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Targeted Interference with Signal Transduction Events

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1 Introduction: The Rationale for the Development of Targeted Drugs in Cancer Therapy

Bernd Groner

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Cancer remains a leading cause of death in the developed world. Survival rates for patients with common cancers detected at an advanced stage are still low. Only about 10% of patients with metastatic colon cancer and about 5% of patients with pancreatic cancer survive for more than 5 years. Cancer therapies are still largely chosen on the basis of diagnostic categories, and all patients of a particular tumor type and stage of disease receive the same treatment. Biological heterogeneity among patients has long been recognized, but the significance of these differences with respect to the course of disease and drug responsiveness is just starting to be understood. In addition, the limited repertoire of available drugs has made it difficult to exploit these differences for different treatment strategies.

Cancer is being considered as a genetic disease. Multiple mutations are thought to be present in tumor cells that alter the gene functions responsible for the manifestation of the transformed phenotype. Although the analysis of mutated genes has already become useful for diagnostic and therapeutic applications, the number of relevant mutations and the identity of the affected genes still have not been determined (Futreal et al. 2004). Cancer cells are constantly subject to mutations in their DNA. These changes occasionally produce cells that can escape their normal growth constraints and form a tumor. The tumor cells are being selected for their ability to divide, trigger the growth of vessels to provide for their blood supply, and invade the bloodstream and other tissues to form metastases. Defects in their cell cycle and apoptosis regulation are due to mutations in proto-oncogenes and tumor suppressor genes. Genomic instability due to defects in DNA repair enzymes increases the rate of mutations and contributes to cancer evolution. More than 350 human genes have been found to be mutated in cancer cells: 90% of these exhibit mutations in somatic cells, 20% can be found mutated in germline cells and thus contribute to the predisposition to cancer, and 10% are mutated both in somatic and in germline cells (Futreal et al. 2004). Loss of gene functions can not only be caused by changes in the primary DNA sequence, but also by epigenetic control mechanisms of gene expression. Secondary modifications of DNA, histones, or transcription factors can underlie such events (Esteller 2006).

To obtain a comprehensive view of the genetic alterations causing and accompanying the emergence of tumor cells in a particular tissue, it is necessary to derive global sequence information. Sjöblom and colleagues (Sjoblom et al. 2006) analyzed the protein coding sequences in 13,023 genes from 11 breast cancer samples and 11 colon cancer samples and found that individual tumors accumulate about 90 mutated genes on average and that at least 11 of them are thought to be cancer promoting. Altogether the number of 189 «candidate» cancer genes that affect gene transcription, cell adhesion, and invasion might not seem too encouraging

when we are looking for the general principles of cancer etiology and a small number of promising drug targets. The cancer genes differed between colon and breast cancers, and each tumor had a different pattern of mutations. This complexity in mutational patterns and the differences among tumors of a distinct histological type have important implications for the variability of current treatment regimens and for the design of new drugs.

The heterogeneity in the genetic changes and the context dependence by which these mutations are causally involved in cancer development and progression makes it difficult to design effective drugs. In addition, the possibility that only a subset of cancer cells with stem cell properties is really relevant for effective eradication of the disease further complicates their design. Tumor cells constantly communicate with normal, neighboring host cells in reciprocal interactions. Factors secreted into the microenvironment of cancer cells by host cells can promote the proliferation of tumor cells, and factors secreted by tumor cells can impede the host immune response (Sawyers 2004).

Which phenotypes are affected in cancer cells, and which mutations can be linked to a particular phenotype? The signaling pathways that control cell cycle progression and cell growth, apoptosis, replicative potential and senescence, motility and invasiveness, metabolic activity, and genome integrity are often deregulated in cancer cells, and the activities of many oncogenes and tumor suppressor genes have been associated with these functions (Vogelstein and Kinzler 2004). The identification of activated oncogenic pathways in particular tumor cells yields a signature that might act as a guide for targeted therapies (Bild et al. 2006).

The concept that the cooperation of oncogenes and tumor suppressor genes, augmented by signals from the tumor microenvironment and stress signals such as DNA damage, can be regarded as the molecular basis of cancer provides the framework for the design of new drugs. These drugs are selected on the basis of their ability to interfere with specific molecules, believed to have a limiting role in the emergence, growth, or progression of tumors. The identification of the appropriate targets for such drugs very much depends on detailed understanding of the molecular alterations causing cancer. The description of cancer in molecular terms will also have profound effects on prevention measures, the early detection of tumors, the improvement of diagnosis complementing histopathological criteria, and the monitoring of treatment.

