QUANTITATIVE PCR ANALYSIS INDICATES THAT ~25% OF CML HAVE HEMIZYGOUS DELETIONS ASSOCIATED WITH THE PHILADELPHIA REARRANGEMENT AND POOR OUTCOME

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Using FISH methods we have previously determined that ~ 10% of CML patients with poor outcome have cryptic deletions resulting in loss of sequences adjacent to 5′*ABL* and 3′ *BCR*. Since previous studies were performed using large (500 kb) commercially available FISH probes, patients with smaller deletions would remain undetected. Therefore a cohort of 71 patients previously tested positive (i.e.no deletion) but nevertheless having an adverse outcome was identified and helped to define the MDR critical for adverse outcome. We used a combination of real time quantitative PCR (Q-PCR), RT-PCR and FISH analysis to identify CML samples in this cohort that have deletions in the region of *ABL/BCR* reciprocal fusion gene. In total, 36% of the patient samples tested (26/71) were shown to have a deletion close to the breakpoints on derivative chromosome 9. Then we used the patient cohort with small deletions in the area of *ABL/BCR* fusion gene for the mapping study. The Q-PCR analysis allowed us to perform fine mapping of the extent of each deletion. We defined for the first time a minimal deleted region on derivative chromosome 9. The biological role of sequences within this region was examined. One of the strongest candidate genes is the *PRDM12* gene which maps within the minimal deleted region. Several lines of investigation strongly suggest that *PRDM12* may function as a tumor suppressor gene and its haploinsufficiency may directly or indirectly impact on oncogenesis and be responsible for modification of disease phenotypes.

TRISOMY 8 IN PHILADELPHIA CHROMOSOME NEGATIVE CELLS IN THE COURSE OF STI-571 THERAPY FOR CHRONIC MYELOCYTIC LEUKEMIA.

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Trisomy 8 is a common marker for clonal evolution and disease progression in chronic myelogenous leukemia. A 50-year-old male with Philadelphia chromosome (Ph1) positive chronic myelogenous leukemia was treated with STI-571. Six months after treatment a complete remission was acheived with the concurrent appearance of another clone with trisomy 8 in ph1 negative cells, as evaluated by interphase FISH and cytogenetic analysis. in the light of this observation, the patent's first sample was reavaluated using CEP 8 probe. trisomy 8 was observed in 18% of the interphase nuclei. Only one mitosis with trisomy 8 was fond in this sample and it was Ph1 negative, suggesting that this clone was not derived from the Ph1 positive population of cells. These finding are difficult to interpret, but the possibility that the patient is constitutionally mosaic for a cell line with trisomy 8 is likely.

ONE ADDITIONAL CASE OF MYELODYSPLASTIC SYNDROME WITH T(17;21)(Q11.2;Q22) AND A NEW TRANSLOCATION THAT DISRUPT THE AML1 LOCUS

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The AML1 gene at 21q22 is a transcription factor regulating a number of genes involved in hematopoiesis. Rearrangement of this gene by translocation has been associated with acute myeloid leukemia, acute lymphoid leukemia and myelodysplastic syndrome. To date, twenty three 21q22/AML1 translocations have been documented with 15 chromosome partners including chromosomes 1, 2, 3, 5, 8, 12, 14, 16, 17, 18, 19 and 20. We report two new cases involving AML1 locus rearrangement. One individual had acute myeloid leukemia type M2 with translocation t(17;21)(q11.2;q22). Two cases with this translocation have been reported. The second individual had therapy related myelodysplastic syndrome with translocation t(17;21)(q22;q22). This is the first case reported with this translocation. Both translocations were defined with GTG banding and the AML1 rearrangement was confirmed by interphase FISH analysis using AML1 specific probes. The breakpoints identified by karyotypic analyses define two different putative fusion partners of AML1 at band 17q11.2 and band 17q22.

