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The Role of Chromosomal Alterations in Human Cancer Development

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Abstract Cancer cells become unstable and compromised because several cancer-predisposing mutations affect genes that are responsible for maintaining the genomic instability. Several factors influence the formation of chromosomal rearrangements and consequently of fusion genes and their role in tumorigenesis. Studies over the past decades have revealed that recurring chromosome rearrangements leading to fusion genes have a biological and clinical impact not only on leukemias and lymphomas, but also on certain epithelial tumors. With the implementation of new and powerful cytogenetic and molecular techniques the identification of fusion genes in solid tumors is being facilitated. Overall, the study of chromosomal translocations have revealed several recurring themes, and reached important insights into the process of malignant transformation. However, the mechanisms behind these translocations remain unclear. A more thorough understanding of the mechanisms that cause translocations will be aided by continuing characterization of translocation breakpoints and by developing in vitro and in vivo model systems that can generate chromosome translocation. J. Cell. Biochem. 102: 320–331, 2007. © 2007 Wiley-Liss, Inc.

Key words: chromosomal rearrangements; fusion genes; fragile site; genomic instability; double strand break; gene deregulation

In 1914, Theodor Boveri proposed the somatic mutation theory of cancer, suggesting that cancer develops from a single cell that acquires a genetic alteration. At that time Boveri's hypothesis could not be proved or confirmed due to the lack of appropriate technologies. Nowell and Hungerford [1960] reported the first consistent chromosomal abnormality, a small marker chromosome (Philadelphia chromosome) associated with a single cancer type, chronic myelogenous leukemia, CML. It was not until 1973, with the use of new banding techniques [Caspersson et al., 1970], that the Philadelphia chromosome was characterized as a reciprocal translocation involving chromosomes 9 and 22 [Balmain, 2001; Rowley, 2001]. Although the Philadelphia chromosome was the first translocation discovered, the first one to actually be molecularly characterized was the t(8;14) responsible for Burkitt's lymphoma and in the early 80's the c-MYC oncogene was molecularly located at the breakpoint region of chromosome 8 [Rowley, 2001].

Cancer is a genetic disease caused by gene alterations, most commonly acquired as opposed to inherited. Furthermore, cancer cells becomes unstable and compromised because several cancer-predisposing mutations affect genes that are responsible for maintaining the integrity and the number of chromosomes during cell division [Jefford and Irminger-Finger, 2006]. Cytogenetic analysis of hematological malignancies, revealed unique chromosomal aberrations, especially balanced translocations, while in solid tumors a more complex karyotype is identified, not only between different tumor cases but also between cells from the same tumor [Mrozek et al., 1997, 2001; Mitelman et al., 2007]. The presence of unique aberrations in a karyotype indicates the regions to be studied in order to find critical genes relevant in tumorigenesis. Tumor development, however, is a result of a multistep process driven by an accumulation of genetic changes, primary and secondary rearrangements, therefore, can be identified in tumor cells. Primary abnormalities, often the only rearrangement present, are perhaps pathogeneti-

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cally significant as the initiating event. On the other hand, secondary rearrangements, mainly unbalanced rearrangements, monosomies, trisomies, deletions, and duplications, are acquired during tumor development and may have a critical role in the progression of the disease. The presence of unique aberrations and simple karyotypes make hematological malignancies the most studied neoplasia with the most genes identified as important for malignant transformations. However, recent data suggest that recurrent balanced translocations are also found in epithelial tumors, although difficult to be identified within a complex karyotype [Tomlins et al., 2005; Aplan, 2006].

CAUSES OF TRANSLOCATIONS

Cancer arises from a single precursor cell as a result of the accumulation of multiple genetic and epigenetic alterations, which can be caused by chemical and physical carcinogens, oncogenic viruses, errors in replication, or as the effect of aging. Genetic alterations and changes in DNA methylation may affect a variety of genes. The initiating event for a translocation is the formation of a DNA double-strand break (DSB), induced either by physiological situations, such as during the development of the immune system, or by exogenous DNA damaging agents. Chromosomal translocations can be a consequence of inappropriate action of a DSB repair pathway [Agarwal et al., 2006; Raghavan and Lieber, 2006; Brugmans et al., 2007].

Usually DNA breakage occurs in two major repair pathways: homologous recombination and non-homologous end-joining. In some situations these pathways can function improperly and rejoin ends erroneously produce genomic rearrangements. Homologous recombination is generally an error-free pathway of homology-directed repair. In mitotic cells, specifically in the S/G2 phase, the template for DSB repairs through homologous recombination is preferentially the sister chromatid. However, in the human genome, the presence of highly repetitive sequences can lead to the ectopic recombination, resulting in DNA rearrangements including translocations. This can undergo homology-promoted replication slippage or homology-mediated illegitimate DSB repair within and between sister chromatids, homologous, or heterologous chromosomes [Agarwal et al., 2006; Brugmans et al., 2007].

On the other hand, non-homologous end-joining is the simplest way of repairing DSBs: the straightforward relegation of ends without the requirement for a template. Non-homologous end-joining plays a major role in the removal of DSBs during G1 phases of the cell cycle since homologous recombination is not efficient in this phase due to the lack of sister chromatids. The process of breakage and rejoining in V(D)J recombination, that is utilized in the physiological process of mature antigen receptor generation in immune cells, can be misused to create aberrations. The consequence of this might be the joining of a proto-oncogene locus with the elements of the antigen receptor locus, bringing the oncogene under an active gene promoter [Agarwal et al., 2006; Brugmans et al., 2007].

