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Mechanisms of Disease: oncogene addiction—a rationale for molecular targeting in cancer therapy

I Bernard Weinstein* and Andrew K Joe

SUMMARY

There has been considerable progress in the systemic treatment of cancer because of the rapid development and clinical application of molecular targeted agents. Although patients with a particular type and stage of cancer are often treated as a single group, more-specific therapy is being considered, as subsets of these patients who are more likely to benefit from treatment with particular agents are being identified. We previously introduced the concept of 'oncogene addiction' to explain how some cancers that contain multiple genetic, epigenetic, and chromosomal abnormalities are dependent on or 'addicted' to one or a few genes for both maintenance of the malignant phenotype and cell survival. Thus, reversal of only one or a few of these abnormalities can inhibit cancer cell growth and in some cases translate to improved survival rates. This review summarizes current experimental and clinical evidence for the concept of oncogene addiction and describes molecular mechanisms that may explain this phenomenon. In addition, we discuss how high-throughput screening methods, including gene-expression profiling and proteomics, and emerging methods for analyzing complex cellular networks can be used to identify the state of oncogene addiction, i.e. the 'Achilles' heel,' in specific cancers. Finally, we discuss the use of molecular targeted agents in combination with other anticancer agents as a strategy to optimize therapy and prevent disease recurrence.

KEYWORDS carcinogenesis, cell circuitry, combination therapy, molecular targeting, oncogene

REVIEW CRITERIA

A formal literature search for this review was not performed; this review includes a summary of the authors' own work and knowledge based on reading the oncology literature. Knowledge gained from regular attendance at conferences, workshops, and other national and international meetings was also included.

IB Weinstein is the Frode Jensen Professor of Medicine, Genetics and Development, and Public Health at the College of Physicians and Surgeons, Columbia University, New York. AK Joe is Florence Irving Assistant Professor of Clinical Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA.

***Correspondence**
Frode Jensen Professor of Medicine, Genetics and Development, and Public Health, College of Physicians and Surgeons, Columbia University, Herbert Irving Comprehensive Cancer Center, 701 West 168th Street, Room 1509, New York, NY 10032, USA
ibw1@columbia.edu

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MULTISTAGE CARCINOGENESIS AND THE CONCEPT OF ONCOGENE ADDICTION

It is now an axiom in oncology that human cancers often evolve through a multistage process that extends over decades. The marked increase in molecular biology studies within the past three decades has revealed that this multistage process is driven by the progressive accumulation of mutations and epigenetic abnormalities in expression of multiple genes that have highly diverse biochemical functions. Malignant carcinomas of the lung, colon, breast, and other organ sites often display mutations in multiple oncogenes and tumor suppressor genes, harbor epigenetic abnormalities that result in increased or decreased expression of hundreds of genes, and contain chromosomal abnormalities that include aneuploidy and loss of heterozygosity at numerous loci; this topic has been comprehensively reviewed.[1,2] It is therefore surprising that despite this extensive disruption in the genomes of cancer cells, there are several examples in both experimental systems (Tables 1 and 2) and in patients with cancer (Table 3) whereby the reversal of only one or a few of these abnormalities can profoundly inhibit the growth of cancer cells and, in some cases, lead to improved survival rates. A few years ago we described this phenomenon as 'oncogene addiction,' to emphasize the apparent dependency of some cancers on one or a few genes for maintenance of the malignant phenotype.[2,3] The purpose of this review is to summarize current experimental and clinical evidence for the concept of oncogene addiction and to describe molecular mechanisms that may explain this phenomenon. In addition, we discuss its relevance to the development of more effective and specific forms of cancer prevention and therapy, by targeting the specific genes that are critical for maintenance of the malignant phenotype of specific types of human cancer. This approach merges with the current development of novel molecular targeted agents for cancer chemoprevention and therapy. Related reviews on this subject have recently been published.[4,5]

Table 1 Examples of oncogene addiction: studies in mice.

