APPLICATIONS OF SKY IN CANCER CYTOGENETICS
Jane M. Bayani and Jeremy A. Squire*

Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, and Department of Laboratory Medicine and Pathobiology, and Medical Biophysics, Faculty of Medicine, University of Toronto, Ontario, Canada.

*Corresponding Author:
JA Squire, Ph.D.
Ontario Cancer Institute
Division of Cellular and Molecular Biology
610 University Ave. Room 9-721
Toronto, Ontario, Canada
M5G 2M9
E-mail: jeremy.squire@utoronto.ca

Key words: FISH, CGH, marker chromosomes, M-FISH, molecular cytogenetics

Acknowledgements:
The authors express their gratitude for the contributions of Ajay Pandita, Paula Marrano, Ben Beheshti, Jana Karaskova, Paul Park and Zong Mei Zhang at the Ontario Cancer Institute as well as Margaret Skokan, George McNamara and Randy Knudtson at Applied Spectral Imaging for technical information. Our gratitude also to Mark Israel for permission to cite recent findings concerning the murine oligodendrogioma model.
Abstract:

Clinical and cancer cytogenetics is a rapidly evolving discipline. The past decade has seen a dramatic change in molecular biology and fluorescence microscopy. The use of fluorescence in situ hybridization (FISH) technologies has enabled the rapid analysis of cytogenetic specimens as an adjunct to classical cytogenetic analysis. Spectral Karyotyping (SKY) is a 24-color, multi-chromosomal painting assay that allows the visualization of all human chromosomes in one experiment. The ability for SKY analysis to detect equivocal or complex chromosomal rearrangements, as well as to identify the chromosomal origins of marker chromosomes and other extrachromosomal structures, makes this a highly sensitive and valuable tool for identifying recurrent chromosomal aberrations. SKY has been applied to various tumor groups including hematological malignancies, sarcomas, carcinomas and brain tumors, with the intent of identifying specific chromosomal abnormalities that may provide insight to the genes involved in the disease process as well as identifying recurrent cytogenetic markers for clinical diagnosis and prognostic assessment. SKY has also been applied for the mouse genome, enabling investigators to extrapolate information from mouse models of cancer to their human counterparts. This review will address the advances that SKY has facilitated in the field of cancer cytogenetics, as well as its variety of application in the cancer research laboratories.

Introduction

Cytogenetics is a discipline, like so many in molecular biology today, that is undergoing dynamic change in both technology and application. Hungerford et al. (1), made the first karyotype from peripheral blood cultures in 1959 using, by present standards, relatively crude techniques, but demonstrated the value of chromosome analysis in both the clinical and research laboratories. Over the years the use of cytogenetic analysis has enabled the identification of recurrent chromosomal abnormalities that are diagnostic markers for cancers including hematological malignancies and solid tumors (2). Early karyotype analysis of many types of malignancies identified complex structural rearrangements and extra-chromosomal structures that were left unidentifiable and simply termed "marker chromosomes" (Figure 1). By the late 1980s, cytogenetics was a mature discipline and underwent more extensive technological change as molecular genetic technologies were applied to cytogenetic preparations.

Fluorescence in situ hybridization (FISH) quickly replaced radioactive in situ assays by the late 1980s (3,4). The hybridization of fluorescently labeled or fluorescently detectable probes from known genes and specific chromosomal loci enabled the genome to be studied in a completely new way. The advancement of recombinant DNA technology permitted newly identified genes to be mapped to chromosomal regions, and mapped relative to other genes (5).
Figure 1. G-banding analysis of an astrocytoma cell line (U373). Arrows indicate abnormal chromosomes detected by banding analysis as well as marker chromosomes. The banded karyotype is as follows:

73,XY,+Y,+Y,+Y,der(1)t(1;?)p11;?),t(?)(q43;?),1,der(1)t(1;?)p11;?),t(?)(q43;?),2,add(5)(p10),-6,+7,+der(7)t(7;?)q32;?),
der(9)t(9;?)q10;?),-10, dup(10)(?p12p15),+der(12)t(12;?)p10;?),+13,-14,del(15)(q26.1),der(16);?(p10;?),der(18)t(6;18)(p11.2;q11.2),-22,mar1,mar2,mar3.

