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Two unusual rearrangements in hematological disorders studied by RT-PCR and/or FISH. *H.E. Wyandt[1,2], R.V. Lebo[1,3], X.-L. Huang[1], B. Loose[1], L. Oshry[4], L. Weintraub[5].* 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Pathology, Boston Univ Sch of Medicine, Boston, MA; 3) Pediatrics, Boston Univ Sch of Medicine, Boston, MA; 4) East Boston Neighborhood Health Center, Boston, MA; 5) Hematology/Oncology, Boston Univ Sch of Medicine, Boston, MA.

Fluorescence in situ hybridization (FISH) reveals an unusual karyotype, 46,XX,inv ins(9;22)(q34; q11.2q11.2)in bone marrow (bm) from a 56 y.o. female with chronic myelogenous leukemia (CML). Probes for bcr and abl-ass (Vysis, Downers Grove, IL) revealed 98% of interphases with a bcr/abl fusion. Metaphases showed juxtaposition of the bcr signal distal to the abl signal on der(9) and no bcr signal on der(22). Partial painting for chromosome 22 (pcp22subtel, Rainbow Scientific, Windsor, CT) revealed the subterminal region of der(22) to be intact. The bcr region of der(22) was thus inserted into der(9). RT-PCR of extracted mRNA amplified a 305 bp fragment, corresponding to a M-bcr/abl 210 kDa fusion product. In order for this product to be transcribed, both bcr and abl must be inverted. The patient had a complete hematological response and a minor cytogenetic response (reduction to 70% percent cells with bcr/abl fusion) with interferon therapy. Bm from a 49 y.o. male with acute promyelocytic leukemia (APL) and associated disseminated intravascular coagulation has the karyotype, 46,XY,t(17;15;17) (q11;q22;q25). FISH using probes for PML in 15q22 RARA in 17q22 (Oncor, Gaithersburg, MD) revealed distal 17q and RARA region of a chromosome 17 to be fused to part of the PML region on a chromosome 15. Distal 15q including the distal part of PML is translocated to the 17 homolog (breakpoint in 17q25), and distal 17q is translocated to the proximal part of the first 17. This complex rearrangement resulted in an inverted partial PML/RARA fusion on der(15), loss of RARA from der(17) and partial PML and intact RARA signals on der(17). The patient was treated with all-trans-retinoic acid, daunorubicin and cytosine arabinoside. He is now in complete hematologic and cytogenetic remission. Both patients have thus responded to treatments suggestive of typical CML and APL, respectively, despite their unusual chromosomal rearrangements.

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Cytogenetics finding in Breast Ductal Carcinoma. *M.L. Soto-Alvarez, A. Rojas-Atencio, F. Alvarez-Nava, K. Urdaneta, L. Gonzalez, A. Boscan.* Unidad de Genetica Medica, Universidad del Zulia, Maracaibo, Zulia, Venezuela.

The feminine breast cancer it is one of the old problems of public health in our society, from the past decade an improvement in him has been observed diagnosis, as well as also in the identification of parameters prognostic that permit a better evaluation of these patients. The objective of this work is to report, the chromosomal anomalies in 32 breast ductal carcinoma (BDC) . In this report, we present the chromosomal abnormalities found in 32 primary breast ductal carcinomas. The tumor samples were studied using the technique for short-term culturing and cytogenetic analysis with G-banding. Only one tumor with normal karyotype was observed. Thirty one (99%) of the tumors had chromosomal abnormalities including 21/ (65,6 %) in which chromosome 1 was involved (trisomy, monosomy or structural abnormalities of the type t(1q;2p) and del(1q42). Other recurrent anomalies such as del(12p); del 4(p); +7; +8; -7; -3; were observed. The significance of these findings and their role in tumorogenesis will become more evident with close follow-up of women who have tumors with an abnormal karyotype.

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A "static" karyotype in epithelial cancer cell lines despite ongoing chromosomal instability. *A.V. Roschke[1], K. Stover[1], G. Tonon[1], A.A. Schaffer[2], I.R. Kirsch[1].* 1) Genetics Department, Medicine Branch, NCI, Bethesda, MD; 2) Computational Biology Branch, NCBI, Bethesda, MD.

Most human tumors and tumor cell lines exhibit numerical and structural chromosomal abnormalities. The goal of this study was to determine the ongoing rates of structural and numerical chromosomal instability in selected cancer cell lines and to investigate the consequences of these rates to karyotypic progression. Our approach was to make single cell subclones of two colorectal (HCT-116 and HT-29) and two ovarian (SKOV-3 and OVCAR-8) cell lines and to delineate rates over time of numerical and structural chromosome changes. We performed SKY, CGH and FISH with chromosome-specific centromeric probes on cell lines and their subclones. The karyotypes of all four cell lines showed evidence of genome destabilization at some previous moment or period in their history. While significant ongoing structural and/or numerical chromosomal instability could be demonstrated in all cell lines during our period of observation, there was a relative stability of the consensus karyotype over many generations. No new clonal structural chromosomal reconfigurations emerged and the few numerical changes of karyotypes were restricted to losses of abnormal chromosomes. This implies a kind of genomic optimization under the present conditions of cell culture and suggests a link between genomic stabilization and cell propagation. We have been able to support this possibility by computer modeling.