Genetic Instability

Another important aspect regarding the occurrence of translocations is the stability of the genome itself. Genetic instability is a persistent state that causes several mutational events leading to gross genetic alterations. This genomic instability can be observed by the heterogeneity in karyotypes within each tumor of the same type and in different parts of the same tumor. Additionally, some tumor cells can possess dynamic instability with continuous transformation capabilities that may undergo clonal selection. Also genetic instability has been demonstrated to be a factor in tumor development. Furthermore, recent data support the possibility that genetic instability actually initiate tumorigenesis [Jefford and Irminger-Finger, 2006].

Fragile Sites

As DNA damage accumulates (point mutations, chromosomal translocations, amplifications, and deletions) the early clonal cancer cells acquire the potential for unlimited, self-sufficient growth, and for resistance to normal homeostatic regulatory mechanisms. Various genes associated with cell growth, senescence, apoptosis or with the maintenance of genomic integrity are implicated in cancer development. In particular, tumor suppressor genes are negative regulators of cell proliferation and, with the exception of leukemias and lymphomas, are frequently mutated, deleted, or hypermethylated in human cancers. In contrast, overexpression or amplification of proto-onco-

genes promotes cell proliferation. Molecular and cytogenetic evidence has demonstrated that, in addition to cancer-specific chromosome translocations, deletions of tumor suppressor genes, oncogenes amplification, and viruses integration, are frequently the consequence of DNA strand breakage at fragile sites [Bishop, 1987; Popescu, 2003].

Fragile sites are regions of the genome that are prone to form gaps or breaks after DNA synthesis is partially perturbed. Known as "the weakest link" fragile sites are indeed weak and are vulnerable targets for various oncogenic agents, and their damage may potentially result in deleterious consequences for genomic integrity and function [Arlt et al., 2006]. At least four fragile sites, involved in recurrent tumor specific translocations, have been identified: fragile site 8C (FRA8C), fragile site 3B (FRA3B), fragile site 16D (FRA16D), and fragile site 2G (FRA2G).

In details, Burkitts lymphoma is characterized by translocations involving the oncogene c-MYC locus at FRA8C to several immunoglobulin gene loci on chromosome 2, 14, 22. Although these translocations result in deregulation of MYC expression, the position of the breakpoints vary among individuals, in any case they cluster within or near the MYC gene. In addition to chromosomal translocations, both regional DNA amplification and HPV integration frequently occur at the FRA8C site [Ferber et al., 2004].

Moreover FRA3B, located on chromosome 3p14.2 is the most highly expressed in various common types of cancer, is the most "fragile" site in the genome and can be induced to break to form gaps in the majority of cells exposed to specific agents or culture conditions [Arlt et al., 2006]. The fragile site contains the locus of the fragile histidine triad gene FHIT which is abnormally expressed in various common types of epithelial cancer and particularly in lung cancer [Sozzi et al., 1996]. FRA3B is also a frequent site for balanced chromosome translocations that affect FHIT in a variety of tumors: the constitutional t(3;8) associated with hereditary renal cell carcinoma (RCC) interrupts FHIT and results in its fusion with the patched-related genes TRC8, t(3;20) identified in breast tumor cell lines, t(3;12) associated with pleomorphic adenoma of the parotid gland, t(3;16) and t(3;4) in esophageal adenocarcinoma [Keck et al., 1999; Popovici et al., 2002; Arlt et al., 2006]. As for unbalanced translocations, they usually result in the loss of genetic material and FHIT alterations have been demonstrated in hepatocellular carcinoma. Overall, translocations involving FRA3B do not necessarily form a gene fusion but directly inactivate FHIT by disruption of the gene [Popescu, 2003; Arlt et al., 2006].

Furthermore, FRA16D covers the WWOX gene, also known as FOR, involved in the recurrent translocation t(14;16) found in multiple myeloma cell lines. This specific rearrangement results in the deregulation of MAF expression, and produces the truncation of at least one allele of WWOX, which might contribute to carcinogenesis [Bednarek et al., 2000; Arlt et al., 2006].

Lastly, FRA2G was recently characterized in members of a family in which multifocal clear RCC segregates with a balanced t(2;3). The genomic map of chromosome 2 revealed the molecular breakpoint to include the gene DIRC1, expressed at low level in various tissues, however it role in RCC remains unclear [Podolski et al., 2001].

Jumping translocations and segmental jumping translocations constitute a distinct class of unbalanced translocations that involve the fusion of a donor chromosome arm or chromosome segment with multiple recipient chromosomes. The breakpoints of these types of translocations have been demonstrated to within fragile sites. In general, deletions and translocation at fragile sites appear simply to inactivate associated genes, rather than deregulate their expression or create fusion genes with altered functions. As such, there may not be any difference in selective advantage conveyed by these two types of chromosomal rearrangements [Popescu, 2003; Arlt et al., 2006]. Furthermore, fragile sites have been associated with gene amplifications, often involving the mechanism of breakage-fusion-bridge cycle, which accumulates intrachromosomal amplicons.

Although not all fragile sites may be equally important in cancer development, the cloning of additional fragile sites associated with recurrent genomic alterations will likely lead to the identification of new oncogenes, tumor suppressor genes, and chimeric genes with oncogenic potential. It also may provide key insight into the mechanism of fragile sites instability on gene function as well as into

effects of oncogenic agents on fragile sites expression.

