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Advances in Cancer Cytogenetics

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Abstract As the end of the millenium approaches, recognition of the milestones achieved in the field of cancer cytogenetics is mandatory. With regard to cancer cytogenetics, the turning century can be divided in three main era: the pre-banding period that has posed important hypothesis and technical premises, the fruitful banding era that led to the discovery of the critical chromosomal rearrangements and cloning of cancer genes and the more recent revolutionizing era of molecular cytogenetics where technological advances permit a global visualization and high-level resolution of chromosomal alterations. J. Cell. Biochem. Suppls. 32/33:173–182, 1999.

Key words: cytogenetics; cancer; genes

In this article, we recall the relevant steps of cancer cytogenetics by outlining the most critical examples in hematologic and solid neoplasia, in which chromosomal rearrangements led to the identification of cancer genes, to determine the mechanisms responsible for their alteration, as well as the functional understanding of the genes involved in these rearrangements. The clinical applications of cytogenetics for diagnostic and prognostic assessment of cancer disease, and for the development of innovative targeted cancer therapy, are also discussed. Finally, we present the more recent technological improvements, such as fluorescence in situ hybridization (FISH) and spectral karyotyping, which promise "long life" to cancer cytogenetics.

HISTORICAL ASPECTS

The suggestion of a significant relationship between chromosomal alterations and pathogenesis of human cancer came in the late nineteenth century from Theodor Boveri [1929], who hypothesized the importance of somatic genetic changes in tumor development. He suggested that mammalian tumors might be associated with aneuploid complement and introduced the concept of genetic instability and of a possible monoclonal nature of tumors. However, all these observations remained unproved until the mid-1950s, when the procedures of cell cultures and metaphase harvesting, by the so-called "squash" technique of the time, permitted the establishment of the number of human chromosomes as 46 and the beginning of the "modern" era of cytogenetics. The first relevant finding was the identification of a characteristic tiny chromosome in patients with chronic myeloid leukemia (CML) [Nowell and Hungerford, 1960], which, in accordance with the suggestions of the First International Conference on Cytogenetics in 1960, was named Philadelphia chromosome (Ph). Scientists then had to wait a decade until the development of chromosome banding techniques demonstrated that this and other clonal chromosomal changes observed in leukemia were not epiphenomena. The breakthrough of banding proved the nonrandom nature of these changes and led to the discovery that the Ph chromosome was indeed the result of a translocation between chromosomes 9 and 22 [Rowley, 1973]. In 1977 the translocation t(15:17) was detected as specifically associated with acute promyelocytic leukemia (APL), suggesting that specific chromosomal changes are an essential component of hematologic malignancies [Rowley et al., 1977]. Other recurring translocations were since identified in leukemia and also in lymphoma and sarcoma. Meantime, the discovery in 1976 of a constitutional chromosomal deletion of the long arm of chromosome 13 (13q14) in retinoblastoma patients posed the basis for the concept of recessive traits in onco-

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genesis and for cloning the first tumor suppressor gene: Rb1 [Friend et al., 1986].

A revolutionizing event in cancer genetics occurred in 1982, when the breakpoint of the translocation t(8;14), previously identified by cytogenetics, was first cloned and the genetic consequences of the translocation elucidated [Dalla Favera et al., 1982]. The concept that human protooncogenes become activated by chromosomal rearrangement that lead either to deregulated expression or to structural modifications through fusions with other chromosomal sequences, as showed later in 1984 in CML, became a dogma of cancer genetics and guaranteed "immortality" to cancer cytogenetics.

CHROMOSOMAL ALTERATIONS CAUSE SPECIFIC GENETIC CHANGES

Epidemiological, clinical, and experimental evidences suggest that cancer is a genetic disease characterized by the accumulation of somatic genetic lesions. These alterations—whether initiation- or progression-associated events—may be mediated by chromosomal translocations. As a corollary to this idea, the molecular characterization of chromosomal rearrangements could lead to the identification of genes relevant to tumorigenesis.

