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TEL/ABL gene fusion shown by FISH using commercially available probes in a patient with CML, eosinophilia and a t(9;12)(q34;p13). *M.J. Pettenati[1], M. Beaty[2], Yi-K. Keung[3].* 1) Depts Pediatrics/Medical Genentics; 2) Pathology; 3) Internal Medicine/Hematology and Oncology, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

CML is a myeloproliferative disorder characterized by cytogenetic or molecular genetic evidence of a t(9;22). We identified a case of Ph- CML with eosinophilia and a t(9;12)(q34;p13) with a TEL/ABL gene fusion shown by commercially available FISH probes.

The individual presented with persistent leucocytosis after an episode of lobar pneumonia. The marrow was 100% cellular composed largely of neutrophilic and eosinophilic precursors and was pathologically consistent with CML. An initial RT-PCR analysis of BCR/ABL showed a positive gene rearrangement. Karyotypic analysis revealed a 46,XX,t(9;12)(q34;p13). FISH with BCR/ABL probes failed to detect any fusions. A repeat RT-PCR analysis failed to detect a bcr/abl fusion. A follow-up cytogenetic study was unchanged. There are 7 reported cases of TEL/ABL gene rearrangements by DNA analysis, one with a chromosome abnormality. By using commercially available FISH probes, LSI BCR/ABL and TEL/AML1, we were able to demonstrate fusion of the ABL and TEL at 9q34.

The TEL(ETV6) gene is a member of the E26 transformation-specific family of transcription factors located at 12p13. Its role of leukemogenesis has been shown as a fusion partner in cases of t(5;12)(q33;p13), t(12;22)(p13;q11) [MN1/ETV6], t(3;12)(q26;p13) [ETV6/EVI1], and t(12;21)(p13;q22) [ETV6/AML1]. TEL(ETV6) is a very promiscuous gene involved in with over 40 different chromosome rearrangements. TEL is the only non-BCR fusion partner for ABL in human leukemia reported to date. Cytogenetically, this 9;12 chromosomal translocation can be difficult to detect. One can demonstrate the fusion of ABL and TEL using commercially available FISH probes in combination. This combination of probes may prove useful in the cytogenetic evaluation of cases with a similar cytogenetic abnormality or CML cases lacking the 9;22 translocation/fusion.

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Gain of Chromosomes 7/7q and 11p in neuroblastoma. *R.L. Stallings[1,2], M. Mullarkey[1], C. Breen[1], J. Howard[1], A. Dunlop[1], M. McDermott[3], F. Breatnach[3], A. O'Meara[3].* 1) National Ctr Medical Genetics, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Faculty of Medicine, University College Dublin, Dublin Ireland; 3) Our Lady's Hospital for Sick Children, Dublin, Ireland.

Loss of chromosome 11q material is a common genetic abnormality of advanced stage neuroblastoma and represents a distinct genetic subtype of tumour. This abnormality is frequently accompanied by unbalanced gain of the 17q region (>90%) and loss of chromosome 3p material (~70%). Gain of 11p material has also been reported in neuroblastoma with 11q loss, but at a considerably lower frequency (~10%) than the losses and gains for the 3p and 17q regions. Results reported here, however, indicate that gain of 11p may occur more frequently in the 11q- neuroblastoma subtype than was previously realized. CGH analyses of neuroblastoma from eleven patients indicated that six out of eleven tumors (55%) with loss of 11q also possessed gain of chromosome 11p. The shortest region of 11p gain was 11p11.2 to p14. G banding and FISH analysis performed on tumor cells from primary and metastatic sites indicates that gain of the 11p region occurs secondary to an unbalanced t(11;17) that led to 11q loss (and 17q gain). Gain of chromosome 7 (17/43 tumours) or 7q (5/43 tumours) material is a very common abnormality in neuroblastoma, but unlike gain of 11p, it is not restricted to the 11q- subtype and is prevalent in both low and high stage tumours. The significance of chromosome 7 gain is underscored by the fact that it was the sole abnormality detected in one tumour. We conclude that gain of 7/7q and 11p material may contribute to either neuroblastoma tumorigenesis or progression.

