The Great Lakes Chromosome Conference 2007

Session I: Cancer Cytogenetics
Chair: Jean McGowan-Jordan, Ottawa

CLL FISH panel implementation: The Eastern Ontario Experience
Jean McGowan-Jordan and Liz Sinclair-Bourque
Children's Hospital of Eastern Ontario, Ottawa

Over the past several years, many publications have shown that standard chromosome analysis is sub-optimal for the detection of chromosome anomalies in certain hematological malignancies; the low mitotic index of the abnormal cell population can lead to high false-negative rates. In chronic lymphocytic leukemia, interphase FISH can improve the abnormality rate several-fold. We use a commercially available panel of FISH probes on bone marrow and unstimulated peripheral lymphocytes. The abnormality identification rate is consistent with that reported in the literature, with abnormalities detected in over 70% of cases referred. Our findings and their prognostic significance will be reviewed.

Impact of allogenic hematopoietic stem cell transplantation on eradication of genomic aberrations as determined by FISH in patients with CLL
Hélène Bruyère, Vancouver General Hospital, Vancouver

Backgound - Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western countries. Good predictors of survival include cytogenetic abnormalities, such as deletions 11q, 17p and 13q14.3. Although chemotherapy is considered the standard treatment, the only curative option is hematopoietic stem cell transplantation (SCT). However, little is known about patient's outcome after SCT depending on the FISH abnormalities detected prior to treatment.
Methods - We identified pre- and post-SCT bone marrow samples for eighteen patients and used FISH probes to detect the copy number of the ATM and TP53 genes and the 13q14.3 locus on all prior-to-transplantation samples and on fifteen post-transplantation specimens. **Results -** Results for prior-to-transplantation bone marrow specimens showed that none, one, two or three abnormalities were found in respectively two, nine, four and three patients. In total, twenty-six abnormalities were detected in sixteen patients. Results for post-transplantation bone marrow specimens showed that the FISH abnormalities in eleven patients became undetectable. Two patients showed only a reduction of the malignant population. Two patients failed to show cytogenetic improvement.
Conclusions - Our results show that SCT can eliminate FISH abnormalities in a majority of CLL patients. Importantly, for all eight patients with an ATM deletion, post-SCT follow-up no longer detected the abnormal cells. The fact that the patients with 11q deletions did well with SCT, while having done poorly with chemotherapy

may lead to further studies which could change practice for this group of patients – perhaps leading to earlier performance of SCT.

MLL Amplification in AML

Diana Munavish, Viola Freeman, Ron Carter
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DNA amplification is a well-described mechanism leading to up-regulation of oncogene expression in malignancy. It is more commonly documented in solid tumours than in leukemia. Cytogenetically gene amplification presents as homogeneously staining regions (hsr's), double minutes (dmin's) or tandem duplications.

This presentation reviews our findings in 2 recent cases of AML with amplification of the *MLL* gene. The literature cites c-*MYC* as the predominately amplified gene in haematological malignancies, while *MLL* gene amplification is rarely observed. Gain of one ore more intact copies of MLL is often detected in hyperdiploid cell lines (e.g. trisomy 11), but massive amplification is quite unusual.

Patient #1 presented with 90% myeloblasts, and immunophenotyping cell markers were consistent with AML. Chromosome analysis showed a near tetraploid cell line with a complex karyotype including multiple structural aberrations. The primary finding was massive amplification of apparently intact MLL signals presenting as a jumping translocations involving chromosomes 2, 11 and 20. Other predominant rearrangements were deletions of 9q, 16q and 20q as well as unidentified marker chromosomes.

Patient #2 presented with 45% myeloblasts and similar immunophenotyping markers, suggesting AML. Cytogenetics findings showed a near diploid cell line characterized by multiple structural and numerical anomalies. The major findings were amplification of MLL (~4-15 signals/cell) as well as loss of chromosome 5 and marker chromosomes present.

FISH analysis with the Vysis (Abbott) LSI MLL probe examined on both interphase and metaphase cells confirmed the unidentified chromosomal material consisted of amplified *MLL* signals in both cases.

