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Chapter 14

Activating Mutations and Targeted Therapy in Cancer

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Additional information is available at the end of the chapter

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1. Introduction

Neoplasia, the accumulation of abnormal cells, occurs because tumor cells often lose control of proliferative signaling, escape growth suppression, can become invasive and metastasize and grow in abnormal environments, induce angiogenesis, withstand cell death, deregulate cellular energetic constraints, avoid immune destruction, promote inflammation and enhance genome instability and mutation (Hanahan and Weinberg 2011). Understanding the mechanisms underlying both the sensitivity and the resistance of tumor cells to anticancer agents first requires understanding the global view of the cancer genome (genetic, genomic, and epigenetic alterations) to identify driver events that decisively influence the viability and clinical behavior of a given tumor. This knowledge, together with an understanding of the mechanism of action of drugs, will lead to the identification of novel targets and the development of targeted therapeutics in the appropriate patient subpopulation.

By 1982, mutations and chromosomal translocations had been established as key genetic mechanisms that are capable of driving cancer. Then, the *MYC* proto-oncogene was found to be activated by translocation as well as amplification, and amplification thus became recognized as an additional cardinal mechanism of cancer gene deregulation (Collins and Groudine 1982; Taub, Kirsch et al. 1982; Vennstrom, Sheiness et al. 1982; Alitalo, Schwab et al. 1983). Epigenetic modifications of genomic DNA or histones by methylation or acetylation also became recognized as key mediators of the cancer phenotype (Esteller 2007).

One of the first pivotal discoveries of activating mutations was within *BRAF* (Figure 1), which encodes a serine/threonine kinase oncogene that transmits proliferative and survival signals downstream of RAS in the mitogen-activated protein (MAP) kinase cascade (Davies,

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Bignell et al. 2002). This was after the discovery of *HRAS* mutations (Reddy, Reynolds et al. 1982; Tabin, Bradley et al. 1982) and similar mutations within *KRAS* (Capon, Seeburg et al. 1983; Shimizu, Birnbaum et al. 1983), *NRAS* (Bos, Toksoz et al. 1985), and other genes. Some of the driver mutations were found to be targets for therapy, whereas others play crucial roles in resistance to therapy. Here, we focus on activating mutations, small molecules that have been used to target mutated genes, and mutations that play crucial roles in resistance to certain therapeutic agents.

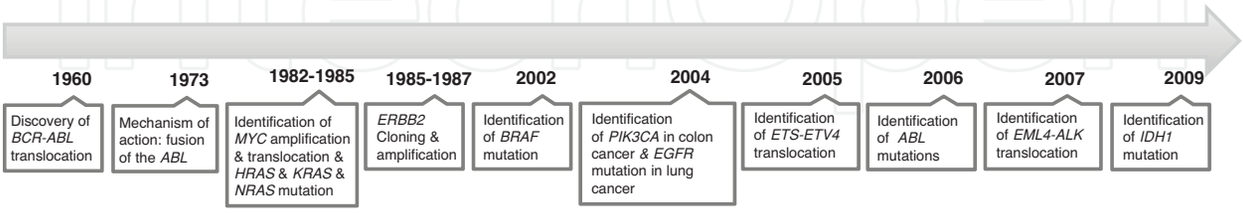


Figure 1. The historical timelines for discovery of driver translocation, mutation and amplification.

2. Types of mutations

Oncogenesis results from mutations or alterations of genes that regulate cell functions such as proliferation, growth, invasion, angiogenesis, metastasis, death, energy metabolism, genome stability, and replication. Simple mutations can be induced in DNA by exposure to a variety of mutagens, such as radiation and chemicals, or by spontaneous errors in DNA replication and repair. Genes with mutations that cause cancer can be grouped into two classes: oncogenes and tumor suppressor genes.

Oncogenes are the mutant form of proto-oncogenes, a class of normal cellular protein-coding genes that promote the growth and survival of cells. Oncogenes encode proteins such as:

a. Growth factors (e.g., *PDGF* and *IGF1*);
b. Growth factor receptors (e.g., *ERBB2*, *EGFR*, and *MET*);
c. Intracellular signal transduction factors (e.g., *RAS* and *RAF*);
d. Cell cycle factors (e.g., *CDK4*);
e. Transcription factors that control the expression of growth promoting genes (e.g., *FOS*, *JUN*, and *MYC*); and
f. Inhibitors of programmed cell death machinery (e.g., *BCL2*).

Tumor suppressor genes, which control cell growth, can be grouped into two classes: gatekeeper and caretaker tumor suppressor genes. Gatekeeper tumor suppressor genes (e.g., *RB1* and *TP53*) block tumor development by controlling cell division and survival, and caretaker tumor suppressor genes (e.g., *MSH2* and *MLH1*) protect the integrity of the genome.

Activation of proto-oncogenes (activating mutations) can occur either by large-scale alterations, such as gain/amplification, insertion, or chromosome translocation, or by small-scale mutations, such as point mutation. Inactivation of tumor suppressor genes

(inactivating mutations) can occur either by small-scale mutation or by large-scale alterations, such as loss of region of tumor suppressor gene or whole chromosome.