Despite the discouraging complexity of the genetic basis of cellular transformation, therapeutic advances have been made exploiting insights into genes that have causal and limiting roles in the cancer process. The integration of such genes into signaling pathways that regulate cell growth and cell fate and the development of agents that interfere with such components in a targeted fashion have led to significant gains for cancer patients. Hormones, antibodies, and low-molecular-weight compounds acting as enzyme inhibitors have been used to target oncogene products. Intuitively, it appears reasonable to interfere with the function of cellular components that are distinguishable in amount or functional properties between normal and tumor cells. The selective estrogen receptor modulators, partial agonists of the natural ligand (Ariazi et al. 2006); trastuzumab (Herceptin), an antibody that interferes with the action of the ErbB2 growth factor receptor (Pegram et al. 2000); imatinib (Glivec), a low-molecular-weight tyrosine kinase inhibitor that blocks the activity of the abl kinase (O'Hare et al. 2006); and gefitinib (Iressa), a tyrosine kinase inhibitor of the EGF receptor (Mendelsohn and Baselga 2006), serve as pioneering examples for the benefits that are emanating from targeted drugs. These drugs are not necessarily curative, and only selected subpopulations of patients respond to them. However, they show that a combination of molecular diagnostics, which reveals the gene defects underlying the transformation process, and the deployment of drugs aimed at individual deregulated signaling components emerges as a viable and promising therapeutic strategy.

Can the lessons from these examples be extrapolated? Other target structures are already

being exploited in a comparable fashion (Dietel and Sers 2006), but it remains to be determined how many limiting components there are that are druggable (Keller et al. 2006). The development of new, powerful agents able to interfere with cell surface growth factor receptors, intracellular signaling kinases, and signal transduction components that are described in this book embodies the hope of many tumor patients and will lead the way to further improvements in treatment.

References

Ariazi EA, Ariazi JL, Cordera F, Jordan VC (2006) Estrogen receptors as therapeutic targets in breast cancer. Curr Top Med Chem 6:181–202

Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, et al. (2006) Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 439:353–357

Dietel M, Sers C (2006) Personalized medicine and development of targeted therapies: The upcoming challenge for diagnostic molecular pathology. A review. Virchows Arch 448:744–755

Esteller M (2006) Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. Br J Cancer 94:179–183

Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR (2004) A census of human cancer genes. Nat Rev Cancer 4:177--183

Keller TH, Pichota A, Yin Z (2006) A practical view of 'druggability'. Curr Opin Chem Biol 10:357–361

Mendelsohn J, Baselga J (2006) Epidermal growth factor receptor targeting in cancer. Semin Oncol 33:369–385

O'Hare T, Corbin AS, Druker BJ (2006) Targeted CML therapy: controlling drug resistance, seeking cure. Curr Opin Genet Dev 16:92–99

Pegram MD, Konecny G, Slamon DJ (2000) The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Cancer Treat Res 103:57–75

Sawyers C (2004) Targeted cancer therapy. Nature 432:294–297

Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314:268–274

Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10:789–799

2 Identifying Critical Signaling Molecules for the Treatment of Cancer

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2.1 Overview

Tumorigenesis is a multistep process whereby an individual cell acquires a series of mutant gene products. These genetic changes culminate in proliferation, growth, blocked differentiation, induction of angiogenesis, tissue invasion, and loss of genomic stability. Given the genetic complexity of tumorigenesis, it is perhaps surprising that there are circumstances in which cancer can be reversed through the repair or inactivation of individual mutant genes. However, recent experiments in transgenic mouse models and clinical results with new pharmacological agents demonstrate that cancer can be treated through the targeted repair and/or inactivation of mutant proteins. Hence, cancers appear to be dependent upon particular oncogenes to maintain their neoplastic properties, thus exhibiting the phenomenon of "oncogene addiction."

We will focus on the notion that critical oncogenes mediate signaling processes that underlie the etiology of cancer. These mutant oncogenes are likely to represent the best targets for the treatment of cancer. We will summarize the major signaling pathways that may be most effectively targeted for the treatment of cancer. Then, we will describe how conditional transgenic model systems have been exploited as innovative avenues for discovery and validation of drug targets and therapeutic agents. Next, we will explore the successes to date of targeted therapeutics and possible approaches to the successful targeting of transcription factors. Finally, we will discuss current thoughts on why the targeted inactivation of specific cell signaling molecules results in tumor regression.

2.2 Critical Signaling Pathways

At least four different classes of signaling molecules are commonly involved in the pathogenesis of cancer including receptors such as ErbB, small GTPases such as Ras, kinases such as BCR-ABL, and transcription factors such as MYC. The proteins in these interacting signaling pathways have been some of the most intensely studied as potential targets and in many cases successfully targeted for the treatment of cancer (Fig. 2.1).

2.2.1 Receptor Signaling

Cell surface receptors are the starting point for all signaling cascades, so it is not surprising that receptors for growth factors were some of the first proto-oncogenes discovered (Olayioye et al. 2000). A multitude of cell surface receptors have been implicated in tumorigenesis including the epithelial growth factor receptor (EGFR), the platelet-derived growth factor receptor (PDGFR), and the insulin-like growth factor receptor (IGFR) (Tibes et al. 2005).