Targeted oncogene[a]	Cancer type	Reference
c-*myc*	T-cell and acute myeloid leukemia	Felsher and Bishop (1999)[6]
Bcr-Abl	Leukemia	Huettner *et al*. (2000)[7]
H-ras	Melanoma	Chin *et al*. (1999)[8]
K-ras	Lung	Jackson *et al*. (2001)[9]
c-*myc*	Pancreas β-cell	Pelengaris *et al*. (2002)[10]
c-*myc*	Osteogenic sarcoma	Jain *et al*. (2002)[11]
Her-2/neu	Breast	Moody *et al*. (2002, 2005)[12,13]
c-*myc*	Breast	D'Cruz *et al*. (2001)[14]
Wnt-1	Breast	Gunther *et al*. (2003)[15]

[a]Switching off the indicated oncogene led to growth inhibition, differentiation, apoptosis and/or tumor regression.

Table 2 Examples of oncogene addiction: studies in human cancer cell lines.

Targeted oncogene	Cancer cell line[a]	Reference
Her-2/neu	Breast	Colomer *et al*. (1994)[16]
Cyclin D1	Esophagus Colon Pancreas Squamous Nasopharynx	Zhou *et al*. (1995)[17] Arber *et al*. (1997)[18] Kornmann *et al*. (1999)[19] Sauter *et al*. (1999)[20] Hui *et al*. (2005)[21]
K-ras^{mut}	Pancreas	Aoki *et al*. (1997)[22]
*K-ras*v^{12}	Pancreas	Brummelkamp *et al*. (2002)[23]
β-Catenin	Colon	Verma *et al*. (2003)[24]
Cyclin E	Liver	Li *et al*. (2003)[25]
Mutant B-Raf	Melanoma	Sharma *et al*. (2005)[26]
MITF	Melanoma	Miller *et al*. (2004)[27]

[a]Treatment of these cell lines with an antisense oligonucleotide or an RNAi directed to the respective oncogene caused growth inhibition, and in some cases decreased tumorigenicity and increased chemosensitivity.

EVIDENCE FOR THE CONCEPT OF ONCOGENE ADDICTION

Evidence that supports the concept of oncogene addiction has been obtained in three diverse systems: genetically engineered mouse models of human cancer (Table 1), mechanistic studies in human cancer cell lines (Table 2), and clinical trials involving specific molecular targeted agents (Table 3). Several investigators have generated transgenic mice that overexpress an oncogene in a specific target tissue under conditions in which the oncogene can be switched on or off (Table 1). Felsher and Bishop[6] used this model system and found that switching on the c-*myc* oncogene in the hematopoietic cells of mice led to the development of T-cell and myeloid leukemias; however, when this gene was subsequently switched off the leukemia cells stopped dividing and displayed differentiation and apoptosis. Dependence on continued expression of a single oncogene for maintenance of the neoplastic state has also been seen in similar murine models of other tissues (Table 1), including: myelocytic leukemia induced by the *Bcr-Abl* oncogene;[7] melanoma induced by the *H-ras* oncogene;[8] lung tumors induced by the *K-ras* oncogene;[9] pancreatic β-cell tumors and osteogenic sarcoma induced by the c-*myc* oncogene;[10,11] breast (mammary) tumors induced by the Her-2/neu oncogene;[12,13] breast tumors induced by the c-*myc* oncogene;[14] and breast tumors induced by the Wnt oncogene.[15] It is of interest that in the c-*myc* breast cancer model, when the c-*myc* oncogene was switched off, although 50% of the breast tumors regressed, the remaining 50% showed only partial regression. Furthermore, breast tumors that recurred

Table 3 Clinical evidence of oncogene addiction.