PRODUCTS OF CHROMOSOMAL TRANSLOCATIONS

Cancer cytogenetic has quickly become a powerful way to identify and study the genes involved in the molecular breakpoints of translocations. In the past two decades, the number of reported tumor specific chromosomal rearrangement has reached 50,000 published in more than 11,500 articles. In details a total of 358 gene fusions, involving 337 different genes are known at present and have been described in all the main subtypes of human neoplasia. Of the 358 fusion genes, 267 have been identified in acute myeloid leukemia, 155 in acute lymphoblastic leukemia and only 75 in solid tumors [Mitelman et al., 2004, 2007].

Fusion Genes and Deregulation of an Oncogene

Molecularly, the genes located at the breakpoints of a chromosomal translocation may be structurally changed with dramatic effects on their products. In details two events of structural aberrations can be generated: "promoter swapping" or fusion genes. In details, as represented in Figure 1A, "promoter swapping" occurs when the regulatory elements of a gene (promoter and/or enhancer) becomes aberrantly juxtaposed to a proto-oncogene, thus driving deregulated expression of an oncogene. Molecularly, the breakpoints of the rearrangements occur upstream the coding region of the partner gene resulting in two chimeric genes which have exchanged their promoter regions, and less frequently non-coding exons. At the genomic level, the 3′ end of the partner gene B is placed downstream of the 5′ end of gene A promoter region. The chimeric transcript contains 5′ untranslated regions (5′ UTR) from the A gene and a coding region B that is intact and encodes a normal protein B [Aman, 1999]. This mechanism can be exemplified by the three translocations that characterize Burkitt's lymphoma: t(8;14), t(8;22), t(2;8). All these rearrangements lead to the activation of MYC, located on 8q24, by juxtaposing the coding sequences of the gene to one constitutively active immunoglobulin (Ig) genes promoter or regulatory regions (IgH at 14q32, IgK at 2p12, and IgL at 22q11) [Aman, 1999; Mitelman, 2000].

On the other hand, as Figure 1B illustrates, fusion genes arise when the coding regions of the two genes are juxtaposed, resulting in a chimeric transcript that produces a fusion protein with a new altered activity. In details, in the majority of cases, fusion genes are formed when DNA breaks occur within two different genes mainly within the introns, A and B, and the gene fragments are joined in erroneous combinations. In most cases, the results are two fusion genes: A–B and B–A. On genomic level, the 3′ partner gene B is placed under the 5′ gene A promoter control region which dominates the transcription control of the fusion gene. As a result, in the fusion protein the functional domains from the A and B proteins are brought together in a new abnormal combination. In cancer, the genes that are often interrupted by a chromosomal rearrangement are oncogenes, thus generating fusion oncogenes [Aman, 1999, 2005; Mitelman, 2000].

Formation of Fusion Oncogenes

As mentioned above, several factors influence the formation of chromosomal rearrangements and consequently of fusion oncogenes and their role in tumorigenesis. Firstly, the rates at which fusion genes are formed are important. Literature suggests that at least some fusion genes are found in healthy individuals, implying that at least some gene fusions emerge at notable rate. The mechanisms behind fusions are unclear but the occurrence of several DSBs that coincide in time and space are important. The proximity of damaged partner genes at the moments of repair is critical and the localization of chromatin and genomic regions in the interphase nuclei may be critical. Secondly, the presence of a fusion gene in a cell is not enough to cause cancer. Additional genetic or epigenetic changes are also needed and the risk for these additional events to occur affects the outcome. Thirdly, once the fusion is formed, its penetrance, that is the proportion of fusion carriers that develop tumors, is determined by selected mechanisms [Aman, 1999, 2005]. Interestingly, many fusion oncogenes demonstrate a strict specificity for tumor type. The risk of getting a certain translocation could depend on cell type specific processes that make the specific genes or DNA regions involved vulnerable to the translocation. It is clear that tumor development in different cell types

