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CYTOGENETICS (CL MARTIN, SECTION EDITOR)

Using Cytogenetic Rearrangements for Cancer Prognosis and Treatment (Pharmacogenetics)

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Abstract Chromosomal rearrangements including translocations, deletions, inversions, and insertions are common genetic alterations in cancer. Over 1,000 recurrent chromosome rearrangements have been reported so far in different human tumors (http://cgap.nci.nih.gov/Chromosomes/Mitelman). Most of these chromosome rearrangements are associated with specific tumor types and bear distinctive diagnostic and prognostic significance. Molecular characterization of these rearrangements has revealed numerous cancer genes, including novel fusion genes, and their normal and aberrant interactions involved in tumorigenesis, and has identified myriad therapeutic targets. With the help of advanced high-throughput technologies, many cryptic chromosome rearrangements undetectable by conventional cytogenetics have recently been discovered and delineated. The understanding of the mechanisms responsible for the formation of recurrent chromosome rearrangements and their biological functions has led to novel treatment regimens that target tumor cells specifically, with minimal impact to normal cells. Here, we review common recurrent chromosome rearrangements in both hematopoietic malignancies and solid tumors, and their clinical significance, with a focus on acquired fusion genes and their therapeutic implications (i.e., pharmacogenetics).

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Chromosome Rearrangement in Leukemia

The list of recurring reciprocal translocations in acute and chronic leukemia detectable by classic cytogenetics and/or fluorescent in situ hybridization (FISH) continues to expand. Many of these translocations have well-established diagnostic and prognostic implications. A few translocations are known to be solely causative of a specific leukemia; others initiate leukemogenesis but additional genetic abnormalities are required for transformation. The role of some translocations in the causation and/or propagation and maintenance of disease has yet to be determined.

There are at least four categories of underlying molecular abnormalities of acquired translocations in leukemia. The two most common are activation of a proto-oncogene by transposition of the coding region to a promoter or enhancer of another actively transcribed gene, and creation of a new hybrid or fusion gene which alters normal cell function. Other mechanisms include inactivation of a tumor suppressor gene and upregulation of miRNAs. Often, especially in acute leukemia, other genes and pathways are activated which increase cell proliferation, block apoptosis, and/or block cell differentiation leading to an accumulation of immature blasts or to aberrant self-renewal.

The revised 2008 WHO classification of tumors of the hematopoietic and lymphoid tissues lists eight major subgroups of myeloid and lymphoid neoplastic diseases, including myeloproliferative neoplasms (MPN); myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1;

myelodysplastic/MPN; myelodysplastic syndrome; acute myeloid leukemia and related neoplasms; acute leukemias of ambiguous lineage; B-lymphoblastic leukemia/lymphoma; and T-lymphoblastic leukemia/lymphoma. Several specific diseases within three of these subgroups, the acute myeloid leukemia and related neoplasms, the acute leukemias of ambiguous lineage, and B lymphoblastic leukemia/lymphoma, are designated by their recurrent chromosomal abnormality, most of which are translocations [1••].

Myeloproliferative Neoplasms (MPN)

Chronic myelogenous leukemia, *BCR–ABL* positive, is the first disease listed in the 2008 WHO classification under MPN [1••]. Detectable by conventional cytogenetics and FISH, a simple reciprocal translocation between the long arms (q) of chromosomes 9 and 22 is the genetic lesion in over 90 % of cases; a minority of patients carry a more complex variant translocation with a third chromosome partner, while others have an insertion of one gene next to the other without reciprocity. Overall, this gene rearrangement is seen in ~95 % of CML patients [2••] and is considered causative of CML without additional genetic abnormalities [3]. The resulting protein product in CML is a 210-kDa chimeric *BCR–ABL* protein with more potent tyrosine kinase activity as compared to the non-chimeric c-*ABL* protein found in non-malignant cells. Enhanced activity of the mutant tyrosine kinase promotes growth and replication through downstream pathways such as *RAS*, *RAF*, *JUN* kinase, *MYC*, and *STAT* [4, 5••].

Targeted therapies against the *BCR–ABL* fusion protein came with the development of small molecule tyrosine kinase inhibitors (TKIs) which block the aberrant protein in some manner; this approach has increased the 10-year overall survival (OS) from ~20 to 80–90 % [5••]. Three protein kinase inhibitors, imatinib mesylate (Gleevec), dasatinib (Sprycel), and nilotinib (Tasigna), are currently in use as front-line treatment options for patients with newly diagnosed CML. Imatinib, the first drug to be approved by the FDA, inhibits phosphorylation of the chimeric protein at the ATP binding site, thus blocking signal transduction downstream. Not only does it inhibit BCR–ABL kinase but it also blocks *PGDFR* and *C-KIT* tyrosine kinase. Up to 30 % of patients either do not respond or become resistant to imatinib. Dasatinib is more potent than imatinib in vitro and also inhibits the Src family of kinases; it was shown in a randomized trial to induce more molecular responses than imatinib. Nilotinib is also more potent than imatinib; thus, it can also elicit responses in patients who have proven resistant to imatinib. The FDA has also approved nilotinib and dasatinib for the accelerated phase of CML following TKI therapy [5••].

While some patients who are resistant to imatinib respond to either dasatinib or nilotinib, others also experience either primary or secondary resistance to these two drugs. A major factor influencing resistance is development of additional mutations; one such common mutation within the hybrid gene is the T315I substitution which blocks access to the enzyme's ATP binding site by all three current drugs. A third generation drug, ponatinib, is in phase II clinical trials for patients who have relapsed or are affected with resistant CML, as well as patients with Ph+ ALL. To date, this new drug appears effective against all known mutations. Specifically, its novel triple-bond linkage is able to overcome the steric hindrance produced by the isoleucine residue at position 315. It also appears to inhibit new mutations at a dosage of 40 nM [5, 6], (http://www.cancer.gov).

Other drugs in development include DCC-2036, a switch pocket inhibitor, which blocks the conformational change needed to transform the oncogenic protein from inactive to active. Omacetaxine is a non-TKI agent which acts by disrupting protein synthesis and inducing apoptosis. Experimentation is also ongoing with aurora kinase inhibitors, farnesyl transferase inhibitors, hedgehog inhibitors, and hypomethylating agents [5••].

Other MPNs, such as polycythemia vera, have recurrent chromosome abnormalities; however, the most common abnormalities are deletions and duplications, not reciprocal translocations.