The SKY karyotype (data not shown) is as follows: 73,XXY,+Y,+Y,+Y,inv(1)(p21;q44),+der(1)del(1)(p22)t(1;8)(q41;?),der(4)t(4;5)(p11;p12)t(4;15)
(q31;q24),der(5)t(5;12)(p12;p12),6,+7,+der(7)del(13;15)t(7;8)(q32;?),t(9;16)(p21;p11),-10, dup(10)(?p12;p15),+der(12;17)(p12;q12),+13,-14,del(15)(q26.1),+del(17)(q12),der(18)t(6;18)(p11.2;q11.2),-22.

Chromosome descriptions in bold describe the revised SKY/cytogenetic description. Underlined descriptions indicate aberrations determined by SKY analysis, not previously detected by banding.
As interphase FISH techniques were applied, the genomic information sequestered in the nuclei could now be accessed, and provide important information on the status of locus/gene specific probes when metaphase spreads were few or not available (6,7), thus overcoming one of the many limitations of classical cytogenetics: the requirement for good quality metaphase preparations (Figure 2).

**Figure 2.** Fluorescence *in situ* hybridization (FISH) on a primary neuroblastoma specimen. **A.** An interphase nucleus showing amplification signals using the *MYCN* gene. The amplification signal pattern seen in this nucleus is indicative of double minute chromosomes as seen in **B.** **B.** A metaphase preparation from the same case showing the paired extra-chromosomal structures hybridized with the *MYCN* probe.

Interphase FISH also enabled retrospective study of large archives of histopathologic paraffin sections for analysis of aneusomies and for subsequent correlative comparisons with established tumor markers (8-10). One of the technically most demanding of the various new FISH technologies is Comparative Genomic Hybridization (CGH) (11-14). Since only DNA is required, CGH is ideal for large retrospective surveys of genomic imbalance and has the potential to locate regions that harbor potential oncogenes in regions of gain, and novel tumor suppressor genes in regions of loss (15,16). Furthermore, it has also been shown to be a useful adjunct to classical cytogenetic methods.
Spectral Karyotyping (SKY)

The advent of the various multicolor FISH techniques, generically termed M-FISH, (17-19) has greatly improved the certainty with which cytogeneticists are able to identify abnormal chromosomes. Presently, there are two methods of performing M-FISH, one based on the use of specific filter sets (17) and the other based on the spectral signature of the fluorochromes or dyes used and termed Spectral Karyotyping (SKY) (18,19). Currently the most popular method of performing this type of analysis is Spectral Karyotyping (SKY) (19). SKY involves the use of 24-color, whole chromosome-painting permitting visualization of each chromosome in one experiment. This technology is based on the principles of spectral imaging (20) and Fourier spectroscopy (21). Flow sorted chromosomes are PCR-labeled (22), either directly or indirectly, with fluorochromes or haptens. Five pure dyes that are spectrally distinct are used in combination to create the unique chromosome cocktail of probes. This probe cocktail is hybridized to metaphase preparations and detected using sophisticated image analytical methodologies. Image acquisition is accomplished by conventional fluorescence microscopy and the use of a specially designed triple filter (SKY CUBE™, Applied Spectral Imaging). In this design, light passes through a Sagnac interferometer focussed on a charged-coupled device (CCD) (Figure 3). Spectral images are acquired and analyzed with the commercially available SD 200 Spectral Bio-Imaging System (ASI Ltd., MigdalHaemek, Israel). The generation of a spectral image is achieved by acquiring ~100 frames of the same image that differ from each other only by the optical path difference (OPD). The collected images are Fourier Transformed and the data sorted in the software (SKYVIEW™). Each chromosome has a unique spectral "signature", generated by the specific combination of one or more of the five pure dyes. Once a spectral image is acquired, the SKYVIEW™ software compares the acquired spectral image against the combinatorial library containing the fluorochrome combinations for each chromosome and generates a "classified" image. The classified image pseudo-colors the chromosomes to aid in the delineation of specific structural aberrations where the RGB (Red-Green-Blue) display image, which displays the fluorescent colors of the chromosomes may appear quite similar. For every chromosomal region, identity is determined by measuring the spectral emission pixel by pixel. Regions where sites for rearrangement or translocation between different chromosomes occur are visualized by a change in the display color at the point of transition.
Figure 3. Schematic representation of image acquisition using an interferometer housed in the optical head (Applied Spectral Imaging, Carlsbad, CA). Images from the hybridized specimen are collected, Fourier transformed and processed.