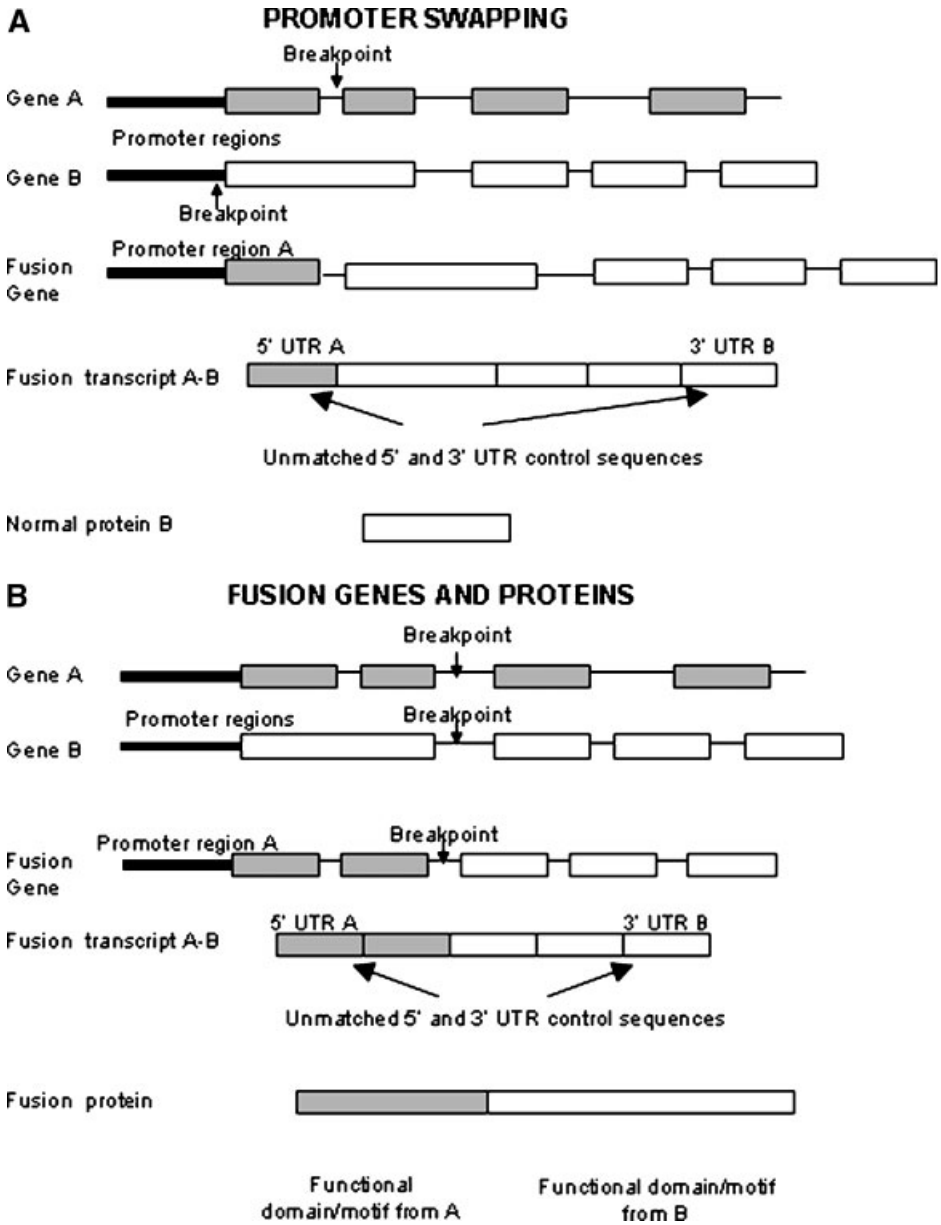

Fig. 1. A schematic representation of rearrangements of genes in tumors: (**A**) promoter swapping and (**B**) fusing genes.

and tissues locations involves many pathways, distinct genes and also exogenous factors. A common mechanism for early genetic changes can however be distinguished in a number of different tumor types by specific chromosome rearrangements [Aman, 1999, 2005].

Moreover, the transcriptional orientation of fusion partner genes is essential in order to harbor functional fusion genes. At times, the partner genes are not oriented in the correct direction with regards to their transcriptional orientation, and more complex rearrangements are needed to fuse the partner genes into functional fusion genes. For instance, the EWS-ERG fusion is found in about 10% of Ewing sarcomas and it is the result of a complex rearrangement, a translocation and an inversion, given that the genes involved are not transcribed in the same centromeric/telomeric direction [Aman, 1999, 2005; Xia and Barr, 2005]. This requirement and the necessary presence of critical functional protein parts seem to influence how frequently variant fusion genes are present in tumors. Moreover, to

produce a functional fusion gene, it is necessary that the exons flanking the breakpoints give rise to splicing events that maintain their reading frames. Overall, the factors that generate double strand breaks are largely unknown.

CLINICAL RELEVANCE OF TRANSLOCATIONS

Studies over the past decades have revealed that recurring chromosome rearrangements leading to fusion oncogenes are specific features not only of leukemias and lymphomas, but also of certain epithelial tumors. Presently, over 600 recurrent balanced tumor-associated chromosomal rearrangements have been molecularly characterized. However, the data is strongly biased in favor of hematologic malignancies and sarcomas [Mitelman et al., 2004, 2007].

Hematological Malignancies

To date, 264 genes fusions, involving 238 different genes, have been identified in hematological disorders including malignant lymphomas. These represent the 75% of all gene fusions presently known in human neaoplasia [Mitelman et al., 2004, 2007]. An appropriate example of a fusion oncogene is BRC/ABL characterizing chronic myelogenous leukemia which is driven by t(9;22)(q34;q11). In particular, the translocation fuses the ABL gene normally located on 9q34, with the BCR gene at 22q11. The BCR/ABL fusion created on the derivative chromosome 22 encodes a chimeric protein with an increased tyrosine kinase activity and abnormal localization. An important example of a recurrent rearrangement which leads to the development of a targeted therapy is the t(15;17)(q22;q21) in acute promyelocytic leukemia which fuses the PML gene (15q22) with RARα gene at 17q21. The PML protein contains a zinc-binding domain called a "ring" finger that may be involved in protein–protein interaction. RARα encodes the retinoic acid alpha-receptor protein, a member of the nuclear steroid/thyroid hormone receptor superfamily. Although retinoic acid binding is retained in the fusion protein, the PML/RARα may confer altered DNA-binding specificity to the RARα ligand complex [Nervi et al., 1992]. Leukemia patients with the PML/RARα gene fusion have an excellent response to the all-*trans* retinoic acid treatment, which stimulates the differentiation of promyelocytic leukemia cells. Similarly, the molecular characterization of the t(9;22)(q34;q11) in chronic myelogenous leukemia, which generates the fusion oncoprotein BCR/ABL, leads to the development of a successful targeted treatment with imatinib. In details, imatinib mesylate (STI-571, Gleevec; Norvartis) is a small molecule adenosine triphosphate analog, which selectively inhibits PDGFB, ABL, and KIT kinases and is effective in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors (GIST), which have aberrantly activated ABL and KIT kinases, respectively [Rubin et al., 2002; Mizutani et al., 2004]. In Table I, a list of recurrent balanced translocations described in hematological malignancies can be found.

