Validating cancer drug targets

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A cancer drug target is only truly validated by demonstrating that a given therapeutic agent is clinically effective and acts through the target against which it was designed. Nevertheless, it is desirable to declare an early-stage drug target as 'validated' before investing in a full-scale drug discovery programme dedicated to it. Although the outcome of validation studies can guide cancer research programmes, strictly defined universal validation criteria have not been established.

Rapid advances in elucidating the molecular basis of cancer, as well as the availability of the complete sequence information of the human genome, have led to the expectation that the discovery and development of cancer drugs might become more predictable and efficient, and less serendipitous, than in the past. The key to such success is believed to lie in the concept of targeted therapy — that is, the development of drugs that influence the action or activity of a specific signalling pathway or constituent thereof. Consequently, three interrelated elements are crucial to implementing such targeted drug discovery projects. First, there must be good reason to believe that a given target (a specific gene or protein against which a drug will be developed) has causal relevance to cancer — that is, a hypothesis must be formulated with respect to the target. Second, data relevant to this hypothesis must be collected and evaluated; this includes evaluating the effects of modulating the activity of a given target in available experimental models. Third, the impact of intervention via the target must be clinically assessed. The process of evaluating potential cancer targets in this manner can be termed 'validation', and the targets that emerge might be considered, in general terms, to be 'validated'.

The manner and extent to which these strategies are integrated into cancer drug discovery and therapy are continuously evolving. This article provides a close and critical evaluation of the available tools and current rationales that might be used to tackle the challenging task of validating cancer drug targets. We initially review the conceptual framework of cancer therapy and define four tracks for the classification of cancer targets. We then scrutinize the utility of validation methods by analysing their impact on the cancer drug discovery process. We finally conclude that many different criteria might be applied when defining a validated target, and that our ability to predict the efficacy of a targeted cancer drug in the clinic holds great, but largely unfulfilled, promise.

Concepts for cancer drug therapy

The overarching hope of cancer drug discovery is to design effective and non-toxic therapies. Over time, the concept of what constitutes a cancer target has become more refined, and has been driven by both a deepening understanding of cell biology and the development of new technologies. Metabolic enzymes were the focus of drug discovery projects in the mid-twentieth century, leading to the development of folate and methotrexate as 'targeted' therapies of their day. Subsequent understanding of DNA structure and the molecular basis of DNA replication allowed therapies directed against DNA polymerases and topoisomerases to be developed. Insight into hormone signalling guided the design of cancer therapies targeting nuclear hormone receptors in breast and prostate cancer.

The deepening molecular understanding of signalling pathways has directly affected the development of targeted therapeutics. Elucidation of the roles of many kinase signalling pathways in cancer, including growth factor receptors and their effectors, along with the identification of kinases as a druggable target class, has recently been the focus of productive target-based oncology drug discovery. Moreover, discerning specific changes in cancer cells has led to more sophisticated hypotheses about the differences between them and their normal counterparts, and the relevance of these differences to the aetiology of the cancerous phenotype (Fig. 1). The quest to identify such distinguishing characteristics — whether dictated by observations of gene rearrangements or mutations, stable epigenetic changes, lineage legacies and identities, or other accrued genetic (or metabolic) liabilities — has become synonymous with modern cancer research and therapeutic development; genes for which activity, expression or dependence is thought to have increased are prime candidates for therapeutic intervention. Moreover, it is expected that future insights will be more comprehensive, accurate, sophisticated and useful than ever before. On the basis of these major principles of cancer dependencies, we can define four different subtypes of cancer target: genetics, synergy, lineage and host (Fig. 2 and Table 1).