Small-scale mutations can be grouped into the following classes on the basis of the effect of the mutation on the DNA sequence:

a. **Base substitution mutation** is the replacement (exchange) of a single nucleotide by another. Base substitutions can be either a **transition**—substitution of a pyrimidine by a pyrimidine (C↔T) or a purine by a purine (A↔G)—or a **transversion**—substitution of a pyrimidine by a purine or vice versa (A↔G, A↔C, G↔T, T↔C). Single nucleotide mutation can lead to qualitative rather than quantitative changes in the function of a protein. The biological activity can be retained, but the characteristics may differ, such as optimum pH and stability. Mutations that occur in coding DNA can be grouped into two classes:

i. **Synonymous (silent) mutations.** In this type of mutation, even if the sequence changes, the amino acid is not altered due to the degenerate genetic code, except if the mutations affect splicing by activating a cryptic splice site or by altering an exonic splice enhancer sequence. Because silent mutations usually confer no advantage or disadvantage to the organism in which they arise, they are also called neutral mutations.

ii. **Non-synonymous mutations.** In this type of mutation, the altered sequence changes the amino acid, which can be a polypeptide (gene product) or functional non-coding RNA. Non-synonymous mutations may have a harmful effect, no effect, or a beneficial effect in the organism. Non-synonymous mutations can be grouped into **nonsense** mutations, where the altered amino acid is replaced by a stop codon, which results in premature termination and is likely to cause loss of function or expression because of degradation of mRNA, and **missense** mutations, where the altered codon specifies a different amino acid, which may affect protein function or stability. **Splice site mutations** are likely to cause aberrant splicing, such as exon skipping or intron retention, and mutations in **promoter** sequences can result in altered gene expression. Finally, some mutations alter the normal stop codon, which terminates mRNA transcription so that a longer or shorter amino acid than normal is translated.

b. **Deletions.** In this type of mutation, one or more nucleotides are lost from a sequence.

i. Deletion of multiple codons (three bases) may affect protein function or stability.

ii. A frameshift mutation—not of a multiple of three bases (codon)—is likely to result in premature termination with loss of function.

iii. A large deletion—partial- or whole-gene deletion—is likely to result in premature termination with loss of function or expression.

c. **Insertions.** In this type of mutation, one or more nucleotides are added into a sequence.

i. Insertion of 3 nucleotides (a codon) or of multiple codons may affect protein function or stability.

ii. A frameshift mutation, which occurs when either <3 or >3 nucleotides are inserted, is likely to result in premature termination with loss of function.

iii. A large insertion, which is partial-gene duplication, is likely to result in premature termination with loss of function. Whole-gene duplication may have an effect because of increased gene dosage.
iv. A dynamic mutation, which is the expansion of a dinucleotide or a trinucleotide repeat, may alter gene expression or may alter protein stability or function.

Whereas mutations in coding DNA have a phenotypic effect, mutations in non-coding DNA are less likely to have a phenotypic effect, except when the mutation occurs in a regulatory sequence such as a promoter sequence and miRNAs. Mutations exert their phenotypic effect through either gain of function or loss of function. Loss-of-function mutations result in either reduced activity or complete loss of the gene product. Gain-of-function mutations can result in either an increased level of expression or the development of a new function of the gene product.

Important progress has been made in developing new technologies for identifying mutations. One of these is next-generation sequencing. This technology enables the identification of copy number changes, chromosomal alterations such as translocations and inversions, and point mutations.

3. Activating mutations and targeted therapies

Recent advances in molecular oncology and discoveries in genetic alterations have yielded new treatment strategies that target specific molecules and pathways in the cancer cell and thereby shed light on personalized therapy. In the past, treatment decisions were based on pathologic results. Now, diagnostic or therapeutic decisions are often also based on genetics/genomic alterations. Currently, the genomic view effectively guides cancer treatment decisions and predicts therapeutic response. Early clinical success was achieved with all-*trans* retinoic acid therapy in patients with acute promyelocytic leukemia (characterized by chromosomal translocations involving retinoic acid receptor α, the target of all-*trans* retinoic acid) (Huang, Ye et al. 1988; Castaigne, Chomienne et al. 1990), Herceptin (trastuzumab, a monoclonal antibody) and in patients with breast cancer in which *ERBB2* is amplified and/or overexpressed (Baselga, Tripathy et al. 1999; Slamon, Leyland-Jones et al. 2001; Vogel, Cobleigh et al. 2002). Also, imatinib mesylate and, subsequently, nilotinib (a selective ABL tyrosine kinase inhibitor [TKI]) have proved effective in patients with the *BCR-ABL* fusion gene, including most individuals (95%) with chronic myeloid leukemia (CML), which constitutively activates the ABL tyrosine kinase (Mauro, O'Dwyer et al. 2002). These successes motivated the discovery of new targets and selective inhibitors for those targets. Currently, targeted therapeutics are used to target receptor tyrosine kinases (*EGFR*, *ERBB2*, *FGFR1*, *FGFR2*, *FGFR3*, *PDGFRA*, *PDGFRB*, *ALK*, *c-MET*, *IGF1R*, *c-KIT*, *FLT3*, and *RET*), non-receptor tyrosine kinases (*ABL*, *JAK2*, and *SRC*), serine-threonine-lipid kinases (*BRAF*, *Aura A and B kinases*, *mTOR*, and *PIK3*), and DNA damage and repair genes (*BRCA1* and *BRCA2*), however not all therapeutics are selective inhibitors. Here, we focus on activating mutations that are targeted by selective inhibitors to inhibit only mutated genes; *EGFR, ALK, c-KIT, BCR-ABL, JAK2, BRAF, IDH1, IDH2*, *FLT3* and *PIK3CA* (Table 1).