Amplification of chromosome material demonstrates genetic instability and is associated with poor prognosis. Reported cases of *MLL* amplification are predominantly males of advanced age with a short survival of approximately 1 month. Both patients expired soon after diagnosis.

Development of a hyperdiploid leukemic cell line in a child constitutionally mosaic for a cell line with t(15;21) Robertsonian translocation and a cell line with isochromosome 21.

Rosemary Mueller, Edmonton Cytogenetics Laboratory*
Dr. Maria Spavor, Dept of Pediatric Oncology,
Dr. Norma Jean Leonard, Medical Genetics Clinic
Stollery Children's and University of Alberta Hospital,
Edmonton, Alberta.

A specimen of peripheral blood from a 2 year old girl was received with the indication of query acute lymphoblastic leukemia but where bone marrow aspirate sampling was inadequate.
Karyotype analysis of the unstimulated blood cells showed three abnormal cell lines:
45,X,der(15;21)(q10;q10)[17]/
54~55,XX,+X,+4,+6,+14,+17,+i(21)(q10)x2, inc[3]/
46,XX,i(21)(q10)[2]
The child was treated for pre B ALL; a week later we requested and received a peripheral blood specimen for analysis with mitogen stimulation. Two cell lines were detected:
45,XX,der(15;21)(q10;q10)[18]/46,XX,i(21)(q10)[12]
A month later, another mitogen stimulated peripheral blood specimen and subsequently two skin specimens were received and analysed.
Results in skin were: 45,XX,der(15;21)(q10;q10)[15]/46,XX,i(21)(q10)[5].
This child has constitutional mosaicism for two structurally abnormal cell lines; the leukemic cell population arose from the i(21)(q10) cell line. Once remission from the leukemia was established, the child was determined to have evidence of developmental delay consistent with Down Syndrome.
**Cytogenetic analyses were performed by Audrey O'Neill, Lynne Faist, Dawn Redford, Julie Schultz, Emily Chan, Melanie McKay.*

Unusual Karyotypes in Pediatric ALL and AML

Mary Shago, Daniel Antinucci, Wendy Cockett, Lihua Ji, Shanthini Kangesan, Julie Malone, Kim Mellick, Xiaoyan Wu, Eul Kyong Kim, Assya Pavlova, Shaoxian Zhu, Gloria Nie and Ikuko Teshima.
Cytogenetics Laboratory, The Hospital for Sick Children

The predominant form of cancer diagnosed in children is acute leukemia. Acute lymphoblastic leukemia (ALL) accounts for ~80% of cases, while acute myelogenous leukemia (AML) comprises ~20% of cases in children. The most common subtype of ALL is precursor B-cell ALL. In this category of leukemia, there are several expected recurrent karyotypes: hyperdiploidy, hypodiploidy, and rearrangements of TEL/AML1, E2A/PBX and MLL genes. The recurrent cytogenetic abnormalities detected in pediatric AML patients are similar to those seen in adult AML.
At the Hospital for Sick Children Cytogenetics Laboratory, we analyze approximately 60 new cases of pediatric leukemia each year. Although the majority of these cases display expected recurrent cytogenetic abnormalities, approximately 5-10% of the cases harbour 'unexpected' abnormalities. Pediatric leukemia cases with hidden hypodiploidy, *MYC*, *BCL-2* and concurrent *CBFB-BCR/ABL* gene rearrangement will be presented.

UroVysion: the new kit on the block

Capua E, Rasiuk G, Chang W, Chang C, Noakes J, Spevak L, Buckley R and Chun K. Genetics Program and Department of Urology, North York General Hospital, Toronto, Canada.

