Radiological Terrorism: Assessing genomic damage and estimating health risk for emergency medical response.

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This presentation will discuss new developments in emergency biological dosimetry and the CRTI National Biological Dosimetry Response Plan (NBDRP). Biological dosimetry is used to estimate the biological consequences of a radiation exposure and is largely based on chromosome aberration detection. The NBDRP will establish a national network of laboratories to respond to a nuclear event. The goal will be to provide rapid radiation dose estimation and long term health risk assessment for exposed individuals. In the event of a large scale radiation event, the dose estimations will guide the actions of emergency officials, emergency responders and health care personnel. The presentation will outline techniques used in biological dosimetry including the dicentric assay, fluorescence in situ hybridization (FISH), premature chromosome condensation-FISH (PCC-FISH), apoptosis and spectral karyotyping (SKY). PCC-FISH in the nuclei of unstimulated lymphocytes is an early assay that could potentially yield important information regarding personal exposure within 4 to 12 hours post-irradiation. Apoptosis and SKY in lymphocytes can estimate the level of damage, and subsequently the magnitude of the dose, 24-48 hours following exposure. These analyses will be used by the medical personnel to triage and treat exposed individuals. In addition, SKY can also be used in follow-up investigations to monitor future health risk. The overall purpose of this presentation is to provide a brief introduction to radiobiology and radiation-induced DNA damage. The health risks associated with radiation exposure and cytogenetic techniques used to estimate the risk will be discussed. Applications of cytogenetic biological dosimetry for emergency and accidental exposure and the NBDRP will be presented.

Unexpected FISH Results in a Patient Referred For Query Lymphoma.

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A lymph node biopsy from a 45 year-old female, whose microscopic examination showed that it was an anaplastic CD30-positive large cell lymphoma, T/null cell type and cytoplasmic ALK-positive, was received for FISH analysis. FISH performed on the sample using the Vysis dual colour break-apart ALK probe did not reveal the classic t(2;5) signal pattern of one fusion (1F), one red (1R) and one green (1G) but rather 1F, 3R, and 2G. Classical G-banding analysis was performed to determine the actual karyotype of the lymphoma. All metaphases examined were near tetraploid with no t(2;5). Metaphase FISH analysis revealed a normal chromosome 2 (1F), two inv(2)(p23q35) (2R and 2G), and a marker chromosome that carried one red signal. The cryptic inv(2) is a variant rearrangement of the classic t(2;5) commonly found in anaplastic large cell lymphoma ALCL), and results in the ATIC-ALK fusion protein that accumulates in the cytoplasm. This is in contrast to the t(2;5), which results in the typical accumulation of the NPM-ALK fusion protein in both the cytoplasm and the nucleus.

An Unbalanced Reciprocal Insertional Translocation as a Cause of Retinoblastoma in a Newborn Baby

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Retinoblastoma, malignancy of the retina, occurs due to the deletion of both copies of the *Rb* tumour suppressor gene, at 13q14.1-2, in retinal tissues. Most cases are unilateral but 20-30% of cases are bilateral and these show familial inheritance. A case was referred to the Birmingham Regional Cytogenetics Laboratory of a family with a history of Retinoblastoma. In particular we describe a case of a one month old baby with bilateral retinoblastoma and dysmorphism.
This patient had been previously karyotyped in another laboratory along with maternal chromosomes and both reported as having a deletion of 13q14 although the mother did not show any evidence of retinoblastoma. On closer analysis the child's chromosomes did indeed show an abnormal chromosome 13. Maternal chromosomes, however, showed the presence of an unusual balanced rearrangement between chromosomes 13 and 14.
This rearrangement was partly delineated by fluorescent *in situ* hybridisation studies using a locus specific probe which hybridised to the retinoblastoma gene. This showed one copy of the gene present in the baby confirming deletion of this region of chromosome 13 and explaining the retinoblastoma observed. Maternal analysis showed two copies of the gene with one copy seen on chromosome 14 at approximately band q12, identified by reverse DAPI banding. Further tests using sub-telomeric probes, alpha-satellite probes and whole chromosome paints for chromosomes 13 and 14 confirmed the rearrangement as an insertional reciprocal translocation. Only one other case of such a rearrangement has been reported in the literature. This abnormality appears to be of great clinical importance posing a high reproductive risk.