Three main structural chromosomal alterations are known: deletions, translocations, and inversions. Specific translocations and inversions have been discovered in different tumor types of solid and hematopoietic tumors and the molecular analysis of cancer breakpoints led to the demonstration of two possible mechanism of genetic perturbation. The first is through juxtaposition of a protooncogene with either immunoglobulin or T-cell receptor genes, which causes deregulated expression of the prooncogene without structural modifications. The second is through the formation of a fusion gene by juxtaposing sequences located on different chromosome or chromosomal bands. As anticipated, the first two chromosomal translocations characterized at a molecular level have been described in tumors of the hematologic compartment (i.e., Burkitt's lymphoma and CML) and exemplify these two models. Subsequently, it has been shown that fusion genes, whose function is often related to transcriptional control, are the most represented genetic rearrangement caused by the chromosomal translocations and inversions reported in solid tumors which constitute about 80% of all human neoplasia. These findings suggest that, overall, altered transcription is a common consequence of chromosomal translocations.

DEREGULATION OF PROTOONCOGENES IN LYMPHOMA

Burkitt's lymphoma is a B-cell tumor characterized by the presence of three chromosomal translocations. The most frequent, the t(8;14)(q24;q32), was discovered in 1976 by Lore Zech. It occurs in about 75–85% of cases. At a molecular level, it was shown in 1982 that this rearrangement juxtaposes the MYC protooncogene at 8q24, with the immunoglobulin heavy chain gene at 14q32 [Dalla Favera et al., 1982]. In 15–25% of cases, variant translocations t(8;22)(q24;q11) or t(2;8)(p12;q24) can occur. In these cases, κ- and λ-chain immunoglobulin genes located at the breakpoints become juxtaposed to MYC on chromosome 8 [Croce et al., 1983]. Deregulated and inappropriate expression of MYC are the principal consequences of these rearrangements [Rabbitts, 1994].

Translocations of 14q32 are the single most frequent common change in lymphoid neoplasia; of these, the t(14;18)(q32;q21) is the most common in lymphoma, and in particular in the follicular type. The bcl2 gene at the breakpoint at 18q21 was cloned in 1984 [Tsujimoto et al., 1984], and its overexpression as an effect of the translocation with the IGH gene at 14q32 soon became evident. This was a crucial example in which the analysis of genes involved in translocation opened up a new era of mammalian cell biology. In fact, bcl2 represents a novel category of genes with specialized activity in the control of cell lifespan, preventing programmed cell death (apoptosis) [Hockenbery et al., 1991]. Bcl2 does not act alone in apoptosis: it is the founding member of a family of related proteins that act in concert to control cell death. It appears that by binding with factors that promote cell death, such as bax, regulatory influence on cell death decision can occur by means of modulation of the ratio bcl2/bax within the cell [Oltvai et al., 1993]. A critical role for bcl2 in tumorigenesis is not restricted to lymphomas, in which it was first described, but extends to a broad majority of human tumors as recently reported in synovial sarcoma, in which the large majority of cases show a constitutive expression of this gene product [Pilotti et al., 1998].

FUSION GENES IN HEMATOLOGICAL NEPLASIA

The first documented example of the formation of fusion genes encoding chimeric proteins after a chromosomal translocation, was the BCR-ABL on chromosome 22. In fact, the standard t(9;22)(q34;q11) [Rowley, 1973], and the complex or variant translocations involving three or more chromosomes, including 9 and 22, move the ABL protooncogene on chromosome 9 next to a gene on chromosome 22 known as BCR [Le Beau, 1997]. The resulting chimeric protein is a tyrosine kinase located in the inner face of cell membrane, which transmits growth regulatory signals from cell surface receptors to the nucleus through the RAS signal transduction pathway. Very recent findings in CML illustrate how the functional understanding of the genes involved in translocations, by providing specific downstream targets, has been fruitful. In fact, pharmacological molecules that have inhibitory activity on tyrosine phosphorilation proved effective in the reversion of in vitro and in vivo tumorigenicity of CML cancer cells [le Coutre et al., 1999]. This is clear-cut evidence linking chromosomal translocation to the development of a cancer-specific therapy. Similar models have also been described in a solid soft tissue tumor, the dermatofibrosarcoma protuberans, as discussed later.