Acute Myeloid Leukemias (AML) with Recurrent Genetic Abnormalities

AML with t(8;21)(q22;q22); RUNX1–RUNX1T1

Of the recurring translocations in AML, t(8;21)(q22;q22) is the most frequent with an incidence of approximately 7 % of all chromosomally abnormal AMLs; it is the sole abnormality in 40 % of cases and is easily detectable by conventional cytogenetics in most cases. Up to 13 % of cases may have a complex or cryptic rearrangement which requires the use of FISH for detection of the gene fusion [7]. It is also one of the most common structural rearrangements in childhood AML and is usually seen in M2, both in children and adults [8]. The molecular result of the 8;21 translocation is the creation of a hybrid gene by the fusion of *RUNX1* on 21q with *RUNX1T1* on 8q. The hybrid *RUNX1/RUNX1T1* gene product disrupts normal RUNX1 protein function and thus inhibits differentiation of myeloid cells, specifically granulocytic and erythroid cells, promoting self-renewal. These data are consistent with the hypothesis that the translocation initiates leukemogenesis [8].

The presence of an 8;21 translocation is sufficient datum on which to make a diagnosis of AML regardless of blast percentage in the marrow or peripheral blood. It is

considered a favorable cytogenetic marker in adults as well as children, although the presence of other abnormalities/mutations may have an adverse effect on response to treatment and OS. It has been established that 8;21 translocation positive patients who also harbor a *FLT3* or *KIT* mutation have a higher rate of relapse and lower OS [1••].

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB–MYH11

As with 8;21 translocation patients, AML patients who are positive for either the inv(16)(p13.1q22) or t(16;16)(p13.1;q22) are classified as having a core binding factor AML [9]. These chromosome abnormalities together are observed in up to 7 % of AML patients, with ~95 % having a pericentric inversion and 5 % having the reciprocal 16;16 translocation. Both chromosome rearrangements result in fusion of *MYH11* gene on the short arm of chromosome 16 with *CBFB* on the long arm of chromosome 16 [10]. The fusion product inhibits *RUNX1* function, thereby changing expression of other genes and blocking differentiation [7].

Both are considered favorable cytogenetic prognostic indicators; however, 30–40 % will relapse. While additional cytogenetic abnormalities do not seem to confer a worse prognosis, the presence of a *FLT3* or *KIT* mutation does [10].

APL with t(15;17)(q22;q23); PML–RARA

Two fusion or hybrid genes created by two different reciprocal translocations are associated with acute promyelocytic leukemia (APL). The most common translocation (~98 % of cases) occurs between the *PML* gene on chromosome 15 and the *RARA* gene on chromosome 17, creating a chimeric retinoic acid receptor transcription factor; a smaller number of APL cases occur as the result of a translocation between the *PLZF* gene on chromosome 11 and the same domain of the *RARA* gene on chromosome 17. While the exact biological function of these new hybrid proteins has yet to be determined, normal differentiation is blocked in cells expressing these proteins and they gain the capacity for aberrant self-renewal.

Administration of all-trans retinoic acid (ATRA) overcomes the differentiation block in *PML–RARA* positive cells but is not effective in patients with *PLZF–RARA* gene fusion. ATRA disrupts the formation of the high molecular weight complexes formed by PML–RARA through the "coiled-coil" region of PML. Once these complexes are disrupted, differentiation can proceed. PLZF/RARA forms the high molecular weight complexes through another domain (BTB/POZ) which is not disrupted by the ATRA [11••]. Arsenic trioxide also induces differentiation of promyelocytes.

AML with t(6;9)(p22;q24); DEK–NUP214

This translocation occurs mainly in children and young adults, and the majority of cases are de novo. The translocation joins almost the entire *DEK* gene on chromosome 6 with two-thirds of the *NUP214* gene on chromosome 9. However, the mechanism by which this fusion protein contributes to the development of AML appears to be different from other fusion proteins which affect transcription. Ageberg et al. [12] showed that *DEK–NUP214* positive myeloid cells show increased total protein synthesis, which occurs, not by transcription deregulation, but by enhanced mRNA translation; mRNA levels remain unchanged. Response to conventional chemotherapy has been reported to be poor, and thus this translocation is considered an adverse prognostic indicator [7].

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1–EVI1

As with the inverted 16 and t(16;16), the inverted 3 occurs much more frequently than the reciprocal translocation; an insertion translocation involving the same breakpoints is also seen on rare occasions. The molecular result of all three rearrangements is to place the promoter of *RPN1* (3q21) next to *EVI1* (3q26.2), a nuclear transcription factor involved in proliferation and maintenance of hematopoietic stem cells, the result of which is over-expression of the normal EVI1 protein product. Considered poor prognostic indicators, this group of rearrangements occurs in up to 2.5 % of AML patients, as well as in MDS and the blast phase of CML [7].

Mixed Lineage Leukemia (MLL) Rearrangements Including AML with t(9;11)(p22;q23); *MLLT3–MLL*

The *MLL* gene located on chromosome 11q23 codes for a transcription regulatory protein which functions via histone methyltransferase activity; the wild-type protein is essential for normal hematopoiesis. MLL fusion products have been shown to activate otherwise inactive genes downstream as well as to silence or cause under-expression of other genes; both play a role in leukemic development [13].

Recurrent rearrangements of *MLL* are seen in 3–4 % of adult AML patients, 3–7 % of adult ALL patients [14], 14–22 % of infant and childhood AML patients [15], and in 4–6 % of childhood patients (>1 year of age). The vast majority of *MLL* rearrangements are reciprocal translocations, with more than 80 partner genes having been described in the literature. Many are quite rare, especially in adults [14]. In the revised WHO classification of 2008, the entry for AML with *MLL* abnormalities was changed to AML with t(9;11)(p22;q23); *MLLT3–MLL*, since this is the

best characterized of the translocations. It was noted that other *MLL* translocations should be specified in the diagnosis, i.e., t(11;19)(q23;p13.3); *MLL–ENL* [1••].

The prognostic effects of MLL rearrangements are dependent upon the partner gene. While the t(11;19) and the t(6;11)(q27;q23) are associated with a poor prognosis, the t(9;11) confers an intermediate risk in adults [14]. In children, t(9;11) is considered by most to be a favorable prognostic factor, especially when it is the sole abnormality. The t(6;11)(q27;q23), the t(10;11)(p12;q23), and the t(11;19) are all associated with a poor prognosis in children [15].