Bladder cancer is the fourth most common type of cancer in men and the twelfth most common in women in Canada. More than 5,000 men and 1700 women are diagnosed with bladder cancer each year. Urothelial carcinoma (UC) of the bladder accounts for the majority of primary bladder cancers, with squamous cell carcinoma and adenocarcinoma accounting for less than 10%. The majority of bladder primary UCs are low-grade lesions and are typically treated with simple surgical excision. However, UC is associated with a high rate of recurrence (70%) and a distinct rate (10%-30%) of progression to high-grade lesions, carcinoma in situ or invasive UC, which necessitate frequent patient surveillance or cystectomy. The current standard for UC surveillance is cystoscopy in conjunction with urine cytology every 3 months during the first 2 years with decreasing frequency thereafter. Limitations of this surveillance algorithm include the need for frequent invasive evaluation of the bladder mucosa by cystoscopy, the relatively low sensitivity of urine cytology (around 34%) and the frequent occurrence of cytologically equivocal ("atypical") results. This has led to the development of many adjunctive assays for the detection of UC recurrence. UroVysion, which is a molecular-based test, is one of these assays. It is a multitarget FISH assay designed to detect chromosomal alterations associated with both low- and high-grade UC lesions. UryVysion is an FDA approved kit that detects aneuploidy for chromosomes 3, 7 and 17 as well as homozygous deletions of the 9p21 locus. It is intended for use in conjunction with current standard diagnostic procedures, as an aid for initial diagnosis of bladder carcinoma in patients with hematuria and subsequent monitoring for tumour recurrence in patients previously diagnosed with bladder cancer. We present the first Canadian clinical experience with UroVysion, with validation both manually and on the BioView, an automated FISH system.

Multi-colour karyotyping (MFISH) and array CGH investigation of ALL, AML and MDS cases with a normal karyotype.

Doug Horsman, Director, Cancer Genetics Laboratory, Department of Pathology and Laboratory Medicine, BC Cancer Agency (dhorsman@bccancer.bc.ca)

Introduction: Prognostic assessment and clinical management of acute hematologic leukemias and myelodysplasia syndromes are critically dependent on standard karyotype analysis. A large proportion of cases yield an apparently normal karyotype and novel investigations may be useful to identify chromosomal alterations as indicators of clinical outcome. We have undertaken multi-colour karyotyping (MFISH) and tiling-path array comparative genomic hybridization (array CGH) to determine if these new technologies can identify genomic alterations not visualized by standard karyotype analysis.

Cases and Methods: Twenty-five cases each of ALL and AML where studied by MFISH analysis; 26 cases of AML with normal or simple karyotype were studied by array CGH; 44 cases of predominantly low risk MDS (Refractory Anemia and RA with Ringed Sideroblasts with normal or simple karyotypes were assessed by array CGH.

Results: Intensive slide making and MFISH analysis of the ALL cases revealed an abnormal clone in the majority of cases. MFISH analysis of the AML cases did not

detect any abnormal clones. Array CGH analysis of AML cases revealed cryptic alterations in a minority of cases, whereas in the MDS cases a significant proportion of cases revealed cryptic chromosomal alterations.
Conclusions: MFISH analysis may be useful to detect clonal karyotypes in selected cases of ALL where the quality and quantity of metaphases is severely compromised. Conversely, MFISH analysis of AML does not provide additional information over standard karyotype analysis. Array CGH analysis may be a useful supplemental test to detect cryptic chromosomal alterations in myelodsyplasia.

Session II: Clinical Problems
Chair: Janette van den Berghe, Saskatoon

To the End of 8p and Back

Janette van den Berghe, Cytogenetics Laboratory, Royal University Hospital, Ellis Hall, 103 Hospital Drive, Saskatoon, Saskatchewan, S7S 0W8.

The short arm of chromosome 8 deserves careful attention by clinical cytogeneticists due to the large number of constitutional abnormalities in this region.
The inv dup(8)(p11.2p23) will be reviewed in which the genetic material reaches almost to the end of the chromosome and then turns back creating an area of terminal deletion and interstitial duplication.. The second most common abnormality in man the t(4;8)(p16.1;p23.1), reported to be mediated by olfactory receptor probes, is also in this region; likewise an interstitial duplication of 8p22p23.1, which is without clinical significance. Most cases with a terminal deletion of 8p do exhibit a phenotypic effect but one of the ones presented does not do so all the time.
The association of Kabuki syndrome with a molecular duplication in 8p will be discussed.