EGFR	*BRAF*	*KRAS*	*PIK3CA*	*c-KIT*	*BCR-ABL*	*IDH1*	*JAK2*
G719A[12]	M117R[13]	G12C[5, 12]	E542K[4, 5, 6, 28, 29]	K642E[13, 22]	M244V[10]	R132C[2, 5, 8, 9, 19, 20, 21, 27]	V617F[8, 19, 20]
G719C[12]	I326T[4]	G12R[12]	E545K[4, 5, 6, 9, 12, 28, 29]	L576P[13]	L248V[10]	R132H[1, 2, 9, 21, 26, 27]	K539L[19]
G719S[12]	K439Q[12]	G12S[5, 12]	E545Q[4]	W557R[13]	G250E[10]	R132S[2, 9, 26]	N542-E543del[19]
T790M[12]	K439T[12]	G12A[5, 9, 12]	E545A[4]	V559A[13]	Q252H[10]	R132G[2, 9, 26]	F537-K539delinsK[19]
L858R[4, 12]	T440P[12]	G12D[5, 12]	E545G[4, 5, 29]	V560D[22]	Y253F[10]	R132L[2, 9, 26]	H538-K539delinsL[19]
L858Q[12]	V459L[12]	G12V[5, 12]	E545V[4]	D816H[13, 22]	Y253H[10]	R132V[2, 9]	F537-I546dupF547L[19]
L858L[21]	G469A[14]	G13C[5, 12]	Q546K[4, 6]	F504L[13]	E255K[10]	R132G[2]	E543del[19]
D761Y[12]	R462I[5]	G13R[12]	Q546E[4]	S502-Y503insFA[13]	E255V[10]	V71I[14, 24]	H538QK539L[19]
L747S[12]	I463S[5]	G13S[12]	Q546P[4]	K550N[13]	D276G[10]	G123R[24]	I540-E543delinsMK[19]
T854A[12]	G464E[5, 11, 17]	G13A[12]	Q546R[4, 6]	Y553N[13]	E279K[10]	G97D[9, 25]	F547V[19]
P782L[21]	G464V[5, 11, 17]	G13D[5, 12, 14]	Q546L[4]	556insL[13]	V299L[10]		H538DK539LI540S[19]
F788L[21]	G464R[5, 11, 17]	Q61K[10, 12, 13]	D549N[4]	K558N[13]	F311L[10]	***IDH2***	F537-F547dup[19]
R748K[21, 22]	G466A[6, 12, 13]	Q61L[5, 10, 12]	H1047L[4, 6, 29]	G565V[13]	T315I[10]		I540-N542delinsS[19]
L747–S752del	G466E[12, 13]	Q61R[12, 13]	H1047R[4, 5, 6, 9, 28, 29]	N566D[13]	T315A[10]	V294M[13]	V536-F547dup[19]
E746-A750del[4, 17, 28]	G466R[12, 13]	Q61H[5, 10, 12, 14]	Q1064R[6]	V569G[13]	F317L[10]	R172K[2, 9]	V536-I546dup[19]
S752-I759del[4]	G466V [12, 13]	A146T[5, 14]	A1066V[6]	R634W[13]	F317V[10]	R172M[2, 9]	
L707S[25]	F468C[5]	Y64D[14]	Y1021C[6]	V654A[13]	F317C[10]	R172G[2, 9]	***FLT3***
T710A[25]	G469A[3, 5, 10, 11, 12, 13,16]	L19F[14]	G12-R19del[6]	N655K[13]	M351T[10]	R172S[2]	
E711V[25]	G469E[3, 5, 10, 11, 12, 13,16]	K117N[14]	R38H[6]	D816H[13]	E355G[10]	R172W[2, 9]	
E749K[25]	G469R[3, 5, 10, 11, 12, 13,16]	E63K[14]	R88Q[4, 5, 6, 9]	D816V[2, 13]	F359V[10]	R140Q[2, 9, 19, 20]	Y592A[2]
E762G[25]	G469S[3, 5, 10, 11, 12, 13,16]	K147N[28]	G106A[6]	D820V[13]	F359C[10]	R140W[2]	
A767T[25]	G469V[3, 5, 10, 11, 12, 13,16]	G12F[10, 12]	E109del[6]	D820Y[13]	V379I[10]	R140L[2, 26]	Y599F[2]
K745R[28]	K475E[13]		V344G[6]	N822I[13]	L384M[10]		F691L[2]
G735S[24]	N581S[5]		E309NfsX10[6]	N822K[2, 13]	L387M[10]		
R108K[9]	E586K[17]		E453K[4, 6]	Y823D[13]	H396R[10]		
T263P[9]	D587A[5]		M1043V[6, 9]	A829P[13]	H396P[10]		D835N[2]
A289V[9]	D594G[5, 13, 16, 23]		M1043I[6]	I841V[13]	F486S[10]		D835Y[2]
G598V[9]	D594K[5, 13, 16, 23]		E81K[6]	S864F[13]	E459K		D835A[2]
L861Q[9]	D594V[5, 13, 16, 23]		H1048R[6]	V120F[29]			D835E[2]
R680G[9]	F595L[5, 11]		G1049R[6]	V560D[22]			D835H[2]
G136A[9]	G596R[5]		E418K[6]	Y503-F504insAY[22]			D835V[2]
G136C[9]	L597Q[11, 12, 13, 17]		C420R[6]	Y570-L576del[22]			D835F[2]
G323A[9]	L597R[11, 12, 13, 17]		H701P[6]	A599T[15]			I836F[2]
A787C[9]	L597S[11, 12, 13, 17]		LWGIHLM10del[9]	V833L[10, 30]			I836S[2]
C866A[9]	L597V[11, 12, 13, 17]		P18del[9]	P577S[10, 30]			M837P[2]
G865A[9]	T599I[5]		N345K[9]	V825A[10, 30]			Y842H[2]
C866T[9]	T599-ins(T-T)[13]		C420R[9]	L576P[30]			Y842D[2]
G971T[9]	V600D[3, 4, 5, 7, 9, 11, 12, 13, 17, 22, 23, 24]			E562K [30]			
G988A[9]	V600E[3, 4, 5, 7, 9, 11, 12, 13, 17, 22, 23, 24]			N564S[30]			
D1006Y[14]	V600G[3, 4, 5, 7, 9, 11, 12, 13, 17, 22, 23, 24]			D816V[2, 10, 17]			
M178I[28]	V600K[3, 4, 5, 7, 9, 11, 12, 13, 17, 18, 19, 23]			D816H[2, 10, 17, 26]			
I475V[28]	V600M[3, 4, 5, 8, 9, 11, 12, 13, 17, 22, 23, 24]			D816I[10]			
S492R[25]	V600R			D816G[10]			
F712S[25]	V600-K605 ins[13]			D816F[10]			
T725T[25]	K601E[5, 13, 22]			V825A[2]			
V742V[21, 25]	K601N[5, 13, 22]			D816Y[2, 17, 26]			
F795S[25]	R682Q[6]			R634R[30]			
G796S[25]	A728V[1]			D820G[19]			
G796V[21]				V825I[19]			
T751I[21]				E839K[19]			
R748K[21]				I957T[19]			
R836R[25]				P31L[19]			
T847I[4]				R956Q[19]			
Q820R[21]				T22A[19]			
E804G[21]				G961S[19]			
L828M[21]				K642E[13]			
F856Y[21]				V559D[22]			
F856L[21]				W557R[22]			