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Comparison of cancer cell lines by karyotype and comparative genomic hybridization. *S.L. Wenger[1], J.R. Senft[2], L.M. Sargent[2], S.G. Grant[3].* 1) Dept Pathology, West Virginia Univ, Morgantown, WV; 2) NIOSH, Genetic Susceptibility Lab, Morgantown, WV; 3) University of Pittsburgh, Dept Environmental and Occupational Health, Pittsburgh, PA.

Two cancer cell lines, MCF7 and ISHIKAWA, were each obtained from two sources, which maintained cells in different culture media. MCF7 was derived from breast tumor: #1 was obtained from ATCC at passage 149 and passaged an additional 16 times; #2 was cultured in another laboratory for unknown passages and subcultured an additional 95 times. Cell line #2 had a baseline HPRT mutation frequency 10-fold higher than #1. ISHIKAWA was derived from an endometrial tumor: #3 was obtained from European Collection of Cell Cultures passaged more than 3 times, was ER positive, and subcultured 130 more times; #4 was subcultured unknown times in another laboratory, was ER negative, and subcultured an additional 132 times. Cell line #4 had a baseline HPRT mutation frequency 1400-fold higher than #3. MCF7 cultures had complex karyotypes, however, similarities included up to 83 chromosomes, additional chromosomes 2,3,4,6,6,7,8,9,10,12,13,14,15,17,19,20, structural abnormalities add(3)(p25),del(6)(q21),add(22)(q13), and at least 1 of up to 17 marker chromosomes in common. The ISHIKAWA cultures each had up to 60 chromosomes with 4-6 markers, but only a missing X in common. CGH studies were performed using different colored fluorochromes to label each of the two MCF7 or ISHIKAWA cell lines, which were then co-hybridized to normal metaphases. Differences seen between the pairs of MCF7 and ISHIKAWA cultures reflect karyotype differences. Some initial DNA polymorphic data for the ISHIKAWA cell lines (Dr. R. Bamezai,New Delhi, India) suggest that they may not be derived from the same established cell line. Our studies demonstrate the utilization of CGH for comparing cell lines originating from the same specimen, but undergoing karyotypic and mutation rate changes due to different culture conditions and passage numbers.

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Is a 20-cell cytogenetic analysis necessary for neoplastic bone marrow specimens? *B. Roland[1,2].* 1) Dept Pathology & Lab Medicine, University of Calgary, Calgary, AB, Canada; 2) Calgary Laboratory Services.

Bone marrow specimens in neoplastic disorders sometimes have a minority of cells with a chromosome abnormality. In order to detect small abnormal clones, North American guidelines require analysis of 20 or more cells from bone marrow and neoplastic blood specimens when all cells are normal. The goal of this study is to determine the effect of analysing fewer than 20 cells on the rate of detection of abnormalities.

Records from 4081 bone marrow specimens that had been analysed in one laboratory between 1991 and 2001 were examined retrospectively. At diagnosis, 20 cells had been analysed routinely; at follow-up, 30 cells had been examined for previously-identified abnormalities. 890 samples had an abnormal karyotype, and 479 of those were mosaic, with both normal and abnormal cells present. For each mosaic abnormal specimen, the following data were recorded: referring and final diagnosis, new diagnosis vs. treated, % abnormal cells, the cell at which an abnormality was first detected, and the cell at which the abnormality was confirmed as clonal.

Of the 479 mosaic abnormalities, 6 were first detected after cell #15. Therefore 6/479 (1.2%) of mosaic abnormalities, or 6/890 (0.7%) of all abnormalities that were detected by a 20-cell analysis would have been missed by a 15-cell analysis. Subgroups of data were also analysed, with similar results for samples at diagnosis, after treatment, and for most referring diagnoses and final diagnoses.