The other critical example of fusion genes created by translocation is that provided by the t(15;17)9q22;q11–12), which is unique to acute promyelocytic leukemias (APL). The frequency of this aberration is close to 100% of APL patients. In rare cases, variant translocations, including a t(11;17) and t(5;17), can occur. The affected genes are the retinoic acid receptor (RARa) gene on chromosome 17 and PML on chromosome 15, PLZF on chromosome 11, and NPM on chromosome 5. These rearrangements produce chimeric transcription factors with novel properties of transcription regulation that produce important effects in the phenotype of affected cells. The fusion gene in the prevalent t(15;17) is formed on the derivative chromosome 15. It consists of the 5′ end of PML and of the 3′ of RARa, including DNA binding and retinoic acid response domains [de The et al., 1990]. The resulting protein retains the capability to dimerize and to bind DNA, as well as to activate retinoic acid responsive gene transcriptions upon exposure to all trans-retinoic acid. It is likely that the PML/RARa chimera inappropriately represses or activates target sequences that affect myeloid cell maturation and block differentiation [Grignani et al., 1993]. Molecular characterization of the chromosomal rearrangements in APL has prompted novel therapeutic approaches, since pharmacologic dosages of retinoic acid make it possible to overcome this differentiation block and induce clinical remission of the disease in APL patients.

Whereas most chromosomal abnormalities are specific for a given hematologic disorder, as promiscuous role of alterations of 11q23 chromosomal region is observed in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) with a prevalence in infant cases. Patients with abnormalities in 11q23 generally exhibit aggressive clinical features and have a poor prognosis with conventional chemotherapy. The ALL1 (also called MLL) gene cloned out of the breakpoint cluster region of chromosome 11q23 [Cimino et al., 1991] is in fact involved in more than 25 recurring translocations [Le Beau et al., 1997]. ALL1 is a regulator of transcription, composed of a zinc-finger domain homologous to the *Drosophila tritorax* product (a potent regulator of homeotic genes), a DNA-binding AT-hook, and a DNA methyltransferase domain. The multiplicity of partner genes with ALL1 in acute leukemia indicate that the disruption of ALL1 within the N-terminal domain may be the critical defect for leukemogenesis, in accordance with the observation that ALL1 can contribute to leukemogenesis not only by generating fusion genes but also by tandem duplications [Schichman et al., 1994] and interstitial deletions. In addition, the partial duplication of ALL1 is the first consistent defect discovered in AML patients with normal cytogenetics and in most patients with trisomy 11 [Strout and Caligiuri, 1997]. Overall, these results suggest that ALL1 may be the most frequent rearranged gene in AML. In conclusion, chromosomal rearrangements provide us with the most important clues regarding the pathogenesis, treatment, and prognosis of acute leukemia.

CHROMOSOMAL REARRANGEMENTS IN SOLID TUMORS

Solid tumors constitute more than 80% of total human neoplasia and contribute more significantly to morbidity and mortality with respect to hematologic malignancies. Nonetheless, the characterization of specific chromo-