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Physical and hematopoietic transcript map of a 5q31 "critical subregion" associated with the 5q- syndrome. *S. Kamakari[1], V. Konstantinopoulou[1], N.P. Anagnou[1,2].* 1) Institute of Molecular Biology and Biotechnology; 2) University of Crete School of Medicine, Heraklion, Greece.

The 5q- syndrome represents a preleukemic state, exhibiting an acquired interstitial 5q23-31 deletion. Four critical subregions seem to be involved in leukemogenesis. To delineate the role of one of these subregions, we constructed a YAC contig between the GM-CSF/IL-3 and TCF-1 genes. Extensive PCR screening of the CEPH and ICI YAC libraries, resulted in the isolation of twelve YACs: three YACs (854G6, 679B9, 14DG10) positive for the GM-CSF/IL-3 genes, five YACs (624G4, 969H4, 969G11, 698D8, 12BE7) positive for the TCF-1 gene and four YACs (14BA6, 15AE2, 14EB12, 28CB1) positive for seven STSs (5157S1, 5250S, bac5060S, bac5177S, 5322S, 5342T and 5328S). STS content mapping of all twelve YACs resulted in the construction of the first complete YAC contig of this subregion and documented that the GM-CSF/IL-3 and TCF-1 genes are linked. The precise size of the genomic region is being investigated by generating a long-range restriction map. Furthermore, in order to develop a hematopoietic transcript map of the subregion, twenty-six chromosome 5-specific ESTs were selected, on the basis of their origin and assignment. PCR analysis and *in silico* searches, resulted in sublocalisation of nine ESTs to the identified YACs and documented their expression in a bone marrow cDNA library. Two of them, namely T77830 and W92884, mapping proximal and distal to the IL3 gene, respectively, were further analysed. Human RNA dot blot analysis of the EST T77830, exhibited expression in kidney, liver, small intestine and lung. Northern blot analysis also documented its expression in a variety of tissues, including bone marrow, with a transcript size of approximately 4 kb. Following the technique of 5' and 3' rapid amplification of cDNA ends (RACE) for this EST, using a bone marrow cDNA library as a template, two overlapping clones, covering a total length of 3.8 kb were isolated and sequenced. Characterisation of the full-length cDNA is in progress. RACE amplification of the EST W92884 resulted in a 500 bp PCR product, currently used as a probe in a bone marrow cDNA library hybridisation screening.

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Non-random X-inactivation suggests that juvenile hemangiomas are monoclonally derived. *J.W. Walter[1], P.E. North[2], A. Mizeracki[2], M. Waner[3], D.A. Marchuk[2].* 1) Department of Genetics, Duke University Medical Center, Durham, NC; 2) Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR; 3) Department of Otolaryngology, University of Arkansas for Medical Sciences, Little Rock, AR.

Juvenile hemangiomas are the most common tumors of infancy, and occur in as many as 10% of all births. These benign vascular lesions enlarge rapidly during the first year of life by hyperplasia of endothelial cells and attendant pericytes, then spontaneously involute over a period of years, leaving a paucicellular fibrofatty residuum. In the present study, six sporadic proliferative-phase hemangiomas were collected following surgical resection, and dissected to enrich for the endothelial cell component of each sample.

To determine if hemangiomas represent a monclonal expansion from a single progenitor cell, we assayed X-inactivation patterns for each lesion (all were obtained from female patients) using the polymorphic X-linked human androgen receptor-A (HUMARA) locus. Three of six hemangiomas, all from patients less than five months of age, demonstrate a significant degree of allele loss, in one case almost complete, following methylation-sensitive restriction digest and PCR amplification, implying a non-random X-inactivation pattern, and a monclonal origin to one or more cellular components of the lesion.

These results suggest that one or more genetic events in a single cell may contribute to hemangioma development. Eventually, accumulation of non-clonally derived cells would mask the clonal nature of these lesions. The identification of clonal, proliferative samples provides an opportunity to characterize genetic events that may precede or modulate hemangioma development, and to identify genes involved with hemangioma formation.

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Characterization of a Complex Chromosome Rearrangement involving 6q in a Melanoma Cell Line: Isolation of a Candidate Tumor Suppressor Gene Interrupted by the Breakpoint at 6q16. *X.Y. Guan[1], H. Zhou[2], J.S.T. Sham[1], H. Zhang[2], J.M. Trent[2].* 1) Dept Clinical Oncology, Univ Hong Kong, Hong Kong, China; 2) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

The incidence of human malignant melanoma has increased dramatically in many parts of the world. Deletion of 6q is one of the most frequent chromosomal alterations in malignant melanoma with a breakpoint cluster at 6p11-q21. Recently, we used G-banding analysis and micro-FISH technique to detect a complex chromosomal rearrangement involving 6q and17p in a melanoma cell line UACC-930. The rearrangement includes an inversion involving 6q, inv(6q;6q)(q16;q27), and a translocation involving the inverted 6q and 17p13. A BAC clone covering the breakpoint 6q16 was isolated by FISH screen. A novel gene (named BIM1, broken in melanoma 1) interrupted by the breakpoint was isolated by partial sequencing analysis of the BAC clone. Full-length cDNA of BIM1 with two isoforms has been isolated. Sequence analysis identified a significant similarity of BIM1 and prenyl transferase gene, which is required for the cancer-causing activity of Ras gene. Loss of BIM1 was also detected in 10/12 melanoma cell lines. Our results indicate BIM1 may play an important role in the development of malignant melanoma.