A839V[21]				V559G[22]			
G863D[12, 21]				V559D[13, 22]			
V851I[12, 21]				V540L[2]			
I821T[21]				M541L[2]			
I789I[21]							
H870N[21]							
V834A[21]							
T725M[17]							
L858R[17]							
R832C[17]							
A868D[17]							
T852M[17]							
T725A[17]							
L703P[17]							
S720F[17]							
N700S[17]							
R836S[17]							
G721S[17]							
L703P[17]							
K708G[17]							
P772-H773insV[12]							
R108K[9]							
L62R[9]							
V651M[9]							
R222C[9]							
T263P[9]							
A289T[9]							
A289V[9]							
A597P[9]							
G598V[9]							
C620Y[9]							
S703F[9]							

[1]Acute lymphocytic leukemia; [2]acute myleloid leukemia; [3]Barret's adenocarcinoma; [4]breast carcinoma; [5]colon carcinoma; [6]endometrial carcinoma; [7]ependymoma; [8]essential thrombocyte; [9]glioma; [10]leukemia; [11]hepatocellular carcinoma; [12]lung cancer; [13]melanoma; [14]multiple myeloma; [15]neuroblastoma; [16]non-hodgkins lymphoma; [17]ovarian carcinoma; [18]pancreas cancer; [19]polycythemia vera; [20]primary myelofibrosis; [21]prostate cancer; [22]sarcoma; [23]stomach cancer; [24]thyroid cancer; [25]colorectal cancer; [26]myelodysplastic syndromes; [27]paraganglioma; [28]H&N (head and neck) cancer; [29]esophageal cancer, [30]lymphoma.

Table 1. Mutations have been reported at *EGFR, BRAF, KRAS, PIK3C, c-KIT, ABL, IDH1, IDH2* and *JAK2* in variety of cancers (Garnett and Marais 2004; Lee, Vivanco et al. 2006; Loeffler-Ragg, Witsch-Baumgartner et al. 2006; Thomas, Baker et al. 2007; Balss, Meyer et al. 2008; The Cancer Genome Atlas Network 2008; Bleeker, Lamba et al. 2009; Hayes, Douglas et al. 2009; MacConaill, Campbell et al. 2009; Yan, Parsons et al. 2009; de Muga, Hernandez et al. 2010; Gravendeel, Kloosterhof et al. 2010; Green and Beer 2010; Reitman and Yan 2010; Yen, Bittinger et al. 2010; Chapman, Lawrence et al. 2011; Konopka, Janiec-Jankowska et al. 2011; Metzger, Chambeau et al. 2011; Murugan, Dong et al. 2011; Passamonti, Elena et al. 2011; Peraldo-Neia, Migliardi et al. 2011; Stransky, Egloff et al. 2011; Tanaka, Terai et al. 2011; The Cancer Genome Atlas Network 2011; Teng, Tan et al. 2011; Montagut, Dalmases et al. 2012; Weisberg, Sattler et al. 2010; *Catalog of Somatic Mutations in Cancer*: www.sanger.ac.uk/genetics/CGP/cosmic/)

3.1. Activating mutations at *BCR-ABL*

In a normal cell, ABL protein is located in the nucleus, but in cancer cells the BCR-ABL fusion protein is found in the cytoplasm and is constitutively active (Goldman and Melo 2008). Studies have shown that *BCR-ABL* is oncogenic in hematopoietic cells, promoting leukemic cell proliferation and inhibiting apoptosis (Lugo, Pendergast et al. 1990; Stoklosa, Poplawski et al. 2008). Notably, *BCR-ABL* activity has also been found to stimulate the

generation of mutagenic reactive oxygen species and to inhibit DNA repair mechanisms (Koptyra, Falinski et al. 2006; Fernandes, Reddy et al. 2009).