The genetics track

The recent ability to analyse the sequence, copy number and expression levels of individual genes within cancer cells, and to simultaneously interrogate many genes in multiple independent tumours versus normal tissues, has led to the belief that such data will facilitate the robust identification of therapeutically exploitable differences between cancerous and normal cells. Such changes are believed to occur at a finite frequency in mammalian cells; serendipitous mutations confer a selective advantage to a subpopulation of cells, leading to cancer. The current conception of cancer targets assumes that tumour cells have undergone such stable changes, that at least some of the alterations are causally related to the cancer state itself (that is, they occur in oncogenes or tumour-suppressor genes), and that they are heritable between sequential cell divisions.

The first evidence of genetic alterations as causal agents in cancer was indirect, with the observation of an increased incidence of tumours in individuals or animals exposed to ionizing radiation or known mutagens. Later, the association of gross chromosomal rearrangements with leukaemias was the first direct evidence that genetic lesions might have a causal role in cancer. The first such rearrangement that was fully understood at the molecular level was a reciprocal translocation associated with chronic myeloid leukaemia (CML), which eventually became known as the Philadelphia chromosome. This translocation creates a transcript

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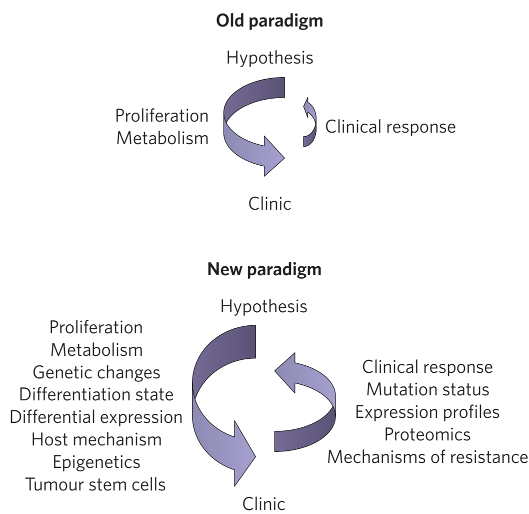

Figure 1 | Evolution of target-validation paradigms. 'Older' paradigms were driven by relatively empirical concepts of differences in metabolism or proliferation rates of tumour cells versus normal cells. In the absence of robust representative models, clinical response was often the sole determinant of target validation. The deepening understanding of the molecular basis of cancer, the availability of more robust experimental models and the ability to evaluate more extensively the characteristics of tumours have led to current concepts of discovery, validation and exploitation of molecular targets in cancer treatment. Importantly, such 'new paradigms' incorporate recent analytical technologies applicable to clinical samples, leading to greater and more insightful feedback from the clinic in evaluating available therapies. In addition, the application of such technologies now drives hypothesis formulation itself; facile identification of changes in the genetic or proteomic status of cancer cells has become a means of generating new hypotheses and of nominating promising novel therapeutic targets.

encoding a novel fusion protein known as BCR-ABL, a misregulated Abl (Abelson murine leukaemia viral oncogene) kinase in which the translocation causes replacement of the endogenous autoregulatory domain with erroneous coding sequences from BCR (breakpoint cluster region). Specific targeting of this aberrant activity led to the development of what might be considered the most notable success in the post-molecular era of cancer drug discovery: imatinib mesylate (STI571 or Gleevec). This inhibitor of the novel kinase has been highly successful in the treatment of CML, resulting in up to 80% response rates in newly treated patients[1]. Whereas many translocations have subsequently been identified as genetic factors associated with leukaemias, few appear to have occurred in kinases or other proteins with enzymatic activity that would be readily addressable by small-molecule inhibitors. Moreover, no such translocation patterns have been identified that appear to be frequently associated with solid tumours.

In addition to genetic changes that alter the encoded proteins in cancer cells, stable changes in gene expression, often through the amplification of specific genes, have successfully served both as a guide for therapeutic development and as a means to identify patient cohorts that might benefit from such treatments. For example, the observation that the epidermal growth factor receptor-2 (*ERBB2*) gene is amplified, and that its encoded receptor protein is aberrantly expressed in some breast cancers triggered the development of trastuzumab (Herceptin), which is an effective antibody therapy[2].