For infants less than 1 year of age, *MLL* rearrangements are associated with very aggressive leukemia; the most common of the translocations in infants is the t(4;11)(q21;q23), which is seen in ~50 % of *MLL* rearrangement positive infants with ALL. Stumpel et al. [13] have recently shown that histone deacetylase inhibitors induced cell death in t(4;11)-positive cells in vitro; they also saw downregulation of specific oncogenes as well as of the MLL-AF4 fusion product. In addition, DNA methylation was restored to suppress inappropriate expression of genes.

Multiple Myeloma

With an estimated 21,700 new cases in 2012 (http://www.cancer.gov), MM is the second most common hematopoietic malignancy in the US. Monoclonal gammopathy of undetermined significance (MGUS) occurs in ~4 % of Caucasians over the age of 50; it may progress to MM [16]. Genetically speaking, MM is extremely heterogeneous, showing chromosome abnormalities, epigenetic changes, and mutations, all of which lead directly or indirectly to dysregulation of a cyclin D gene. Chromosome abnormalities seen in MM are numerical, more precisely hyperdiploidy or hypodiploidy, or structural, including translocations, deletions, and duplications. Translocations involving the immunoglobulin (Ig) heavy chain gene on chromosome 14 and a partner putative oncogene on another chromosome are seen in ~40 % of MM patients. Either the t(4;14)(p16.3;q32) or the t(11;14)(q13;q32) are seen in ~30 % of patients; ≤5 % show one of the following less common translocations: t(14;16)(q32;q23), t(6;14)(p21;q32), t(8:14)(q24;q32), or t(14;20)(q32;q11.2) [17].

The 4;14 translocation, which must be detected by FISH, places *FGFR3*, a tyrosine kinase receptor, and *MMSET*, which has histone methyltransferase activity, under constitutive control of *IgH*. Thus upregulation of both genes is seen in these patients. This translocation carries an adverse prognosis [17, 18]. The t(14;16) which upregulates *c-MAF* and the t(14;20) which upregulates *MAFB* are rare translocations which have been associated with impaired clinical outcome and short survival, although the prognostic significance of t(14;16) has been questioned. MEK inhibition has been shown in one study to result in downregulation of *MAF* in patients with t(14;16) and t(4;14) [18]. Two translocations result in upregulation of other cyclin D genes, which are cell cycle regulator genes: the 11;14 translocation (17 % of patients) moves *CCND1* under the control of *IgH*, and the 6;14 translocation (2 % of patients) results in upregulation of *CCND3*.

Data from the MRC Myeloma IX trial implied that no one genetic lesion by itself, but instead a combination of genetic lesions, defines the high risk group [18]. Other types of genetic abnormalities are also important.

Acute Lymphocytic Leukemia (ALL)

In both pediatric and adult ALL, genetic aberrations are a major determinant in clinical outcome, including risk of relapse [19]. The 2008 WHO classification lists five translocations under "B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities" [1]. The t(v;11q23); MLL rearranged was discussed above, and the rare t(5;14)(q31;q32) translocation leading to *IL3–IGH* fusion in B lymphoblastic leukemia/lymphoma, which should be considered in the differential diagnosis of peripheral blood eosinophilia, will not be discussed in detail.

B Lymphoblastic Leukemia/Lymphoma with t(12;21)(p13;q22); ETV6–RUNX1

The *ETV6–RUNX1* gene fusion produced by a reciprocal translocation between the short arm of chromosome 12 and the long arm of chromosome 21 is seen in up to 27 % of childhood ALL, usually B cell precursor ALL. The hybrid protein alters self-renewal and differentiation capacity of hematopoietic stem cells [20].

This translocation is generally considered to confer a favorable prognosis. Some studies have indicated a higher risk for later relapse in these patients, but Moorman et al. [19] failed to confirm that risk. They suggested that treatment differences could account for the lack of late relapse in their patients.

B Lymphoblastic Leukemia/Lymphoma with t(9;22)(q34q11.2); BCR–ABL1

In addition to CML, the 9;22 translocation fusing the *BCR* and *ABL* genes is observed in 25 % of adult ALL and in 3-5 % of pediatric ALL cases. Cytogenetically, the translocations in CML and ALL look the same; however, they are different at the molecular level due to the presence of

two breakpoint cluster regions within the *BCR* gene which result in two different size proteins. Whereas the fusion gene produces a 210-kDa protein in CML patients, the majority of pediatric ALL patients carry a 190-kDa protein; adult ALL patients may have either form [3]. Both proteins have constitutive tyrosine kinase activity increasing cell proliferation and inhibiting cell differentiation [2••].

The presence of a *BCR–ABL1* gene fusion is considered a poor prognostic indicator in pediatric and adult ALL patients with a high risk of relapse [21, 22]. Schultz et al. [23] showed that a combination of intensive imatinib dosing and intensive chemotherapy improved the 3-year event-free survival rate for children with minimal toxicity as compared to either imatinib or conventional chemotherapy alone. Similar results have been found in adult ALL patients [22]. However, both pediatric and adult patients can develop resistance to imatinib, as has been seen in CML patients, hence the importance of second generation TKIs [22].

B Lymphoblastic Leukemia/Lymphoma with t(1;19)(q23;p13.3); TCF3–PBX1

The t(1;19)(q23;p13.3) is seen in up to 3–5 % of childhood B cell precursor ALL. The translocation creates a fusion gene between *TCF3*, a transcription factor necessary for early lymphoid development, and *PBX1*, a homeobox gene also important in lymphoid precursor development; the activating fusion transcript is on the translocated 19, hence the presence of disease even with loss of the derivative chromosome 1. Historically, this translocation has been considered a poor prognostic indicator; however, it is associated with good prognosis with modern intensive protocols [24].

Chromosome Rearrangement in Lymphomas

Specific genetic rearrangements have proven important in influencing lymphoma prognosis and to some extent in defining or refining lymphoma classification. These rearrangements often result in altered expression of rearranged gene products and/or altered regulation of downstream pathways that affect cell proliferation and survival.

A comprehensive review of lymphoma rearrangements is beyond the scope of this article. This portion of this review will focus on lymphomas with commonly rearranged genes with potential therapeutic targets.