Target	Disease	Agent	Regimen	Reference
HER-2	Breast[a]	Trastuzumab	Combination	Slamon *et al*. (2001)[28,b], Piccart-Gebhart *et al*. (2005)[29,b]
BCR/ABL	Chronic myeloid leukemia[a]	Imatinib	Monotherapy	Hughes *et al*. (2003)[31]
C-KIT	Gastrointestinal stromal tumor[a]	Imatinib	Monotherapy	Demetri *et al*. (2002)[33]
EGFR	NSCLC[a]	Gefitinib, erlotinib	Monotherapy	Shepherd *et al*. (2005)[32,b], Taron *et al*. (2005)[35], Lynch *et al*. (2004)[36]
EGFR	Head and neck, colorectum[a]	Cetuximab	Combination	Baselga *et al*. (2005)[39], Cunningham *et al*. (2004)[40]
EGFR	Pancreas[a]	Erlotinib	Combination	Moore (2005)[34]
VEGF	Breast, colorectum[a], kidney	Bevacizumab	Combination	Miller *et al*. (2005)[41], Hurwitz *et al*. (2004)[42,b], Yang *et al*. (2003)[43]
VEGFR, B-Raf	Kidney[a]	Sorafenib	Monotherapy	Stadler (2005)[55]

Treatment regimen indicates agent alone (monotherapy) or in combination with cytotoxic agents (combination). [a]FDA-approved; [b]Phase III evidence demonstrates improved disease-free or overall survival rates. Abbreviations: NSCLC, non-small-cell lung cancer; VEGFR, vascular endothelial growth factor receptor.

were found to be c-*myc* independent and some of these displayed an activated *K-ras* oncogene.[14] Similarly, in the Her-2/neu breast tumor model[12] (Table 1) tumors that recurred were found to be Her-2/neu independent, and this was recently found to be caused by increased expression of the transcription factor Snail.[13] In the Wnt-1 murine model (Table 1), even though downregulation of Wnt-1 resulted in rapid and extensive regression of aneuploid and invasive breast tumors and pulmonary metastases, a number of breast tumors recurred that were Wnt-independent. Apparently, recurrence was caused by acquisition of mutations in the p53 tumor suppressor gene.[15] The relevance of these examples of 'escape from oncogene addiction' will be discussed later with respect to the themes of oncogene addiction in human cancers and combination therapy.

A variety of studies using human cancer cell lines also indicate that although these cells are aneuploid and carry numerous genetic and epigenetic abnormalities, they can also be highly dependent on the activity of a single oncogene for maintaining the malignant phenotype (Table 2). Blocking expression of the oncogenes for HER2, cyclin D1, K-ras, β-catenin, cyclin E, B-Raf, or microphthalmia transcription factor (MITF) using either antisense DNA or RNA interference (RNAi) strategies can markedly inhibit the *in vitro* growth of various types of human cancer cells.[16–27] In some cases blocking oncogene expression also increases the sensitivity of these cells to specific chemotherapy agents and inhibits their tumorigenicity in mice.[19] As a result of the efficacy of the RNAi method for inhibiting the expression of specific genes, the list of such examples of oncogene addiction is now rapidly expanding.

The most convincing and clinically relevant evidence for the concept of oncogene addiction comes from the increasing number of examples (i.e. prospective randomized trials) of the therapeutic efficacy of antibodies or drugs that target specific oncogenes in human cancers (Table 3). One of the earliest examples is the use of the antibody trastuzumab,[28] which targets the receptor tyrosine kinase HER2. This membrane associated receptor is overexpressed in 20–30% of breast cancers and it is now established that use of trastuzumab in these patients can markedly inhibit tumor growth and prolong patient survival in both the adjuvant and metastatic settings.[28,29] We should, however, emphasize that the therapeutic effects of trastuzumab may be mediated at least in part via immune mechanisms.[30] Within the past few years several low molecular weight drugs have been developed that target and inhibit the activity of other specific protein kinases that have key roles in the growth and survival of human leukemia and