MLL Amplification

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Bone marrow from a 48-year old female was referred to cytogenetics for acute leukemia /myelodysplastic syndrome. The patient had anemia/thrombopenia for the last 12 months and a history of breast cancer (1998) treated with XRT and 9x CMF. The peripheral blood, bone marrow aspirate, biopsy and flow cytometry results were consistent with therapy related secondary myelodysplastic syndrome (refractory anemia with excess blasts).
Classical G-banding was performed and all metaphases examined were abnormal and bore multiple numerical and structural abnormalities, including a derivative 11 chromosome rearrangement with a breakpoint at 11q23. FISH was performed using the Vysis MLL dual color break-apart probe set to determine if MLL was involved in this 11q23 rearrangement. Metaphase FISH analysis showed four copies of the intact MLL gene: one on the normal 11 chromosome, one on the der(18) and one each on 2 markers. The der(11) did not retain a copy of the MLL gene. Interphase FISH analysis using the MLL probe set showed a signal pattern consistent with the presence of 2-6 copies of the MLL gene, where most abnormal

nuclei had 4 copies. Duplication or amplification of the MLL gene is a rare phenomenon in hematologic disorders, but is significantly associated with t-MDS and t-AML, a complex karyotype and a shorter survival.

Fluorescence in situ hybridization of nuclei isolated from paraffin-embedded lymphoid tissue.

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FISH analysis of histological sections from paraffin-embedded tissue is often difficult to accomplish because of overlapped nuclei and truncated cells that interfere with accurate scoring of individual nuclei. In this report, we describe a technique to isolate individual nuclei from needle cores and 10 micron thick sections of paraffin-embedded tissue. This method allows for successful FISH analysis on a variety of lymphomas, including Burkitt, mantle cell, follicular and diffuse large B-cell.

Philadelphia Positive Acute Lymphoblastic Leukemia With An Additional Philadelphia Chromosome And A Deletion On The Derivative Chromosome 9.

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We report a unique case of an adult patient with Philadelphia chromosome positive (Ph+) B-cell acute lymphoblastic leukemia (B-ALL). Interphase fluorescence in situ hybridization (FISH) analysis with the Vysis *BCR*/*ABL* extra signal (ES-FISH) probe set showed a signal configuration consistent with the presence of two Ph chromosomes and a deletion on the derivative 9 chromosome. No metaphases were available for metaphase FISH analysis. Although the extra fusion signal did not look like the result of a translocation involving the minor breakpoint cluster region (m-*BCR*), RT-PCR confirmed that it was indeed a translocation involving the major breakpoint cluster region (M-*BCR*), ie. a p210 transcript. Further FISH analysis using the Vysis *BCR*/*ABL* dual colour dual fusion (D-FISH) probe set confirmed that this patient carried an M-*BCR* rearrangement with an extra Ph chromosome along with a deletion 5' of the *ABL* gene on the derivative 9 chromosome. Although this type of deletion is found in ~15-20% of CML patients and associated with a poor prognosis, to the best of our knowledge, this is the first ALL patient found to carry this deletion. The clinical implications, if any, are not known at this time.

Homozygous deletion of p16 gene in childhood acute lymphoblastic leukemia : clinical outcome and prognostic significance.

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Inactivation of the tumor suppressor gene, $p16^{INK4A}$ (CDKN2), either by deletion (homozygous or heterozygous) or methylation, plays an important role in tumorigenesis. This gene is located in 9p21 and has been found to be deleted in many types of tumors, including leukemias. Cytogenetic abnormalities involving chromosomal band 9p21-22 has been reported in 7% to 13% acute lymphoblastic leukemia (ALL). Homozygous deletion of both p16 and p15 occurs in at least 60% of T- and 20% of B-cell lineage cases, and is the major means of gene inactivation in childhood ALL. It is mainly associated with higher relapse rates and poor outcome. The overall survival is reported to be significantly worse and are classified as higher risk group.
We present here two cases : a case of childhood B-ALL with homozygous deletion of p16. At age 3 1/2 years, she presented with WBC count of 27,000/uL, hemoglobin level 77gm/L, CNS negative, no mediastinal mass, and was treated according to the standard risk protocol of DFCI 95-001(Dana Farber Cancer Institute), with no cranial irradiation. Cytogenetic analysis at initial diagnosis showed a normal karyotype. She responded well to the therapy which ended in Sept 2001. RT-PCR for t(9;22), t(12;21), t(4;11), t(1;19) and FISH for 11q23 were negative. In Nov 2001 she presented with facial palsy and relapse in both bone marrow and CNS was diagnosed. Chromosome analysis showed presence of del(9p) in 7 out of 24 cells. She was then treated according to the high-risk arm of DFCI 2000-01. Remission was attained within a month of treatment and was consolidated with a fully matched unrelated bone marrow transplantation. She did well for 124 days post transplant, but again had a marrow and CNS relapse. FISH analysis with LSI p16/CEP9 probe (Vysis) showed homozygous deletion of p16 in 61% cells and heterozygous deletion in 13% cells. The patient died in Sept 2002 at age 6. The second case is a 23 month old female with pre B-cell. Her karyotype at diagnosis is 45,XX,i(9)(q10),der(20). FISH with p16 probe shows homozygous loss. She is under investigation.
The clinical presentations and the genetic findings in our patients are consistent with those reported earlier showing a high frequency of 9p abnormalities in higher risk patients (Ursula et al, 1997; Heerema et al, 1999).The present Molecular Cytogenetic results suggest that homozygous deletion of p16 is a significant prognostic factor in childhood ALL, and is important in the identification of this subgroup of standard risk patients with increased risk of treatment failure.