SKY has enabled the elucidation of complex or equivocal structural aberrations that may otherwise have been left undetected by classical cytogenetics or FISH alone (Table 1).

Each assay has particular strengths, but many also possess limitations in certain situations. One of the most obvious limitations of SKY is its inability to readily detect deletions or other intrachromosomal structural changes such as inversions. However there are some compelling reasons for the systematic use of SKY in the analysis of abnormal chromosome preparations of the type encountered in cancer cells. The ease of interpretation of the clearly assigned color patterns means that it is not essential to have a highly experienced metaphase analyst to perform the microscopy. In addition the acquired images can be analyzed objectively so that subtle translocations as small as ~2 megabases of DNA can be detected (23). Such a small chromosomal region would be difficult to detect with certainty by conventional banding analysis. SKY therefore provides a method for rapid high-resolution screening of the cancer karyotype and has applications both in the research and clinical cytogenetics laboratory. In the four years since the first SKY report (19), there have been almost 100 publications illustrating how SKY has helped delineate aberrations and improve our understanding of human disease processes.
**Table 1. Comparison between molecular cytogenetic techniques.**
The advantages and limitations of conventional banding, Spectral Karyotyping, Comparative Genomic Hybridization and Fluorescence *in situ* Hybridization.

<table>
<thead>
<tr>
<th>Description</th>
<th>G-Banding Analysis</th>
<th>FISH*</th>
<th>SKY</th>
<th>CGH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identifies chromosomal aberrations</strong></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>• translocations (balanced)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• translocations (unbalanced)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• copy number changes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• additions (&gt;10MB)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• deletions (&gt;10MB)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• deletions of specific genes/loci</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>sometimes</td>
</tr>
<tr>
<td>• inversions</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• insertions</td>
<td>sometimes</td>
<td>yes</td>
<td>sometimes</td>
<td>no</td>
</tr>
<tr>
<td>• identifies presence of markers</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>sometimes</td>
</tr>
<tr>
<td>• distinguishes between double minutes and homogeneously staining regions</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>• identifies chromosomal origins</td>
<td>sometimes</td>
<td>yes</td>
<td>yes</td>
<td>sometimes</td>
</tr>
<tr>
<td>• identifies specific p/q arms/bands</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>sometimes</td>
</tr>
<tr>
<td>Requires specifically labelled probes</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>• more than 2 probes at one time</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Requires viable material</td>
<td>yes</td>
<td>sometimes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Requires metaphase spreads</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Applicable to interphase nuclei</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Applicable to DNA extracted from paraffin embedded tissue</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Affected by normal tissue contamination</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Identifies tumour heterogeneity</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Applicable for studies in other species</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

*FISH requires the use of loci specific probes to detect the aberration of interest.*

**Hematological Malignancies**

The vast majority of cytogenetic data derived from human diseases has come from the study of hematological malignancies (2,24,25). With the relative ease of access to blood and bone marrow samples, for both initial diagnosis and monitoring, a wide variety of different analyses can be performed. The cytogenetic study of hematological malignancies is particularly important since the presence of recurrent and specific chromosomal aberrations are etiologically, diagnostically and prognostically significant (2,25). Among the first papers, describing SKY analysis, to be published was a study of hematological malignancies, including CML, AML, MDS, and ALL (26). In this report, SKY
analysis confirmed initial rearrangements, identified the chromosomal origins of marker chromosomes as well as uncovered translocations not detected by banding analysis. The improved sensitivity in detecting these subtle chromosomal translocations demonstrated the immediate value of SKY analysis.