THE USE OF CLASSICAL AND MOLECULAR CYTOGENETICS IN CANCER PATIENTS.

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Patient #1: refered for aplastic anemia follow up study after immunosuppression therapy. Initial bone marrow chromosome study done elsewhere showed the presence of iso(11). Classical and molecular cytogenetic studies revealed that the patient had the following karyotype: 46,XY,t(2;11)(p21;q23).isht(2:11)(MLL+;MLL-). Patient #2: referred for acute leukemia, pancytopenia and AML. Cytogenetic examination showed the presence of two cell lines, 46,XY,del(11q23)[12]/46,XY[8]. G to FISH analysis showed an insertion of the 5' end of the MLL gene into 10p12 and the 3' end was deleted.

TOPOISOMERASE IIα LEVELS IN HIGH GRADE HER2-POSITIVE VERSUS HER2-NEGATIVE BREAST TUMORS

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Background:Topoisomerase IIα is an essential enzyme that alters DNA conformation during DNA replication. Anthracyclines, drugs that target topoisomerase II α, are commonly used to treat breast cancer in adjuvant therapy. Clinical studies have suggested that breast tumors with HER2 amplification are particularly sensitive to anthracyclines although molecular mechanisms for this remain unclear.
Design: Histologic grade and HER2 status were confirmed for a group of formalin fixed, paraffin embedded, invasive breast tumors. Samples were evaluated for topoisomerase IIα expression and cell proliferation using immunohistochemical staining. Samples were assessed for amplification of the HER2 and TOP2A genes using quantitative PCR and FISH.
Results: Controlling for histologic grade, there was no statistically significant difference between the HER2 positive and HER2 negative groups for topoisomerase IIα or the Ki-67 antigen. Expression of the two markers was strongly correlated. There were highly significant differences between the Grade II and III tumor groups. Twenty-nine samples showed amplification for HER2 alone while five also had TOP2A amplification. Only a weak association was seen between the TOP2A gene copy number and its protein expression.

Conclusions: Topoisomerase IIα expression is highly correlated with cell proliferation but not directly associated with HER2 status.

MOLECULAR CYTOGENETIC EVALUATION OF METASTATIC BREAST CANCER DEMONSTRATING LOSS OF HER-2/*neu* AND CHROMOSOME 17 POLYSOMY: A CASE REPORT.

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HER-2/neu amplification and overexpression is considered to be a key prognostic and predictive factor in breast cancer. While immunohistochemistry (IHC) is widely used to assess HER-2/neu overexpression, molecular cytogenetic evaluation using fluorescence in situ hybridization (FISH) is commonly used to detect gene amplification in metastatic breast cancers with equivocal IHC results. We present a case of a 66 year old woman who had a left modified radical mastectomy for a 10 cm poorly differentiated infiltrating carcinoma of no special type, with metastases to 15 of 18 axillary lymph nodes. Immunohistochemistry for HER-2/neu oncoprotein using monoclonal antibodies CB11 and Tab 250 showed weak to moderate intensity partial cell membrane staining around a small minority of the tumour cells and was interpreted as equivocal for overexpression. Replicate FISH experiments and analysis of paraffin-embedded, formalin-fixed tissue sections using the PathVysion HER-2 Probe Kit (Vysis) identified the HER-2/neu to chromosome 17 copy number ratio to be 0.47 and 0.5. With a ratio of less than 2, gene amplification is not observed. A ratio of less than one would be consistent with the interpretation of HER-2/neu monosomy and chromosome 17 polysomy. A single HER-2/neu hybridization signal was observed in approximately half of the analyzed cells. Approximately half of the cells presented with two or more chromosome 17 centromere signals (3-6 hybridization signals), suggestive of chromosome 17 polysomy. HER-2/neu monosomy or chromosome 17 polysomy has been reported in breast cancer. The clinical significance of finding both imbalances together is not clear. A review of the current literature suggests that their simultaneous presentation may be a rare occurrence.