B Cell Lymphomas

B cell lymphomas harbor a variety of gene rearrangements. There are extensive data suggesting that the mechanisms that enable physiologic rearrangement of B cell Ig genes are the same mechanisms that predispose B cells to undergo lymphoma-associated gene rearrangements. Activation-induced cytidine deaminase (AICD) is a mutagen that initiates physiologic somatic hypermutation (SHM) and class switch recombination (CSR) of Ig genes in normal B cells, resulting in Ig diversity. AICD can also initiate pathologic translocations and mutations in Ig and non-Ig genes associated with B cell lymphoma and other malignancies [25]. Expression of AICD has been reported in Burkitt lymphoma (BL), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), mucosa-associated lymphoid tissue (MALT) lymphoma [25], and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [26]. Increased expression of AICD has been shown to confer poor prognosis in CLL/SLL and is associated with higher proliferation index, increasing cytogenetic complexity, and a higher incidence of p53 deletion compared to AICD-negative cases CLL/SLL [26]. SHM with features characteristic of AICD-mediated SHM has been identified in lymphoma-related oncogenes such as *BCL2*, *BCL6*, and *cMYC* [27]. The implications of gene rearrangements and somatic mutations and their downstream effects provide potential therapeutic targets.

FL and DLBCL and IGH–BCL2 fusion

Approximately 80 % of FL and 25 % of DLBCL have t(14;18)(q32;q21) or its variants, resulting in a fusion of the *BCL2* gene on chromosome 18 to the promoter region of *IgH* gene on chromosome 14 or *IgK* on chromosome 2 or *IgL* on chromosome 22 [28]. BCL2 inhibits apoptosis and can be overexpressed in virtually any B cell lymphoma. These fusion genes do not produce fusion proteins; it is the Ig gene enhancer that stimulates the overexpression of BCL2. Several BCL2 inhibitors including a BCL2 antisense molecule oblimersin, a BH3/BAD mimetic ABT-263, a BH3-bim mimetic obatoclax, and a BH3 only mimetic AT-101, have been shown to be of variable clinical benefit when used in combination with chemotherapy drugs and/or monoclonal antibodies in relapsed or refractory CLL/SLL, MCL, DLBCL, and FL [29•].

B Cell Lymphoma and BCL6-Associated Fusion Genes

BCL6 is a transcription repressor expressed by germinal center (GC) B cells. *BCL6* undergoes somatic mutation in normal GC B cells. The detection of *BCL6* mutation is useful in identifying lymphomas of GC or post-GC origin.

Translocations involving *BCL6* on 3q27 are common in DLBCL (30–40 %), FL (5–10 %) [30], primary mediastinal large B cell lymphoma PMBL (~33 %) [31], and

nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (48 %) [32]. The translocation partner may be an Ig or a non-Ig gene. The Ig partners include *IgH* in t(3;14), *IgK* in t(2;3), and *IgL* in t(3;22), and non-Ig partners are *RHOH* in t(3;4), histone *H1F1* in t(3;6), *OBF1* in t(3;11), and *LCP1* in t(3;13). *BCL6* expression is generally associated with GC origin and better prognosis in DLBCL; however, the level of expression does not always correlate with the presence or absence of a translocation. In a recent series of DLBCL, *BCL6* translocation was not found to correlate with prognosis [33]. In FL, *BCL6* rearrangements have been shown to be associated with a higher incidence of progression to DLBCL [34]. Small molecule BCL6 inhibitors, such as 79-6, have shown in vitro and in vivo activities against BCL6-positive DLBCL [35].

MCL and CCND1-Associated Fusions

CCND1 on chromosome 11 encodes for Cyclin D-1, which is needed for cells to progress from Gap1 (G1) to Synthesis (S) phase of the cell cycle. The vast majority of MCL have t(11;14)(q13;q32) translocation, resulting in overexpression of Cyclin D-1. Variant translocation partners include *IgK* in t(2;11) and *IgL* in t(11;22). Preclinical studies have yielded conflicting results regarding whether agents targeting cyclin D-1 may be effective [36, 37]; however, one study showed that decreasing cyclin D-1 expression in vitro via cyclin D-1 RNA interference with cycD1–siRNA (siD1) and cycD1-dicer substrate resulted in decreased proliferation and increased apoptosis in two MCL cell lines [37].

BL and cMYC-Associated Fusions

The *cMYC* gene on chromosome 8 is a transcription factor that regulates numerous target genes involved in cell cycle, metabolism, and protein synthesis. *cMYC* is invariably rearranged in BL. The most common translocation partner is *IgH* on chromosome 14, resulting from t(8;14)(q34;q32) and less commonly either *IgK* on chromosome 2 or *IgL* on chromosome 22. Simple translocations involving Ig genes and *cMYC*, with few or no additional cytogenetic abnormalities, are a hallmark of BL. *cMYC* Translocations occur in approximately 14 % of DLBCL and are associated with additional cytogenetic abnormalities and significantly worse prognosis [38].

B cell lymphomas with translocations involving the *BCL2* and/or *BCL6* in addition to *cMYC* translocation are now recognized as particularly aggressive lymphomas with a poor OS and poor response to standard chemotherapy as well as intensive chemotherapy [39]. These lymphomas are also termed "Double hit" or "Triple hit" lymphomas or as B cell lymphoma unclassifiable with morphologic features intermediate between DLBCL and BL.

A recent preclinical study showed inhibition of *MYC* transcription by bromodomain and extra terminal domain (BET) inhibitor (+)-JQ1 in a BL cell line with t(8;14) in vitro and significant antitumor activity in xenograft models of BL and acute myeloid leukemia in vivo [40].

T Cell Lymphomas

Anaplastic Large T Cell Lymphoma and ALK-Associated Fusions

T cell lymphomas have far fewer characteristic gene rearrangements than B cell lymphomas. A notable exception is anaplastic lymphoma kinase-positive anaplastic large T cell lymphoma (ALK + ALCL). ALK + ALCL have a translocation involving *ALK* on chromosome 2. In 75–80 % of cases, the translocation partner is nucleophosmin (*NPM1*) on chromosome 5 [41]. The t(2;5)(p23;q35) results in the *NPM1–ALK* fusion protein. Variant translocations found in ALK + ALCL are t(X;2)(q11;p23), t(1;2)(q25;p23), inv(2)(p23q35), t(2;3)(p23;q21), t(2;17)(p23;q23), t(2;19)(p23; p13.1), and t(2;22)(p23;q11.2), resulting in fusions of *ALK* gene to partner genes *MSN*, *TPM3*, *ATIC*, *TFG*, *CLTC*, *TPM4*, and *MYH9*, respectively.