carcinoma cells.[31,32] The remarkable therapeutic efficacy of some of these compounds (Table 3) provides direct evidence for the concept of oncogene addiction. Examples include imatinib, which targets the oncogenic BCR/ABL protein in chronic myeloid leukemia,[31] and also targets the product of the oncogene c-*kit* in gastrointestinal stromal tumors,[33] and the EGFR-targeted drugs gefitinib and erlotinib in non-small-cell lung carcinoma (NSCLC), pancreatic cancer, and glioblastoma.[32,34–38] Recent studies suggest that cetuximab, a monoclonal antibody that targets the EGFR, could have significant antitumor activity in head and neck and colorectal carcinomas;[39,40] and that bevacizumab, a monoclonal antibody to VEGF, might have significant antitumor activity in carcinomas of the breast, colon and kidney.[41–43] These clinical studies also provide insights into the phenomenon of oncogene addiction. For example, in a subset of patients with chronic myeloid leukemia who initially responded to imatinib but later suffered a relapse, examination of the leukemic cells showed a *de novo* mutation in the kinase domain of the BCR/ABL protein, which blocked the inhibitory activity of imatinib.[44] Likewise, it was recently found that the tumor from a patient with NSCLC, who relapsed 2 years after an initial dramatic response to gefitinib, had acquired a second point mutation in the kinase domain of the EGFR, which blocked the binding of gefitinib.[45] The strong selective pressure for emergence of cells that carry *de novo* mutations in the respective oncogenes indicates the remarkable dependence of these neoplastic cells on these oncogenes, and provides further evidence for the concept of oncogene addiction. At the same time these findings reveal the emergence of resistance mechanisms to molecular targeting agents. Studies in progress indicate that, in the case of the *Bcr/Abl* oncogene, there are other drugs that can inhibit the kinase activity of the mutant BCR/ABL protein,[46] and it could be possible to develop similar drugs that act on resistant mutants of the EGFR and resistant forms of other protein kinases. Furthermore, it might be possible to suppress the emergence of these types of resistant cells by combining a specific protein kinase inhibitor with an agent that inhibits cell proliferation via a different mechanism; this approach would limit the likelihood of the emergence of mutant clones. This aspect is further discussed later in this review in the section on combination therapy.

MECHANISMS OF ONCOGENE ADDICTION

We have previously proposed that the phenomenon of oncogene addiction is a consequence of the fact that the multistage process of carcinogenesis is not simply a summation of the individual effects of activation of multiple oncogenes and inactivation of multiple tumor suppressor genes.[2,3] This proposal is consistent with the fact that the proteins encoded by these genes often have multiple roles in complex and interacting networks, which display both positive and negative feedback control. The function of these proteins is also influenced by their levels of activity and the context in which they are expressed. Thus, a given oncogene can enhance cell proliferation but it can also enhance apoptosis. Furthermore, throughout the multistage carcinogenic process, the evolving cancer cell must maintain a state of homeostasis between positive-acting and negative-acting factors in order to maintain structural integrity, viability, and the capacity to replicate. For these reasons, the intracellular circuitry or 'wiring diagram' that regulates signal transduction and gene expression in cancer cells is very different, i.e., 'bizarre,' when compared to that of normal cells.[2,3] In cancer cells a given oncogene may play a more essential and qualitatively different role in a given pathway or 'module' compared with its role in normal cells. Thus, cancer cells may be much more dependent on the activity of a specific oncogene than normal cells.[3]

Within the context of disordered cell circuitry, specific mechanisms have been proposed to explain why inactivation of an oncogene might lead to selective growth inhibition, differentiation and/or apoptosis in cancer cells but not in normal cells that express the same oncogene. One explanation is that, in order to maintain homeostasis, the proliferation-enhancing effects of a specific oncogene in cancer cells might be partially buffered through negative feedback mechanisms, through increased expression of proliferation-inhibitory factors.[2] If this oncogene is then inactivated the cancer cells might suffer a relative excess of the latter inhibitory factors and thus undergo apoptosis, before a new level of homeostasis can be achieved. The apparent propensity of some cancer cells to undergo apoptosis when stressed[47] could enhance this process.

