinoma

Figure 2. Genotypic-Phenotypic Correlations in Cancer

(A) Convergent phenotypes: tumors of similar, if not identical, phenotypes may be driven by distinct genetic aberrations. Convergent phenotypes are, perhaps, best exemplified by gastrointestinal stromal tumors, which have remarkably similar histological characteristics and clinical behavior but are driven by mutations in either *KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog*) or *PDGFRA* (platelet-derived growth factor receptor, peptide A), or more rarely by mutations in *SDHB* (succinate dehydrogenase complex, subunit B, iron sulfur [Ip]) or *SDHC* (succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa) (Janeway et al., 2011). In addition, mouse models have demonstrated that inactivation of *Brca1* and *Tp53* in lumenal progenitor and basal cells of the mouse mammary gland lead to the development of tumors with similar histological features, immunohistochemical characteristics, and transcriptomic profiles (Liu et al., 2007; Molyneux et al., 2010).

(B) Divergent phenotypes: tumors of distinct phenotypes (originating either in the same cell or from cells of different cell lineage) and having distinct clinical behaviors may possess identical driver genetic aberrations. For example, subgroups of gastrointestinal stromal tumors, mucosal and acral melanomas, as well as mast cell disorders are all driven by activating *KIT* mutations. Likewise, the oncogenic *ETV6-NTRK3 (ETS variant 6-neurotrophic tyrosine kinase, receptor, type 3*) fusion gene is one of the defining features of congenital fibrosarcomas, cellular mesoblastic nephromas, secretory carcinomas of the breast, acute myeloid leukemias, tumors from distinct anatomical sites, distinct differentiation lineages, and different clinical behaviors (Lannon and Sorensen, 2005).

(C) Correlations between prevalence, phenotypic diversity, and genetic heterogeneity of epithelial malignancies. Common epithelial malignancies are phenotypically diverse, given that they comprise operational subgroups of multiple diseases and are not discrete biological entities. These cancers are often genetically unstable they often harbor a multitude of mutations that are usually private (i.e., unique to an individual cancer) and that may affect several molecular pathways. Tumors with phenotypes that are relatively homogeneous usually have low-to-moderate levels of genetic instability and are driven by a limited repertoire of genetic aberrations, which are relatively recurrent. Rare cancer types are phenotypically homogeneous and are often driven by pathognomonic genetic aberrations. Not all rare cancer types have low levels of genetic instability, but even when they are more genetically unstable, the repertoire of genetic aberrations found in rare and phenotypically homogeneous cancers appears to be more limited than that found in common cancer types.

In addition to the genetic heterogeneity between cancers, strong evidence now indicates that genetic heterogeneity also occurs within individual tumors (Anderson et al., 2011; Ding et al., 2010; Mullighan et al., 2008; Shah et al., 2009b). A substantial proportion of cancers may be composed of a mosaic of nonmodal populations of cells (i.e., populations that account for <50% of all cancer cells) that, although they share the same initiating event, evolve through the acquisition of additional genetic aberrations. This evolution may explain not only the phenotypic diversity within a given cancer (Geyer et al., 2010) but also the cancer's proclivity to metastasize to distinct anatomical sites (Ding et al., 2010; Shah et al., 2009b) as well as its resistance to both chemotherapy and targeted therapies (Mullighan et al., 2008).

Genetic differences between primary tumor and metastatic deposits and the existence of genetic heterogeneity with a tumor raise some interesting questions about the universal importance of the phenomenon of tumor self-seeding by circulating cancer cells (Kim et al., 2009) and "cancer stem cells" (Anderson et al., 2011). The phenotypic diversity within cancers has been ascribed to the existence of cancer stem cells and their ability to differentiate down multiple lineages (Reya et al., 2001). However, in one study, sorted populations of both putative cancer stem cells and more differentiated cells displayed similar genetic heterogeneity found in the bulk of the tumor (Anderson et al., 2011). Moreover, at least in some cases, phenotypically distinct areas within a given tumor are underpinned by distinct patterns of genetic alterations (Geyer et al., 2010). Finally, leukemias that were genetically heterogeneous at the time of diagnosis maintain this genetic heterogeneity upon serial transplantation (Anderson et al., 2011). These results do not necessarily refute the case for cancer stem cells, but at least in these situations, a Darwinian or clonal cancer evolution mechanism for cancer heterogeneity appears much more likely.