somal and the subsequent genetic rearrangements has been hampered by the complexity of the cytogenetic changes and the karyotypic heterogeneity that may obscure the primary alterations relevant to neoplastic development. However, the last several years have witnessed the identification of highly specific rearrangements, mainly in soft tissue sarcomas, as well as in several epithelial tumors. Most translocations and inversions reported in solid tumors reflect, at the molecular level, the creation of chimeric transforming sequences by disrupting and fusing unrelated genes positioned at each of the involved chromosome breakpoints. Despite the fact that recurrent translocations were described in 1984 as unique abnormality in specific tumor types such as the t(11;22) in Ewing's sarcoma (20) and the t(X;18) in synovial sarcoma [Turc-Carel et al., 1986], the first cytogenetic alteration characterized at the molecular level was a paracentric inversion of the long arm of chromosome 10, inv(10)(q11.2q21), a hallmark of thyroid papillary carcinoma. The molecular characterization of this rearrangement led in fact to the discovery of the chromosomal mechanisms of activation of the RET protooncogene, localized in 10q11.2, by truncation and fusion of its 5′ end (containing the tyrosine kinase domain) with a donor gene located in 10q21 (D10S170) [Pierotti et al., 1992]. An interchromosomal rearrangement, a translocation t(10;17) (q11.2;q23), has also been described that lead to the fusion of RET with RIα gene at 17q23 [Sozzi et al., 1994]. As a consequence of these fusions, (1) RET, whose tissue-specific expression is restricted to a subject of neural cells, becomes expressed in the epithelial thyroid cells; (2) it undergoes dimerization that triggers a constitutive, ligand-independent trans-autophosphorylation of the cytoplasmic domains, inducing a constitutive mitogenic pathway; and (3) the relocalization of its intrinsic biochemical activity in the cytoplasm permits potential interaction with unusual substrates. Thus, the oncogenic activation of RET can be defined as an ectopic, constitutive and topologically abnormal expression of its tyrosine kinase activity [Pierotti et al., 1996]. It is worth noting that, the inv(10) was the first evidence of oncogene activation by an acquired specific chromosomal abnormality in a human solid tumor [Rabbitts, 1994].

In soft tissue sarcoma, the cytogenetic analysis of different histotypes revealed tumor type-specific chromosome rearrangements. The t(11;22)(924;912) is a hallmark of primitive neuroectodermal tumors (pPNET) that include Ewing's sarcoma, peripheral neuroepithelioma, and Askin's tumor. It occurs in 85% of cases; in the remaining 15% variant translocations, t(21;22); t(7;22), t(2;22) are detected (Table I). The EWS gene in 22q12 is fused in frame with different members of the ETS family of transcription factors. Products of these fusion are chimeric transcription factors that retain domain-specific DNA binding capability in combination with EWS transactivation properties. The oncogenic potential of these chimeric genes is based (1) on the inappropriate expression of target genes via strong transcriptional activation domain, and (2) on novel protein-protein interaction.

It has become then apparent that the EWS gene is promiscuously involved in several other tumor-associated gene fusions occurring in sarcomas of children and adults (Table I). Two examples are EWS/ATF in malignant melanoma of the soft parts with t(12;22), EWS/CHOP in myxoid liposarcoma with t(12;22), and EWS/WT1 in intra-abdominal desmoplastic small round cell tumors (IDSRCT). The latter is particularly interesting, as IDRSCT is an aggressive malignancy involving the abdominal serosal surfaces, which appears to have a predilection for young males. The molecular analysis of the recurrent t(11;22)(p13;q12) has shown that the NTD domain of EWS is fused to the last three zinc fingers of the WT1 DNA binding domain [Ladanyi and Gerald, 1999]. WT1 is a tumor suppressor gene that encodes a transcription factor that binds DNA through a series of zinc fingers and represses transcription of specific target genes. Mutations that lead to loss of function of the zinc-finger domains abrogate such a repression effect. The chimeric protein EWS/WT1 also acts as a transcription activator of the WT1 target gene. Therefore, the loss of the proximal zing finger in the fusion gene may convert WT1 from a transcriptional repressor to a transcriptional activator. This observation constitutes the first and unique example of conversion of a tumor suppressor into an oncogene by a chromosomal translocation.

A recent study in a soft tissue tumor, dermatofibrosarcoma protuberans (DFSP), further il-