A second mechanism is based on the concept of 'synthetic lethality' originally derived from studies in lower organisms.[5] According to this

concept, two genes are said to be synthetic lethal if mutation of one of the two genes is compatible with survival but mutation of both genes causes cell death.[5] For example, certain cancer cells might be highly dependent on a given oncogene because during their development they lost the function of another gene that normally performs a similar function. A drug that inhibits the activity of the oncogene would, therefore, selectively target the cancer cells and spare the normal cells. Furthermore, because of the bizarre circuitry of cancer cells, pairs of genes in cancer cells that have a synthetic lethal relationship may differ from those in normal cells, thus increasing the dependence of tumor cells on a specific oncogene. A related explanation for oncogene addiction is that, during the multistage carcinogenesis process, cancer cells become highly dependent on specific oncogenes and their related pathways because of the large numbers of mutated and inactivated genes that normally function in other pathways. This dependence could render cancer cells less adaptable than normal cells.[48]

It is of interest that only a subset of patients with NSCLC (about 10–20%) display favorable and often impressive clinical responses to the EGFR inhibitor gefitinib, and this response is often associated with tumors that have specific activating mutations in the kinase domain of EGFR. For reasons that are not understood, patients with these activating mutations are also more likely to have adenocarcinomas, be female, nonsmokers, and of Japanese origin.[35–37] Thus, addiction to a specific oncogene might occur only in a subset of specific types of cancers with a distinct etiology, and only when that oncogene is mutated and not simply activated. Normal EGFR activation results in induction of multiple downstream signaling pathways, some of which enhance cell proliferation while others enhance cell survival (i.e. inhibit apoptosis). An experimental study indicated that mutations in the EGFR can preferentially enhance activation of the survival, Akt-associated pathway.[49] This could explain why NSCLC cells that harbor this mutation in EGFR are highly dependent on this activated oncogene for survival. Similarly, the presence of specific deletion mutations in the *EGFR* gene in glioblastoma was recently shown to correlate with clinical responses to an EGFR inhibitor.[38] These data fit the paradigm that oncogene addiction can be caused by the establishment of distorted pathways of signal transduction (i.e. bizarre circuitry) during tumor development. Recent studies suggest that, in addition to point mutations in the gene encoding the EGFR, other factors could influence the sensitivity of NSCLC tumors to EGFR inhibitors, including *EGFR* gene amplification, the activation state of the EGFR protein, specific downstream signaling pathways, and pharmacologic factors.[50,51]

FUTURE DIRECTIONS AND CLINICAL APPLICATIONS

Identification of the critical oncogene or 'Achilles' heel' in specific human cancers

As emphasized earlier, human cancers display multiple genetic and epigenetic abnormalities. Furthermore, it is now apparent that these abnormalities frequently differ between different types of cancer, and also between subsets of the same type of cancer. In view of this complexity, how can we identify the specific oncogene or oncogenes that have a critical role in maintaining the malignant phenotype in these different cancer types, or within individual cases? In other words, how do we identify the Achilles' heel in specific cancers so that each patient can be treated with the appropriate molecular targeted agent? At the present time there are no methods to fully assess the total circuitry that controls cell proliferation, differentiation and apoptosis in normal or cancer cells. Advances in network theory, systems biology, and computer modeling, which are discussed below, may eventually make this possible.