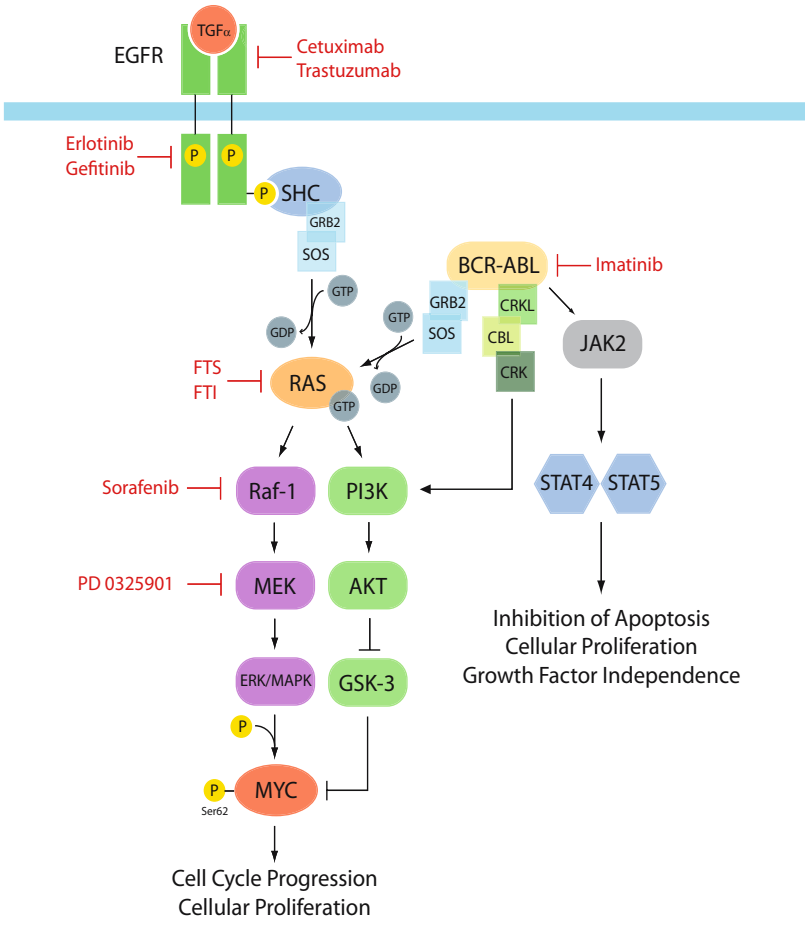

Fig. 2.1 Critical signaling pathways as drug targets for cancer therapy. A Surface receptors dimerize and activate downstream effectors such as *RAS* (a *GTP*ase). Many surface receptors are targeted by antibodies, and *EGFR* has been targeted through cetuximab and trastuzumab. B *RAS* activation is caused by *SOS*-mediated exchange of *GDP* for *GTP* on RAS. G protein signaling molecules, such as *RAS*, have been targeted by farnesyltransferase inhibitors (FTI) and S-farnesylthiosalicylic acid (FTS). C The BCR-ABL fusion protein activates *RAS*, PI3K, and other oncogenic signaling molecules. Tyrosine kinases are often targeted with small molecules. *BCR-ABL* has been targeted by imanitib mesylate. D Transcription factors are often the terminal effectors of a pathway. To date, transcription factors have yet to be successfully targeted. All drugs are in red at their sites of action

A prototypical example of receptors is the ErbB family of transmembrane tyrosine kinase receptors, which includes four family members: EGFR (ErbB1), HER2/NEU (ErbB2 hereafter referred to as HER2), ErbB3, and ErbB4. Ligands have been identified for each of the ErbB family members except for HER2, which likely heterodimerizes with EGFR or HER3. Downstream effectors of these molecules include the MAPK pathways, PI3K/AKT pathways, Janus signaling, RAS signaling, and STAT signaling. Activation of these receptor pathways induces cellular proliferation, survival, and motility (Bianco et al. 2006). Each of these receptors exhibits tissue-specific expression. Correspondingly, mutated receptors have been implicated in particular types of human cancer (Yarden and Sliwkowski 2001). For example, EGFR is commonly overexpressed in lung carcinoma (Hirsch et al. 2003), while Her2 overexpression is more commonly associated with breast cancer (Ross et al. 2004).

There are several reasons why cell surface receptors are attractive candidates for drug targets. First, receptors are specific to particular tissue types, thus allowing for target specificity. Second, pharmacologically blocking a ligand-binding site is an obvious and frequently successful strategy to inactivate a receptor. Finally, drugs that target receptor molecules do not need to be able to transit through cellular membranes.