The presence of *NPM1–ALK* fusion is associated with good prognosis. NPM–ALK activates multiple signaling pathways. Many clinical trials have shown response of non-small cell lung cancer to ALK-inhibitors (ALKi). A recent case study reported two patients with refractory ALK + ALCL who obtained complete response with the ALKi crizotinib [42]. There is ongoing recruitment of ALK + ALCL patients for clinical trails of crizotinib and other ALKi (http://www.clinicaltrials.gov).

It is important to note that many leukemia- and lymphoma-associated fusion gene transcripts have been identified in apparently healthy individuals; the expression levels of these fusion genes in healthy individuals are often indistinguishable from those in patients with minimal residual diseases (MRDs) [43•]. Therefore, when interpreting sensitive real-time PCR-based MRD tests, the baseline expression level should be considered and sequential MRD testing should be adopted.

Chromosomal Rearrangements in Solid Tumors

Cytogenetic studies of solid tumors have fallen behind those of hematopoietic malignancies. In addition to the technical challenges associated with studying solid tumor tissues, the difficulty of obtaining fresh tumor tissue for cytogenetics studies is one of the main reasons fewer such studies exist. Recent developments of new technologies, such as microarray and next generation sequencing, have

revealed a new list of recurrent and often cryptic cytogenetic rearrangements in solid tumors. As in hematological malignancies, recurrent genomic aberrations in solid tumors are often driver mutations, which can be used as biological markers for cancer diagnosis and prognosis, and are ideal therapeutic targets. Here, we review the common recurrent cytogenetic rearrangements in solid tumors, including mesenchymal tumors and carcinomas, and their prognostic and therapeutic significance.

Mesenchymal Tumors

Mesenchymal tumors are solid tumors with mesenchymal differentiation. Recurrent balanced chromosomal rearrangements are present in approximately 10 % of mesenchymal tumors [44]. These translocations give rise to chimeric fusion genes that function as aberrant transcription factors, such as fusion gene *EWSR1–FLI1* resulting from t(11;22)(q24;q12) in Ewing sarcoma, or activate growth factor tyrosine kinase, such as fusion gene *COL1A1–PDGFB* resulting from t(17;22)(q22;q13) in dermatofibrosarcoma protuberants (DFSP).

Ewing Sarcoma (EWS) and EWSR1–FLI1 Fusion

EWS is a group of sarcomas with small blue round cells that display features of both mesenchymal and neuroectodermal properties. Almost 100 % of Ewing sarcomas bear a *EWSR1* gene fusion to a member of the ETS family of transcription factors, with 85 % of them to the *FLI1* gene resulting from t(11;22)(q24;q12) [45, 46]. The translocation results in a hybrid transcript and an oncogenic chimeric protein [47]. Approximately 15 % of cases exhibit variant translocations, such as t(2;22)(q33;q12), t(7;22)(p22;q12), t(17;22)(q12;q12), and t(21;22)(q22.3;q12) with fusion of the *EWSR1* gene to *FEV*, *ETV1*, *E1AF*, or *ERG*, respectively [48•].

The identification of *EWSR1–FLI1* fusion gene has made the differential diagnosis of Ewing sarcoma from other small blue round cell tumors (SBRCT) significantly easier [49]. Commercial FISH probes are available for the detection of *EWSR1*-associated translocations (*EWSR1* Break-Apart probes; Abbott Molecular, Abbott Park, IL, USA, and CytoCell, Cambridge, UK) and the *EWSR1-FLI1* fusion gene (dual color dual fusion probe; CytoCell). Real-time PCR-based tests have also been used to detect *EWSR1–FLI1* chimeric transcripts, which offer higher sensitivity but may miss rare forms or variant chimeric transcripts [50].

The EWSR1–FLI1 fusion protein forms a transcriptional complex with RNA helicase A (RHA), and this complex has been implicated in the pathogenesis of Ewing sarcoma [51, 52]. Therefore, the EWSR1–FLI1 fusion protein is an ideal target for specifically treating Ewing sarcoma without affecting normal cells. Mithramycin, an inhibitor of the EWSR1–FLI1 oncogenic transcription factor, has shown anti-Ewing sarcoma activity both in vitro and in vivo [53]. YK-4-279, a small molecule that blocks oncogenic protein EWSR1–FLI1 interaction with RHA, induces apoptosis and tumor regression in Ewing sarcoma models [54]. It has been documented that the EWSR1–FLI1 fusion protein modulates numerous biological pathways. These modifications include activation of IGFR, PDGFR, VEGFR, and SHH pathways, and repression of Wnt and TGFβRII pathways, which lead to the malignant phenotype of Ewing sarcoma: proliferation, angiogenesis, immune system escape, metastatic potential, and treatment resistance [51]. In addition to blocking the EWSR1–FLI1 fusion protein itself, inhibition of EWSR1–FLI1 targets may be helpful in controlling the disease. Recent studies combining the IGF1R antibody cixutumumab with the mTOR inhibitor temsirolimus showed preliminary evidence of durable antitumor activity in heavily pretreated EWSR1 family tumors [55]. A randomized phase 2 trial (AEWS1221) is being planned by the Children's Oncology Group to assess the feasibility of adding AMG479, an IGF1R antibody, to the standard intensively timed 5-drug chemotherapy regimen for the treatment of EWS [56]. Other targeted therapies that are currently in clinical trials include TKIs, such as imatinib; CDK inhibitors, such as nutlin-3; antiapoptotic molecule inhibitors, such as navitoclax, a BCL2 inhibitor; and inhibition of angiogenesis, such as bevacizumab, etc. [57••].

DFSP and COL1A1–PDGFB Fusion

DFSP is a rare neoplasm of the dermis layer of skin. The disease behaves as a benign tumor in most cases, but metastasis can happen in 2–5 % of cases. More than 90 % of DFSP tumors display the recurrent t(17;22)(q22;q13) translocation or supernumerary ring chromosomes containing material from chromosomal 17q22 and 22q13 accompanied by simple chromosome trisomies [58]. The translocation fuses the growth factor *PDGFB* to the promoter of the *COL1A1* gene. The fusion gene is a transcriptionally upregulated *PDGFB* gene that constitutively activates *PDGFRB* gene, an intracellular tyrosine kinase, leading to tumor formation [59]. Targeted treatment using the tyrosine kinase inhibitor imatinib has demonstrated striking efficacy in advanced cases of DFSP [60].