Solid Tumors

The cytogenetic and molecular study of solid tumors and the identification of the complex rearrangements has been improved by the implementation of advanced molecular cytogenetic techniques such as SKY (Spectral Karyotyping Imaging, ASI, Israel) and conventional or array Comparative Geneome Hybridization (CGH) [Speicher and Carter, 2005]. These molecular techniques rely on a genomic approach so that with a single experiment the genome can be studied for translocations, or deletions and amplifications. The fact that so many gene fusions have been found in hematological malignancies has led to the hypothesis that, in contrast to solid tumors, they are caused by such genes fusions. All solid tumors, benign and malignant, make up the 27% of the total number of cases with an abnormal karyotype reported in literature [Mitelman et al., 2004, 2007]. However, recent discoveries make us believe that also certain solid tumors are driven by fusion genes but that the major dilemma is the difficulty in molecularly identifying them. Cytogenetically, the chromosome morphology is often poor, which renders difficult and problematic their karyotype's characterization. Additionally, the karyotypes are usually so complex that even when the quality is satisfactory, it is impossible to identify and study all complex rearrangements solely with conventional cytogenetics (Fig. 2). Also, the 80% of carcinomas present cytogenetically unrelated clones within the same karyotype thus making it difficult to identify the critical recurrent balanced translocations and, therefore, gene fusions. So far,

TABLE I. Recurrent Balanced Rearrangements in Hematological Malignancies

Hemapoietic tumors		
Lymphoid		
Disease	Affected gene	Rearrangement
Anaplastic large cell lymphoma	NPM-ALK	t(2;5)(q23;q35)
	TPM3-ALK	t(1;2)(q25;p23)
	TFG-ALK	t(2;3)(p23;q21)
	ATIC-ALK	inv(2)(p23q35)
	MSN-ALK	t(X;2)(q11–12;p23)
	CLTCL-ALK	t(2;17)(p23;q23)
Burkitt's lymphoma, B-cell ALL	MYC (relocation of IgH locus)	t(8;14)(q24;q32)
	MYC (relocation of IgK locus)	t(2;8)(p12;q24)
	MYC (relocation of IgL locus)	t(8;22)(q24;q11)
B-cell precursor acute lymphoid leukemia	E2A-PBX1	t(1;19)(q23;p13)
	E2A-HLF	t(17;19)(q22;p13)
	TEL-AML1	t(12;21)(p12;q22)
	BCR-ABL	t(9;22)(q34;q11.2)
	MLL-AF4	t(4;11)(q21;q23)
	IL3-IgH	t(5;14)(q31;q32)
Diffuse large B-cells lymphoma	BCL2-IgH	t(14;18)(q32;q21)
	BCL6-variant partners	t(3;v)(q27;v)
	BCL8-IgH	t(14;15)(q32;q11–13)
	FCGR2-Igλ	t(1;22)(q22;q11)
	MUC1-IgH	t(1;14)(q21;q32)
	NFKB2-IgH	t(10;14)(q24;q32)
Extranodal mucosa-associated lymphoid tissue	MALT1-API2	t(11;18)(q21;q21)
	MALT1-IgH	t(14;18)(q32;q21)
	BCL10-IgH	t(1;14)(p22;q32)
	BCL10-Igκ	t(1;2)(p22;p12)
Plasma cells myeloma	FGFR3-IgH and MMSET	t(4;14)(p16;q32)
	MAF-IgH	t(14;16)(q32;q23)
	MAF-Igλ	t(16;22)(q23;q11)
	CCND1-IgH	t(11;14)(q13;q32)
	MUM/IRF4-IgH	t(6;14)(p25;q32)
Pre-T cell lymphoblastic leukemia, lymphoma	MYC (Relocation to TCR α/δ locus)	t(8;14)(q24;q11)
	LYL1 (Relocation to TCRB locus)	t(7;19)(q35;p13)
	TAL2 (Relocation TCRβ locus)	
	SCL (Relocation to TCR α/δ locus)	t(1;14)(p32;q11)
	OLIG2 (Relocation to TCR α/δ)	t(14;21)(q11;q22)
	LMO1(RBTN1) (Relocation to TCR α/δ)	t(11;14)(p15;q11)
	LMO2 (RBTN2) (Relocation to TCR α/δ)	t(11;14)(p13;q11)
	HOX11 (Relocation to TCR α/δ)	t(10;14)(q24;q11)
	HOX1–1L2	t(5;14)(q35;q32)
	CALM-AF10	t(10;11)(p13;q21)
	NUP98-RAP1GDS1	t(4;11)(q21;p15)
Myeloid		
Acute promyelocytic leukemia	PML-RARα	t(15;17)(q21;q21)
	NPM-RARα	t(5;17)(q35;q21)
	PLZF-RARα	t(11;17)(q23;q21)
Acute myeloid leukemia or CMML	ETV6-variant partners	t(12;v)(p13;v)
Acute myeloid leukemia	NUP98-variant partners	t(11;v)(p13;v)
	MLL-variant partners	t(11;v)(q23;v)
	AML1-ETO	t(8;21)(q22;q22)
	CBFB-MYH11	inv(16)(p13q22)
	FUS-ERG	t(16;21)(p11;q22)
	CEV14-PDGFRB	t(5;14)(q33;q32)
	P300-MOZ	t(8;22)(q33;q32)
	MOZ-TIF2	inv(8)(p11q13)
	MOZ-CBP	
	DEK-NUP214	t(6;9)(p23;q34)
	RBM15-MKL	t(1;22)(p13;q13)
	MLF1-NPM1	t(3;5)(q25;q34)
	AML1-EVI1	t(3;21)(q26;q22)