Introduction of QF-PCR as a rapid aneuploidy screen for all women undergoing amniocentesis: A pilot project

D. Allingham-Hawkins[1], E. Winsor[2], D. Chitayat[2], V. Cirigliano[3], A. Summers[1], K. Chun[1]
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Aneuploidies of chromosomes 13, 18, 21, X and Y are the most common abnormalities detected in prenatal specimens, representing ~70% of all chromosome abnormalities detected and ~77% of unbalanced abnormalities. Given that conventional chromosome analysis typically takes 10-14 days due to the need for cell culture, it is desirable to have a rapid, cost effective method of ruling out these common abnormalities thereby reducing patient anxiety. We are conducting a pilot study investigating the use of quantitative fluorescent PCR (QF-PCR) as a rapid aneuploidy screen for all women undergoing amniocentesis at two large prenatal diagnostic centres in Toronto, Canada. An initial validation study was performed on 200 blind specimens using the Aneufast^{TM} QF-PCR kit from Genomed (UK). These

included a variety of normal and abnormal specimens. We are subsequently performing QF-PCR on 1000 prospective specimens to assess the (1) sensitivity and specificity of QF-PCR compared to karyotyping; (2) failure rate of QF-PCR due to maternal cell contamination, ambiguous results or other factors; (3) turnaround time of QF-PCR from specimen receipt to report and; (4) cost of QF-PCR compared to FISH as a rapid prenatal aneuploidy screen. To date, analysis has been completed on >700 specimens. The results of the validation study will be presented as well as data on the specimens analysed to date with specific emphasis on the above stated outcome measures.

A large-scale evaluation of QF-PCR for the rapid prenatal screening of Down syndrome in Korea

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Quantitative fluorescent PCR (QF-PCR) using short tandem repeats (STRs) has been introduced as an alternative approach for the rapid detection of aneuploidies. This assay allows high sample throughput and is cost effective and efficient. In the recent studies, application of QF-PCR on a large number of prenatal samples has proved the reliability of the method for prenatal screening of common aneuploidies. Here, we report our experience with QF-PCR as a screening tool for rapid prenatal diagnosis of trisomy 21 on 4700 uncultured amniotic fluid samples.

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Case example: Nullisomy Xq or Monosomy 21?

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A peripheral blood specimen was received from a newborn girl with clinical indications listed as dysmorphism, isolated thrombocytopenia and hypoglycemia.
Karyotyping revealed 45,X, dic(X;21)(q13;p11.2). Further FISH studies confirmed the presence of both the X and 21 centromeres and the presence of XIST sequences in the dic(X;21) chromosome.
Parents' peripheral blood karyotypes were normal.
X-inactivation of the dic(X;21) would lead to functional monosomy of 21 whereas X-inactivation of the normal X homologue would lead to functional nullisomy of Xq in

these cells. Either of these might be expected to have a profound effect on phenotype.
A clinical examination of the child showed her birth weight, length and head circumference were all <5% centile. She had micropthalmia of the left eye, dysmorphic facial features including a slightly prominent glabella, mild brachcephaly, a small upturned nose with a low nasal bridge, short philtrum with full lips. She also had hand anomalies, namely, tapered fingers, small fingernails, an adducted right thumb.
However, thus far, this child has experienced relatively few health problems and is currently alive and well at nine months of age.
**Cytogenetic analyses by John Duck, Emiliya Demianuk.*

Marker Chromosome - inv dup(15): Review Cases, Potential Mechanism and Candidate Genes

Catherine Fen Li, Ron Carter, Jack Wang, Viola Freeman, Walter Scott, Thomas Fisker and Melodie Rathbone
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Marker chromosome is not uncommon, it occurs 1 in 2500 live births. Although marker chromosome can be derived from any human chromosomes, the majority is derived from chromosome 15. Inverted duplication of chromosome 15 is the most common marker chromosome in human. The clinical presentation of this marker chromosome ranges from normal individual to inv dup (15) syndrome, presented as developmental delay, mental retardation, seizures, and autistic behaviour. We will report a prenatal case of this marker chromosome in a 39-year-old woman undergone amniocentesis due to late maternal age, review published cases for this marker chromosome, and discuss the potential mechanism and candidate genes for this marker chromosome.