TABLE I. Fusion Genes in Sarcomas

Tumor	Translocation	5′/3′ Fusion Gene	Type
Ewing's sarcoma/pPNET	t(11;22)(q24;q12)	EWS/FLI-1	RNA binding/ETS TF
	t(21;22)(q22;q12)	EWS/ERG	RNA binding/ETS TF
	t(7;22)(p22;q12)	EWS/ETV1	RNA binding/ETS TF
	t(2;22)(q33;q12)	EWS/FEV	RNA binding/ETS TF
Ewing's sarcoma/undifferentiated sarcoma of infancy	t(17;22)(q12;q12)	EWS/EIAF	RNA binding/ETS TF
Clear cell sarcoma (melanoma of soft parts)	t(12;22)(q13;q12)	EWS/ATF1	RNA binding/bZIP TF
Intra-abdominal desmoplastic small round cell tumours	t(11;22)(p13;q12)	EWS/WT1	RNA binding/Zn finger TF
Myxoid chondrosarcoma	t(9;22)(q22–31;q11–12)	EWS/CHN	RNA binding/steroid thyroid receptor gene
Liposarcoma (myxoid and round cell)	t(12;16)(q13;p11)	TLS(FUS)/CHOP	RNA binding/bZIP
	t(12;22)(q13;q12)	EWS/CHOP	RNA binding/bZIP
Alveolar RMS	t(2;13)(q35;q14)	PAX3/FKHR	PB&HD/FD
	t(1;13)(p36;q14)	PAX7/FKHR	
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SYT/SSX1 SYT/SSX2	?/?
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	PDGFB/COL1A1	Platelet-derived growth factor/collagene type 1α1
Congenital fibrosarcoma	t(12;15) (p13;q25)	ETV6(TEL)/TRK3	HLH TF/neurotrophin-3 receptor

lustrates how molecular unraveling of chromosomal rearrangements can lead to a functional understanding of the genes involved and to design possible novel targeted therapies. In a collaborative study with C. Turc-Carel, we focused on the characterization of the ring chromosome that was consistently reported in DFSP and demonstrated that supernumerary ring chromosomes indeed derived from a t(17;22) chromosomal translocation [Minoletti et al., 1996]. Cloning of the translocation breakpoint led to the finding that these rearrangements fuse the plateled-derived growth factor β-chain (PDGFB) gene on chromosome 22 with the collagen type 1A1 (COL1A1) on chromosome 17 [Simon et al., 1997]. During this fusion process, sequences upstream of PDGFB exon 2 and the elements controlling PDGFB transcription and translation, as well as those encoding normal signal peptide, are removed. The characterization of the transforming activity of this chimeric sequence came with the demonstration, by DNA transfection into the NIH3T3 cell line, of the induction of an autocrine mechanism by the rearranged PDGFB gene involving the activation of the endogenous PDGF receptor [Greco et al., 1998]. These findings open up possible new avenues for therapeutic intervention by interrupting this autocrine growth circuit by specific molecules that inhibit receptor phosphorylation.

Other specific chromosome rearrangements, such as t(X;18) in synovial sarcoma (SS), t(12;16) in myxoid liposarcoma (MLS), t(2;13), and t(1;13) in alveolar rhabdomyosarcoma (ARMS), are described. Their detection in tumor specimens represents an invaluable tool for diagnosis. In fact, improved diagnosis has been reported for undifferentiated Ewing's sarcoma, pPNETs, rhabdomyosarcoma, and neuroblastomas, which are morphologically indistinguishable. In such cases, conventional molecular cytogenetic analysis by FISH (Fig. 1), as well the use of reverse transcription-polymerase chain reaction (RT-PCR) for direct amplification of fusion transcripts are essential for an accurate diagnosis [McManus et al., 1996].

CHROMOSOMAL DELETIONS

Deletions of chromosomal bands and loss of a whole chromosome are more common in carcinoma and often lead to inactivation of tumor suppressor genes (TSG) that are critical in the pathogenesis of these tumors. Inactivation of TSG usually involves mutation of one allele and deletion of a chromosomal region in the other allele. These mutations lead to the absence of the protein product, suggesting that these genes