Cancer cells might be dependent upon such changes for their survival; indeed, the therapeutic successes outlined above suggest that this is the case. The dependence might occur early in the evolution of a tumour, or could be contingent upon changes that occur during tumour establishment. This hypothesis of continuous dependence has become known as oncogene addiction[3], and is a reasonable interpretation of the successful targeted treatments exemplified above. Methods now exist to identify with relative ease genetic changes that have occurred in cancer cells. However, identifying the particular changes to which tumour cells have become addicted, as well as the timing of onset of such dependence, remain key challenges in applying this hypothesis to cancer therapy.

The synergy track

Synthetic lethal genetic interactions are classically defined as significantly deleterious or lethal phenotypes resulting from the combination of two or more mutations that do not produce such phenotypes individually. Gain-of-fitness changes that occur in cancer cells, allowing their survival or conferring a growth advantage, might also inadvertently sensitize the cells to other stresses that would have no consequence in normal cells but have lethal consequences in combination with the tumorigenic changes. Cells bearing such unique combinations would have distinctive liabilities that might be exploited therapeutically[4,5]. Analogous concepts are widely applied in yeast genetics, and might also have important implications in developing specific cancer therapies.

Chemical inhibition of the function of a gene product can be considered as equivalent to a genetic loss-of-function mutation. As such, the identification of specific drug targets in cancer cells that are synthetic lethal in combination with mutant genes might allow the specific destruction of cancer cells while leaving normal cells intact. A recent example of such a scenario is the preliminary observation that inhibiting poly(ADP-ribose) polymerase (PARP) in combination with mutations that inactivate the breast cancer (*BRCA*) gene, results in tumour cell death in experimental settings[6]. Because the PARP and BRCA proteins play important roles in different DNA damage-repair processes, loss of both of these functions might have disastrous consequences for the cell. Thus, cancer cells that have undergone homozygous somatic loss of BRCA function are distinct from other cells, and might be characteristically susceptible to PARP inhibition. Rapamycin sensitivity of cells in which loss of PTEN (phosphatase and tensin homologue) or gain of phosphatidylinositol-3-OH kinase (PI(3)K) function has occurred is another example of a synthetic lethal phenotype that is applicable to cancer therapy[7]. This is consistent with the previously suggested convergence of PTEN and FRAP/mTOR (mammalian target of rapamycin) signalling in pathways involving the AKT and S6 kinases.

Along similar lines, many cancer cell lines have lost essential protective cellular mechanisms in the process of becoming tumorigenic. For example, loss of apoptotic signals, such as those mediated by B-cell leukaemia/lymphoma 2 (BCL2) family members, is necessary for survival of pre-malignant or tumour cells. In cases where cancer cells are tenuously poised to defy apoptotic signals, restoring the function of apoptotic signalling pathways might result in selective death of such cells. One major challenge in this area will be to determine, in a clinical setting, which tumours would be susceptible to such intervention[8].

Although purely speculative, another aspect of synergy in cancer therapeutics relates to agents that have specific cellular targets but selectively induce the death of cancer cells by as yet unknown mechanisms. Examples include histone deacetylase (HDAC) inhibitors and heat-shock protein 90 (HSP90) inhibitors, such as geldanamycin. The observed therapeutic effects might derive from a combination of the targeted effect with other collateral changes the cancerous cell has undergone that are not present in normal cells. Although such effects are poorly defined at present, systematic exploration of such treatment modalities and their mechanisms might have great value in guiding the therapeutic use of these agents.