PRENATAL DIAGNOSIS BY NUC ISH: CRITERIA FOR SELECTION OF PATIENTS AND AN ANALYSIS OF FINDINGS

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Nuclear fluorescence in situ hybridization (nuc ISH) is now a commonly used technique for the rapid detection of fetal aneuploidies in amniotic fluid cells. However, the increased expense, technical time and equipment consumption required to perform this test is very great. To conserve laboratory resources, we developed a strategy for selection of patients who could benefit most from this procedure. The Genetics clinic at the Credit Valley Hospital established the following criteria for the selection of patients for nuc ISH. Criteria 1: Late gestation (>21 weeks at counselling) with routine late maternal age (LMA) or maternal serum screen (MSS) associated risk. Criteria 2: A risk of 3% or greater for a chromosome abnormality based on LMA or MSS. Criteria 3: Ultrasound findings suggestive of a chromosome abnormality. Over a six month period, nuc ISH was performed on amniotic fluid specimens from 49 qualifying patients, representing 8% of the total amniotic fluids tested during that period. Nine (18.4%) patients qualified under category 1; 30 (61.2%) patients qualified under category 2 and 10 (20.4%) patients qualified under category 3. The abnormal nuc ISH findings were: 0/9; 5/30 (16.7%) and 4/10 (40%) respectively. The use of strict selection criteria is a valuable tool, ensuring consistency, fairness, and increased predictability of impending abnormal results.

A CASE REPORT: UNEXPECTED KARYOTYPE IN A BOY WITH FEATURES OF ANGELMAN SYNDROME

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A child was referred for a genetic assessment due to developmental delay. At two years of age, he presented with features of Angelman syndrome. Chromosome studies were ordered, revealing a 46,XX/47,XX,+idic(Y)(q11.1) mosaic karyotype. Fluorescence *in situ* hybridization (FISH) failed to detect a 15q12 deletion. Methylation studies by Southern analysis were normal. Therefore, a cytogenetic diagnosis of Klinefelter syndrome was assigned. A genetic follow-up was performed when the patient was 14 years of age. The child lacked the characteristics typical of Klinefelter syndrome. Instead, his behaviour and phenotype were consistent with Angelman syndrome. Chromosome studies were again ordered, with a request to perform FISH to try to detect a SNRPN deletion. The karyotype was the same as before, and routine metaphase FISH failed to reveal a deletion. However, nuclear ISH showed low level mosaicism for a deletion at the SNRPN locus (10% of nuclei). Further molecular cytogenetic analysis revealed that the deletion occurred in cells lacking the idic(Y) chromosome. A model for the sequence of events leading to mosaicism in this patient is presented. This case illustrates the importance of correlating phenotype with laboratory results to arrive at the ultimate, correct diagnosis.

ORIGIN OF FAMILIAL R(19) MOSAICISM: MOLECULAR EVIDENCE OF RING OPENING

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A familial non-supernumerary r(19) was revealed in a mother and her fetus following prenatal diagnosis for advanced maternal age. The r(19) was present in 4% of cultured leukocytes from the mother, and the amniotic fluid cells showed 100% r(19) at diagnosis. However, loss of the r(19) and an emerging normal cell line became evident during passaging, leading to 46,XY,r(19)/46,XY mosaicism. Mechanisms for this include: chimerism; loss of the ring followed by duplication of the remaining, normal chromosome, and telomeric fusion with sporadic ring opening. Cytogenetic and molecular evidence were collected to further characterize the structure of the r(19) and to determine the origin of the emerging normal cell line. The molecular cytogenetic evidence supported telomeric fusion as the mechanism of ring formation in this case. To determine the origin of the normal cell line, cultured amniotic fluid cells were dilution cloned and a non-ring containing, normal cell line was obtained. Microsatellite markers were used to determine the parental origin of the chromosome 19 homologues in the normal culture, as compared to the ring containing culture. The molecular evidence obtained suggested that the normal cells arose due to ring opening as opposed to chimerism or uniparental disomy. This study confirms the mechanism of telomeric fusion with ring opening as a mode of transmission of ring mosaicism in families.