2.2.2 GTPases

Receptor signaling is frequently mediated through small GTPases (Bourne et al. 1991). A characteristic feature of GTPases is that they must first be modified to localize to the plasma membrane, where they are active (Downward 1996). The RAS family represents a prototypical example of small GTPases consisting of three members: H-RAS, K-RAS, and N-RAS (Bourne et al. 1991). RAS family members are some of the most commonly mutated genes associated with human cancers. At least 25% of human tumors exhibit activating point mutations of a RAS gene (Bos 1989). RAS proteins are known to regulate many cellular processes including cellular growth, proliferation, apoptosis, and angiogenesis (Lowy and Willumsen 1993; Downward 1996).

2.2.3 Kinases

Kinases are among the most abundant signaling molecules, with approximately 500 members, many of which when mutated function as oncogenes (Manning et al. 2002). The ability to readily pharmacologically target the ATP-binding domains of kinases has made these gene products attractive drug targets (Schlessinger 2000; Ventura and Nebreda 2006). The most well-known example of a kinase associated with neoplasia is the BCR-ABL fusion protein, which results from a chromosomal translocation between the ABL proto-oncogene and the BCR locus. BCR-ABL overexpression has been implicated in the pathogenesis of chronic myelogenous leukemia (CML) and ALL. The targeted inactivation of BCR-ABL through the drug imatinib mesylate (Gleevec, STI-571) is the most cited example of a successful targeted therapeutic (Sawyers 2002; Daley 2003; Druker 2004; Deininger et al. 2005).

2.2.4 Transcription Factors

Nuclear transcription factors are among the proteins most frequently implicated in cancer. The family of MYC proto-oncogenes (c-, n-, l-MYC) are overexpressed in up to half of all human cancers (Nesbit et al. 1999). MYC has been shown to regulate the transcription of thousands of target genes (http://www.myc-cancer-gene.org; Dang et al. 1999; Oster et al. 2002), suggesting that these gene products function as grand coordinators of gene expression programs.

c-MYC was the first family member to be discovered (Bishop 1982). c-MYC is expressed in most cell types and is found to be overexpressed in most types of human cancers. In particular, c-MYC is found to be activated through chromosomal translocation in Burkitt lymphoma. n-MYC is expressed in neuronal cells and is often amplified in neuroblastoma. l-MYC was first identified in lung tissue and is associated with small cell lung carcinoma (Nesbit et al. 1999). To date, transcription factors have yet to be successfully targeted with small molecules.

2.3 Conditional Transgenic Mouse Models

Although the last couple of decades have led to a remarkable amount of insight into the molecular etiology of cancer, to date most conventional therapies for cancer are purely empiric. Only recently have targeted strategies become incorporated into the treatment of cancer. The development of transgenic mouse models has provided the unprecedented opportunity to model the contribution of specific gene products to the pathogenesis of neoplasia Italics. Moreover, the

recent development of conditional transgenic models has made it possible to directly interrogate when and how the inactivation of oncogenes can result in tumor regression. Many reviews have extensively described the application of conventional transgenic mouse models for the development of therapeutics for cancer (Van Dyke and Jacks 2002; Weiss and Shannon 2003; Gutmann et al. 2006). Here we will focus on the use of conditional transgenic models to define when and how oncogenes can be used as targets for the treatment of cancer.

2.3.1 Experimental Approaches

Three different strategies have been most commonly utilized to conditionally regulate gene expression in transgenic mouse models: the Tet system, the tamoxifen system, and the TVA system (Jonkers and Berns 2002; Van Dyke and Jacks 2002; Giuriato et al. 2004).

2.3.1.1 The Tet System

The tetracycline regulatory system (Tet system) was developed as a strategy to regulate the transcription of genes in eukaryotic cells by utilizing prokaryotic transcriptional regulatory proteins (Gossen and Bujard 1992; Gossen et al. 1994). There are two variations of this system: One activates transgene expression in the presence of a tetracycline such as doxycycline (Tet-On), while the other system shuts off transgene expression upon doxycycline addition (Tet-Off). In both variations, two different transgenes are generated. The first transgene uses a tissue-specific promoter to drive the expression of a tetracycline transactivator (tTA or rtTA). The second transgene contains a tetracycline response element (Tet-O) adjacent to a target gene of interest. The tTA or rtTA protein binds to the Tet-O promoter regulating gene transcription. The presence of doxycycline prevents binding of the tTA protein to the Tet-O element, turning off gene expression (Tet-Off), or promotes the binding of the rtTA protein to the Tet-O element, turning gene expression on (Tet-On). The Tet system facilitates monitoring of transgene expression at the transcription level in specific tissues within the mouse.

2.3.1.2 The Tamoxifen System

The tamoxifen system also has been employed to conditionally regulate gene activation post-transcriptionally. MYC fused with the estradiol receptor exhibited conditional oncogene activation (Eilers et al. 1989). A mutant version of the estradiol receptor, which binds tamoxifen, is utilized to prevent endogenous estradiol from activating gene function (Littlewood et al. 1995). Upon addition of tamoxifen, MYC is active, and withdrawal leads to an inactive product.