Currently, several empiric approaches can be used to help identify the Achilles' heel of specific types of human cancer. One approach is to use high-throughput screening of thousands of compounds in chemical libraries to identify specific compounds that preferentially inhibit *in vitro* growth or induce apoptosis in specific types of human cancer cells. A related approach that is being rapidly expanded is to use a library of siRNAs—low-molecular weight RNAs that are taken up by cells and inhibit the expression of specific genes—to identify which genes are required to maintain the proliferation and/or survival of specific types of cancer cells. Once such genes are identified, drugs can be designed to target the related proteins.[52] A recent study in mice suggests that it might become feasible to administer to patients a specific siRNA preparation that knocks down the expression of a critical oncogene in the tumor, thus providing a novel approach for delivering cancer therapy.[53]

In addition, specific criteria might be used to assist in identifying genes that are most

likely to be critical for maintaining the malignant phenotype. For example, oncogenes that are mutated early in the multistage process of tumor development might be favored candidates because they had a critical role in determining subsequent aspects of the abnormal circuitry in the evolving cancer cells. Oncogenes that are mutated, and not simply overexpressed, might also be more likely targets for therapy since they reflect the 'hard-wiring' of cancer cells, rather than epigenetic abnormalities. Mutated oncogenes might therefore be more likely to be present in the stem-cell population of tumors rather than just in the progeny cells. In addition, mutated oncogenes might be more likely to have qualitatively different roles than oncogenes that are only overexpressed. This aspect is exemplified by the properties of a mutated EGFR in NSCLC cells.[49] Specific cancers display increased expression of genes that normally have critical roles in stem cells, or during normal tissue development and differentiation. These include the genes that encode proteins involved in the Wnt, Hedgehog, TGFβ/BMP, Notch, Snail, Slug, and MITF signaling pathways.[54–58] Specific cancers could be highly dependent on, (i.e. addicted to) one of these factors. For example, inhibition of the Hedgehog pathway in murine medulloblastoma blocked proliferation and inhibited tumorigenesis.[57] The transcription factor MITF is involved in normal melanogenesis and is overexpressed in human melanoma; inhibition of its expression in melanoma cell cultures markedly inhibited cell growth.[56] Recent studies indicate that Snail, a transcription factor that has a major role during embryonic mesoderm development, also has a role in the recurrence of human breast cancers.[13] It might, therefore, also be a critical target for therapy in these cancers. It is possible that dependence on a specific oncogene might be different in the stem cells than in the progeny cells in a given tumor, because of differences in their intracellular circuitry. Optimal therapy would then require developing molecular targeted agents that specifically target the critical oncogene in the stem cells of a specific cancer. Further characterization of stem cells in specific tumors should clarify this aspect of oncogene addiction, and the potential limitations of specific molecular targeted agents.

'Network theory' and the emerging field of systems biology may eventually provide methods for conceptualizing and analyzing the entire circuitry of specific types of normal and cancer cells, and thus facilitate identification of specific pathways of oncogene addiction in different types of cancer cells. Networks in mammalian cells share similar principles with networks that are relevant to engineering and other disciplines. Thus, by viewing the cancer cell as a complex or complicated system,[59] genes and proteins as 'nodes',[60,61] and groups of interacting proteins in a signal transduction pathway as 'modules', engineering tools and concepts can be used to analyze large datasets to generate an holistic view of the regulatory network of normal and cancer cells. Furthermore, a concept called Boolean genetic network theory can be used to study the cancer cell as a dynamic system.[62] By analyzing the myriad of individual and interacting genes in a cancer cell in a Boolean fashion (i.e. active = 'on', inactive = 'off'), the overall activity state ('attractor state') of specific cancer cells (e.g. proliferation, apoptosis, or differentiation) might be determined or predicted. Cells are described as being in a dynamic equilibrium between these attractor states, and changes in cell state ('hysteresis') can be described as responses to various external and internal stimuli. Thus, in the future it could be possible to use network theory to describe and predict the existence of an 'addicted' attractor state, and to also predict the specific oncogene responsible for this addicted state.[59,62]

Advances in network theory and systems biology are being facilitated by powerful microarray and proteomic methods that compare expression profiles of thousands of genes and proteins between normal tissues, cancers, and subtypes of specific cancers. Recent data are providing insights into signaling pathways and networks characteristic of specific types of cancer; this is discussed in a review by Baak *et al.*[63] Hopefully, this information will facilitate the identification of pathways of oncogene addiction characteristic of specific cancers or their subtypes, and thus guide the use of specific molecular targeted agents in specific patients. A more detailed understanding of these networks might also provide insights into tumor resistance and the appropriate choice of combination therapy for specific patients.