Recent publications describing the cytogenetics of chronic myeloid leukemia (CML) have shown the presence of other cytogenetic abnormalities in addition to the hallmark Philadelphia translocation (9;22) (26-28). Markovic et. al. (27), examined a series of variant Philadelphia translocation cases by cytogenetics, FISH and SKY. Among the patients studied, none indicated the presence of the classic (9;22) translocation together with a variant translocation, suggesting that these variant rearrangements developed de novo and concurrent with the onset of disease. Figure 4 shows an example of a variant CML case.

Studies of acute myelogenous leukemia (AML) by SKY have revealed the frequent involvement of chromosomes 5, 7 in rearrangements and translocations (26,29,30). Interestingly, a similar result was found among myelodysplastic syndrome (MDS) cases (26,31-33). MDS is a myeloid disorder usually resulting as a secondary malignancy following chemo- or radiotherapy (34,35). Giemsa banding (G-banding) analysis of MDS has detected the frequent losses of chromosomes 5 or 5q as well as losses of 7 or 7q. The application of SKY analysis to these tumors, have revealed that these losses, while frequent, are also involved in unbalanced and cryptic translocations. Kakazu et al.. (31), published 20 MDS cases comparing banding analysis and SKY. G-banding analysis revealed the expected frequent losses of 5/5q and 7/7q. However, SKY analysis revealed that in some samples, the deletions were not simple interstitial losses, but were shown to be cryptic, unbalanced translocations or additions, leading to deletion of 5q or 7q.

![Figure 4](image_url)

**Figure 4.** SKY analysis of a variant CML case. A. Shown is the Red-Green-Blue (RGB) display of the hybridized metaphase spread. Arrows indicate aberrations detected by SKY. B. The SKY karyotype using the classified (pseudo-colors) colors and the detected aberrant chromosomes.
The use of SKY has been applied to other hematological malignancies including the B- and T-cell neoplasms (36-39) and multiple myelomas (40,41). Sawyer et al. (41) identified 6 multiple myeloma patients with a novel recurrent t(14;16)(q32;q22 approximately 23). In a similar study, Rao et al. (40) also identified the recurrent breakpoint site at (14)(q32) in their patient group. The use of SKY analysis, therefore, demonstrates the ability to refine karyotypic analysis and identify potentially new recurrent translocations.

**Sarcomas**

Sarcomas are defined as tumors arising from bones, muscles, fibrous tissues, and some organs. As with hematological malignancies, specific chromosomal aberrations are often associated with a particular histological subtype leading to fusion oncoproteins. The ability to detect additional acquired and/or recurrent aberrations associated with recurrence, survival, response to therapy or stage is invaluable. To date, SKY analysis has only been applied to a small number of sarcomas.

Rhabdomyosarcoma (RMS) is a tumor derived from skeletal muscle and is comprised of two major subtypes (42). Pandita et. al.(12), compared established embryonal and alveolar cell lines by SKY and determined that the alveolar cell line SJRH30 exhibited a higher degree of chromosomal rearrangement than the embryonal cell line RD. This suggested that the alveolar cell line possessed an increased level of genomic instability, possibly explaining the less favorable outcome in this subtype.

More rare tumors such as alveolar soft part sarcomas (ASPS) have also been studied using SKY. The literature cites chromosome 17q25 as a site of frequent cytogenetic classification of an ASPS with the sole abnormality of an add(17)(q25) to der(17)t(X;17)(p11.2;q25) following SKY analysis. A previous report by Heimann et.al(44) also identified a t(X;17)(p11;q25) in an ASPS, using a series of two and three-color FISH experiments to confirm their results. This example highlights the advantages of SKY in enabling the identification of all translocations in one experiment, rather than in a series of experiments that are both time consuming and exhaustive of precious cytogenetic samples.