2.3.1.3 The RCAS-TVA-Tet System

The Tet system can be combined with the RCAS-TVA system (Lewis et al. 2003; Pao et al. 2003). In this approach a tissue-specific promoter is used to drive the expression of the avian retroviral receptor (TVA) in transgenic mice. The cells of these mice also contain a Tet-O regulated transgene, but lack the rtTA protein. Avian retroviral vectors (RCAS) are used to deliver the rtTA transactivator to cells that express the TVA. The successfully infected cells now contain a transgene whose expression can be regulated by doxycycline.

2.3.2 Defining When Cancer Is Reversible

Conditional transgenic models have been used to evaluate the consequences of oncogene inactivation italics. From these studies, several general themes emerge regarding the role of oncogenes in the initiation and maintenance of tumorigenesis, as we have described (Felsher 2003, 2004a, 2004b; Giuriato et al. 2004; Bachireddy et al. 2005; Shachaf and Felsher 2005a, 2005b). Oncogene inactivation can reverse tumorigenesis by inducing sustained tumor regression through differentiation, proliferative arrest, and/or apoptosis (see Table 2.1). The specific consequences of the inactivation of an oncogene depend on the type of tumor. In some cases, even briefly in-

Table 2.1 Consequences of oncogene inactivation in transgenic mouse models

Oncogene	Model	System	Tumor type	Response to inactivation	Mechanism of tumor regression	References
BCL2	MMTV-tTA Tet-O-BCL-2 Eμ-MYC	Tet-off	Lymphoblastic leukemia	Regression	Apoptosis	Letai et al. 2004
BCR-ABL	MMTV-tTA Tet-O-BCR-ABL	Tet-Off	B-cell leukemia	Regression[a]	Apoptosis	Huettner et al. 2000
	SCL-tTA Tet-O-BCR-ABL	Tet-Off	CML	Regression	ND	Koschmieder et al. 2005
FGF-10	CCSP-rtTA or SPC-rtTA Tet-O-CMV-FGF10	Tet-on	Pulmonary adenomas	Regression	ND	Clark et al. 2001
HER2/NEU	MMTV-rtTA Tet-O-NeuNT	Tet-On	Mammary carcinomas	Regression[a]	Decreased proliferation and apoptosis	Moody et al. 2002
MET	LAP-tTA Tet-O-MET	Tet-On	Hepatocellular carcinoma	Regression	Decreased proliferation and apoptosis	Wang et al. 2001
c-MYC	EμSRα-tTA Tet-O-MYC	Tet-Off	T- and B-cell lymphoma, acute myeloid leukemia	Regression[a]	Cell cycle arrest, differentiation and apoptosis	Felsher and Bishop 1999a, 1999b; Marinkovic et al. 2004
	EμSRα-tTA Tet-O-MYC	Tet-Off	Osteosarcoma	Regression	Differentiation	Jain et al. 2002
	MMTV-rtTA Tet-O-MYC	Tet-On	Breast adenoma	Partial Regression	ND	D'Cruz et al. 2001; Boxer et al. 2004
	LAP-tTA Tet-O-MYC	Tet-Off	Hepatocellular carcinoma	Regression	Apoptosis and differentiation	Beer et al. 2004; Shachaf et al. 2004
	Plns- MycER^{Tam}	Tamoxifen	Pancreatic islet cell	Regression	Growth arrest, differentiation, cellular adhesion, vascular collapse	Pelengaris et al. 2002
	Involucrin-MycER^{Tam}	Tamoxifen	Papillomas	Regression	Growth arrest and differentiation	Pelengaris et al. 1999; Flores et al. 2004
RAS	Tyr-rtTA H-Ras$^{(V12G)}$ Ink4a$^{-/-}$	Tet-On	Melanoma	Regression[a]	Apoptosis, EGFR expression required	Chin et al. 1999; Wong and Chin 2000
	SP-r-rtTTA RtTA-KiRas$^{(G12C)}$	Tet-On	Lung adenoma	Regression	ND	Floyd et al. 2005
	CCSP-rtTA Tet-O-KiRas$^{(G12C)}$	Tet-On	Lung adenoma	Regression	ND	Floyd et al. 2005
	CCSP-rtTA Tet-op-K-Ras4B$^{(G12D)}$	Tet-On	Lung adenoma	Regression	Apoptosis	Fisher et al. 2001
	Nestin-TVA RCAS-tTA RCAS-Akt RCAS-Tet-O-KRas	RCAS	Glioblastoma	Regression	Apoptosis	Holmen and Williams 2005
WNT	MMTV-rtTA Tet-O-WNT1 P53$^{-/-}$	Tet	Mammary adenoma	Regression[a]	ND	Gunther et al. 2003

[a] While most of the tumors regressed on oncogene inactivation, some of the mice relapsed while the oncogene was inactivated

ND, not determined

activating an oncogene may be sufficient to induce sustained tumor regression (Jain et al. 2002; Flores et al. 2004), but in other cases, this has not been observed (Boxer et al. 2004). Oncogene inactivation may uncover the stem cell properties of tumor cells and induce a state of tumor dormancy (Boxer et al. 2004; Jonkers and Berns 2004; Pelengaris et al. 2004; Shachaf et al. 2004; Yu et al. 2005). Finally, the genetic context can affect whether inactivation of an oncogene will induce sustained regression, or whether the tumors can relapse, acquiring additional genetic events (D'Cruz et al. 2001; Karlsson et al. 2003b; Boxer et al. 2004; Moody et al. 2005).