Inflammatory Myofibroblastic Tumor (IMT) and ALK-Associated Rearrangements

IMT is an uncommon mesenchymal neoplasm with a small risk of aggressive behavior and metastasis. It encompasses a spectrum of myofibroblastic proliferation along with varying

amounts of inflammatory infiltrate. Approximately 50–60 % of cases present with a 2p23 rearrangement involving the gene *ALK*, including t(1;2)(q25;p23), t(2;2)(p23;q13), t(2;11)(p23;p15), t(2;17)(p23;q23), and t(2;19)(p23;p13.1) [60]. Fusion partners may be *TPM3* at 1q25, *RANBP2* at 2q13, *CARS* at 11p15, *CLTC* at 17q23, or *TPM4* at 19p13. A recent phase I clinical trial of crizotinib, a selective MET/ALK inhibitor, showed a long-term partial response in an IMT patient with *ALK–RANBP2* rearrangement but not in a patient with *ALK*-negative disease [61]. The use of ALK-directed therapy in IMT has been very limited. Multiple phase I and II clinical trials are currently open to investigate the efficacy of crizotinib and second generation ALK inhibitors in *ALK* rearrangement positive malignancies, including IMT [62].

Tenosynovial Giant-Cell Tumor (TGCT) and Pigmented Villonodular Synovitis (PVNS) and COL6A3–CSF1 Fusion

TGCT and PVNS are related diseases with features of neoplastic proliferation and inflammatory reactions. The majority of patients carry t(1;2)(p13;q37) translocation or its variants. The translocation puts the *CSF1* gene under the control of the *COL6A3* gene in tumor cells, resulting in aberrant expression of the *CSF1* gene and abnormal accumulation of CSF1R protein in nonneoplastic cells [63]. Targeted inhibition of CSF1R with imatinib has shown promising results [64].

Other Treatments Target Mesenchymal Tumor-Associated Rearrangements

Myxoid liposarcoma (MLS) is characterized by t(12;16)(q13;p11) translocation resulting in a fusion gene consisting of the 5′ part of the *FUS* gene and the complete coding region of the *DDIT3* gene [65]. The FUS–DDIT3 fusion protein functions as an abnormal transcription factor acting on a number of downstream target genes. Trabectedin, a potent alkylator that has been approved in Europe as second-line therapy for advanced soft tissue sarcomas, has shown prolonged progression-free survival (PFS) in patients with MLS [66]. In synovial sarcomas, the *SS18–SSX* fusion gene resulting from t(X;18)(p11.2;q11.2) translocation is seen in over 80 % of cases[67]. The fusion protein activates the RAS pathway through upregulating the *FGF* gene [68]. Studies have shown that disruption of the FGF signaling pathway in synovial sarcoma by specific inhibitors of FGF receptor caused cell cycle arrest, leading to significant growth inhibition both in vitro and in vivo [69].

Carcinomas

Carcinomas are cancers of epithelial origin. The significance of chromosomal rearrangements or fusion genes in carcinomas has been largely neglected until recent years. With the development of new technologies, such as microarray and next generation sequencing, new chromosome translocations, inversions, deletions, and insertions have been identified in common carcinomas, such as those of the prostate, breast, and lung. Most known carcinoma-associated fusion gene proteins are involved in signaling pathways that activate cell proliferation or homeostasis [70].

Lung Cancer and ALK-, ROS1-, and RET-Associated Fusions

Lung cancer is the cancer with the highest mortality rate in both men and women in the U.S. [http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf]. Approximately 80 % of lung cancer cases are non-small-cell lung cancers (NSCLC). Although a given chromosomal rearrangement is only seen in a small percentage of lung cancer cases, multiple recurrent gene fusions have been reported in lung cancer cell lines and tumor samples.

In 2007, Soda et al. first reported a recurrent interstitial deletion and inversion within chromosome 2p that fused the N-terminal portion of *EML4* to the intracellular region of *ALK* using a retrovirus-mediated cDNA expression system [71]. They further proved that the fusion protein had tumor transforming capabilities both in vitro and in vivo [71]. Additional studies not only confirmed the recurrent *EML4–ALK* fusion but also identified alternative fusion partners, such as *TFG* and *KIF5B* [72]. Although only present in about 5 % of lung cancer patients, this finding inspired extensive studies of ALK inhibitors. Within 3 years, these studies resulted in encouraging data: patients with NSCLC showed a 57 % overall response rate with an estimated 72 % probability of 6-month progression-free survival when treated with crizotinib, a selective MET/ALK inhibitor [73••]. These studies led to FDA approval of crizotinib (Xalkori; Pfizer) for treatment of late-stage lung cancer patients whose tumors harbor ALK-associated rearrangements. A FISH probe that detects different *ALK*-associated fusions (ALK break apart probe; Abbott Molecular) was also approved by the FDA for companion diagnosis.

The second lung cancer-associated chromosomal rearrangement involves the *ROS1* gene. The most common partner of *ROS1*-associated rearrangements is *CD74*, resulting from translocation t(5;6)(q32;q22.1). Other partners include *SLC34A2*, *SDC4*, *TPM3*, and *FIG*, and the list continues to grow [74]. *ROS1* is an orphan receptor tyrosine kinase that plays a role in epithelial cell differentiation and regionalization of the proximal epididymal epithelium. It activates several downstream signaling pathways related

to cell differentiation, proliferation, growth, and survival, including the PI3 kinase-mTOR signaling pathway. Preclinical data showed that crizotinib displayed dose-dependent inhibition of *ROS1*-translocated NSCLC cell lines [75].

Another fusion gene associated with lung cancer is the *KIF5B–RET* fusion gene resulting from a pericentric inversion of chromosome 10, inv(10)(p11.2q11.2) [76]. The fusion gene was detected by using whole-transcriptome sequencing and has been found in approximately 2 % of lung cancer [76]. The inversion fuses the 5′ *KIF5B* coiled-coil domain to the *RET* kinase domain causing aberrant activation of the *RET* oncogene. Currently, there is no inhibitor available that specifically targets oncogenic RET protein, but trials of kinase inhibitors with anti-RET activity have been conducted in thyroid cancer, leading to FDA approval of vandetanib for the treatment of adults with metastatic hereditary medullary thyroid cancers [77].