Synovial sarcomas, characterized by a t(X;18)(p11.2;q11.2) and Ewings sarcomas, characterized by a t(11;22)(q24;q12) have also been analysed by SKY(47,48) to detect recurrent aberrations in addition to those described. An interesting report by Cohen et al. (47), described a bone tumor that was originally diagnosed as a primitive neuroectodermal variant of a Ewings sarcoma by immunohistochemistry and histopathology. The G-banding analysis revealed an unbalanced translocation between chromosomes 1 and 22 as well as additional material on the X chromosome. SKY confirmed the der(1)t(1;22)(p13;q12?) and identified the aberrant X as a balanced translocation between X and 18: t(X;18)(p11.2;q11.2). SKY analysis, therefore, helped to identify the bone tumor as a synovial sarcoma, rather than the original description of a primitive neuroectodermal variant of a Ewings sarcoma.
The analysis of osteosarcomas and other tumors of the bone will benefit greatly from SKY analysis. It is well known that the karyotypes of osteosarcomas are frequently complex and highly aneuploid (49-51). In many cases, only partial karyotypes can be accomplished due to the presence of numerous structural aberrations and extra-chromosomal structures including double minute chromosomes (dms), homogeneously staining regions (hsrs) and ring chromosomes. The lack of identifiable banding patterns makes traditional cytogenetic analysis challenging. The lack of a consistent chromosomal aberration makes FISH analysis also difficult. SKY studies of osteosarcomas in our laboratory and others (Zielenska et al. submitted, Boehm et al. submitted) have identified numerous complex rearranged chromosomes often involving more than 5 different chromosomes within the structure. It has enabled the identification of ring chromosomes and small marker chromosomes. Although every chromosome appears to be involved in chromosomal translocations, there is an apparent preferential occurrence of rearrangements involving 1q21-22, 1q41-44, 11p15, 12p13, 17p, 19q13 and 22q11-13.

Among the more benign bone tumors, chondromyxoid fibromas are rare tumors that are histopathologically difficult to distinguish from other cartilaginous neoplasms. A cytogenetic and SKY study of two chondromyxoid fibromas by Safar et al. (52), identified clonal abnormalities of chromosome 6 [t(6;9)(q25;q22)] but at a breakpoint on the long arm distal to a pericentric inversion of chromosome 6 [inv(6)(p25q13)], that has been proposed as a specific genetic marker for chondromyxoid fibroma (53).

Carcinomas

Carcinomas, like the sarcomas, reveal highly aneuploid and grossly rearranged karyotypes. The SKY literature of both primary tissues and established carcinoma cell lines is rapidly increasing. A number of publications have used SKY for the study of oral carcinoma (54), hepatocellular carcinoma (55,56), colorectal carcinoma (57), bladder cancer (58), pancreatic carcinomas (59), breast (60,61) and cervical carcinoma (62). The use of cell lines for SKY studies is facilitated by the ease of culture conditions and optimizing sample quality. However many cell lines are intrinsically unstable and clonal isolates may exhibit quite divergent or heterogeneous karyotypes at higher passage numbers.

Prostate carcinoma has been intensively studied over the past few years, particularly in the field of early detection. Chromosomal aberrations that are detected in early stage prostate cancer could serve as markers for diagnostic testing. Many studies make use of established cell lines from high grade and metastatic tumors. Two publications describe the cytogenetic changes in the prostate cancer cell lines PC-3, DU145 and LNCaP (63,64) by SKY. Relatively consistent results were obtained between the two studies and additional changes not apparent by G-banding were present but neither study was able to identify a recurrent or consistent aberration common to all three cell lines. Macoska et al. (65) examined the karyotypes of 4 virally transformed low-grade prostate tumors
using SKY and screened for loss of heterozygosity of 8p by allelotyping. The results indicated the preferential loss of 8p sequences as well as gains or aberrations of 8q among the tumors. These losses of 8p sequences could be directly correlated to the disruption of chromosome 8p as determined by SKY analysis. Other common chromosomal aberrations involving 11q13,q22,q23 were detected in four HPV-immortalized cell lines (normal epithelium and prostate tumors), believed to be a result of the viral transformation.