Deep sequencing of cancer metastases indicates that, as with primary cancers, metastases may be composed of multiple clones that have acquired genetic abnormalities not detectable in the primary tumor (Anderson et al., 2011; Ding et al., 2010; Mullighan et al., 2008; Shah et al., 2009b). Independent studies of leukemias have now demonstrated that the clones harboring the most complex patterns of genetic aberrations are not necessarily the dominant clones or the clones that will be the source of relapse after therapy (Anderson et al., 2011; Mullighan et al., 2008). In fact, instead of outright competition or predatory interactions, a more complex interplay between genetically distinct populations within a cancer may take place (Marusyk and Polyak, 2010). Examples have been documented of both *commensalism*, or a positive interaction in which one clone benefits the other without itself being affected, and *mutualism*, or cooperation between cancer cell populations (Calbo et al.,

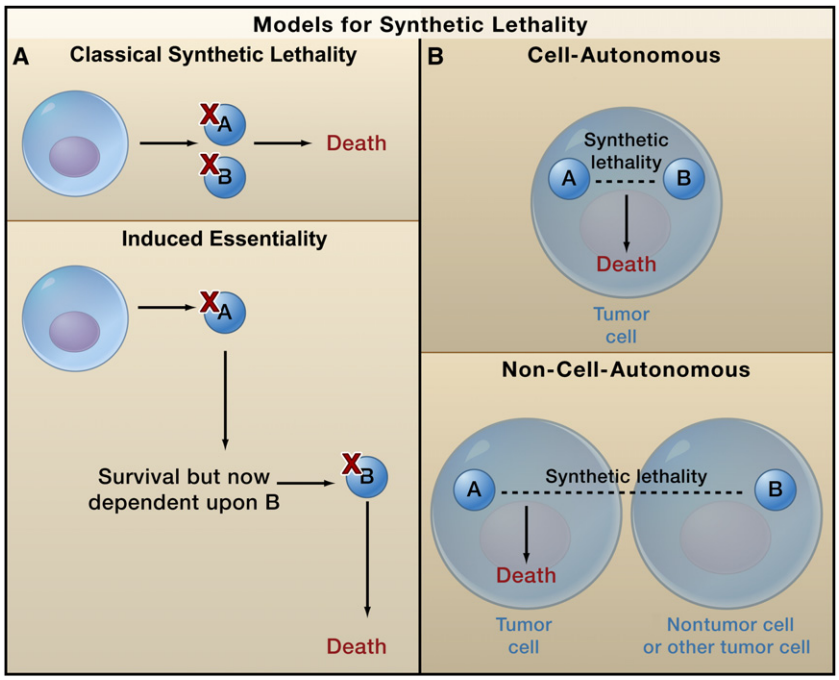


Figure 3. Therapeutic Implications of Gene Interactions

(A) Classical synthetic lethality and induced essentiality (Tischler et al., 2008). Classically, synthetic lethal relationships (shown here between gene A and gene B) have generally been discussed in terms of functional redundancy between genes or proteins that have similar functions (top). This model describes systems where A and B are relatively interchangeable in terms of function. Although this model likely applies to some forms of synthetic lethality, the revised model of induced essentiality is particularly pertinent to cancer (bottom). This model relies upon the inherent plasticity of biological networks and their ability to respond to perturbation. Here, loss of gene A is compatible with viability but only because molecular networks reorder to accommodate this perturbation. In this new state, gene B is now essential, and its inhibition causes cell death (i.e., synthetically lethal with gene A).