2.3.2.1 Conditional Models of Receptor-Induced Tumorigenesis

The Tet system has been used to conditionally overexpress receptors including an oncogenic form of HER2 containing an activating point mutation in its transmembrane domain (Moody et al. 2002, 2005). Expression was directed to the breast by utilizing the mouse mammary tumor virus (MMTV) promoter to drive the expression of the rtTA protein. Within 4 days of HER2 activation by doxycycline administration, the mice developed hyperplastic abnormalities. Six weeks after oncogene activation, all of the mice developed multiple invasive mammary carcinomas. The tumors were solid invasive carcinomas that often metastasized to the lung. After 48 h of HER2 inactivation through doxycycline withdrawal, the tumor cells exhibited proliferation arrest and increased apoptosis. The primary carcinomas rapidly and completely regressed in over 90% of the mice, with a mean regression time of 17 days. Within 30 days, the pulmonary metastases had also completely and rapidly regressed. However, a majority of the mice that had a complete regression upon HER2 repression eventually relapsed. Furthermore, when the primary tumors and metastases were transplanted into syngeneic hosts, they completely regressed only 55%–70% of the time. The relapsed tumors all uniformly lacked both endogeneous and transgene protein expression, indicating that the tumors had all become HER2 independent (Moody et al. 2002). Subsequently, Snail, a transcriptional repressor, was found to be activated in relapsed tumors (Moody et al. 2005). Therefore, although oncogene inactivation can cause tumor regression, some transgenic tumors are capable of becoming independent of their initiating oncogenic event.

2.3.2.2 GTPases and Tumor Regression

The Tet-On system has been used to generate a conditional model of mutant H-RAS-induced melanomas (Chin et al. 1999; Wong and Chin 2000). The tyrosinase gene promoter (Tyr) was used to conditionally overexpress an H-RAS bearing an activating point mutation (V12G) in an Ink4a-deficient background. Approximately 25% of the mice developed melanomas within 60 days of H-RAS activation. The melanomas were invasive, highly vascular, and amelanotic. The tumors exhibited expression of tyrosinase-related-protein-1 (TRP-1), an early melanocyte-specific maker. Within 48 h of H-RAS inactivation through doxycycline withdrawal, the tumors decreased their proliferation and exhibited robust apoptosis. Within 14 days of H-RAS inactivation, the tumors had completely regressed, with only microscopically detectable scattered tumor foci. Notably, melanomas transplanted into SCID hosts also regressed on inactivation of mutant H-RAS. Approximately 30% of the melanomas resumed growth, even in the absence of H-RAS, but relapsed tumors failed to express TRP-1, suggesting that these tumors were phenotypically different from the primary tumors (Chin et al. 1999). Additionally, studies have shown that EGFR signaling is required for maintenance of a tumorigenic phenotype in H-RAS-induced melanomas. A dominant-negative EGFR reduced the tumorigenicity of melanomas, and sustained expression of EGFR can delay tumor regression (Bardeesy et al. 2005).

The Tet system has also been used to generate conditional models of mutant K-RAS-induced lung adenocarcinoma (Fisher et al. 2001; Floyd et al. 2005). The Clara cell secretory protein (CCSP) promoter was used to regulate gene

expression in alveolar epithelial cells. Within 7–14 days after induction of K-RAS overexpression, type II pneumocytes exhibited focal hyperplasia, and after 2 months multiple solid adenomas or adenocarcinomas were present in the lung. The solid adenomas contained a population of macrophages, but lacked invasive growth and stromal elements. The adenocarcinomas had fewer macrophages and cytoplasmic inclusions, but had local invasion of the pleura. Within 3 days of K-RAS inactivation through doxycycline withdrawal, tumors exhibited decreased cellular density and an increased rate of apoptosis. Within 7 days of K-RAS inactivation, only a few patches of hyperplasia were found, and within a month no residual tumor tissue was found in five of five mice. The same mice were generated in either a p53- or an Ink4A/Arf-deficient background. Tumors grew rapidly in these mice after K-RAS induction but regressed with the same kinetics. TUNEL assays revealed that regardless of the genetic context, tumor regression was associated with apoptosis (Fisher et al. 2001). Similarly, CCSP-regulated K-RAS (G12C)-induced lung adenomas regressed upon oncogene inactivation (Floyd et al. 2005). Hence, even aggressive lung tumors in a tumor suppressor-deficient background regress on the inactivation of a single oncogene.