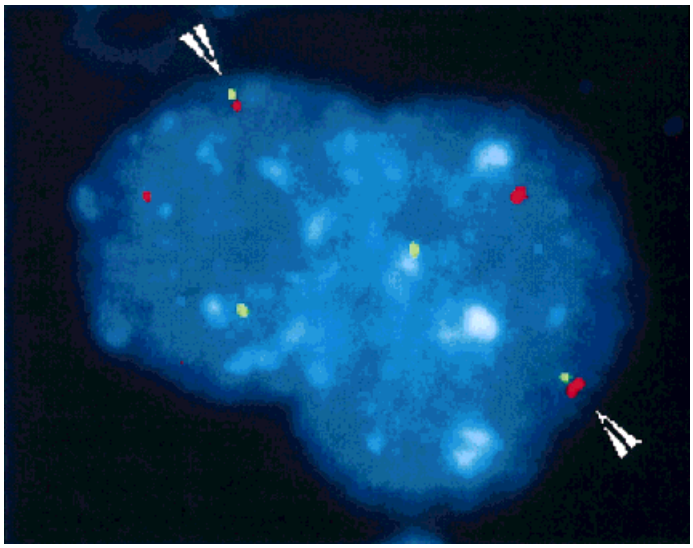

Fig. 1. Digital image of a preparation of isolated frozen nuclei from a Ewing's sarcoma hybridized with digoxigenin-labeled cosmid probe for region 11q24 (red) and a biotinilated cosmid probe for region 22q12. Juxtaposition of signals of bicolor detection indicate the presence of the translocation t(11;22)(q24;12) (arrows).

function as suppressor genes whose normal role is to limit cellular proliferation. Chromosomal deletions can be further restricted using molecular approaches such as restriction fragment-length polymorphism (RFLP), loss of heterozygosity (LOH), and FISH, with a range of probes to the specific chromosomal bands, or with YAC, BAC, or cosmid probes. In fact, FISH represents the most efficient and reproducible approach for precise localization of single sequences within metaphase chromosomes. A recent example of cloning of a TSG starting from a chromosomal deletion is the FHIT gene identified at the chromosomal band 3p14.2. The first cytogenetic report of a chromosomal deletion del(3)(p14.2p29) in cell lines by SCLC occurred in 1982 [Whang-Peng et al., 1982]. Chromosomal deletions and LOH affecting different regions of 3p (3p12, 3p14.2, 3p21, 3p24–25) were then reported in 100% of SCLC and in about 70% of NSCLC [Sozzi, 1998]. The observation of 3p alterations in preinvasive lesions of the bronchus also suggests that one or more tumor suppressor gene(s) may act as gatekeepers for lung carcinogenesis. A number of other very common tumor types, including head and neck, esophageal, breast, cervical, and kidney also show a high frequency of 3p losses. In order to position the critical genes in these regions, studies of LOH and the search for homozygous deletions in primary tumors and cell lines were performed. These studies led to the narrowing of the deleted region 3p14.2 to a few hundred kilobases, very close to a t(3;8)(p14.2;q24) chromosome translocation point of hereditary renal cell carcinoma [Kastury et al., 1996]. By constructing a cosmid contig of the critical region of 3p14.2 and using these cosmids in exon-trapping experiments, we were able to clone an exon (exon 5) and then the entire cDNA of a gene designated Fragile Histidine Triad (FHIT) [Ohta et al., 1996]. The 1-2 Mb FHIT gene is composed of 10 exons, of which 5 are protein coding (exons 5–9); it encodes a small mRNA (1.1 kb) and a small protein (16.8 kDa). Interestingly, the breakpoint at 3p14.2, involved in the t(3;8) translocation observed in the familial renal cell carcinomas, interrupts the third intron of the FHIT gene, inactivating one of the two FHIT alleles. It is also of interest that the most common fragile site of the human genome, FRA3B, maps within the FHIT gene. In this regard, this is the first molecular evidence tying the instability of fragile sites to cancer. Loss of Fhit protein expression as a result of FHIT gene inactivation has been now demonstrated in lung cancer and in a wide variety of other human epithelial tumors, representing one of the most frequently altered TSG in human neoplasia [Croce et al., 1999].