The discovery of this oncogenic fusion protein led to the development of imatinib mesylate. Imatinib, an ABL kinase inhibitor, was the first therapeutically successful treatment for CML and gained U.S. Food and Drug Administration approval in 2001. However, a substantial proportion of patients with CML developed resistance to imatinib because of mutation in *BCR-ABL* fusion gene (>90 mutations that affect >55 amino acid residues in *BCR-ABL*) (Table 1) (Branford 2007). Interestingly, *BCR-ABL* mutations were found in 57% of patients with acquired resistance to imatinib compared with 30% of patients with primary resistance (Soverini, Colarossi et al. 2006). The point mutation(s) in the *BCR-ABL* kinase domain result in the resistance to imatinib by reducing the flexibility of the kinase domain and its binding to imatinib, and inhibiting the activity of the kinase (Burgess, Skaggs et al. 2005; O'Hare, Walters et al. 2005).

T315I is the most common imatinib-resistant mutation in *BCR-ABL*; among the other highly imatinib-resistant mutations are L248V, Y253F/H, E255K/V, H396P/R, and F486S (Houchhaus, La Rosee et al. 2011). These discoveries were followed by the development of second-generation TKIs to inhibit BCR-ABL: dasatinib, and nilotinib. The response rate to these second-generation BCR-ABL inhibitors in patients harboring imatinib-resistant mutations is variable, depending on the mutation: L248V (40%), G250E (33%), E255K (38%), and E255V (36%), but response rates are low in those harboring F317L (7%) or Q252H (17%) (Muller, Cortes et al. 2009). The following imatinib-resistant mutations are sensitive to nilotinib: M351T, G250E, M244V, H396R, F317L, E355G, E459K, F486S, L248V, D276G, E279K, and V299L. The following are sensitive to dasatinib: M351T, G250E, F359V, M244V, Y253H, H396R, E355G, E459K, F486S, L248V, D276G, E279K, Y253F, F359C, and F359I. The following mutations are resistant to dasatinib: V299L, T315A, and F317I/L. The following are resistant to nilotinib: Y253F/H, E255K/V, and F359C/V (Hochhaus, La Rosee et al. 2011). All three these inhibitors inhibit the catalytic activity of BCR-ABL by binding to the ATP-binding pocket of the ABL kinase domain.

3.2. Activating mutations at *BRAF*

One of the discoveries of mutations affecting cancer prognosis is *BRAF* mutations. *BRAF* has been discovered to be the most commonly mutated oncogene in melanoma (50–60%) (Davies, Bignell et al. 2002), papillary thyroid carcinoma (36–53%) (Yeang , McCormick et al. 2008), colon carcinoma (57%), serous ovarian carcinoma (~30%) (Yeang , McCormick et al. 2008), and hairy cell leukemia (100%) (Tiacci, Trifonov et al. 2011). To date, >60 distinct mutations in the *BRAF* gene have been identified (Table 1) (Garnett and Marais 2004; *Catalog of Somatic Mutations in Cancer*: www.sanger.ac.uk/genetics/CGP/cosmic/). The most prevalent mutation is a missense mutation in *BRAF*, which results in a substitution of glutamic acid to valine at codon 600 ($BRAF^{V600E}$) and occurs in 90% of all *BRAF* mutations (Garnett and Marais 2004). *BRAF* encodes BRAF, a member of the RAF family of cytoplasmic serine/threonine protein kinases. BRAF phosphorylates MEK protein and

activates ERK signaling, downstream of RAS, which regulates multiple key cellular processes that are required for cell proliferation, differentiation, apoptosis, and survival. The *RAF* family (*A-RAF, B-RAF, C-RAF*) members are components of a signal transduction pathway downstream of the membrane-bound small G-protein RAS, which is activated by growth factors, hormones, and cytokines (Robinson and Cobb 1997).

MEK inhibitors suppress ERK signaling in all normal and tumor cells. In contrast, the RAF inhibitor vemurafenib inhibits the ERK pathway and cell proliferation only in tumor cells with mutant *BRAF*. Targeted therapy and selective inhibitors for certain altered genes are crucial to enable targeting of tumor cells but not normal cells.

Mutated *BRAF* activates and deregulates the kinase activity of BRAF. The recently developed BRAF inhibitor vemurafenib (PLX4032) inhibits RAF activation selectively only in cells carrying the *BRAF* V600E mutation. Clinically, vemurafenib has an 80% response rate in metastatic melanoma patients harboring the *BRAF* V600E mutation, but 18% of patients treated with vemurafenib develop at least one squamous-cell carcinoma of the skin or keratoacanthoma as an adverse event (Chapman, Hauschild et al. 2010). The remaining 20% of patients who harbor the *BRAF* V600E mutation, and also patients who do not harbor the *BRAF* V600E mutation, are resistant to vemurafenib. Other mechanisms that cause vemurafenib resistance are mutations in *NRAS* and *c-KIT* alterations. *c-KIT* alterations (mutations and/or amplifications) are found more frequently (28-39%) in melanomas from acral, mucosal, and chronically sun-damaged sites (Curtin, Busam et al. 2006), whereas uveal melanomas uniquely harbor activating mutations in the a-subunit of a G proteins of the Gq family, GNAQ and GNA11 (Van Raamsdonk, Bezrookove et al. 2009; Van Raamsdonk, Griewank et al. 2010). *NRAS* mutations are observed in 15–30% of cutaneous melanomas and are mutually exclusive of *BRAF* mutations; the most common change occurs at G12 or Q61 (Brose, Volpe et al. 2002). Currently, no selective inhibitor for those mutations exists. In contrast, *BRAF* mutations are also found in colon cancer (8%) (Hutchins, Southward et al. 2011), papillary thyroid cancer (44%) and anaplastic thyroid cancer (24%) (Xing, Westra et al. 2005), but limited study has reported to date. However, vemurafenib has limited therapeutic effects in *BRAF* (V600E) mutant colon cancers because inhibition of *BRAF* (V600E) causes a rapid feedback activation of EGFR, which induces continued proliferation in *BRAF* (V600E) inhibited cells. Therefore, blocking the *EGFR* by gefitinib, erlotinib or cetuximab has strong synergistic with inhibition of *BRAF* (V600E) by vemurafenib in colon tumor cell *in vivo* and *in vitro* (Prahallad, Sun et al. 2012). The question remains to answer whether the same BRAF selective inhibitor can be effective in other tumor types due to lack of evidence.