LEAN Cytogenetics: Experience from the Hospital for Sick Children

Robert Mulgrew (MLT), Cytogenetics Laboratory, Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto

In early 2006, the cytogenetics laboratory at the Hospital for Sick Children, along with a number of other departments, participated in the LEAN program with the aim of reducing/preventing sample backlogs.
The LEAN process challenges the way we work, asking us to identify and eliminate waste and variation so that each activity carried out creates value from the perspective of the patient. One of the key principles of LEAN is to improve the flow of work through the laboratory by eliminating waste, so that a sample is never just sitting, waiting for the next step in the process.
A focus group was established to identify the demand placed on the laboratory in terms of the numbers and types of tests requested. The entire laboratory process was then mapped, showing every activity carried out between the sample entering

the laboratory and a result being issued. From this process map, we identified areas that could be changed to improve work flow and different working groups were established to develop and implement these changes.
Many of the changes made have had a large impact on the way we all work, particularly in terms of promoting awareness of demand and achievement within the laboratory. Over this time period we have also seen a significant reduction in our blood sample backlog resulting in far quicker turnaround times for patients, despite the observation that the numbers of blood samples received has actually increased.

Prenatal detection of a familial KAL1 duplication by CGH array analysis

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Amniocentesis was performed due to ultrasound anomalies, including bilateral clefts and kidney malformations. Karyotyping and FISH for the KAL1 deletion were ordered by the clinical geneticist. Normal results were obtained. Fetal cultured cells and parental bloods were sent to Signature Genomics for prenatal chip array analysis. A maternally inherited duplication in the KAL1 region of the X chromosome was found in the fetal DNA; however the duplication could not be confirmed by FISH. Interphase FISH analysis of the KAL1 locus in the fetus, mother and maternal grandfather were performed at our hospital and the results, although not straight-forward, were consistent with a familial duplication. Since the maternal grandfather has a normal phenotype, the etiology of the fetal anomalies remains unknown. The parents elected to continue the pregnancy. This case illustrates the difficulty of confirming and interpreting prenatal microarray results. The technical and clinical issues associated with this case will be presented.

Factors Affecting the Growth of Amniotic Fluid Cells in Culture

Judith Reid, Technologist, Cytogenetics Laboratory, Children's Hospital of Eastern Ontario, Ottawa
Geoffrey Rowe, Senior Advisor, Microsimulation, Socio-Economic Analysis and Modeling Division, Statistics Canada
Jean McGowan-Jordan, Head, Cytogenetics Laboratory, Children's Hospital of Eastern Ontario, Ottawa

Many factors are thought to contribute to the success and rate of growth of amniotic fluid cell cultures. These may include gestational age, fluid volume and fluid quality. An investigation was done to determine why some amniotic fluids received by the CHEO Cytogenetics Lab grew more slowly than others, yielded fewer colonies and often required subculturing. Data collected over a 10 week period and analysed will be presented. A review of the current literature will also be included.

Careful what you FISH for!

Eva Cappa, Hana Sroka, Mount Sinai Hospital, Toronto

Case presentation of a 31 year-old primigravida women with increased nuchal translucency (4.1 mm AT 12w 3 days) and positive first trimester screening indicated a greater than 1/8 risk for Down syndrome and 1/36 for trisomy 13/18 (PappA- 0.33 MoM and free beta hCG 4.39). Patient declined CVS, elected to proceed with amniocentesis at 16 weeks gestation. FISH for aneuploidy ordered based on protocol and revealed probe signals compatible with trisomy 21 in a male fetus. Final chromosome analysis revealed....