Tumor–stromal interactions

The concept of oncogene addiction is also relevant to tumor-stromal interactions, tumor

invasion and tumor metastasis. Thus, activated oncogenes like *EGFR* or *Ras* stimulate signaling pathways in cancer cells that cause increased expression of matrix metalloproteinases (which enhance tumor invasion) and of the angiogenic factor VEGF.[64,65] Inactivation of these genes in cancer cells can inhibit tumor invasion, angiogenesis, and even recurrence. The neovasculature associated with tumor-induced angiogenesis is abnormal compared with normal vasculature.[66] The circuitry that regulates the growth and function of endothelial cells in this neovasculature might, therefore, differ from that of normal vasculature[41–43]. Thus, it could be possible to develop agents that preferentially target tumor vasculature; bevacizumab provides a promising example, and its use has led to improved disease-free and overall survival rates in patients with colon cancer.[42]

Combination therapy

It should be emphasized that although the concept of oncogene addiction may apply to a given cancer at a particular time or stage, it is apparent from some of the mouse model experiments[67] (Table 1) and from clinical experience with molecular targeted agents (Table 3) that cancers can 'escape' from a given state of oncogene addiction. Presumably, this finding reflects the genomic instability of cancers, and could also reflect epigenetic changes in gene expression that lead to an altered state of cell circuitry; for example, ongoing changes in DNA methylation and chromatin structure in a cancer cell population. It is not known whether this escape leads to secondary addiction to another oncogene or to the growth of a population of 'non-addicted' cancer cells. For these reasons, as well as the likelihood of heterogeneity within tumors, it is unlikely that the use of a single molecular targeted agent will achieve long lasting remissions or cures in human cancers, especially for late-stage disease. Combination therapy will, therefore, be required, which raises several unresolved questions. Can such combinations be rationally designed? Should the individual agents act on the same molecular target but by different mechanisms, or on different targets in the same pathway, or should each agent target a different pathway or cellular mechanism? The first approach could prevent emergence of the types of resistance recently seen with imatinib[44] and gefitinib.[45] The second approach also seems rational because it is likely that cancer cells may be addicted to specific signaling pathways rather than a single oncogene. A drug combination that targets proteins that function at different stages in the same pathway may, therefore, be more likely than a single drug to inactivate that pathway.[68] The third approach can also be justified because of the disturbance of multiple pathways in cancer cells. It is becoming increasingly apparent that certain molecular targeted agents are actually promiscuous, i.e. they target more than one molecule, and this multiple targeting could enhance their therapeutic efficacy.[69] For example, the drug sorafenib seems to have activity against renal carcinomas by targeting both a mutant *Raf* oncogene and the VEGF receptor, thus inhibiting both cancer cell proliferation and angiogenesis.[55] The compound sunitinib apparently exerts antitumor activity by targeting multiple protein kinases.[55] It may therefore be useful to further exploit this principle by using so-called 'multi-targeting' agents. HSP90 is a molecular chaperone that stabilizes various proteins. Geldanamycin[70] and related compounds inhibit HSP90 and may therefore, increase the degradation of mutant oncogenic proteins. Conversely, inhibitors of the proteosome-ubiquination proteolysis pathway such as bortezomib[71] might stabilize and increase the activity of cellular proteins, including tumor suppressors (e.g. p53). These two types of agents could, therefore, also be relevant to the concept of oncogene addiction and might preferentially target mutant oncogenic and tumor suppressor proteins.