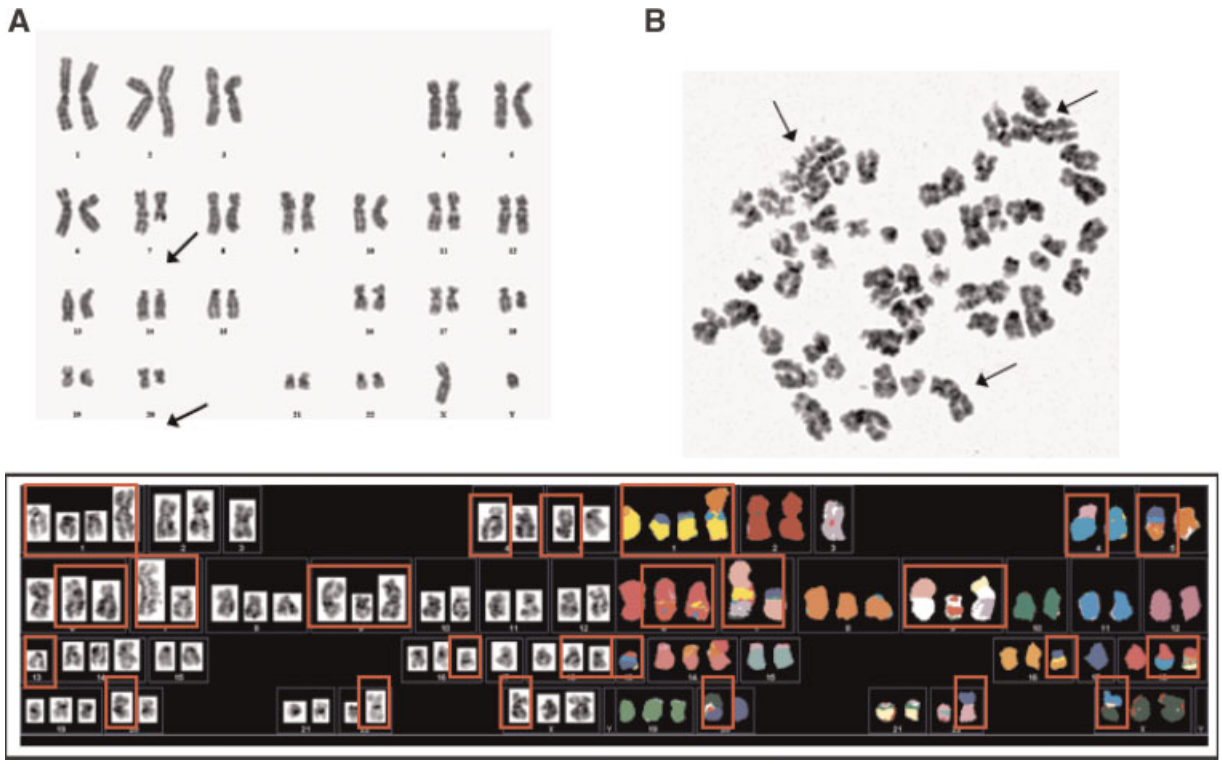

Fig. 2. **A**: A G-banded karyotype of an myelodysplastic syndrome case presenting a simple karyotype with only two chromosomal changes: a del(7q) and del(20q). **B**: A G-banded metaphase of a complex soft tissue sarcoma case. A G-banded karyotype was not sufficient to characterize the complex chromosomal rearrangements (enclosed in the red box). Usin SKY the complex rearrangments were identified.

only 70 gene fusions involving 83 different genes have been identify in malignant solid tumors [Mitelman et al., 2004, 2007]. Recurrent balanced rearrangements identified in solid tumors are enlisted in Table II.

Sarcomas

The most studied malignant solid tumors, are bone and soft tissue sarcomas, a clinically and morphologically heterogenous group of neoplasms of mesenchymal or neuroectodermal origin carrying a relatively simple karyotype. Forty-one known fusion genes are reported in 17 different sarcoma types. The data obtained from sarcomas, indicate that similarly to hematological malignancies, these tumors are driven by fusion genes [Mitelman et al., 2004, 2007].

For instance, dermatofibrosarcoma pertuberans (DFSP) is a fibrohistiocytic tumor of intermediate malignancy. Locally, it is highly invasive and aggressive, though it metastasizes rarely. Local surgical excision is the main therapy; however, it results in a high rate of local recurrence. Cytogentic studies of DFSP have constantly revealed the balanced rearrangement t(17;22)(q22;q13) or a ring r(17;22), interrupting and fusing the genes COL1A1 on chromosome 17q22 and PDGFB on 22q13. The fusion produces the activation of PDGFB receptor tyrosine kinase pathways and contributed to DFSP generation and proliferation [Rubin et al., 2002; Mizutani et al., 2004].