The lineage track

Comparative analysis of gene expression has led to the observation that patterns of gene expression from tumours of the same cell type resemble each other and their normal counterparts more closely than either cancer cells or normal cells derived from different tissue types. Thus, cancer cells often maintain many features of the cells from which they were derived. The possibility that such residual or legacy characteristics might be exploited in targeted cancer therapy has come to be known as lineage addiction. The therapeutic efficacy of oestrogen receptor antagonists, such as tamoxifen and letrozole (Femara), in the treatment

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of breast cancer is a striking example of a relatively non-toxic targeted therapy that supports this concept[9]. The dependence of many prostate tumour cells on androgen receptor (AR) signalling, which is required for the survival of normal prostate secretory epithelial cells, and the therapeutic efficacy of AR antagonists are further examples of the validity of such concepts[10]. In addition, haematopoietic lineage has been effectively exploited in the treatment of non-Hodgkin's lymphoma: the anti-CD20 antibody rituximab (Rituxan), which recognizes a differentiation marker associated with cells of the lineage and a differentiation state characteristic of these cancer cells, has been highly effective in treating this malignancy.

In other cases, lineage status might provide a means of exploiting endogenous differentiation pathways characteristic of the tissue from which a given tumour arose, resulting in either death or re-entry of tumour cells into normal differentiation (for example, terminal differentiation). The successful use of retinoic acid in the treatment of acute promyelocytic leukaemia is an example of such a therapeutic strategy[11]. Recent identification of the microphthalmia-associated transcription factor (*MITF*) gene as an amplified sequence in aggressive melanomas, as well as its previously established role as a differentiation and survival factor in normal melanocytes, suggests that such paradigms might be applicable to other cancers[12].

The host track

Other strategies for targeted therapy do not address tumour cells *per se*, but instead focus on tumour environment or context. The apparent requirement of many tumours to sponsor *de novo* blood vessel development has led to the recognition of angiogenesis mediators as potential therapeutic targets. Bevacizumab (Avastin), which is a monoclonal antibody that inhibits vascular endothelial growth factor (VEGF) receptor, is an example of an angiogenesis-directed therapy that has shown some efficacy in the treatment of colorectal cancers. In addition, clinical efficacy against renal cell carcinoma and gastrointestinal stromal tumours has been observed by small-molecule inhibitors such as sorafenib (Nexavar) and sunitinib (Sutent), which target the tyrosine kinase activity of VEGF receptor[13].

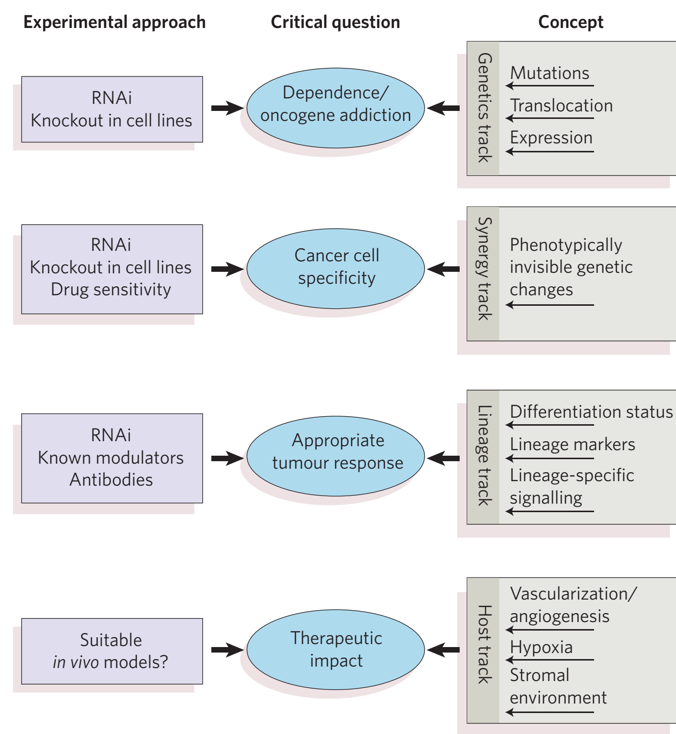

Figure 2 | Schematic overview of the four cancer drug discovery tracks. Each track is defined by the specific features listed. The critical question that must be addressed for target-validation purposes varies by track, and determines the appropriate experimental approaches for the evaluation and validation. RNAi, RNA-mediated interference.