3.3. Activating mutations at *PIK3CA*

Shortly after *BRAF* mutations were found and selective inhibitors of the mutant *BRAF* were developed, activating point mutations were found in *PIK3CA* (Samuels, Wang et al. 2004) in a variety of cancers, including breast (20–30%) (Bachman, Argani et al. 2004; Campbell, Russell et al. 2004), colorectal (Parsons, Wang et al. 2005), endometrial (Samuels and Ericson

2006), ovarian, and hepatocellular cancers and medulloblastoma (Broderick, Di et al. 2004), among others (Kang, Bader et al. 2005; Lee, Soung et al. 2005). *PIK3CA* encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K), a lipid kinase that drives AKT signaling to govern cell growth and survival. PI3Ks are heterodimers, composed of catalytic (p110α; PI3Kα) and regulatory (p85) subunits. Catalytic units include the ABD, RBD, C2, helical, and kinase domains, whereas the regulatory unit comprises the SH3, GAP, nSH2, iSH2, and cSH2 domains. Mutations mostly cluster between the kinase domain and other domains within the catalytic subunit (Huang, Mandelker et al. 2007). The family of receptor tyrosine kinase, together with the MAP kinase and PI3K cascades, forms part of the obsolete growth factor signaling pathway governing tumor cell growth and survival (Samuels, Diaz et al. 2005). Due to complexity and diverse activation of PI3K signaling, such as activating mutations or amplification of *PIK3CA,* or upstream of RTK, loss of *PTEN* or activating mutations of *RAS* in human cancers (Courtney, Corcoran et al. 2010), developing the effective therapeutic agents against PIK3CA might be more challenging (Zhao and Vogt 2008). Hereby, either single agents or combination with other therapeutic agents against to PIK3CA are under development (Courtney, Corcoran et al. 2010).

3.4. Activating mutations at *EGFR*

This finding was followed by the identification of activating point mutations and small insertions/deletions in *EGFR*, an oncogene encoding a receptor tyrosine kinase, which is present more frequently in East Asian individuals with non–small-cell lung cancer (NSCLC) (25%) than in Caucasian people (10–15%) and occurs most frequently in lung adenocarcinomas (Lynch, Bell et al. 2004; Paez, Janne et al. 2004; Pao, Miller et al. 2004). Activating mutations were initially identified in 3 kinase domain exons (18, 19, and 21), encoding G719S and G719C in exon 18 and L861Q in exon 21; the most common mutations are small in-frame deletions in exon 19 and the leucine-to-arginine substitution mutation L858R. L858R mutation causes constitutive activation of the tyrosine kinase of EGFR. Oncogenic mutation of *EGFR* activates downstream signaling pathways of EGFR, which are implicated in tumor cell growth, proliferation, and survival. This discovery led to the development of the selective EGFR TKIs erlotinib and gefitinib. Inhibition of EGFR by EGFR inhibitors blocks the activity of tyrosine kinase, and hence the activation of the downstream cellular pathways. Individuals with lung adenocarcinoma harboring the G719S and L858R mutations are sensitive to gefitinib or erlotinib. Although patients harboring these mutations have a high response rate to the EGFR inhibitors gefitinib and erlotinib, the duration of the response is not long, and patients relapse after about a year of treatment (Pao and Chmielecki 2010).

One of the mechanisms by which resistance to erlotinib or gefitinib develops in 50% of relapsed patients is acquisition of a resistant mutation in exon 20 (T790M) in *EGFR* (Kobayashi, Boggon et al. 2005; Pao, Miller et al. 2005) or activating mutation in *KRAS* (Pao, Wang et al. 2005). A second mutation in *EGFR* (T790M) is also found rarely in the germline to be associated with an inherited susceptibility to lung cancer (Bell, Gore et al. 2005; Vikis, Sato et al. 2007). This mutation has been shown to decrease the affinity of EGFR to gefitinib

in the L858R mutant by increasing the affinity of EGFR to ATP (Yun, Mengwasser et al. 2008). This resistant mutant led to the development of promising new agents as second-generation EGFR inhibitors (Li, Shimamura et al. 2007; Li, Ambrogio et al. 2008; Zhou, Ercan et al. 2009). Another mechanism by which resistance to erlotinib or gefitinib develops is amplification (20%) or mutation (Y1230H) in *MET*, an oncogene encoding receptor tyrosine kinase (Bean, Brennan et al. 2007; Engelman, Zejnullahu et al. 2007). Overexpression of HGF, a specific ligand of MET, is another mechanism by which resistance to EGFR inhibitors develops (Yano, Wang et al. 2008).