2.3.2.3 Tumor Regression in a Kinase Model

A conditional transgenic model for BCR-ABL leukemias was generated by using either the MMTV or SCL (stem cell leukemia) promoter to drive the expression of tTA (Huettner et al. 2000; Koschmieder et al. 2005). Upon induction of BCR-ABL the mice developed B-cell leukemia associated with lymphadenopathy, splenomegaly, and bone marrow infiltration. A third of mice with BCR-ABL under the control of the SCL promoter developed B-cell lymphoblastic disease resembling blast crisis, closely mimicking what is observed in patients with chronic myelogenous leukemia (CML). Inactivation of BCR-ABL induced rapid tumor regression in all mice. BCR-ABL inactivation was associated with the apoptosis of 80% of the tumor cells within 20 h and complete tumor regression within 5 days. Sustained regression of tumors was observed in tumors arising from three of the four founder lines, as long as the mice had BCR-ABL continuously inactivated. Upon reactivation of BCR-ABL the tumors rapidly reoccurred. Interestingly, all the mice derived from the fourth founder relapsed within 4 weeks after complete regression. Relapsed tumors lacked continued expression of BCR-ABL protein and mRNA, suggesting that they had become independent of BCR-ABL expression.

2.3.2.4 Nuclear Transcription Factors

The Tet and tamoxifen systems have been used to demonstrate that MYC inactivation can induce tumor regression in a multitude of different types of cancer (see Table 2.1). The Tet-Off system was used to regulate human c-MYC in lymphoid cells under the regulation of the EμSRα promoter (Felsher and Bishop 1999a, 1999b; Marinkovic et al. 2004). When MYC is constitutively activated, 100% of the mice developed hematopoietic tumors within 5 months. On gross examination, the mice exhibited enlargement of the thymus, liver, spleen, and gastrointestinal lymph nodes. Histological examination revealed that tumor cells had invaded all hematopoietic organs as well as liver, kidney, blood, and the lamina propia of the intestines. In one study, tumors were generally immature CD4+/CD8+ T-cell lymphomas and were rarely acute myeloid leukemias (Felsher and Bishop 1999a). In another study, tumors were either B- or T-cell lymphomas (Marinkovic et al. 2004). In both studies, the resulting hematopoietic tumors exhibited a high degree of genomic instability reflected by chromosomal gains, losses, or translocations (Felsher and Bishop 1999a; Marinkovic et al. 2004). Despite this genomic complexity, the inactivation of MYC resulted in rapid and sustained tumor regression. Upon MYC inactivation, tumor cells arrested, differentiated, and then underwent apoptosis. Over 50% of tumors exhibited sustained regression for over 30 weeks. Thus MYC inactivation can induce sustained regression of hematopoietic tumors.

Table 2.2 Oncogene inactivation in the therapeutic setting

Target	Target type	Drug	Cancer	Clinical efficacy	References
EGFR	Receptor tyrosine kinase	Cetuximab	Colorectal cancer	Synergism with irinotecan in irinotecan-refractory colorectal cancer	Cunningham et al. 2004
		Erlotinib (Tarceva)	NSCLC	Approved for refractory NSCLC; disappointing results of addition to chemotherapy in initial treatment of NSCLC	Shepherd et al. 2005
		Gefitinib (Iressa)	NSCLC	Approved for refractory NSCLC; disappointing results of addition to chemotherapy in initial treatment of NSCLC	Kris et al. 2003; Giaccone et al. 2004; Herbst et al. 2004
ERBB2 (Her2/Neu)	Receptor tyrosine kinase	Trastuzumab (Herceptin)	Breast cancer	Increases response rates and improves survival when added to chemotherapy for metastatic HER2 overexpressing breast cancer	Slamon et al. 2001
VEGF	Receptor tyrosine kinase ligand	Bevacizumab (Avastin)	Metastatic colorectal cancer	Significant prolongation of survival in combination therapy	Hurwitz et al. 2004
RAS	GTPase	Zanestra	Colorectal and pancreatic cancer	No effect	End et al. 2001
		ISIS 2503	Pancreatic adenocarcinoma	Unclear benefit in combination therapy	Alberts et al. 2004
BCR-ABL	Tyrosine kinase	Imatinib mesylate (Gleevec/ STI-571)	CML; GIST	Complete hematologic and cytogenetic remissions in most CML patients; partial response in more than half of GIST patients	Demetri et al. 2002; O'Brien et al. 2003
RAF-1	Tyrosine kinase	Sorafenib	Metastatic renal cell carcinoma; advanced melanoma	Improves time to progression in metastatic renal cell carcinoma and produces partial responses in combination therapy against advanced melanoma	Flaherty 2004; Escudier et al. 2005

Conditional transgenic mice expressing c-MYC under the control of the Eμ-promoter occasionally developed highly metastatic osteosarcomas (Jain et al. 2002). Histological examination of the primary tumor revealed the presence of disorganized bone matrix. MYC inactivation induced rapid tumor regression associated with the differentiation of tumor into mature bone. Continuous video time-lapsed microscopy (CVTL) revealed that upon MYC inactivation tumor cells ceased to proliferate and differentiated. Identically, MYC inactivation in tumors italics was associated with the differentiation of malignant cells into mature osteoid. Upon MYC reactivation fewer than 1% of the cells were able to regain a proliferative phenotype. Surprisingly, MYC reactivation was also associated with the apoptosis of the now differentiated tumor cells. Moreover, even the transient inactivation of MYC was found to increase the survival of mice with these tumors. Hence, at least in some circumstances, even brief oncogene inactivation can induce sustained loss of a neoplastic state.