In conclusion, a 15-cell analysis of bone marrow specimens will detect 98.8% of the mosaic abnormalities and 99.3% of all abnormalities that are detected by a 20-cell analysis. These data should be considered when reviewing guidelines for the extent of cytogenetic analysis of bone marrow specimens. For some specimens at the time of diagnosis, a 15-cell analysis may be adequate, if additional cells are checked to confirm the clonality of any single-cell abnormalities that are detected.

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Chromosomal imbalances and RET rearrangements in radiation-induced thyroid tumors. *H.E. Richter[1], A. Walch[2], J. Smida[2], L. Hieber[1], E. Lengfelder[3], E.P. Demidchik[4], H.-U. Weier[5], H. Zitzelsberger[1].* 1) GSF-National Research Center for Environment and Health, Institute of Molecular Radiobiology, Neuherberg, Germany; 2) Institute of Pathology, Technische Universitaet Muenchen, Germany; 3) Radiobiological Institute, University of Munich, Germany; 4) Center for Thyroid Tumours, Minsk, Belarus; 5) Department of Subcellular Structures, Life Sciences Division, University of California, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

The aim of this study was to investigate cytogenetic changes in radiation-induced papillary thyroid tumors from children exposed to the radioactive fallout following the Chernobyl nuclear accident in 1986. The control group was comprised of tumors without radiation history. Methods used were comparative genomic hybridization (CGH) and RT-PCR analysis for RET expression. Results of this study were expected to provide leads to molecular cytogenetic mechanisms involved in radio-carcinogenesis. Frozen and micro-dissected paraffin-embedded tissues were available for CGH analyses. Ligation-mediated PCR was performed for amplification of whole genomic tumor DNA. Additionally, the specimen RNAs were analyzed by RT-PCR for specific rearrangements of the RET proto-oncogene (RET/PTC1, RET/PTC3) and general expression of the RET tyrosine kinase domain. For cases with insufficient RT-PCR amplification Southern blot analysis of PCR products were performed with radioactive labeled oligonucleotide probes. CGH analysis showed chromosomal imbalances in 23% (8 of 35) of the childhood tumors involving mostly chromosomes 1, 2, 5, 19, 20 and 22. In the non-irradiated control group from Munich, we detected copy number changes in 5/8 cases. At present specific RET rearrangements or expression of the RET domain was found in 9 of 16 childhood and 1 of 3 of the control tumors. Only one radiation-induced thyroid tumor with a rearranged form of the RET proto-oncogene (PTC1) showed additionally chromosomal imbalances detected by CGH suggesting an involvement of additional genes in the carcinogenic process. The authors gratefully acknowledge support from the US NIH (1R01 CA80792, 1R33 CA88258).

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Multiple factors influence aneuploidy-driven chromosomal instability in oral squamous cell carcinoma. *S. Reshmi-Skarja[1], W.S. Saunders[2,3,4], D.M. Kuda[1], S.M. Gollin[1,3,4].* 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 3) The Oral Cancer Center at the University of Pittsburgh, Pittsburgh, PA; 4) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Oral squamous cell carcinoma (OSCC) arises through a complex, multistep process of genetic alterations. Previous studies have shown that OSCC cells exhibit near-triploid karyotypes and contain various clonal structural and numerical abnormalities. We hypothesized that in OSCC cells, chromosomal instability (CIN) is influenced by the state of aneuploidy within a cell. To test this hypothesis, we isolated six clones from two of our OSCC cell lines. Using a panel of centromere probes specific for chromosomes 4, 6, 7, 9, 11, 17, 20, and X, fluorescence *in situ* hybridization (FISH) was carried out on each of the clones. Variations in chromosome number both within clones and between clones of the same cell line were observed. However, despite similar chromosomal copy number, 'homologs' of a chromosome were structurally distinct from cell to cell ("marker chromosome evolution"). Spectral karyotyping (SKY) analysis revealed the segmental origin of these structurally aberrant chromosomes in cells within a clone. Our findings suggest that the aneuploidy observed in OSCC cells may be the result of both intrinsic chromosomal factors and extrinsic cytoskeletal factors, and that CIN is driven by their combined effects.

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