Session III: New Technologies
Chair: Judy Chernos, Calgary

New Technologies in the Clinical Cytogenetics Service

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The history of clinical cytogenetics is relatively short, stemming from the establishment of the human chromosome number in 1956 which launched The Trisomy Period with the identification numerical anomalies associated with syndromes, on through the Banding Era during which structural changes were revealed with associated phenotypes. A period of relative *status quo* persisted until technical breakthroughs launched cytogenetics into the current Molecular period. The imminent demise of clinical cytogenetics was forecast with each new molecular advance. Instead, there has been renewed interest in the human karyotype and clinical cytogenetics has experienced resurgence.

New and evolving technologies are based on the fundamental principle of DNA double-helix complementarity. Basic FISH uses fluorescently labelled DNA probes complementary to target DNA to demonstrate the presence of a region of interest on metaphase or intact nuclei. Combinations of different fluorophore-labelled probes from whole chromosome libraries (M-FISH or SKY) or partial chromosome libraries (mBand) can detect cryptic rearrangements or the origin of additional material, including markers.

Variations on basic FISH include Primed *in situ* labelling (PRINS) which involves hybridization of primers on a slide with PCR amplification to detect and localize targets of interest; and Reverse FISH where DNA from flow-sorted or micro-dissected patient chromosomes is PCR-amplified and hybridized to metaphase chromosomes.

A further extension is based on quantitative differences between patient and control DNA co-hybridized to metaphase preparations (CGH) or DNA-spotted slides (microarray CGH) to detect genetic imbalances.

All these technologies have made cytogenetics a much more vibrant and colourful field in which to work.

Predicting outcome in follicular lymphoma using interactive gene pairs

Cheryl Foster, Tara Baetz, Patricia Farmer, Hong Guo, Roland Somogyi, Larry Greller, Karen Harrison, David LeBrun, Harriet Feilotter
DNA Diagnostic Laboratory, Kingston General Hospital, Queen's Microarray Facility, Queen's University

Follicular lymphoma is a common lymphoma of adults. While its course is often indolent, with long survival times, a substantial proportion of patients have a poor prognosis, often due to transformation to a more aggressive lymphoma that requires intensive treatment. Currently available clinical prognostic scores, such as the Follicular Lymphoma International Prognostic Index (FLIPI), are not able to optimally predict transformation or poor outcomes. We have used a statistically conservative approach, PIA (Predictive Interaction Analysis), to identify pairs of interacting genes that predict poor outcome, measured as death within 5 years of diagnosis. The evidence reported here may provide the basis for an expression-based, multi-gene test for predicting poor FL outcomes.

Microdeletion of distal 15q characterized by FISH and CGH microarray

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A 4 year 10 month old boy presented with short stature and micropenis. He was born at 38 weeks gestation weighing 6 lb. His growth velocity decelerated between 7 to 16 months with a minimal response to growth hormone therapy. His micropenis was responsive to testosterone. He had left cryptorchidism and congenital hip dysplasia. He sat at 10 months, walked at 2 years, and was late to talk. At 4 years 10 month, he was about 1 year delayed in cognitive skills. He had a height age of 2.5 year, bone age of 4 year and head circumference of 1 yr. There was a generous suprapubic fat pad, one café-au-lait spot and bilateral 5th finger clinodactyly. Family history was negative for developmental delay.
Routine G-banding analysis at ~500 band level showed a normal male karyotype. However, FISH using multiple subtelomeric probes (ToTelVysion, Vysis) identified a de novo 46,XY.ish der(15)t(14;15)(qter+,qter-) karyotype with the 15qter replaced by the 14qter. This was evidenced by the presence of the 14qter probe and absence of the 15qter probe in the der(15). CGH array analysis using CytoChip (BlueGnome) at ~0.8 Mb resolution with Roswell Park BACs and 90 regions for constitutional conditions showed no duplication of the 14qter region, which was inconsistent with the FISH result. This discrepant finding indicates that the Vysis 14qter FISH probe is distal to the CytoChip probe with no overlap between the two probe sets. The array detected a 15q26.3 deletion of 7 clones spanning 3,676,560 bp (genomic position: 96495118.00-100171678.00) and ~15 genes, including IGF1R. This study illustrates the importance of using a combination of FISH and CGH array analysis for characterization of submicroscopic rearrangements.