The tamoxifen system also has been used to evaluate the consequences of MYC inactivation in different types of tumors with MycERTAM (see Table 2.1). MycERTAM has been expressed in the skin through the involucrin promoter

(Pelengaris et al. 1999; Flores et al. 2004). MYC activation resulted in increased proliferation and blocked differentiation of the suprabasal epidermis. Sustained MYC activation resulted in hyperplasia, dysplasia, angiogenesis, and papillomatosis. MYC inactivation resulted in regression of blood vessels, restoration of cellular differentiation, and the regression of papillomas. A brief inactivation of MYC in keratinocytes caused the cells to differentiate and become unresponsive to MYC reactivation. MYC reactivation could not restore a proliferative phenotype to the differentiated keratinocytes, and eventually the cells were sloughed off the skin (Flores et al. 2004). Hence, brief inactivation of MYC can induce the sustained loss of neoplastic features in some skin tumors.

MycERTAM was also expressed under the control of the insulin (plns) promoter to induce pancreatic islet cell carcinomas (Pelengaris et al. 2002). Within 24 h of MYC activation, virtually all β-islet cells were rapidly proliferating. By 72 h of MYC activation 4%–7% of β-cells were undergoing apoptosis, and within 6–10 days almost no β-cells were detectable. MycERTAM was expressed in the presence of BCL-xL to address the consequences of MYC activation when apoptosis is repressed. Within 7 days of MYC activation β-cells became hyperplastic, ceased insulin production, and decreased expression of the intercellular adhesion molecule E-cadherin. Within 6 weeks pancreatic islet cell carcinomas had formed highly vascularized tumors. Upon MYC inactivation, these tumors regressed completely. The tumors decreased proliferation, differentiated, increased expression of E-cadherin, and exhibited vascular collapse. While these tumors initially regressed upon MYC inactivation, transient MYC inactivation did not result in sustained tumor regression.

The Tet system has been used to explore the role of MYC in the initiation and maintenance of liver cancer by utilizing the liver activator protein (LAP) promoter to express tTA (Beer et al. 2004; Shachaf et al. 2004). The latency of tumorigenesis was inversely correlated with the age at which MYC was activated (Beer et al. 2004). When MYC was activated during embryonic development, mice would succumb to neoplasia within 10 days of birth. In contrast, if MYC was activated in adult mice, the mean latency of tumor onset was 35 weeks. The tumors generated in adult mice histologically resembled hepatocellular carcinomas (HCC) and/or hepatoblastomas. MYC was found to be able to induce proliferation in embryonic or neonatal liver cells but to induce cellular hypertrophy without cellular proliferation in adult liver cells. In part, this was explained by the observation that MYC induced a p53-dependent arrest in cellular division in adult hepatocytes. Thus it appears that the ability of MYC to induce tumorigenesis depends on epigenetic parameters dictated by developmental state.

The same Tet system model was used to examine the consequences of MYC inactivation in liver tumors (Shachaf et al. 2004). The liver tumors were locally invasive, occasionally metastasized to the lung, and were readily transplantable into SCID mice. Within 4 days of MYC inactivation, tumor cells stopped proliferating, differentiated into normal liver cells, and subsequently underwent apoptosis (Shachaf et al. 2004). Even after 5 months of continuous MYC inactivation, a residual population of tumor-derived cells remained detectable. However, MYC reactivation immediately resulted in resumption of a tumorigenic phenotype. Thus these results were in marked contrast to earlier reports that brief inactivation of MYC can result in a permanent loss of a neoplastic phenotype. One possible explanation for these results is that MYC inactivation uncovers the latent stem cell properties of tumor cells that now can differentiate into normal liver, but some of these cancer stem cells retain the capacity to regain their neoplastic features. In support of this hypothesis, upon MYC inactivation some of the residual cells expressed the liver stem cell marker cytokeratin 19 (CK-19) (Shachaf et al. 2004)

MYC also has been conditionally expressed in mammary epithelium by using a Tet-On system with the MMTV promoter (D'Cruz et al. 2001; Boxer et al. 2004). MYC activation resulted in mammary adenocarcinomas with a mean latency of 22 weeks in approximately 86% of mice. Histologically, the tumors exhib-