Moreover, it is possible that tumours, by evolving within a particular physiological niche, might become dependent upon certain growth factors or other environmental elements (such as stromal cells) for growth or survival. Although intuitively legitimate, such therapeutic concepts have thus far met with only limited success, perhaps because of the absence of experimental models that recapitulate the complex and perhaps idiosyncratic environments of tumours *in situ*.

Whether targets associated with metastasis represent an opportunity for therapeutic intervention is controversial and largely unexplored (see the review in this issue by Christofori, page 444). It is possible that cancer cells at a metastatic site might have dependencies or vulnerabilities distinct from the primary tumour that could be exploited therapeutically. However, as mentioned above, the establishment and validation of legitimate and representative experimental models is an important and so far unsurmounted challenge.

Validation strategies

As outlined above, the definition of a validated cancer target, and the manner in which a given target is identified and vetted, has changed over time. Our current definition of target validation is the experimental evaluation of the role of a given gene or protein in cancer; this serves as the basis for determining whether it is a promising target for cancer therapy. As such, target validation, in the strictest sense, is simply a process of hypothesis generation and testing. Criteria for evaluating the validity of a target might range from observations of altered mutation or expression status in tumours, to evidence that activity of a given target contributes to cancer cell growth in one or more experimental systems. Below, we explore in more detail three strategies for validation — a genetic approach, functional cell-based assays and validation in animal model systems (Table 2) — that can be used to assess the four types of target we have classified (Fig. 2).

Genetic validation

Patterns of somatic mutations in a specific gene in a given tumour type have come to be accepted as compelling evidence that the mutant form of the gene (and perhaps even its wild-type counterpart) has an aetiological role in that tumour type. In addition, when such mutations occur in genes associated with signalling cascades, both the mutated gene product itself and downstream effectors of that pathway are potential therapeutic targets.

One example of a signal transduction pathway in which several targets are mutated in cancer is the Ras–Raf–MEK signalling cascade. *K-RAS* is mutated in approximately 80% of pancreatic cancers. In melanoma, *N-RAS* is activated in 15% of patients, whereas *K-RAS* and *H-RAS* are rarely mutated[14]. The historical inability to identify effective inhibitors of Ras — either directly by targeting its GTPase function or indirectly by inhibiting farnesyl transferases that modify Ras and target it to the plasma membrane — has led to scrutiny of signalling elements downstream of *RAS* as potential targets[15]. A newly emerging therapeutic strategy is based upon the finding of somatic mutations of the *BRAF* gene in several different cancer types, including approximately 65% of sporadic melanomas[16,17]. The presence of these mutations in both precancerous lesions and metastatic melanoma suggests that this genetic change might be an early event in tumorigenesis[18]. The critical question for therapeutic development is whether tumour cells actually depend upon the activity of mutant *RAS* or *BRAF*, and, if so, at which stages of tumour development this occurs. Accordingly, validation beyond the mere presence of somatic mutations is important. Such genetic analysis of the Ras pathway has been promising: although targeting of Ras *per se* has been ineffective, inhibitors of *BRAF* and *MEK* have been developed and show early promise in the clinic[19].