Another tumor group under intensive study, are the lung cancers. Dennis and Stock (66) analysed a primary small cell lung carcinoma (SCLC) and 5 SCLC cell lines by FISH and SKY. Their results describe the consistent breakpoint on the long arm of chromosome 3 at band 3q13.2 in a variety of aberrations. Luk et al. (67) compared a non small cell squamous lung carcinoma (NSCLC) and adenocarcinoma cell line by SKY and identified the presence of more complex chromosomal rearrangements that were detected by CGH or banding alone. The squamous cell line was characterized by more chromosomal aberrations involving chromosomes 3, 5, 6, 7, 8, 10, 11, 13 and 16 as compared to the adenocarcinoma cell line, which generally exhibited numerical changes.

Ovarian carcinoma is an aggressive tumor with a poor prognosis (68,69). Cytogenetic analysis of these tumors demonstrate highly aberrant karyotypes (70-72) with many random and non-random changes. A new molecular cytogenetic study in our laboratory on primary ovarian carcinomas has revealed a pattern of complex clonal chromosomal aberrations consistent with tumors presenting as advanced disease (Figure 5). A study by Bible et. al (73), using an ovarian cell line, investigated the use of Flavopiridol. Currently undergoing Phase II testing, it is the first inhibitor of cyclin-dependent kinases to enter clinical trials. The study attempted to discover the mechanisms of resistance in a high passage (hp) ovarian cancer cell line OV202. SKY was used to confirm the relatedness between the high passage line and the parental line. In a similar experiment, Knutsen et. al. (61) conducted molecular cytogenetic studies on three multidrug-resistant cancer sublines (two derived from MCF7 and one from LS174) that were highly resistant to the chemotherapeutic agent mitoxantrone, and anthracenedione. The amplification of 4q21-q22 and the MXR gene in independently derived mitoxantrone-resistant cell lines was identified as well as the participation of 4q21-22 in translocations.
Figure 5. SKY analysis of a primary ovarian carcinoma specimen. A. Shown is the RGB display of the hybridized metaphase with arrows indicating the rearranged chromosomes. B. This panel illustrates the inverted DAPI banding of the same metaphase to help identify chromosomes in a manner similar to G-banding. The right panel shows the aberrant chromosomes as detected by SKY analysis. Shown from left to right, are the RGB, DAPI, and classified chromosomes as well as their chromosomal origins. The classified colors help to better identify chromosomal aberrations when the RGB display colors are similar (as in the case of the t(4;8)).

Brain Neoplasms and Tumors of Neuronal Origin

Brain tumors are a poorly understood and diverse group of tumors presenting with extraordinary difficulties in management compounded by a relatively rudimentary understanding of their genetic basis. Prognosis is frequently difficult to predict and the diagnosis itself is often equivocal due to the variable nature of presentation. To date, no recurrent chromosomal aberration has been identified in brain neoplasms and tumors of neuronal origin. SKY studies of brain tumors are relatively few (74), but evidence suggests more consistent cytogenetic abnormalities will be detected by this approach. Bayani et al. (75) analysed 19 medulloblastomas and 5 supratentorial PNETs in a retrospective and prospective study using classical banding, CGH and SKY. They revealed that these histologically similar tumors were more heterogeneous than previously thought, with SKY providing clearer evidence of intratumor cytogenetic heterogeneity suggestive of chromosomal instability (see below). The combined data from all three techniques identified chromosomes 7 and 17 as most frequently altered. Chromosomes 3, 6, 10, 13, 14, 18 and 22 were also found to be frequently involved in gains, losses or chromosomal rearrangements. No consistent chromosomal aberration/translocation was detected in this series,
however this study demonstrated the presence of more aberrant karyotypes among the supratentorial PNETs, desmoplastic and large cell variants of medulloblastoma in comparison to the classical medulloblastoma subtype.

Analysis of glial tumours including astrocytomas, glioblastoma multiforme (GBM), glial cell lines, gangliogliomas and an ependymoma has also been carried out by (Squire et al. In press). SKY helped to refine complex karyotypes and identified chromosomes 3, 5, 7 and 11 to be frequently involved in chromosomal rearrangement, although no consistent chromosomal translocation was detected. Furthermore, SKY helped to illustrate that the astrocytic tumours showed more karyotypic complexity as compared to the gangliogliomas and ependymomas.