(B) Some lethal genetic interactions likely occur because of the interaction of genes only within one cell (i.e., cell-autonomous synthetic lethality) (top). However, gene interactions between different cells can also cause lethality (bottom). This could be in cells that directly interact with the tumor cells (e.g., fibroblasts) or distant cells producing growth-promoting hormones such as estrogen.

2011). These phenomena may be related to the high prevalence of genetic heterogeneity within cancers and the observation that, at early and late stages of disease evolution, there may be no absolute dominance of a single clone (Marusyk and Polyak, 2010).

The impact of intratumor heterogeneity on the metastatic ability of a given cancer has been the subject of considerable discussion (Klein, 2009). Data from gene expression profiling studies led to the suggestion that the metastatic behavior of a cancer was determined at an early stage and that all cells within a given cancer would have a similar proclivity to give rise to metastases (Weigelt et al., 2003). Recent sequencing studies have called into question these concepts. In pancreatic cancers, metastatic clones may harbor specific genetic aberrations, and the acquisition of metastatic ability occurs at a later stage. There is evidence to suggest that it may take at least 5 years for a fully malignant but nonmetastatic clone to transform into a metastatic "founder" clone (Yachida et al., 2010). On the other hand, clinical observations suggest that the existence of clones fully capable of metastatic dissemination may be present at the early stage of the development of some types of cancer, such as "triple-negative" breast cancers (Foulkes et al., 2010). It seems likely that in different cancer types, the timing differs for the emergence of a clone fully capable of metastatic dissemination. Moreover, this timing may depend on the size of the cancer cell pool, the levels of genetic instability, intratumor genetic heterogeneity, and proliferation rates of cancer cells (Foulkes et al., 2010).

Therapeutic Implications

Although some of the concepts we present in this Perspective could be viewed as semantic or even speculative, we believe they may have considerable utility in the development of new therapeutic approaches for cancer. Over the past few years, rapid and significant advances have been made in directly targeting the dependency or *addictions* that some tumor cells have on gain-of-function oncogenic mutations, such as the *BCR-ABL* fusion gene, the *ERBB2* amplification, and the *BRAF V600E* mutation (reviewed by Lord and Ashworth, 2010 and Haber, this issue). Conversely, little progress has been made in targeting tumor suppressor gene dysfunction, despite the relatively long-standing ability to identify and characterize these genes. However, by applying synthetic lethal concepts, this is now possible. The first example, at least knowingly, of a synthetic lethal therapy to reach the clinic is the targeting of BRCA1- or BRCA2-deficient tumor cells with PARP (poly(ADP-ribose) polymerase) inhibitors, and this potential success story establishes a new paradigm for drug development (Ashworth, 2008).

In essence, the relationship between BRCA and PARP is a true synthetic lethal one; normally PARP activity is not essential, but in the absence of *BRCA* gene function, PARP activity is critical for cell survival. Rather than representing a strict functional redundancy between BRCA and PARP, this effect is more likely to be an *induced essentiality* effect (Tischler et al., 2008) (Figure 3A) and a form of functional buffering. In clinical trials, PARP inhibitors can elicit significant and sustained antitumor responses in patients with *BRCA1* or *-2* mutant tumors that arise at different sites, such as breast, ovary, and prostate (Fong et al., 2009). Likewise, in vitro BRCA-deficient cells, even those with experimentally imposed BRCA dysfunction, are profoundly sensitive to PARP inhibitors, almost regardless of the histology or genetic background involved. The BRCA-PARP synthetic lethality also appears relatively unaffected by potential compensatory changes in the cell. This may be because there are few buffering mechanisms for this particular gene interaction, and

this interaction thus constitutes what we term a *hard synthetic lethality*.