Gefitinib and erlotinib are first-generation, reversible EGFR inhibitors. Currently being developed are second-generation irreversible EGFR inhibitors, which inhibit EGFR kinase activity even when the T790M mutation is present. Neratinib (HKI-272) (Li, Shimamura et al. 2007; Wong, Fracasso et al. 2009; Sequist, Besse et al. 2010) and afatinib (BIBW 2992) (Eskens, Mom et al. 2008; Li, Ambrogio et al. 2008; Yap, Vidal et al. 2010) are dual inhibitors against EGFR and HER2, and PF-00299804 is a multi-inhibitor against EGFR, ERBB2, and ERBB4 (Engelman, Zejnullahu et al. 2007). For *MET* gene amplification, the MET inhibitor PHA-665752 has been developed (Engelman, Zejnullahu et al. 2007). Recently, new EGFR inhibitors (WZ4002, WZ3146, and WZ8040) have been reported that suppress the growth of *EGFR* T790M-containing cell lines by inhibiting phosphorylation (Zhou, Ercan et al. 2009). Erlotinib has a statistically significantly higher response rate than chemotherapy (83% vs 36%) (Friedrich 2011). In fact, some activating mutations, like those of *KRAS*, may not be drug targets but may rather govern the resistance to selective inhibitors of EGFR (Allegra, Jessup et al. 2009). Activating mutations of *EGFR* are also present in glioma, breast, endometrial and colorectal carcinomas. *KRAS* mutations at G12 and G13 are associated with resistance to erlotinib or gefitinib in *EGFR* mutated lung adenocarcinoma parients (Pao, Wang et al. 2005) and metastatic colorectal carcinoma (Allegra, Jessup et al. 2009).

Shortly after the discovery of *EGFR* mutations, somatic activating mutations of *ERBB2* were found in 2–4% of patients with lung adenocarcinoma. *ERBB2* is a receptor tyrosine kinase, one of the members of ERBB family, and the only one that does bind to any known ligand but activates downstream signaling pathways by homo- or hetero-dimerization with other ERBB family members. Small in-frame insertion mutations span exon 20 of the kinase domain of *ERBB2*, and these are analogous to the mutations in the paralogous exon 20 in the *EGFR* gene that confer resistance to erlotinib or gefitinib. ERBB2 is a receptor tyrosine kinase that heterodimerizes or homodimerizes with EGFR and other members of the ERBB family, ERBB3 and ERBB4, to activate downstream signaling pathways (Hynes and Lane, 2005)..

3.5. Activating mutations at *JAK2*

The discovery of the somatic gain-of-function mutation (V617F) in Janus kinase 2 (*JAK2*) in >90% of individuals with polycythemia vera, 50% of individuals with primary myelofibrosis, and 60% of those with essential thrombocytopenia (Levine, Wadleigh et al. 2005), all of which are Philadelphia chromosome -negative myeloproliferative neoplasms, generated interest in developing JAK2 inhibitors. The JAK kinases (JAK1, JAK2, JAK3, and JAK4) were first identified in 1989 (Wilks 1989). Structurally, all members of the JAK family

contain seven distinct domains: JAK homology (JH) domains 1 to 7 (JH1–7). The tyrosine kinase domain (JH1) is located at C-terminus of the protein and is responsible for the kinase activity. The pseudokinase domain (JH2) has no kinase activity, but deletion of the JH2 domain leads to increased kinase activity. JH3 and JH4 are similar to the SH2 domain, and their roles are still unclear (Wilks, Harpur et al. 1991; Lindauer, Loerting et al. 2001; Giordanetto and Kroemer 2002; Saharinen and Silvennoinen 2002). JH5, JH6, and JH7 are located at the amino-terminus of the protein and play a role in binding the JAK molecule to the cytokine receptor and in maintaining receptor expression at the cell surface (Huang, Constantinescu et al. 2001). JAK2 is a nonreceptor tyrosine kinase that mediates signals between cytokine receptors and downstream targets.

An activating mutation of *JAK2*, a valine-to-phenylalanine substitution at position 617 (V617F) (Scott, Tong et al. 2007), leads to constitutive activation of STAT5. The JAK inhibitors INCB01824, TG101348, and lestaurtinib (CEP701), which inhibit JAK1 and JAK2, results in a marked reduction (>50%) in massive splenomegaly (Verstovsek, Kantarjian et al. 2010).

3.6. Activating mutations at *c-KIT*

Other kinase activating mutations have been found in the oncogene c-*KIT* in gastrointestinal stromal tumors (GIST), acral or mucosal melanoma, endometrial carcinoma, germ cell tumors, myeloproliferative diseases, and leukemias, which is the mutations cause constitutive activation of *c-KIT* (Malaise, Steinbach et al. 2009). c-KIT is a transmembrane cytokine receptor tyrosine kinase that is expressed on the surface of hematopoietic stem cells. Most GIST patients who harbor *c-KIT* mutations have a response to imatinib mesilate (80%). This raises the question of whether imatinib or nilotinib (TKIs) may elicit clinical responses in *KIT*-mutant melanoma or endometrial carcinoma or in other cancers that harbor *KIT* mutations. Acquired resistance to imatinib commonly occurs via secondary gene mutations in the *c-KIT* kinase domain in GIST. For example, the V560G mutation in *KIT* is sensitive to imatinib, although the D816V mutation is resistant to imatinib (Mahadevan, Cooke et al. 2007).