THE REVOLUTION OF FISH, COMPARATIVE GENOMIC HYBRIDIZATION, AND SPECTRAL KARYOTYPING

Chromosome banding resolution has limits, as many subtle abnormalities involving small chromosome segments or that do not lead to a characteristic alteration of banding pattern cannot be resolved. FISH is the most powerful molecular cytogenetic procedure for detecting

genetic alterations in cancer cells. A variety of probes have been developed that permit analysis of individual chromosomes, chromosome bands, centromeric or telomeric regions, and single loci. The limit of resolution of conventional FISH is localization of a 1-kb probe, but a number of procedures based on hybridization of DNA fibers released from nuclei, named fiber-FISH, now allow for increased resolution of mapping with a range of 1–300 kb. FISH approaches with a battery of DNA probes now available can resolve most type of complex and cryptic chromosomal aberrations (Fig. 2).

In cancer research and diagnosis, "interphase cytogenetics" by FISH is used to detect numerical chromosome changes and structural aberrations and can be performed in isolated nuclei, cytological preparations as well as in sections of paraffin-embedded tissue. Among the applications of interphase FISH that are already performed on a routine basis is the detection of chromosomal changes in leukemias. Nuclei from CML patients hybridized with abl and bcr probes to detect the translocation (9;22) show juxtaposition of signals of bicolor detection. Quantification of bcr/abl-positive cells is helpful in monitoring CML patients receiving therapy, as this defect can be assessed in single cells. In acute nonlymphocytic leukemias (ANLL) subtypes M3 and M4E0, characterized by t(15;17) and inv(16), respectively, FISH can establish rapid, reliable diagnosis, thus selecting patients requiring treatment intensification. Interphase cytogenetics has been applied to several soft tissue tumors for diagnosing reciprocal translocation specific for these entities. For instance, t(X;18) of sinovial sarcoma, can be detected by using probes against the centromeres of chromosome X or 18, together with two probes against the chromosome X-specific sequences SSX1 and SSX2 [Janz et al., 1995]. In small round cell tumours of childhood, FISH has also been described for the detection of specific translocation such as t(11;22) of Ewing's sarcoma and pPNETs [Nagao et al., 1997]. Other applications include the assessment of oncogene copy number, using locus-specific probes such as amplifications ErbB-2 (Fig. 3), considered prognostically significant in breast carcinomas [Mezzelani et al., 1999]. Unfortunately, FISH requires previous knowledge of the chromosomes and genes involved in the aberrations. Among the most recent additions to the FISH repertoire are comparative genomic hybridization (CGH) and spectral karyotype (SKY).

The CGH technique, based on hybridization of differentially labeled normal and tumor DNA on normal chromosome spreads, is powerful for the detection of DNA amplification and deletion [Kallioniemi et al., 1994]. The sensitivity of CGH for the detection of deletion or amplification is within the range of 10–12 Mbp. However, quantitative detection of copy number aberration affecting much smaller regions is possible (2 Mbp) [Kallioniemi et al., 1994; Bentz et al., 1998]. A major limitation of the CGH technique is that chromosomal rearrangements, like reciprocal translocation or inversions, are not detectable. A recent study on primary breast cancer indicates that certain chromosomal imbalances are very often selected in preferential order, during the progression of this tumor.