MEWO: A "CLASSICAL STUDY" OF CHROMOSOME REARRANGEMENTS

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Karyotypic abnormalities, including numerical changes, structural rearrangements, and amplification of specific chromosomal regions morphologically detected as homogeneously staining regions (HSR) or double minutes (DM), are commonly found in cancer cells. The observation that specific chromosomal changes may be characteristic of particular malignancies has led to a continued interest in identifying those specific changes that occur during tumor growth and metastasis. An analysis of the karyotypic changes occurring during the progression of disease is of value in determining which changes predispose a cell to progress to a more malignant phenotype and may be useful in monitoring the course of the disease and the effects of treatment. Chromosome instability is also apparent in cell lines derived from tumors, and an analysis of the changes may identify the types of DNA sequences and processes that are involved in the generation of abnormal karyotypes.

The human melanoma cell line has been used to study chromosomal changes occurring during in vitro passaging as well as during tumor growth and metastasis in nude mice. Some of the chromosomal changes seen (including multiple rearrangements of chromosomes 13 and 15, amplification and translocation of chromosome 15p sequences, and response of the cells to increased doses to methotrexate) will be presented. The findings appear to be an exaggeration of instability seen in both cancer cells and normal cells.

UNRAVELING THE MYSTERY OF AUTISM: FROM GENETICS TO PROSPECTIVE IDENTIFICATION AND PREVENTION

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Autism spectrum disorders (ASDs) are characterized by impairments in reciprocal social interaction and communication, and stereotypic activities, and affect at 1/250-1/500 children. A group of more than 60 researchers, clinicians, and parents from Canada and the US have formed the Autism Spectrum Disorders Research Team (ASD-RT), an interdisciplinary group of investigators with a broad range of expertise & a mandate to undertake research that will result in a better understanding of the etiology, pathogenesis, and effects of treatment of persons with ASD. ***The ultimate aims of our research program are to develop methods for very early identification of children at risk, and intervene by appropriate means to prevent overt symptoms of autism.*** To achieve these goals, we are undertaking the following projects: 1) A Research Registry, in which ~5000 families provide information on different aspects of ASD enabling subgrouping of families; 2) A Genetics and Phenotyping Study, which involves careful phenotypic and behavioural studies on families with two or more cases of ASD, coupled with molecular (DNA), cytogenetic and biochemical studies – aimed at identifying culprit genes; 3) A Prospective Study of ASDs to identify the earliest signs of atypical development heralding an ASD, and an Intervention/Prevention Study aimed at preventing the development of overt ASD symptoms; 4) An Epidemiological Study of ASDs in Canada, to determine the incidence and prevalence of these conditions and identify etiologic factors.

A COMPLEX FAMILY HISTORY AND UNUSUAL FISH TELOMERE FINDINGS

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The proband was referred to genetics clinic at 3 years and 9 months. She had dysmorphic features, microcephaly, global developmental and language delay. She was similar to her mother who also was dysmorphic, microcephalic and significantly delayed. A four generation pedigree showed an extensive maternal family history of schizophrenia, congenital heart defects, developmental delay and microcephaly. Chromosome, Fragile X and FISH for 22q11.2 results were all normal. FISH using the VYSIS telomere Xp/Yp probe showed an additional signal on the q terminal of one X chromosome. This X chromosome also had a normal Xq telomere. This finding was confirmed with probes from a 2nd manufacturer (Cytocell). This unusual X chromosome was also present in the proband's mother, a cousin (47,XYY) and the cousin's (clinically normal) mother. It is not clear if this finding is significant or a familial variant. Analysis on additional family members and possibly X inactivation studies may be helpful.