Epidermal growth factor receptor (EGFR) represents an interesting example of a genetically validated target. Both RNA expression levels and genetic mutations have implicated *EGFR* as a causal factor in non-small-cell lung cancer (NSCLC), leading to the development of two EGFR

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Table 1 | Targets of approved cancer drugs

Cancer drug	Target	Disease indication	Genetic validation		Functional validation		Model validation	References
			DNA changes	Protein expression	siRNA/shRNA	Exogenous expression	Mouse models	
Genetic dependence								
Gleevec	BCR–ABL	CML, ALL	Translocation	Disregulated protein is expressed	Inhibition of cell growth and colony formation in soft agar, activation of apoptosis	BCR–ABL fusion causes transformation	Transgenic mice develop pre-B leukaemia, lymphoma	43
Gleevec	PDGFR-α, KIT	GIST	Somatic mutation, translocation	Overexpressed	Inhibition of cell proliferation	Constitutive activation of AKT and MAPK	Transplantation of KIT(G559)- and KIT(V814)-infected bone marrow cells leads to acute leukaemia	44–46
Herceptin	ERBB2	Breast cancer	Amplification, increased copy number	Overexpressed	Growth inhibition, induction of apoptosis	Constitutive activation of ERK	ERB-induced mammary tumours	47
Iressa, Tarceva	EGFR	Lung adenocarcinoma, non-small-cell lung carcinoma	Amplification, increased copy number, somatic mutation	Overexpressed	Inhibition of cell growth, induction of cell-cycle arrest, suppression of invasion		Transgenic mice develop cancer	48,49
ATRA	RAR-α	APML	Translocation	Aberrant protein is expressed		Downregulates TNF-α	NPM/RAR-α, hCG-PML/RAR-α, hCG-PLZF/RAR-α transgenic mice develop leukaemia	50,51
Lineage dependence								
Tamoxifen	Oestrogen receptor	ER+ breast cancer		Overexpressed	Reduces RAR-α, EGFR tyrosine phosphorylation and DNA synthesis	Increased proliferation	ER modulators inhibit growth and progression of pre-malignant lesions in a mouse model of ductal carcinoma *in situ*	52,53
Letrazole	Aromatase	ER+ breast cancer		Overexpressed		Increased proliferation and anchor-independent growth	Knockouts show reduced tumour incidence and delayed tumour onset	54,55
Flutamide, Biclutamide	Androgen receptor	Prostate cancer		Overexpressed	Cell-growth inhibition	Prevents TGF-β1-induced growth inhibition and apoptosis	Transgenic mice develop prostate cancer	56
Rituximab	CD20	Non-Hodgkin's lymphoma		B-cell marker		–	–	–
Host dependence								
Avastin	VEGF receptor	Colon cancer, pancreatic cancer		Overexpressed	Inhibition of proliferation, induction of apoptosis	–	–	57

ABL, Abelson murine leukaemia viral oncogene; AKT, protein kinase B; ALL, acute lymphoblastic leukaemia; APML, acute promyelocytic leukaemia; BCR, breakpoint cluster region; CML, chronic myeloid leukaemia; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; ER, oestrogen receptor; GIST, gastrointestinal stromal tumours; KIT, stem cell-factor receptor; MAPK, mitogen-activated protein kinase; NPM, nucleophosmin; PDGFR-α, platelet-derived growth factor receptor-α; PLZF, promyelocytic leukaemia zinc-finger protein; PML, promyelocytic leukaemia protein; RAR-α, retinoic acid receptor-α; shRNA, short-hairpin RNA; siRNA, short-interfering RNA; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

tyrosine kinase inhibitors: gefitinib (Iressa) and erlotinib (Tarceva). However, despite the presence of *EGFR* abnormalities in many NSCLC tumours, therapeutic inhibition of EGFR has resulted in significant tumour regression in only 10–20% of patients[20]. Although recent studies have demonstrated the presence of activating *EGFR* mutations in responsive patients[21,22], more recent studies have documented response in some patients without apparent genetic alterations in *EGFR*[23]. The interpretation of these clinical trials is further complicated by other factors, such as differences in drug scheduling, chemotherapy combinations and patient characteristics. Additional contributing genetic factors, such as the status of *PTEN*[24,25] or ErbB family members[26], might also influence the clinical efficacy of EGFR inhibition. This serves as an important reminder of the fact that, despite the apparent validity of therapeutic hypotheses derived from genetic data, ultimate determination of the applicability of such data to clinical efficacy might occur relatively late in drug development, and could give unexpected or confounding results.