3.7. Mutations at *IDH1 and IDH2*

IDH1 encodes a nicotinamide adenine dinucleotide phosphate (NADP)+-dependent enzyme that converts isocitrate to 2-ketoglutarate in the cytoplasm. Somatic mutations were found to be present in *IDH1* and *IDH2* in 88% of individuals with secondary glioblastomas, 68% of those with grade II glioma (lower grade diffuse astrocytomas), 78% of those with grade III anaplastic astrocytomas, and 69% of those with grade III anaplastic oligodendrogliomas (Dang, Jin et al. 2010; Dang, White et al. 2010) as well as 31% of patients with myeloproliferative neoplasm (Green and Beer 2010) and 10% of those with acute myeloid leukemia (AML) (Dang, Jin et al. 2010; Yen, Bittinger et al. 2010). Mutations in *IDH* were first reported to be activating mutations, but subsequent studies of mutations at arginine R132 (in *IDH1*) and at R140 or R172 (in *IDH2*) in the enzyme showed a gain of new function and the ability to convert alpha-ketoglutarate to 2-hydroxyglutarate (Dang, White et al. 2009). Mutations that have been reported in *IDH1* and *IDH2* are summarized in Table 1. Mutations in these metabolic enzymes uncover novel avenues for the development of anticancer

therapeutics, but specific inhibitors are needed for the mutated forms R132, R140, or R172. It is not clear what the role of this mutation is in cancer and whether it is crucial for tumorigenesis, although the 2-hydroxyglutarate metabolite is a biomarker that can be measured in whole blood and used to select targeted therapy (Yen, Bittinger et al. 2010).

3.8. Fusion genes

Another recent breakthrough was the discovery of translocations or other chromosomal rearrangements between ETS transcription factors (*ERG*, *ETV1*, and *ETV4*) in >40% of prostate cancers (Tomlins, Rhodes et al. 2005; Tomlins, Laxman et al. 2007; Berger, Lawrence et al. 2011) and the fusion of anaplastic lymphoma kinase (*ALK*) with other genes in NSCLC (Soda, Choi et al. 2007; Choi, Soda et al. 2010). Echinoderm microtubule-associated protein-like 4 (*EML4*) is fused to *ALK*, which leads to a fusion-type tyrosine kinase between the N-terminus of *EML4* and the C-terminus of the *ALK* that is a chimeric oncoprotein and is found in 3–5% of NSCLC tumors (Soda, Choi et al. 2007; Choi, Soda et al. 2010). The inversion on chromosome 2p [inv(2)(p21p23)] leads to formation of the *ELK4-ALK* fusion oncogene. The chromosomal inversion occurs in different locations, and multiple *EML4-ALK* variants have been reported; all involve the intracellular tyrosine kinase domain of *ALK* (exon 20) but different truncation of *EML4* (exon 2, 6, 13, 14, 15, 17, 18, or 20), TFG, and *KIF5B*; the most common inversion is in exon 13 of *EML4* (Hernandez, Pinyol et al. 1999; Choi, Takeuchi et al. 2008; Takeuchi, Choi et al. 2009). The amino-terminal coiled-coil domain within EML4 is necessary and sufficient for the transforming activity of *EML4-ALK* (Soda, Choi et al. 2007). This fusion tyrosine kinase may activate downstream signaling pathways of ALK, such as RAS/RAF. This recent discovery of the genetic rearrangement between ALK and the aforementioned genes has led to the development of another targeted agent, crizotinib (PF-02341066), for the treatment of NSCLC. Crizotinib, a TKI that was initially designed as an inhibitor of MET, is currently used to inhibit both tyrosine kinases, MET and ALK in NSCLC. *ALK* rearrangement has been found mostly in younger and more likely to be never or light smoker lung adenocarcinomas and is more frequent in the Asian population than in the American or European population (Sasaki, Rodig et al. 2010). Patients who developed resistance to BRAF inhibitors were found to be harboring the C1156Y (46.6%) and L1196M (15.1%) mutations in the *ALK* gene (Choi, Soda et al. 2010) and also the F1174L mutation (Sasaki, Okuda et al. 2010).

3.9. Activating mutations at *FLT3*

FLT3 encodes a receptor tyrosine kinase that is involved in stem cell development and differentiation, stem and/or progenitor cell survival, and the development of B-progenitor cells, dendritic cells, and natural killer cells in the bone marrow (Small, Levenstein et al. 1994). Two common mutations have been found in AML: internal tandem duplication (ITD) in-frame mutations of 3–400 base pairs in the juxtamembrane region, and point mutations in the tyrosine kinase domain (TKD) D835 (7%). Mutations in the ITD and TKD lead to constitutive activation of tyrosine kinase (Abu-Duhier, Goodeve et al. 2001), and this finding led to the design of the first-generation FLT3 inhibitors lestaurtinib (CEP701) (Smith, Levis et al. 2004), midostaurin (PKC412A) (Stone, DeAngelo et al. 2005), sunitinib

(SU11248)(O'Farrell, Foran et al. 2003), sorafenib (BAY43-9006), and tandutinib (MLN518), followed by the second-generation FLT3 inhibitors KW2449 (Pratz, Cortes et al. 2009) and AC220 (Zarrinkar, Gunawardane et al. 2009).

4. Future directions

Drugs targeting some of these mutations are now either undergoing clinical testing or have protocols in the approval process. The discovery of base mutations through systematic DNA sequencing has provided decisive genetic evidence that these same pathways play crucial roles in tumorigenesis and maintenance and has also opened up new avenues for the deployment of targeted therapeutics. We are just starting to understand the genetic mechanisms that lead to the development of cancer and play a role in treatment. Hence, we are still at the beginning of the road map to targeted therapy. We still need to discover all activating mutations or other chromosomal rearrangements, inactivating mutations, and epigenetic alterations in the genome that drive cells to tumorigenesis for each type and subtype of cancer, and we need to identify resistant and sensitive mutations to find the correct targets for the development of new selective therapeutic agents, and use combination of selective therapeutic agents.

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