The imatinib treatment was shown, as well as in CML, to be effective in the treatment of DFSP proliferation and to induce apoptosis. This demonstrated for the first time that inhibitions of the PDGFB receptor with imatinib mesylate can lead to important anti-tumor effects in at least one type of human solid tumor. In conclusion, given that the PDGFB pathway is well known and several chemical compounds blocking the PDGFB signaling are available, it is therefore reasonable to expect that in the near future therapies specific for DP could be designed [Greco et al., 1998; Rubin et al., 2002].

The fact that most cytogenetically balanced aberrations that have been characterized at the molecular level lead to gene fusions does not mean that all such gene rearrangements are

TABLE II. Recurrent Balanced Rearrangements in Solid Tumors

Disease	Affected gene	Rearrangement
Solid Tumors		
Sarcomas		
Alveolar Rhabdomyosarcoma	PAX3-FKHR	t(2;13)(q3?;q14)
	PAX7-FKHR	t(1;13)(q36;q14)
Alveolar soft-part sarcoma	TFE3-ASPL	t(X;17)(p11;q25)
Angiomatoid fibrous histiocytoma	FUS-ATF1	t(12;16)(q13;p11)
Dermatofibrosarcoma protubeans	COL1A1-PDGFB	t(17;22)(q13;q13)
Desmoplastic small round cell tumor	EWS-WT1	t(11;22)(p13;q12)
Endometrial stromal sarcoma	JAZF1-JJAZ1	t(7;17)(p15;q21)
Ewing's sarcoma	EWS-FLI	t(11;22)(q24;q12)
	EWS-ERG	t(21;22)(q22;q12)
	EWS-ETV1	t(7;22)(q22;q12)
	EWS-E1AF	t(2;22)(q33;q12)
	EWS-FEV	t(17;22)(q12;q12)
	FUS-ERG	t(16;21)(p11;q22)
Infantile fibrosarcoma	ETV-NTRK3	t(12;15)(p13;q25)
Inflammatory myofibroblastic tumour	TPM3-ALK	t(1;2)(q22;p23)
	TPM4-ALK	t(2;19)(p23;p13)
	CLTC-ALK	t(2;17)(p23;q23)
Low grade fibromyxoid sarcoma	FUS-CREB312	t(7;16)(q33;p11)
Myxoid chondrosarcoma	EWS-CHN	t(9;22)(q22;q12)
	TAF2N-CHN	t(9;17)(q22;q11)
	TCF12-CHN	t(9;15)(q22;q21)
Myxoid liposarcoma	FUS-CHOP	t(12;16)(q13;p11)
	EWS-CHOP	t(12;22)(q13;q12)
Synovial sarcoma	SYT-SSX1	t(X;18)(p11;q11)
	SYT-SSX2	
	SYT-SSX4	
Soft-tissue clear cell sarcoma	EWS-ATF1	t(12;22)(q13;q13)
Carcinomas		
Follicolar thyroid carcinoma	PAX8-PPARγ	t(2;3)(q13;p25)
Papillary thyroid carcinoma	H4-RET (PTC1)	inv(10)(q11.2;q21)
	RIa-RET (PTC2)	t(10;17)(q11.2;q23)
	ELE1-RET (PTC3,4)	inv(10)(q11q22)
	RFG5-RET (PTC5)	
	TPM3-NTRK1 (TRK)	inv(1)(q21q22)
	TPR-NTRK1 (TRK-T1)	inv(1)(q21q25)
	TFG-NTRK1 (TRK-T3)	t(1;3)(q21;q11)
Prostate cancer	TMPRSS2-ERG	inv(21)(q22.2;q22.3)
	TMPRSS2-ETV1	t(7;21)(p21.2;q22.3)
	TMPRSS2-ETV4	t(17;21)(q21;q22.3)
Renal-cell carcinoma	PRCC-TFE3	t(X;1)(p11;q21)
	ASPSCR1-TFE3	t(X;17)(p11;q25)
	SFPQ-TFE3	t(X;1)(p11;p34)
	NONO-TFE3	inv(X)(p11;q12)
Salivary gland tumors (malignant)	CTNNB1- PLAG1	t(3;8)(p21;q12)
	TORC1-MAML2	t(11;19)(q21;p13)
Secretory breast carcinoma	ETV6-NTKR3	t(12;15)(p13;q25)

exclusively formed by balanced chromosome abnormalities. There are several gene fusions that are typically detected in the context of an unbalanced cytogenetic rearrangement, such as COL1A1–PDGFB in DFSP with supernumerary ring chromosomes, alveolar part sarcoma chromosome region candidate 1 (ASPSCR1)-TFE3 in an unbalanced der(17) t(X;17)(p11;q25), or NUP2-ABL1 occurring on amplified episomes (submicroscopic extrachromosomal circular DNA structure) in T-cell ALL [Rubin et al., 2002].

Epithelial Tumors

Our knowledge regarding fusion genes in solid tumors, yet constitutes the 10% of known recurrent balanced chromosome rearrangement, is very limited. However, evidence reveals that fusion oncogenes may be more common in epithelial tumors than previously thought. Thyroid papillary carcinoma was the first epithelial tumor type in which a gene fusion between RET and CCDC6 was identified. To date another 14 fusion genes have been reported, 9 of which involve the RET gene, in thyroid carcinomas [Mitelman et al., 2007].