An Overview of Microarrays: Applications in Research and Clinic

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DNA microarray enables us to visualize global gene expression for the first time with the knowledge that the variation in each gene expression is richer than the allelic variation in its sequence. Finding the link between the expression variation and phenotypic variation may provide clues to the biological roles of gene(s) to identify the molecular basis of phenotypic variation among cells and individuals. To generate a gene expression profile of a condition (s) that is to identify the up- or down-regulation of gene (s) expression which can be both the cause and effect in a disease state. To study the global changes involving thousands of genes is not possible without microarray. A large portion of the genome can be interrogated simultaneously to identify the common cluster of genes with similar expression pattern which may reflect the similar function; and the exceptions or unknown members may help to identify functionally important novel genes based on these expression patterns.

Moreover, temporal sequence of gene expression can be followed in a biological event utilizing microarray. Finally, microarray can be used to develop diagnostic tools, either to identify the markers or use the global gene expression information for classifying different stages of the disease state, by using a subset of global candidate genes relevant to the disease. The aggregate database is also useful to develop new drugs based on either novel genes thus identified, or the knowledge of expression levels of mRNA in different pathological states. This presentation will give you an overview of microarray and several applications of microarray both in research and clinical settings, including cytogenetics.

Navigating genomic databases: application to clinical cytogenetics

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The resources generated by the Human Genome Project are having an increased utility in the clinical cytogenetics laboratory. Resources available for high resolution FISH are often limited to commercially available probes, restricting testing to common chromosomal anomalies. The availability of extensive genomic information housed in publicly accessible databases, however, makes it simple to investigate any region of the genome. Included in these databases are the cytogenetic locations of thousands of BAC clones used in the determination of the human genome sequence. These BAC clones are ideal probes in FISH analysis. The 150 kilobase average size makes them large enough to be detected under the microscope using standard labeling procedures. As well, the BAC clones are readily available from many sources, one of which is The Centre for Applied Genomics at the Hospital for Sick Children (http://tcag.bioinfo.sickkids.on.ca). Examples of how to use the UCSC 'GoldenPath' Genome Browser database (http://genome.ucsc.edu) to find probes for 'custom' high resolution FISH analysis will be demonstrated in a tutorial manner.

Going Custom: Using custom probes to characterize difficult cases

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The ability to refine cytogenetic breakpoints has improved dramatically over the past 25 years. In fact, the gold standard has been replaced several times since the advent of G-banded analysis. This includes high resolution G-banding by cell synchronization methodologies and the use of fluorescence *in situ* hybridization as a tool for detection and confirmation of microdeletion syndromes. Genome chip arrays are likely to eventually replace microscopic detection of additions and deletions in the near future. However, balanced rearrangements are likely to always require cytogenetic and molecular cytogenetic analysis. The availability of locus and band specific cloned DNA offers the opportunity for the molecular cytogeneticist to investigate and confirm deletions as well as subtle balanced rearrangements. Cases exemplifying the uses of custom FISH probes to refine breakpoints will be presented and discussed in the context of the clinical cytogenetic laboratory.

Telomeres…beginning to understand the ends

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Over the past several years, an increasing number of laboratories have begun to offer genome-wide telomere FISH screening for patients with unexplained mental retardation. With this new technology, laboratories are able to identify cryptic telomere rearrangements that otherwise might have gone undetected by routine karyotype analysis. During this presentation, the methods used for telomere screening and studies reporting the frequency of unbalanced telomere rearrangements will be reviewed. In addition, the consequences of telomere rearrangements, including not only those that have an associated phenotype, but also those that are familial benign variants, will be discussed.

FISH and cytogenetic characterization of an unbalanced X;13 translocation: Clinical case report and phenotypic implications.