Thyroid Cancer and RET- and PAX8-Associated Fusions

Thyroid carcinoma is the most frequent endocrine cancer. Ninety-five percent of thyroid carcinomas are derived from thyroid follicular cells. Approximately 80 % of the cases are papillary thyroid carcinoma (PTC) and ~15 % are follicular thyroid carcinoma (FTC). Rare thyroid cancers include the very aggressive and almost invariably lethal anaplastic thyroid carcinoma (ATC), as well as medullary thyroid carcinoma (MTC), which is derived from parafollicular C cells. Recurrent chromosomal rearrangements associated with thyroid cancer include a variety of *RET/PTC* fusions in PTC and *PAX8–PPARG* in FTC.

Clonal *RET/PTC* rearrangements occur in about 20 % of PTC cases and are specific to this tumor [78]. There are over a dozen *RET* rearrangement partners. The most common partner is *CCD6*, forming chimeric oncogene *RET/PTC1* through a paracentric inversion inv(10)(q11.2q21), followed by *NCO4* forming chimeric oncogene *RET/PTC3* also through a paracentric inversion inv(10)(q11.2q11.2) [79]. *RET/PTC* fusions are more commonly seen in patients with radiation exposure, particularly *RET/PTC3*, and are more prevalent in children than in adults [80]. The identification of *RET/PTC* fusions in thyroid cancer is of diagnostic significance, but their prognostic role is yet to be determined.

Translocation t(2,3)(q13;p25) has been reported in about 35 % of follicular thyroid carcinomas. The translocation fuses the *PAX8* gene with transcription factor *PPARG*, resulting in the production of a *PAX8–PPARG* fusion gene protein PPFP, which appears to have dominant negative effects over PPARG. In vivo studies have predicted that PPFP agonist pioglitazone could be therapeutic in patients with PPFP-positive carcinomas [81].

Renal Cell Carcinoma (RCC) and TFE3- and ALK-Associated Fusions

RCC constitutes a group of epithelial tumors that are highly heterogeneous with respect to morphology and genetic characteristics. Clear cell RCC accounts for 70–75 % of the cases, papillary RCC 10–15 %, chromophobe RCC ~5 %, and oncocytoma ~5 %. Rare types of RCC include collect duct, multilocular cystic, tubular, mucinous and spindle cells, and Xp11.2 translocation RCC [82]. RCC-related chromosomal rearrangements include Xp11.2-associated translocations leading to different *TFE3* fusion genes and the recent discoveries of rearrangements involving 2p23 resulting in *ALK*-associated fusions.

Xp11.2 translocation RCCs comprise approximately 1 % of all primary kidney epithelial tumors and approximately 40 % of childhood RCCs. These chromosomal rearrangements lead to the expression of *TEF3* fusion genes. The most common translocation is t(X;1)(p11.2;q21), resulting in a PRCC–TFE3 fusion protein with increased transactivating activity [82]. Other rearrangements include t(X;1)(p11.2;p34) leading to *PSF/TFE3* fusion, inv(X)(p11.2q12) leading to *NONO/TFE3* fusion, der(X)t(X;17)(p11;q25) or t(X;17)(p11;q25) leading to *ASPSCR1/TFE3* fusion, and t(X;17)(p11;q23) leading to *CLCT/TFE3* fusion. The chimeric TFE fusion proteins upregulate the *MET* tyrosine kinase receptor, which in turn triggers dramatic activation of downstream signaling pathways and leads to a neoplastic cascade in normal cells. A recent publication reported a persistent complete response to sunitinib, a multitargeted tyrosine kinase inhibitor, in a child with *TFE* translocation-positive relapsed metastatic RCC [83]. A few retrospective studies of adult patients with metastatic *FTE3* fusion RCCs showed plausible response to TKIs and mTOR inhibitors [84].

In addition to *FTE3*-associated rearrangements, *ALK*-associated rearrangements in renal cancer have been recently described. Reported fusion partners include *ELM4*, *TPM3*, and *VCL* [85]. Although *ALK*-associated rearrangements only account for ~1 % of all RCCs, they are of great significance in selecting RCC patients who would benefit from ALK inhibitor therapy.

Other Recurrent Chromosomal Rearrangements Associated with Carcinomas

The majority of prostate cancers display fusion of *TMPRSS2* gene with oncogenic EST transcription factors *ERG*, *ETV1*, *ETV4*, *ETV5*, and *ELK4* [86]. *TMPRSS2–ERG* fusion is the most prevalent chromosomal rearrangement, occurring in about 50 % of prostate cancer cases in western countries, and is associated with a more aggressive phenotype [87]. In breast cancer, almost all secretory breast cancer (SBC)

Table 1 Selected FDA approved and investigational targeted agents in chromosome rearrangement-associated tumors