From a network perspective, BRCA-PARP gene interaction may be part of a relatively small network with few synthetic viability interactions that can rescue the BRCA-PARP synthetic lethality. Regardless of whether this is the case, one mechanism of resistance to PARP inhibitors in cells with *BRCA2* mutations are secondary mutations that reinstate a functional BRCA2 isoform, resulting in one of the first examples of synthetic lethal resistance (Edwards et al., 2008). If this mechanism turns out to be pervasive, then it supports the hypothesis that few synthetic viability interactions exist that can rescue PARP inhibitor resistance.

In contrast to the hard synthetic lethality observed between BRCA and PARP, some of the recently identified gene interactions involving *RAS*, although still de facto synthetic lethalities, seem more susceptible to changes in cell type, context, and/or genetic background (Barbie et al., 2009; Luo et al., 2009a; Scholl et al., 2009); this effect we term *soft synthetic lethality*. This indicates that the resilience of any synthetic lethal or gene addiction effect must be stringently addressed on multiple genetic backgrounds before consideration as a therapeutic approach. Experimentally, these areas are now relatively straightforward to address. With improvements in genetic screening approaches, such as RNA interference, it is now feasible to systematically identify synthetic lethal interactions and test how influenced these are by compensatory network modifications (Ashworth and Bernards, 2010).

Cancer therapy applies a highly stringent form of selective pressure upon the tumor cell; the molecular mechanisms that functionally buffer the inhibition of a cancer drug target and that cause synthetic viability are most likely selected for and have the potential to lead to therapy resistance. This is perhaps most obviously seen in the numerous reports that describe resistance to signal transduction kinase inhibitors that target the mitogenic, prosurvival, and antiapoptotic functions of oncogenes like *BRAF* (Johannessen et al., 2010; Nazarian et al., 2010; Villanueva et al., 2010). After a strong initial response, the therapeutic effect of BRAF inhibitors appears abrogated by compensatory changes in the activity of other signaling pathways. From a genetics perspective, this could be seen as synthetic viability between the drug target and the gene(s) that leads to the compensatory mechanism. Hopefully, targeting these compensatory mechanisms and the use of combinatorial therapies, which simultaneously hit the primary target and the likely buffering and resistance mechanisms, will prove useful.

Regardless of whether this turns out to be the case, clearly it is not sufficient only to identify cancer drug targets that are tumor selective; these targets must also be robust in the face of genetic interactions and network compensation. Such networks of genetic interactions are being comprehensively catalogued in model organisms for which technical methods and many mathematical and computational approaches are already established. It is debatable whether a significant number of these gene interaction effects directly translate across species. Therefore, identifying global networks in human cells, although technically challenging, is a major objective and potential outcome from the burgeoning field of systems or network biology.

Given the growing understanding of the interplay between tumor cells and their microenvironment, and also between individual tumor cells themselves (e.g., *commensalism* and *mutualism*), it may be possible to exploit gene interactions that occur between cells rather than within individual cells as a therapeutic approach. For example, a drug could induce changes in the microenvironment or even macroenvironment that are synthetically lethal with a mutation or gene addiction in a tumor cell. Such *non-cell-autonomous synthetic lethality* (Figure 3B) could already be represented by the interaction between *CYP19A1* (the gene-encoding aromatase) and genes encoding estrogen receptors, like *ESR1.* Aromatase inhibitors, such as anastrozole and letrozole, inhibit the production of estrogens by targeting aromatase; this inhibition of hormone production targets the genetic dependency of some breast tumor cells upon *ESR1* and estrogen signaling (Johnston and Dowsett, 2003). The identification of additional heterotypic cell-cell interaction networks will require the development of appropriate in vitro or in vivo screening methodologies that better reflect the biology of cancer.

Here, we have tried to make the case that viewing cancer through the prism of genetic interactions may help us better understand cancer biology and the development of new therapeutic approaches. However, it is important to emphasize that these concepts by themselves merely generate hypotheses, and their practical application will require a thorough understanding of the molecular mechanisms involved.

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