The examples cited above point out both the tremendous promise and the challenges of using genetic data in target validation. It remains difficult to predict response to therapy solely by examining expression levels and/or the genetic status of a particular gene. However, knowing that a gene is genetically altered and selected for in cancer cells might be evidence that is compelling enough to initiate a drug discovery project. Further validation using experimental models has an important complementary role (or at least a parallel path) for understanding the function and role of genetically identified targets in cancer.

Functional target validation in cell-based systems

Experimental systems for the discovery, validation and functional analysis of tumour genes have played an important role in cancer research for several decades. The ability to introduce and express exogenous genes in non-transformed cells led to the discovery of the first transforming mutation in human cancer[27]. The ability to express and knockout genes in mice has led to the development of model systems that have greatly advanced our understanding of the biology of cancer in a physiological setting. The more recent advent of RNA-interference (RNAi) technologies allows the 'knockdown' of expression of specific genes transiently by short-interfering RNA (siRNA), stably by short-hairpin RNA (shRNA) or in an inducible fashion via regulatable shRNA[28]. Conceptually,

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gene-specific loss of function in appropriate experimental systems should predict the outcome of using a specific inhibitory compound in tumour cells[29,30], and can be extended to testing of combinations of several genes or compounds in cell lines with various defined genetic backgrounds. Such approaches would ideally be used before starting a small-molecule therapeutic discovery programme, and even enable exploration of the synthetic-lethality concepts described above.

Each of these strategies has notable advantages and drawbacks. For example, in the case of knockdown-mediated target evaluation, the inability to observe any phenotype has the caveat that the threshold of lowered protein levels required to manifest a phenotype might not have been achieved. In addition, when selecting tumour cell lines that have undergone directed knockout or stable shRNA-mediated knockdown of candidate oncogenes, adaptive responses might occur (or even be selected for), leading to potentially erroneous results. Transient siRNA-mediated knockdown is neither suitable for long-term tissue-culture studies nor compatible with *in vivo*-implantation tumorigenesis models, where it might be necessary to maintain knockdown of the targeted gene for weeks or months in order to assess phenotype. In these contexts, inducible expression, homologous recombination-mediated gene knockout or shRNA systems — although challenging to implement — are particularly informative.

Cancer cell lines in tissue culture are widely utilized in early-stage evaluation of potential cancer targets, and can be used to test criteria such as growth rate, immortalization, loss of contact inhibition, two-dimensional colony formation, colony formation in soft agar and reliance upon growth factors. However, the inability to establish many tumour-derived cells in tissue culture limits the scope of these techniques. Moreover, the cells that do grow in the laboratory might have undergone adaptive changes, raising concerns as to whether they are truly representative of the cancer phenotype. Finally, conventional cell-culture systems are limited in their ability to recapitulate many aspects of the *in situ* tumour microenvironment, including hypoxia, stromal cell interaction and vascularization. Nevertheless, these systems have been valuable tools for the discovery and evaluation of potential cancer targets.

Animal model systems

Transgenic mouse models have been crucial in the study of cancers of the lymphoid and haematopoietic systems, as complex and diverse lineages of cells and cancers in these systems cannot be replicated in the laboratory. Such mouse models have served as the platform for seminal work implicating stem cells in cancer[31].

One drawback of conventional transgenic models (that is, knockout or knock-in systems) is that the deletion of many cancer-relevant target genes leads to embryonic lethality. Moreover, although constitutive ectopic oncogene expression might lead to tumours in mice, such models do not necessarily represent initiation and progression of tumours as they arise in humans. To overcome such limitations, inducible mouse models have been developed in which the expression of certain genes can be altered at specific times.