Interstitial Deletions of Proximal Chromosome 6q: A Study of 8 Patients Across Canada

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Objective: Interstitial deletions of chromosome 6q have been rarely reported. We report on eight cases of interstitial deletion of proximal 6q.
Subject: Eight patients from 7 families with interstitial deletion at 6q11-16 regions were collected across Canada. Five patients had *de novo* rearrangements; three patients from two families had recombinant chromosome 6, derived from between-arm intrachromosomal insertion of 6q13-14.2 to 6p21 in their parents.
Methods: Karyotyping and FISH analysis using a panel of 70 BAC probes were performed on the cultured peripheral blood.
Results: The molecular breakpoints differed from the breakpoints obtained from conventional karyotyping. Five patients with *de novo* deletions had varying breakpoints; however, the 3 patients with recombinant chromosome 6 possessed exactly the same breakpoints. The carrier parents in two independent families also revealed the same, but unexpected FISH signals pattern not previously identified. These findings suggested a common mechanism is responsible for this type of rearrangement. This study also supported previous findings that umbilical hernias and upslanting palpebral fissures are strongly associated with proximal 6q deletions. We narrowed down the critical region for umbilical hernia to a 7-Mb DAN region at 6q13-14.1.
Conclusions: Our studies demonstrate the utility of FISH analysis using labeled BAC probes in uncovering novel chromosomal rearrangements in intrachromosomal insertion, and its potential effect on recombination during meiosis. The high incidence of umbilical hernias in our patients suggests further investigation is required in order to identify potential gene(s) responsible for this phenotype.

Genetic Profiling of Brain Tumours

Karen Harrison, Dept of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario

Brain tumors remain a major cause of morbidity and mortality among patients with cancer. The clinical variability of gliomas is not fully captured using current histological parameters alone, leading to problems for accurate diagnosis and consequently, prognosis and therapeutic management. As a result, there has been a turn towards identifying and using molecular genetic markers that are specific to each type and grade of glioma. This is a promising area of research since many tumours are associated with non-random genetic alterations. New molecular technologies such as FISH, array CGH, expression microarray analysis and microsatellite analysis can all be used to identify genetic alterations. Research

investigating the use of genetic markers as a diagnostic technique not only aids in the further evolution of an objective classification system, but it also provides insight regarding the origin of gliomas. It is currently unclear whether the different prognostic outlooks for the tumour subtypes are a result of differences in underlying tumour development, or whether the subtypes simply represent stages in tumour progression or degree of genetic alteration. The importance of this distinction lies in the ability to tailor treatment to reflect our understanding of the genetic events that underlie the tumour phenotype.

Inherited Balanced Translocation: Unexpected Imbalance at the Molecular Level

Mary Shago[1,2,4], Lynette Penney[2,4], Christian Marshall[2,3], Jennifer Skaug[3], Chao Lu[3], Rosanna Weksberg[2,4], Stephen W. Scherer[2,3,4].
Cytogenetics Laboratory[1], Research Institute[2], and The Centre for Applied Genomics[3], The Hospital for Sick Children, and the University of Toronto[4].

When an apparently balanced translocation is inherited from a normal parent, the risk for phenotypic abnormality in the child is considered to be extremely low.
An apparently balanced chromosome rearrangement, t(9;12)(q22;q13) was identified in a developmentally delayed child referred for karyotype analysis. An identical rearrangement was detected in her clinically normal father. Analysis of the child's DNA on a 500K Affymetrix chip revealed a ~1Mb loss at the 9q22 translocation breakpoint. Follow-up FISH and quantitative PCR analyses confirmed the deletion in the child. FISH analysis of the father's chromosomes revealed a complex rearrangement that was not detectable by G-band analysis. The child inherited an unbalanced version of her father's chromosome rearrangement. Although the majority of inherited balanced translocations are likely simple, two-break rearrangements, detailed investigation may be warranted in some cases.