Usually, translocations in solid tumors result in gene fusions that encode chimeric oncoproteins. The first chromosome abnormalities to be molecularly characterized in solid tumors were an inv(10)(q11.2;q21.2), as the more frequent alteration, and a t(10;17)(q11.2;q23), in papillary thyroid carcinoma [Pierotti et al., 1992]. These two abnormalities represent the cytogenetic mechanism which activate the proto-oncogene RET on chromosome 10, by generating the fusion genes forming the oncogene RET/PTC1 and RET/PTC2, respectively [Sozzi et al., 1994; Bongarzone et al., 1994]. Moreover, other chromosomal rearrangements leading to RET activation were recently described and listed in Table II, along with the other recurrent balanced translocations characterized in epithelial tumors. A great impact in the study of solid tumors is foreseen by the recent identification of a large subset of prostate cancers harboring the fusion genes TMPRSS2/ERG, TMPRSS2/ETV1, and TMPRSS2/ETV4, generated by inv(21) (q22.2;q22.3), t(7;21)(p21.2;q22.3) and t(17;21)(q21;q22.3), respectively. These fusion genes were identified with the use of newly advanced technical bioinformatics approaches have been used to study genes with a very high expression in microarray analysis [Tomlins et al., 2005]. In particular, the gene fusion of the 5′ UTR of TMPRSS2 (a prostate-specific gene) to ERG or ETV1 (genes of the ETS family), was identified in the majority of prostate cancers. Although the clinical significance of those fusions is unknown, recent investigations indicate that the expression of TMPRSS2/ERG among prostate cancer patients is a strong prognostic factor for disease progression [Tomlins et al., 2005].

Reciprocal Products of Translocations

Although a considerable number of cancers are associated with reciprocal chromosomal translocations, the detection of the molecular products encoded by both derivatives is not frequent. However, the reciprocal product is more often expressed in hematological malignancies, in particular CML and APL. On the other hand, among solid tumors the translocation t(X;17) represent an ideal example of the importance of the reciprocal products. For instance, an unbalanced translocation is formed with the gene sASPL and TEF3 and it is associated with alveolar soft part sarcoma. On the contrary, if a balanced translocation is formed involving the same loci, leading to the expression of both genes, it is associated to a morphologically distinct subset of papillary renal adenocarcinoma. Although the details of the molecular role are still to be elucidated, it is becoming more apparent that, when expressed, these molecules often play an important role in tumorigenesis and/or modulating response to therapy [Rego and Pandolfi, 2002].

Chromosomal translocations are typically reciprocal, therefore leading to the generation of two chimeric genes. Usually only one of the products is considered to be essential to the oncogenic process based on the fact that, (1) it is detected in the majority of the patients, in contrast with the reciprocal product; (2) it retains most of the functional domains of the parental proteins; or (3) rare complex chromosomal translocations have been identified in which one of the reciprocal products is not formed, nevertheless the tumor/leukemia exhibit the same phenotype. The third point could be misleading since additional genetic events might functionally complement the missing oncogenic activity normally contributed by the reciprocal fusion protein [Rego and Pandolfi, 2002]. In many chromosomal translocations, one of the two fusion transcripts is never detected thus suggesting that the expression of the reciprocal product is probably not required for tumorigenesis in many cases. Experimental evidence obtained in tumorigenic mouse model of APL suggests that reciprocal product might potentiate and/or modify the leukaemogenic activity of the APL-associated fusion proteins PML-RARa and PLZF-RARa. Also in sarcomas, the reciprocal product seems to play an important role in determin-

ing the occurrence of tumors with distinct histopathological features [Rego and Pandolfi, 2002].

CONCLUSIONS

Overall, cytogenetic aberrations have been reported in over 45,000 human neoplasms. The study of chromosomal translocations have revealed several recurring themes, and reached important insights into the process of malignant transformation. The mechanisms behind these translocations, however, remain unclear. A more thorough understanding of the mechanisms that cause translocations will be aided by continuing characterization of translocation breakpoints and by developing in vitro and in vivo model systems that can generate chromosome translocation [Aplan, 2006].

To date, all balanced rearrangements that have been characterized molecularly act by deregulating a gene in one of the breakpoint or by creating a fusion gene. Published data are strongly biased in favor of the hematological malignancies, which constituted 74% of the cases, all solid tumors made up only the 26% [Mitelman et al., 2007]. Because most recurrent aberrations and rearranged genes have been found in hematological disorders, whereas numerous genomic imbalances have been identified in solid tumors, it has become generally accepted that there are pathogenetic differences between these neoplasms. Overwhelming evidence supports the hypothesis that the neoplastic phenotype is caused by an accumulation of a number of genetic and epigenetic alterations. It is believed that deletion of tumor suppressor genes is increasingly regarded as a preferred initiating event in epithelial tumorigenesis.

In conclusion, epithelial tumors may be characterized by several, but individually rare, pathogenetically important gene rearrangements that have not been identified. Some of these may be even be cryptic and submicroscopic. As epithelial tumors denote the dominant cause of cancer morbidity and mortality, the implications are trivial since the development of target treatment for the product of pathogenetic gene fusions in malignant tumors is making great and critical progress [Mitelman et al., 2004]. Lastly, it may provide the basis of a system for the detection, prediction, and determination of the prognosis of cancer as well as for identifying new targets for cancer therapy. The detection of the intracellular targets of these fusions will harbor new and important insights into molecular pathways that underlie tumor development. Ultimately, a combination of these approaches with conventional treatments may provide a powerful new approach to treat these fusion-positive tumors.

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