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We report on a prenatally diagnosed female infant with severe abnormal phenotype and a trisomy of the short arm of X chromosome (Xp11.2→pter) due to de novo unbalanced X;13 translocation with the karyotype 46,XX,der(13)t(X;13)(p11.2;q10).The trisomy of Xp11.2→pter was confirmed with fluorescence in situ hybridization (FISH), using an X chromosome painting probe and telomeric FISH probes specific for the short arm of chromosome X. FISH analysis with an XIST probe showed that the derivative chromosome 13 does not include the XIST locus at the X-inactivation center. Based on current understanding, it is assumed that the X chromosome short arm would not be subject to X inactivation. The patient has craniofacial dysmorphism, heart defects, and urogenital anomalies. Functional disomy of Xp11.2→pter most likely accounts for the abnormal phenotype in this patient.

Cytogenetic Study in Human Congenital Anomalies

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As per available record, the incidence of human congenital anomalies is 1% and the rate of abortion of abnormal fetuses is much higher. Congenital anomalies might arise due to chromosomal aberrations or gene mutations. Some of these aberrations/mutations are incompatible with intra uterine development of fetus while in other cases the fetus survives full term and is born abnormal. During three years study (1997-2001) a total of 300 children born with congenital anomalies were referred to the Human Genetic Counseling and Research Centre for chromosome study. Majority of the referred children were found to have chromosomal abnormalities. Trisomy 21 was found to be the main kind of

chromosomal aberration affecting both the sexes. Following trisomy 21, was the monosomy of X-chromosomes and its mosaics and next to this chromosomal aberration was the XXY chromosome constitution and its mosaics. Only one child was found to have Trisomy 13. The work is going on and attempts are being made to carry out the population screening programme.

Robertsonian Translocation (Rob t):Genotype - Phenotype Correlation

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The incidence of Rob t is around one in 10,000 livebirths . Rob t occurs within and between the D and G group chromosomes. Among them, the commonest is Rob t 13;14, 14;21 and 21;21. At Division of Human Genetics, from 1976, sixty six Rob t have been confirmed. The breakdown : 14;21(29,43.93%) , 21;21 (20, 30.30%); 13;14 (11;16.66%); 15;21 (2, 3.04%); 13;13 (1, 1.52%); 13;15 (1, 1.52%); 14;15 (1,1.52%); 15;21 (1, 1.52%); 15;22 (1, 1.52%). Male (34) to female (32) ratio was 1.06:1. On correlation with the chief complaints, in 51cases, Rob t was associated with Down Syndrome (77.26%); 11 with bad obstetric history (16.66%) and one each with mental retardation, multiple congential anomaly, ambiguous genitalia and primary amenorrhea. Thus, it is seen that Rob t seemed to be phenotypically associated mostly with Down Syndrome and its pathology and in couples with recurrent abortions / livebirths / mental retardation /multiple congenital anomaly ie bad obstetric history.Normal karyotype was found in 70 parents (53.03%). In 52 parents (39.40%) karyotype is yet to be done. Parental origin was determined in 10 (7.57%); it was maternal in 8 and paternal in 2. It has been suggested that due to sequence homology 13;14 and 14;21 may have the disposition for Rob t and mostly it is maternal in origin. Accordingly, counselling is being provided to the patients and their family with emphasis on follow – up.

The Perils of Additional Testing: a prenatal marker case

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A healthy 34 year old female presented at the genetics clinic because of a positive integrated prenatal screen: elevated Beta hCG (8.17 MOM) and slightly elevated AFP (3.16 MOM). Family history was negative. Fetal ultra sound showed an apparently normal fetus with mild right hydronephrosis. Fetal echocardiogram at 21 weeks showed a normal fetal heart. Chromosome results on amniotic fluid was 47,XX,+mar in all cells examined. One chromosome 22p- variant was noted. The marker was C-Band positive and bisatellited. Parental karyotypes were normal. Various FISH probes, including the DiGeorge/VCF were used for marker identification and to rule out Cat Eye Syndrome. The marker was negative

for all probes except the Alpha satellite 14/22. Unexpectedly, the additional FISH workup detected cryptic abnormalities on the "normal" 22p- chromosome. The DG/VCF probe showed a duplication of the ARSA locus. The 22qTel probe also detected a duplication of the 22q telomere on the "p" terminal. The patient was counseled for risks associated with the de novo abnormalities of chromosome 22. Termination was performed in the United States because of advanced gestation. The fetal X-ray was normal. The fetus (from photographs) appeared to have micrognathia, abnormal position of fetal fingers and a broad nasal bridge. This case illustrates the perils of additional testing: the more we do the more we find. The abnormal 22 in this case was a serendipitous finding and not detectable with routine G-Banding.