Microscopic Work Causing Microscopic Trauma

Stephanie Freeman, BSc. Kin
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Geneticists and Genetics technologists spend countless hours focusing on detecting microscopic pathologies yet rarely consider the microscopic trauma their job may be causing them. Due to the repetitive nature of many occupations, it is not uncommon for those who perform similar work tasks (microscopy and pipetting, for example) to present with similar injuries (such as those to their neck and upper extremity).
On the other hand, not all workers present identically, nor do all workers become injured.
Taking a closer look at these injuries assists in explaining these initially confusing findings by examining their mechanism of injuries and the corresponding pathological processes that occur. After identifying these injuries and reasons for their development, options for therapy and expected outcomes can be considered, respectively.

Finally, the goal of any therapy should contain a prevention component to avoid development of new injuries and recurrences of those previously experienced.

H. Allen Gardner Memorial Lecture

A "Bullseye" Approach: Targeting Plasma Cells in Multiple Myeloma

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Multiple myeloma (MM) is a B cell malignancy characterized by clonal expansion of plasma cells. Many MM patients achieve a complete remission by conventional criteria; however, patients eventually relapse as a consequence of residual disease (RD). Current approaches for the measurement of RD in bone marrow are based on morphologic assessment of bone marrow aspirate and biopsy, immunohistochemistry, flow cytometry, molecular studies and conventional cytogenetics (CC)/FISH. Morphologic assessment of MRD is often difficult due to the fact that normal plasma cells may also be present in the bone marrow and CC studies are hampered by the low proliferative nature of malignant cells. Clonal abnormalities of -13/del(13q), 14q32/IGH, del(17p), and hyperdiploidy (+5,+9,+15) have been reported in >80% of newly diagnosed MM cases; however, detection of these abnormalities post treatment by CC or standard FISH has proven to be very difficult in samples with less than 20% BM involvement. In this study, we investigated 114 post treatment samples collected from 88 MM patients (21 pts with multiple studies), all with less than 20% BM involvement, using a sequential May-Grünwald Giemsa (MGG) (morphology)/FISH approach to determine the plasma cell genotype (target or T-FISH). Cytospin slides were made using 200 l of BM and stained with MGG for morphologic classification on a Duet Image Analyzer (Bioview Ltd., Rehovot, Israel). After identifying and mapping the plasma cells, the slides were destained and hybridized with one of four FISH probe sets corresponding to the chromosome aberrations listed above. T-FISH results were correlated with CC, the BM pathology, which quantified the percentage of plasma cells in the BM aspirate, and BIOMED-2 PCR analysis for *IGH* (FR1, 2 and 3) and *IGK* gene rearrangements (InVivoScribe Technologies, San Diego, CA). Target FISH identified MM aberrations in 100/114 (87.7%) samples, a finding higher than the 56/71 (78.9%) positivity detected by combined molecular *IGH* and *IGK* PCR studies. The T-FISH aberrations observed were *IGH* in 58 samples, -13/del(13q) in 38, hyperdiploidy in 30, hypodiploidy in 6, and del(17p) in 2, with 30 samples showing more than one abnormality. Only 8 samples showed clonal karyotypic aberrations by CC; an additional 3 samples showed a presumed stemline with only one abnormal cell (9.8%). A comparison with the percentage of plasma cells in the BM smears showed T-FISH detected residual disease in all 42 samples with 6% plasma cells, 7 of 8 hemodilute samples, one smoldering MM sample and 77.8% (49/63) of the samples with 1-5% plasma cells. Our data indicate target FISH is a quick and robust assay to quantitate the number of neoplastic plasma cells in low-level disease. Furthermore, target FISH is an universally applicable, effective means of detecting MM regardless

of treatment status providing an exceptionally sensitive molecular cytogenetic strategy for investigating other low proliferative malignancies in the near future.