Disease	Fusion gene	Chromosome rearrengement	Targeted therapy
Chronic myelogenous leukemia	BCR–ABL1	t(9;22)(q34;q11.2)	Imitanib[a], dasatinib[a], nilotib[a], ponatinib[a]; bafetinib, phase I; bosutinib and imatinib, phase III
Acute promyelocytic leukemia	PML–RARA	t(15;17)(q24;q21.1)	ATRA
	PLZF–RARA	t(11;17)(q23;q21.1)	
Acute lymphoblastic leukemia	BCR–ABL1	t(9;22)(q34;q11.2	Imitanib[a], dasatinib[a], nilotib[a], ponatinib[a]; bafetinib, phase I; bosutinib and imatinib, phase III
Alveolar soft part sarcoma	TFE3–ASPSCR1	t(X;17)(p11.2;q25)	Crizotinib, phase II
Clear cell sarcoma	ATF2–EWSR1	t(2;22)(q31.3;q12)	Crizotinib, phase II; sirolimus and dasatinib, phase I
	DDIT3–EWSR1	t(12;22)(q13;q12)	
Desmoplastic small round cell tumor	WT–EWSR1	t(11;22)(p13;q12)	Imatinib, phase II
Dermatofibrosarcoma protuberans	COL1A1–PDGFB	t(17;22)(q22;q13)	Imatinib, phase II; pazopanib, phase II
Extraskeletal myxoid chondrosarcoma	NR4A3–TCF12	t(9;15)(q22;q21)	Pazopanib, phase II
	NR4A3–TAF15	t(9;17)(q22;q11)	
	NR4A3–EWSR	t(9;22)(q22;q12)	
Endometrial stromal sarcoma	PHF1–JAZF1	t(6;7)(p21;p15.2)	Dasatinib and ipilimumab, phase I
	PHF1–EPC1	t(6;10)(p21;p11)	
	JAZF1–SUZ12	t(7;17)(p15.2;q11.2)	
Ewing sarcoma and pPNET with EWSR1 translocations	FEVEWSR1	t(2;22)(q36;q12)	Cixutumumab, phase II; mithramycin, phase I/II
	ETV1–EWSR1	t(7;22)(p22;q12)	
	NR4A3–EWSR1	t(9;22)(q22;q12)	
	FLII–EWSR1	t(11;22)(q24;q12)	
	ATF1–EWSR1	t(12;22)(q13;q12)	
	ETV4–EWSR1	t(17;22)(q21;q12)	
	ERG–EWSR1	t(21;22)(q22.3;q12)	
Inflammatory myofibroblastic tumor	TPM3–ALK	t(1;2)(q21;p23)	AP26113, phase I/II; crizotinib, phase II; cixutumumab, phase I/II; cixutumumab, phase I/II
	ALK–RANBP2	t(2;2)(p23;q13)	
	ALK–CLTC	t(2;17)(p23;q23)	
	ALK–TPM4	t(2;19)(p23;p13.1)	
Liposarcoma, myxoid and round cell	DDIT3–FUS	t(12;16)(q13;p11.2)	Trabectedin, phase II; pazopanib, phase II
	DDIT3–EWSR1	t(12;22)(q13;q12)	
Rhabdomyosarcoma, alveolar	PAX7–FOXO1	t(1;13)(p36.1;q14.1)	Crizotinib, phase II
	PAX3–FOXO1	t(2;13)(q36.1;q14.1)	
Synovial sarcoma	SSX1–SS18	t(X;18)(p11.2;q11.2)	Everolimus and imatinib, phase I/II; cixutumumab, phase I/II
	SSX2–SS18	t(X;18)(p11.2;q11.2)	
	SSX4–SS18	t(X;18)(p11.2;q11.2)	
	SSX1–SS18L1	t(X;20)(p11.2;q13.3)	
Non-small-cell lung cancer	EML4–ALK	inv(2)(p21p23)	Crizotinib[a]
	KIF5B–ALK	t(2;10)(p23;p11.22)	
	TFG–ALK	t(2;3)(p23;q12.2)	
	TPM3–ROS1	t(1;6)(q21.2;q22)	mTOR inhibitors, preclinic; crizotinib, preclinic
	SDC4–ROS1	t(6;20)(q22;q12)	
	SLC34A2–ROS1	t(4;6)(q15.2;q22)	
	CD74–ROS1	t(5;6)(q32;q22)	
	EZR–ROS1	inv(6)(q22q25.3)	
	LRIG3–ROS1	t(6;12)(q22;q14.1)	
	KIF5B–RET	inv(10)(p11.22q11.21)	NA
	CCDC6–RET	inv(10)(q11.21q21.2)	

Table 1 continued

Disease	Fusion gene	Chromosome rearrengement	Targeted therapy
Salivary gland tumors	CTNNB1–PLAG1	t(3;8)(p21.3∼22;q12)	Dovitinib, phase II
Renal cell carcinoma, papillary	NONO–TFE3	inv(X)(p11q12)	Lapatinib[a], sorafenib[a], sunitinib[a], temsirolimus[a], pazopanib[a], bevacizumab and erlotinib, phase II; everolimus, phase II;
	TFE3–PRCC	t(X;1)(p11.2;q21)	
	PSF–TFE3	t(X;1)(p11.2;p34)	
	CLTC–TFE3	t(X;17)(p11.2;q23)	
	ASPL–TFE3	t(X;17)(p11.2;q25)	
Thyroid carcinoma, follicular	PAX8–PPARG	t(2;3)(q13;p25)	Pazopanib, phase II
Thyroid carcinoma, papillary	RET–CCDC6	inv(10)(q11.2q21)	Pazopanib, phase II
	RET–NCOA4	cryptic inv(10)(q11.2q11.23)	
	RET–RIA	t(10;17)(q11.2;q23)	

[a] FDA-approved

demonstrate the *ETV6–NTRK3* fusion resulting from translocation t(12;15)(p13;q25), which was originally cloned in pediatric mesenchymal cancers, congenital fibrosarcoma, and cellular mesoblastic nephroma [88]. The translocation has also been described in patients with acute myeloid leukemia. It is not surprising to see that the ETV6–NTRK3 fusion protein has potent in vivo and in vitro transforming activity in multiple cell lineages, including fibroblasts, hematopoietic cells, and epithelial cells. Recent studies showed that IGF1R/INSR inhibitors could block ETV6–NTRK3 transformation activities in vitro and significantly reduced tumor growth in vivo [89].

Summary

In this review, we have summarized common chromosomal rearrangements, the resultant fusion genes, and their clinical significance. A list of FDA-approved and investigational targeted agents in chromosome rearrangement-associated tumors are summarized in Table 1. We cannot overstate the importance of fusion genes in cancer. Not only have they served as diagnostic and/or prognostic biomarkers but they have also facilitated the understanding of tumor initiation and maintenance, as well as the development of direct and indirect targeted therapies. However, care must be taken when interpreting highly sensitive MRD tests, as these fusion transcripts may be present in healthy individuals at a level that is indistinguishable from those in patients with MRDs. From the discovery of the Philadelphia chromosome to the development of imatinib for the treatment of CML, the emergence of targeted treatments has begun to challenge the traditional clinical oncology paradigm of diagnosing and treating tumors based on their histology and anatomic locations. A new schema of personalized medicine based on cancer genomic profiles has started to surface. Nevertheless, an effective targeted treatment is yet to be developed for most of these fusion genes; secondary mutations that lead to tumor resistance to existing targeted therapies have been recognized. It is foreseeable that, with the advancement of high-throughput whole genome sequencing technologies, more and more new cancer-associated fusion genes will be discovered. The challenge of understanding the mechanisms of fusion gene formation and their roles in tumor initiation, progression, diagnosis, and treatment is only just beginning.

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