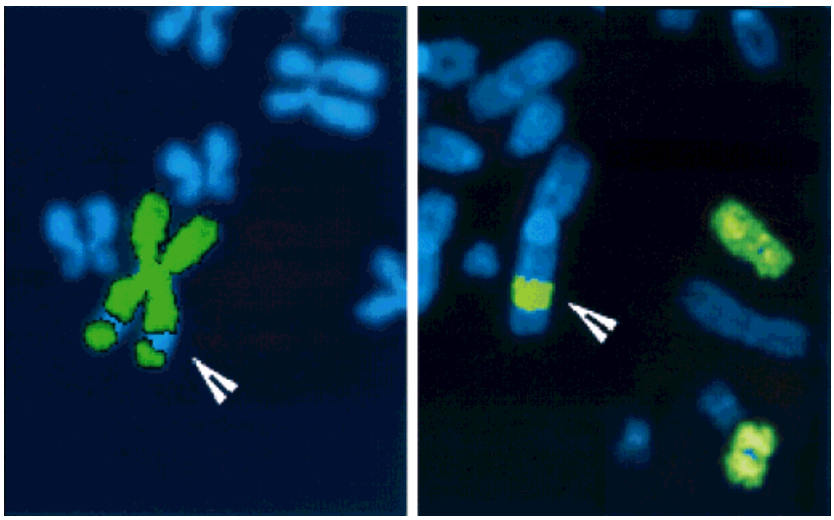

Fig. 2. Illustration of the use of fluorescence in situ hybridization (FISH) in detection of genomic alterations in cancer cells. Detection of a chromosomal insertion by hybridization with painting probes. The segment between bands 10q21 and 10qter is inserted into the long arm of chromosome 1 at band 1q31 [ins(1;10)] (arrows).

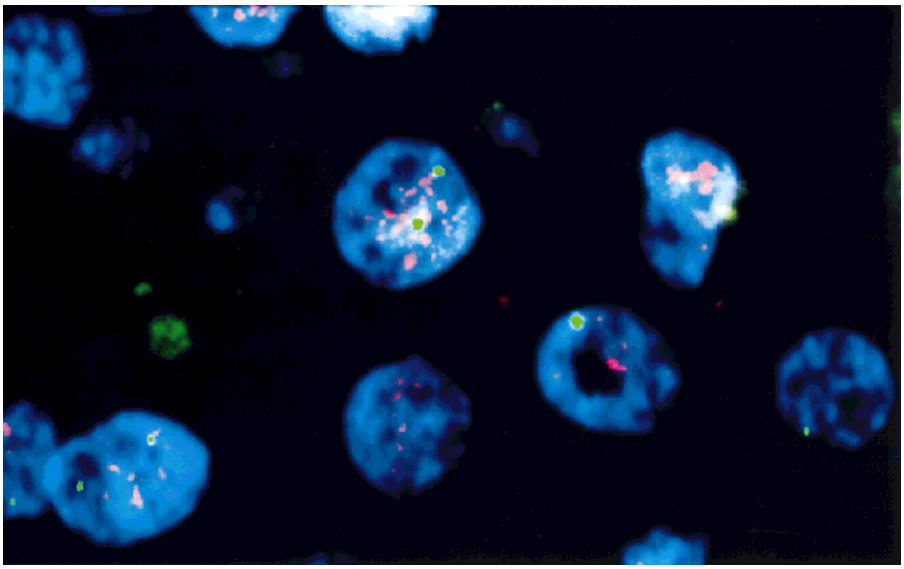

Fig. 3. Nuclei isolated from paraffin-embedded breast cancer tissue cohybridized with ErbB-2 gene probe (red) and chromosome 17 centromeric probe (yellow). Multiple copies of ErbB-2 clustered at the sites of hybridization with chromosome 17 probe.

Gains of 1q and 8q were the most common genetic changes; either one or both of these changes were found in 80% of cases. Further study of such common changes may form the basis for a molecular cytogenetic classification of breast cancer.

SKY permits rapid identification of complex and subtle chromosomal rearrangements without any prior knowledge of the chromosomes involved. By combining Fourier spectroscopy, cooled-charged device imaging, and optical microscopy, this technique enables visualization of all human chromosomes, each pair stained with a different color [Schrock et al., 1996]. Unprecedented accuracy in the characterization of hidden chromosome abnormalities by SKY is predicted and already demonstrated in haematological malignancies [Veldman et al., 1997].

CONCLUSIONS

From the initial findings of recurrent cytogenetic alterations in tumors, this century has witnessed a remarkable number of discoveries that led to a molecular understanding of the genes involved and of their biochemical pathways. The partnership among cytogeneticists, molecular cytogeneticists, cell biologists, biochemists, and physicians has greatly facilitated and enhanced our knowledge of malignant transformation.

The essential goal now is to translate our understanding of cancer cell biology and molecular genetics into more accurate control of cancer disease. Unique tumor markers are provided by the discoveries of fusion genes, mutated tumor suppressor genes, and amplified oncogenes and are useful for improved detection of cancer. According to these changes it will be possible to target cancer cells more specifically and in a near future the promise is that cancer patients could benefit of individually designed therapies.

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