Clinical studies indicate that the efficacy of certain molecular targeted agents can be enhanced by combining them with cytotoxic agents, i.e. agents that often act by inhibiting DNA or chromosomal replication. Trastuzumab that targets HER2 can improve response and survival rates if given in combination with paclitaxel to patients with metastatic breast cancer.[28] The combination of bevacizumab or cetuximab with cytotoxic chemotherapy agents can also improve response rates in patients with metastatic colon and breast cancer, respectively.[40,41] Furthermore, when bevacizumab was added to a combination chemotherapy regimen it improved overall survival rates in patients with metastatic colon cancer.[42] As with chemotherapy, the efficacy of targeted therapy is likely to be greater in patients with minimum residual disease. Thus, treatment

with trastuzumab after adjuvant chemotherapy significantly improves disease-free survival in patients with early-stage breast cancer.[29]

Tumor suppressor gene hypersensitivity

In this review we have emphasized the roles of dominant-acting oncogenes that enhance cell proliferation and cell survival. The disordered circuitry of cancer cells, however, is also a consequence of inactivation or loss of expression of tumor suppressor genes, which normally inhibit proliferation or enhance apoptosis. Experimental studies indicate that reintroducing a wild-type tumor suppressor gene (e.g. those encoding p53, Rb, or APC) into human cancer cells where the respective endogenous gene was inactive usually caused marked inhibition of growth, induction of apoptosis and/or inhibition of tumorigenesis in mice.[2,3] These results would not be expected if cancer cells evolved simply through the stepwise addition of genetic abnormalities, because then correction of just one mutagenic event should have only a modest inhibitory effect. Thus, some cancer cells seem to be hypersensitive to the growth-inhibitory effects of specific tumor suppressor genes. We postulate that, like oncogene addiction, this effect also reflects the bizarre circuitry of cancer cells, and have termed this phenomenon 'tumor suppressor gene hypersensitivity'.[2,3] This phenomenon can also be exploited in cancer treatment by gene therapy, for example by the use of an adenovirus that encodes a normal p53 protein[72] or by targeting a downstream signaling pathway that was activated as a result of loss of activation of a tumor suppressor gene. Alternatively, if a tumor suppressor gene is inactivated by the process of DNA methylation, which frequently occurs in cancer cells (e.g. $p16^{INK4}$ gene), drugs that cause demethylation of cellular DNA could switch such genes back on and thereby inhibit tumor growth.[73] This mechanism seems to be the means by which 5-azacytidine and zebularine inhibit tumor growth.[73,74] Drugs that inhibit histone deacetylase enzymes and thereby enhance gene expression, such as depsipeptide or suberoylanilide hydroxamic acid, might also exert antitumor effects by reactivating silenced tumor suppressor genes.[75] Synergistic or additive effects on tumor growth inhibition might be obtained by combining drugs that exploit both oncogene addiction and tumor suppressor gene hypersensitivity.

CONCLUSION

At the present time, the choice of the best molecular targeted agent and the appropriate combination therapy for a specific patient with cancer is largely empirical. Nevertheless, the rapid development of diverse molecular targeted agents coupled with further mechanistic studies, and advances in profiling the molecular circuitry of specific subsets of human cancer, should make it possible to further exploit the concept of oncogene addiction to achieve more effective and selective therapies for several types of human cancer.

KEY POINTS

- 'Oncogene addiction' describes the phenomenon by which some cancers that contain multiple genetic and epigenetic abnormalities remain dependent on (addicted to) one or a few genes for both maintenance of the malignant phenotype and cell survival
- Evidence that supports the concept of oncogene addiction has now been obtained in three diverse systems: 1) genetically engineered mouse models of human cancer, 2) mechanistic studies in human cancer cell lines, and 3) clinical trials with specific molecular targeted agents
- 'Network theory' and other techniques of systems biology may provide methods for analyzing the entire circuitry of cancer cells and thus facilitate identification of pathways of oncogene addiction in specific types of human cancer
- These insights will guide the development and clinical application of novel molecular targeted agents
- Treatment regimens that combine molecular targeted agents with other anticancer agents could provide the optimal strategy for treating and preventing cancer

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