Table 2 | Validation strategies

Strategy	Examples
Genetic	Somatic mutation, DNA amplification
	RNA expression
	Protein expression
Cell-based	Expression of exogenous genes
	siRNA, shRNA knockdown
	Somatic knockouts/knock-ins in cancer cells
	Tissue culture (plastic, soft agar and so on)
Animal models	Mouse models (knockouts, inducible and transgenic)
	Model organisms

shRNA, short-hairpin RNA; siRNA, short-interfering RNA.

It has become clear that many functions associated with the proliferation or survival of cancer cells are conserved in diverse organisms[32–34]. Such functions range from cell division to regulatory signal transduction pathways. A prime example of the impact that a model organism can have on cancer research is programmed cell death (apoptosis)[35]. Although researchers had characterized a non-necrotic form of death in cancer and other cells, the recognition of programmed cell death as a bona fide biological phenomenon was first achieved in nematodes, in which maps of cellular destiny documented the loss of specific cells at designated stages of development. Discovery of the molecular players that regulate apoptosis (including BCL2), and the finding that such players have a role in the survival of cancer cells, has dramatically changed concepts of how these cells die. Discovery of inhibitors of BCL2, as well as modulation of other apoptotic signalling and execution pathways, has become a very active area in the discovery of novel cancer therapeutics.

Future challenges

The experimental evaluation of a given targeted therapy entails consideration of both the target itself and the manner in which modulation of its function will be evaluated. This question is of overwhelming importance considering the essential roles these models play in predicting the path and outcome of targeted cancer therapy. The intrinsic value of target evaluation in model systems ultimately lies in the extent to which these systems accurately represent relevant properties of human disease, and are therefore predictive of the outcome of exploiting a given target in a therapeutic setting[36]. For example, gene-expression profiles of biopsy samples might be expected to have more intrinsic value (that is, disease relevance) than those of cell lines derived from tumours. Whether phenotypes associated with the ability of cells to form tumours in nude mice have greater predictive value than evaluating proliferation in cell culture or colony formation in soft agar remains unclear. It is clear that current mouse models do not have sufficient predictive value[37], and the relative value of gene-expression data derived from patient samples versus experimental systems has not been explored systematically.

One recent example of cancer gene discovery and validation that captures the range and promise of the activities described here is the PI(3)K signalling pathway. Sequencing of the PI(3)Ks in a panel of cancer samples led to the suggestion that activating mutations in *PIK3CA* have an aetiological role in a range of human tumours[38,39]. Indeed, Samuels and colleagues showed that deletion of activated *PI3K* alleles in colon cancer cell lines leads to reduced survival under low-serum conditions[40]. Expression of such activated alleles also results in transformation of cells in tissue culture[41]. Thus, the finding of *PIK3CA* mutations in tumours, coupled with experimental elucidation of the activities of the encoded gene products and confirmation that mutant cell lines depend on these activities, converge to support the hypothesis that the PI(3)K pathway offers an exciting opportunity for drug discovery and therapeutic intervention in cancer.

In 2004, the US Food and Drug Administration approved only four new small molecules for treatment of cancer. Only one of these therapies came about through target-directed drug discovery; the other three therapeutics were the result of incremental improvements of agents that were originally discovered by opportunistic approaches (for example, testing compounds for effects on cell proliferation). Therefore, despite the great promise and desirability of target-directed cancer drug discovery, efforts to design effective therapeutic strategies based upon hypothesis-driven molecular targets are still in their infancy (Table 2).

The sophisticated tools now available for cancer target discovery and validation, and for subsequent development of therapeutic agents, have significantly improved the quantity and quality of information that can be collected, as well as the speed with which such data can be analysed[42]. Accordingly, the 'cycle times' for evaluating hypotheses associated with every step of cancer drug discovery and therapeutic development are becoming shorter (Fig. 1). The ability to fail or succeed more quickly suggests that progress will continue to accelerate as we learn how better to use the tools we have and to incorporate new technologies into these processes. ■

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