There is a better understanding of the cytogenetics of neuroblastoma but there still remain some uncertainties in assessing prognosis (76). Trakhtenbrot et al. (77), described a patient with stage IV neuroblastoma and bone marrow, liver, bone and subcutaneous metastasis. SKY analysis enabled the refinement of misclassified chromosomes by G-banding, including the detection of an unbalanced translocation +der(1)t(1;12)(p13;q11-12) that was originally described as del(1)(p34.1).

**SKY Analysis of Genomic Instability**

The stability of the genome is governed by cell cycle checkpoint pathways and defects in the genes involved can lead to chromosomal instability. Many of the cytogenetic changes in carcinomas are thought to arise as a result of segregation defects during mitosis leading to an increase in the number of numerical changes in the karyotype (78,79). SKY can provide more precise information concerning both numerical and structural changes when genomic instability is suspected. A recent detailed study of *in vitro* evolution in prostate cancer cell lines, demonstrated that SKY could distinguish considerable tumour heterogeneity and identify *de novo* chromosomal aberrations that arise during maintenance *in vitro* (Beheshti et al. Accepted).

The effects of ionizing radiation, on both tumor cells and surrounding normal tissues, are also well suited for detailed SKY analysis. Zitzelsberger et al. (80) analysed childhood thyroid carcinomas that were induced after the Chernobyl nuclear accident in 1986 as well as those that resulted after radiotherapy. Their study detected chromosomes 1, 2, 9, and 13 to be most commonly affected, with more frequent breakpoints at 1q, 4q, 5q, 6p, 10q, 12q, 13q, and 14q. Similar results were obtained in our laboratory (unpublished), where normal human lymphocytes from a number of individuals were exposed to low-level ionizing radiation. The cells were analysed by SKY to gauge intrinsic levels of radiosensitivity. In many cases, complex chromosomal changes were detected (Figure 6), ranging from balanced and unbalanced translocations, to insertions and deletions. These studies illustrate the growing application of SKY in both sporadic and induced genomic alteration, and the field of radiosensitivity.
Figure 6. SKY analysis illustrating radiosensitivity experiments carried out on normal male lymphocytes. Normal male lymphocytes were cultured, exposed to therapeutic levels of radiation and continued in culture for 42 hours. The cells were harvested and fixed for cytogenetic preparations and hybridized to the SKY Paints™. Shown in the inset are the inverted DAPI and classified chromosomes that were found to be aberrant.

Murine SKY Analysis and Detection of Recurrent Aberrations

SKY procedures and specific probe cocktails is available for murine analysis (81). SKY can be used to analyze any metaphase target, provided pure populations of sorted chromosomes can be obtained. The differential labeling of mouse chromosomes greatly facilitates karyotyping of the similar looking, acrocentric mouse genome (Figure 7). The mouse has been an important model organism for understanding human diseases and cancer. The ability to create transgenic animals to recapitulate the mouse form of specific human diseases has given investigators excellent in vivo model systems for investigating novel therapies and to study the disease process. The progress of the Human Genome Project has also facilitated the sequencing of the mouse genome. Syntenic maps between the mouse and human genomes have been developed and are continuously being refined. Mapping of mouse genes have recognized that gene clusters found in humans have identical gene orthologues in the same configuration in the mouse (82). The manipulation of genes in the mouse can
have profound effects on development as well as on specific tissues and organs often resulting in a greatly increased incidence of cancer. The consequences of these alterations may be alluded to in their karyotypes, thus giving us an insight as to the potential aberrations that could be expected in the human genome. A number of investigators have taken advantage of this technology (81, 83-91) for a variety of studies.

Coleman et al. (84) used SKY analysis to look at the divergent cytogenetic natures of the prototypical bilineage lymphoblastic pre-B lymphoma cell line, P388, and the progenitor macrophage-like tumor line P388D1. The apparently complex and triploid karyotype of the P388D1 cell line was interpreted, by the authors, to indicate that the myeloid differentiatitional program in the bipotential pre-B cell lymphoma P388 is characterized by genomic instability.

Figure 7. SKY analysis of a murine oligodendroglioma cell line. This tetraploid line exhibited clonal aberrations, among them, the loss of chromosomes 7, 8 and a translocation between chromosomes 11 and 15. A. Cytogenetic preparations from the mouse line were hybridized with the Murine SKY Paints™ and analysed using the SKYView software. Arrows indicate chromosomal translocations. B. RGB and inverted DAPI of the aberrant chromosomes. Shown is a non-clonal aberration detected involving chromosomes 5 and 19, and the clonal t(11;15) aberration.

Preliminary studies using an oligodendroglioma mouse model (personal communication, Dr. Mark Israel) suggest that the murine system accurately
represents human oligodendroglioma both histologically and cytogenetically. Using SKY (Figure 7), a mouse oligodendroglioma cell line was analyzed to detect chromosomal aberrations. These abnormalities serve as an entry point for identifying syntenic regions in the human for specific disease loci. Human oligodendrogliomas are characterized by losses of genomic sequences at 1p and 19q(92,93). The corresponding chromosomal locations in the mouse map to mouse chromosomes 4 (human 1p) and mouse chromosome 7 (human 19q). The illustrated cell line is hypotetraploid with clonal losses including the loss of mouse chromosome 7, consistent with the loss of 19q in the human tumor. Additional structural changes were detected, whose significance are yet to be determined.

**Evolutionary Studies**

SKY has not only been used for the cytogenetic analysis of aberrant cell lines, tumors, transgenic mouse cells or for sensitivity to ionizing radiation and chemo-toxins, but also for comparative studies among other primates (94,95). Best et. al. (94) used the human SKY probes for hybridization to baboon metaphase spreads. Their results showed that the SKY results were consistent with the majority of comparative gene mapping data between the two species and that these data were also compatible with earlier studies comparing macaque and human chromosomes. In another comparative study Moore et al. (95), conducted cytogenetic studies of a rheboon, the result of a mating between a male rhesus macaque and a female baboon, to determine whether one numbering system for the cytogenetics of baboons and macaques should be adopted. The rheboon karyotype showed the expected 42 chromosomes with each homologue identical to each other, despite the difference in parentage. Furthermore, hybridization with the human SKY probes was identical to the SKY karyotype generated for baboons by Best et. al(94). This data supported the inferences regarding chromosomal homology between the primates. From an evolutionary standpoint, it is noteworthy that most of the rheboon chromosomes hybridized to probes derived from a single human chromosome with only a few rheboon chromosomes comprised of more than one human chromosome.

**Conclusions**

Cytogenetics is the study of chromosomes and the detection of chromosomal aberrations. In neoplasia, diseases of inheritance and constitutional aberrations, genetic changes occur, and in some cases, these genetic changes can be detected at the resolution of cytogenetic analysis. The use of advanced molecular cytogenetic assays such as FISH, CGH and SKY, have allowed an increased sensitivity for the detection of such aberrations. The use of SKY, is primarily in a research application (Figure 8) with a growing presence in translational research programs. As the costs of equipment and reagents decline, SKY will become an integral part of the routine cytogenetic
diagnostic laboratory. From the selection of diverse publications that have been reviewed, it is obvious that molecular cytogenetic analysis has an impact on the study of human cancer, disease progression/outcome, drug and radiosensitivity. The technology has enabled researchers to view the entire genome at one time and this comprehensive analysis provides important clues concerning genome stability and the acquisition of cytogenetic aberrations at both the initialization and progression phases of tumorigenesis.

8. SKY Analysis and Other FISH-based Technologies in the Research and Clinical Laboratories

**Figure 8.** The use of Spectral Karyotyping and other FISH-based technologies in the research and clinical laboratories. Molecular cytogenetic analysis helps to bridge the findings from varying disciplines within routine diagnostics and research.
References:


