

Characterization of Chromosomal Abnormalities in Cancer by Spectral Karyotyping

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ABSTRACT

Spectral karyotyping is a novel method for the simultaneous visualization of the entire chromosomes of an organism by painting the chromosomes using a combination of fluorochromes. This allows improved identification of chromosomal aberrations that cannot be identified by conventional banding methods. Since introduction of cancer as a disease of the genome, researchers have employed various molecular techniques for a better understanding of malignancies. This review discusses the role and contributions of spectral karyotyping in the study and characterization of both solid and hematological malignancies.

Keywords: Spectral karyotyping; Neoplasms; Chromosomes.

INTRODUCTION

The first report of human chromosomes was made in 1882 by Flemming, in which 22–28 chromosomes in the dividing cells of the corneal epithelium were described (1). In 1922, Painter reported that the number of human chromosomes was 48 and that sex was determined according to the presence or absence of the Y chromosome (1). The report of “ $2n = 46$ ” made in 1956 by Tjio and Levan established the nature of human chromosomes (1). Following this report, the relationships between various congenital disorders and chromosomal abnormalities were being revealed one after another (2). In 1959, Lejeune et al. (3) demonstrated that Down syndrome is caused by the presence of an extra chromosome 21, i.e., trisomy (4). This was a turning point in the subsequent discovery of other trisomy syndromes, including chromosomes 18 (5,6), 13 (7), 8 (8), and 22 (9,10). Besides the chromosomal number aberrations, abnormalities associated with sex chromosomes, such as Turner syndrome (11), Klinefelter syndrome (12) and other chromosomes (6,13) were reported.

Several techniques have been developed for the identification and study of chromosomes. In 1971, Caspersson et al. developed Q-staining using quinacrine mustard, a fluorescent dye that binds to DNA, which was instrumental in the discovery that chromosomes have banding patterns (stripes) (14). Later, improved staining techniques, including R-, T-, C-, and G-staining, were developed (2). The analysis of bands has further advanced into high-resolution chromosome banding (15). Later, the development of fluorescent *in situ* hybridization technique (FISH) enabled detection of chromosomal microdeletions and structural abnormalities (16). More recent FISH-based techniques such as comparative genomic hybridization (17) and spectral karyotyping (SKY) (18) have relatively improved chromosomal analysis capability.

Theodor Boveri projected that cancer is caused by chromosomal derangements that cause cells to divide uncontrollably (19); in modern terms, cancer is a “disease of the genome”. Researchers have established two main types of cancer-causing genes (oncogenes and tumor suppressor genes) and the genomic alterations that give rise to them (e.g., nucleotide substitutions, chromosomal copy number

alterations, and DNA rearrangements) (20). These studies also began to suggest considerable complexity in the mutational origins of cancer, with cancer-causing genes varying across and within tumor types and with multiple genes contributing to tumorigenesis (21).

An increased understanding of malignancies and the advent of improved cell culture techniques led to the discovery of the Philadelphia chromosome, a chromosomal abnormality specific to chronic myelogenous leukemia (22). Subsequently, mutations were discovered in melanoma (23), colon (24) and lung cancer (25). The US National Cancer Institute launched the Cancer Genome Atlas (TCGA) in 2009; concordantly, an International Cancer Genome Consortium was established with contributions from researchers from over 15 countries (26).

Principle of SKY

Two techniques are used as the basic principles of FISH: chromosome painting and multicolor fluorescence. The former involves drawing an entire image of certain chromosomes using fluorescent signals, while the latter involves drawing images of several hybridization signals with different fluorescent dyes. In 1996, Schrock et al. developed the SKY technique by combining these two techniques (18).

In SKY, the color emission of chromosomes is determined by combining painting probes and fluorescent dyes. In this technique, new colors can be developed by extracting a pair of different fluorescent dyes from the five fluorescent dyes used in this technique including Spectrum Orange, Texas Red, Cy5, Spectrum Green and Cy5.5, and mixing each pair together. Thus, it is theoretically possible to create $2^n - 1$ number of colors from n types of fluorescent dyes. Therefore, 31 color types can be created from five types of fluorescent dyes (Figure 1). In reality, however, because some fluorescence has a wavelength close to that within the infrared spectrum, a two-dimensional imaging spectroscopy system (e.g., the Spectral Bio-Imaging System SD-200, Applied Spectral Imaging Ltd. Israel) needs to be used to process spectral images so that 24 macroscopically distinguishable colors can be created (2). The amount of samples required for chromosome testing for regular congenital abnormalities is about 3.0 ml of

heparin-treated blood and about 1.0 ml of bone marrow fluid and about 5.0 ml of blood in the case of blood disorders. Currently, no standardized method is available for describing chromosome karyotypes based on SKY analysis (2).

Overview of methodology of SKY

SKY involves various steps as outlined by Trakhtenbrot (28):

1. A probe cocktail (Applied Spectral Imaging Ltd, Migdal, Israel) consisting of fluorescently labeled probes for each chromosome is made by labeling chromosome specific libraries generated by PCR from flow-sorted chromosomes with specific combinations of one or more of the five spectrally distinct fluorochromes (FITC, Rhodamine, Texas Red, Cy5 and Cy5.5).
2. Metaphase preparations are hybridized with this probe cocktail and then stained with 4,6-diamidino-2 phenylindole (DAPI) in antifade medium.
3. The SpectraCube® Imaging system (Applied Spectral Imaging Ltd, Migdal Ha'emeq, Israel) as shown in Figure 2 is used to discriminate between the different spectral characteristics of chromosomes. The system measures chromosome-specific emission spectra generated by the combinatorially labeled chromosome-specific painting probes.
4. The spectral signature of the fluorochrome combinations is analyzed using SKYVIEW™ software, which classifies the chromosomes by comparing the acquired spectral characteristics to the combinatorial library containing the fluorochrome combinations for each chromosome. In the classified image, the chromosomes appear in a Red-Green-Blue (RGB) display in which FITC is seen as blue, Rhodamine and Texas Red are seen as different shades of green and the infrared dyes not visible to the human eye, Cy5 and Cy5.5, are assigned different shades of red.
5. The chromosomes are then automatically sorted into a karyotype table according to the nomenclature rules for G-bands. Rearrangements, translocations between different chromosomes and components of marker chromosomes are all easily identified because of a color change at the point of transition. Finally, the software assigns a specific classification pseudo-color to each chromosome allowing chromosomal aberrations to be even more easily visualized.

6. The DAPI image is captured separately and inverted to give a G-banding-like pattern (Figure 3). This image may be used to compliment the SKY analysis with chromosome banding information.

Applications of SKY

SKY has proven valuable in clinical cytogenetics for identifying chromosomal rearrangements that cannot be recognized by conventional G-banding, such as translocations that either are subtle or involve regions with similar banding patterns. Analysis of complex chromosomal re-arrangements in solid tumor cytogenetics has also been accomplished as well as identifying de novo balanced and unbalanced translocations, which are occasionally quite small and difficult to identify. Identification of inter-chromosomal aberrations has been a major application of this technique that has a great potential use in comparative cytogenetics. Another application of SKY extends to the multiparameter analysis of cytological preparations (2,18,27-29).

SKY analysis has revealed numerous markers and derivate chromosomes, hidden translocations, chromosomal insertions, homogeneous staining regions and double minutes unidentified or incorrectly identified by G-banding (28). Due to the nature of painting probes, SKY alone cannot detect intra-chromosomal rearrangements, such as paracentric or pericentric inversions, small duplications and deletions. The resolution of SKY (1–3 Mb) depends on the level of chromosomal condensation and on the combination of the fluorochromes involved in structural rearrangements. Thus, SKY should be seen as a complement rather than a replacement of conventional G-banding analysis (28).

Cancer characterization by SKY

SKY is particularly valuable in cancer cytogenetics and provides a much more detailed portrayal of the highly abnormal karyotypes that characterize advanced tumors and cancer cell lines. The detailed definition of markers by the SKY technique leads to the determination of an increased number of aberrations per tumor, identification of more chromosomal regions involved in the karyotype evolution and the analysis of more metaphases, especially polyploid. SKY also enables the discovery of a larger number of sub-clones and the revelation of different

clonal evolution pathways of karyotype alterations in cancer (28,29).

Table 1 shows a chronological outline of findings related to chromosomal aberrations identified using SKY with or without the augmentation of other molecular techniques.

Comparisons, Capabilities And Limitations Of Spectral Karyotyping

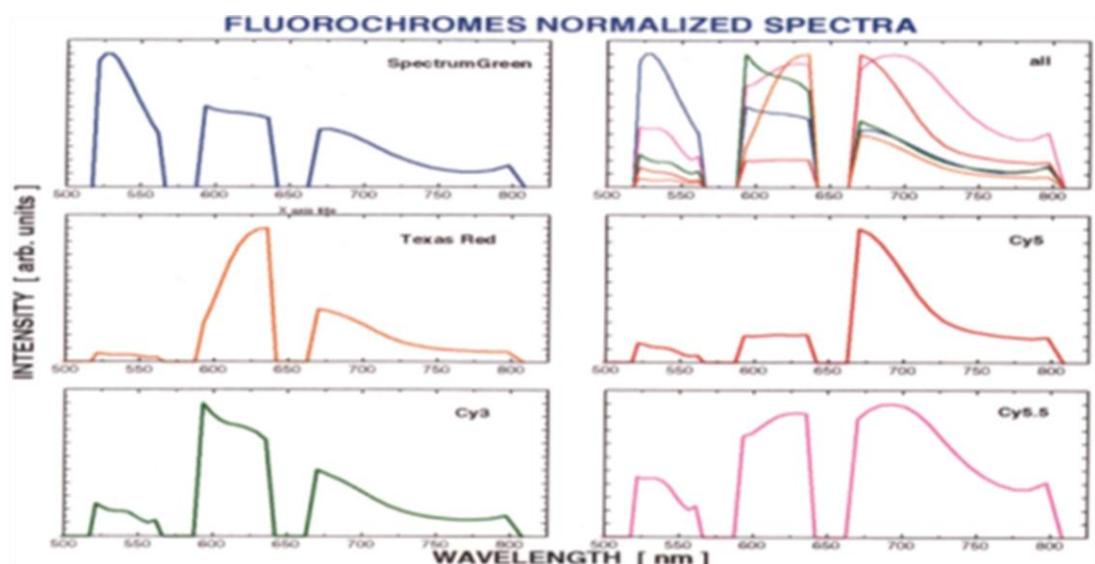
SKY is a FISH-adapted protocol that can be used to detect both chromosome copy number changes as well as gross translocations within the entire genome (2,74).

For FISH, Multiplex FISH (M-FISH), Combined Ratio-FISH (COBRA-FISH) and Spectral Karyotyping (SKY), metaphase chromosome spreads are required [75]. Instead of one labelled probe per chromosome, SKY employs chromosome-specific probe sets consisting of up to five distinct fluorescent dyes, resulting in chromosome-specific,

unique combinations (75). This allows for simple detection of all chromosomes in a metaphase spread. Another main difference between FISH-based methods and SKY is the detection method of the labelled chromosomes: a fluorescence microscope for FISH-based methods and an interferometer for SKY [76].

The key limitation for SKY is the requirement of dividing cells (34). In some cases, dividing cells are not available, e.g. in the case of paraffin-embedded material or primary tumor material. Furthermore, when harvesting primary tumor material, colcemid-mediated enrichment of mitotic cells to obtain condensed chromosomes is not possible, which, together with low proliferation rates, disqualifies metaphase-dependent aneuploid-quantification (34).

Figure 1- The spectra measured for the five chromosomes. The spectra shown are normalized by dividing each spectrum by the transmission spectrum (of the emission band) of the triple-bandpass filter (27)



Another important challenge of SKY is the detection of intrachromosomal arrangements (63). This has been remedied with the introduction of multiplex multicoloured banding (mMCB) and spectral color banding (SCAN) (77). These techniques improve on the advantages of COBRA-FISH, M-FISH and SKY allowing simultaneous visualization of chromosome bands in different colors in a single hybridization.

Other challenges associated with the technique include the need for quality mitoses, a successful hybridization and the high cost (27). The cost might be remedied by reducing the amount of fluorochromes used or perhaps expedient another labeling technique that involves cheaper chemicals. Automation of the technique might be helpful as well as miniaturization of the equipment needed.

Figure 2-Diagram of the spectral imaging system that is connected to a conventional epifluorescence microscope (27)

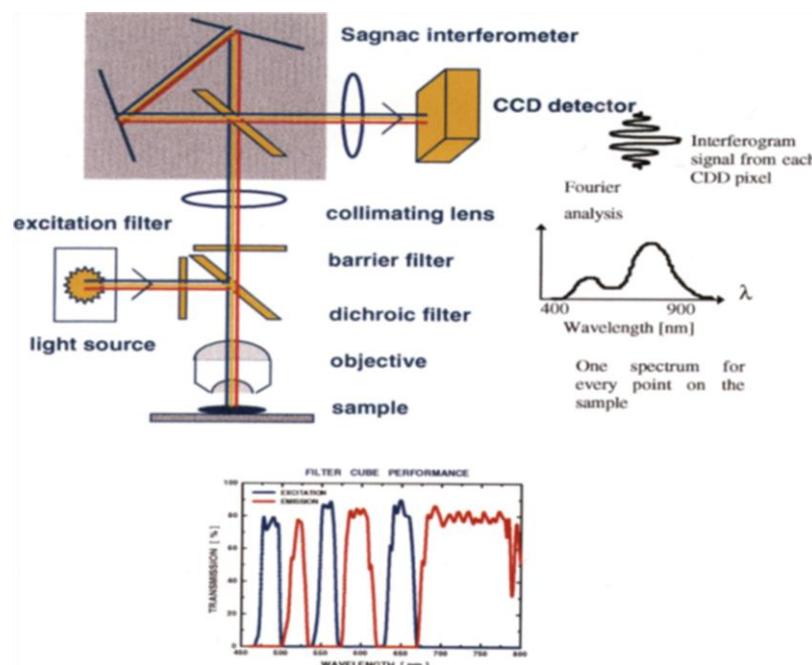


Figure 3- Illustration of karyotypes as displayed by SKY (27)

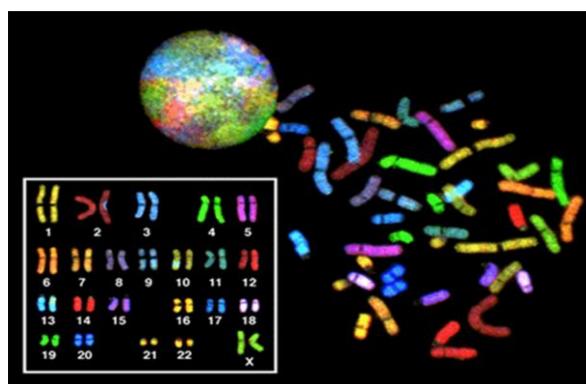


Table 1-Chromosomal characterization of cancer using SKY

Cancer type / cell line	Chromosome(s) affected	Type of aberration	Additional notes
HeLa cell line (30)	1,2,3,5,2,8,9,11,12,13,15,16,19,20 and 22	Chromosomal breakpoints	Identification of 2 sub-clones
Prostate cancer cell lines (31)	1,2,4,6,10,15,16	Balanced translocations and pinpoint rearrangements	New alterations identified
Acute myeloid leukemia (32)	7,11,23	Cryptic 11q23 translocation in 20/20 cells and a minor monosomy 7 clone in 3/21 cells	Previously diagnosed as cytogenetically normal
Colon cancer cell lines (33)	2,5,7,8,12,11,13,14,18,20,22	Complex rearrangements	Redefinition of 6 markers and 13 newly identified markers
Breast carcinoma cell lines (34)	1,4,8,10,11,14,17	Unbalanced Translocations	Chromosome 8 was predominantly affected in 19 cell lines
Breast cancer cell lines (35)	1,7,8,9,11,13,16,17,18,20	Unbalanced translocations	Major translocations in 15 cancer cell lines were outlined with chromosome 8 being the most frequently affected
Medulloblastoma and related primitive neuroectodermal tumours (36)	3,6,7,10,13,14,17,18,22	Aneusomy	Chromosome 7 and 17 are the most commonly affected
Non-Hodgkin's lymphoma (37)	All chromosomes	Translocations	21 previously unidentified chromosomal rearrangements were discovered
Primary breast carcinoma and their lymph node metastasis (38)	1,2,3,6,7,13,14,16,19,20,21,22,X	Complex translocations	Similarities were noted in the karyotype of primary cancers and their metastatic tumors.
Uroepithelial carcinoma (39)	1,4,5,8,9,11,17	Several deletions	Chromosome 5 rearrangement is associated with more aggressive phenotypes
Multiple myeloma (40)	8,14,11	Several translocations	Translocation has been seen to involve the whole arm of 8p
Breast cancer cell lines (41)	8,11,12,21,22,23	Several translocations	Chromosome 8 abnormal in majority of them with chromosome 11 being the most frequent partner in 16 cell lines
Prostate cancer cell lines (42)	8	Aneusomy	Evidence of chromosomal instability in prostate cancer
Colorectal cancer (43)	All autosomes	Structural abnormalities	Additional subsets of colorectal cancer revealed
Pancreatic cancer cell lines (44)	1,5,7,9,11,12,18	Unbalanced structural aberrations	Loss at 11p and gains at 5p and 12p are the most common
Bladder cancer cell lines (45)	8,12,X	Translocation and deletions	Identification of <i>RhoGDI2</i> , a candidate gene for bladder cancer
Epithelial cancer cell lines (ovarian and colorectal cancer) (46)	1,3,7,11,15,17,18	Numerical and structural abnormalities	Obtaining a karyotype despite high rates of chromosomal instability
Acute myeloid leukemia (47)	11,21,22	Structural aberrations	Re-interpretation of acute myeloid leukemia genome previously discovered
Head and neck squamous cell carcinoma (48)	3,5,6,7,8,11,13,14,15,18,22,23	Structural aberrations	Genetic changes in these malignancies were identified
Serous ovarian adenocarcinoma (49)	1,2,3,4,6,8,9,10,11,12,17,18,20,21	Unbalanced translocations, isochromosomes and deletions	Loss of 12p11.2 common and scattered in several chromosomes
Nasopharyngeal carcinoma (50)	2,3,5,6,7,8	Unbalanced and reciprocal translocations	Chromosome 2 was the most predominantly affected
Osteosarcoma (51)	8,17,20	Numerical and structural abnormalities	Chromosome 20 is the most frequently affected
Cervical cancer cell lines (52)	All autosomes	Structural abnormalities	Derivative chromosomes denoting HPV sequences revealed
Lung cancer cell lines (53)	1,3,6,10,12,17,21,24	Unbalanced translocations	Genomic characterization of 10 cell lines
Meningioma (54)	22,23	Duplication, deletion and translocation of chromosome 22	Chromosome 22 abnormality was the hallmark of the tumor
Gastric cancer (55)	1,3,7,8,11,13,1,20	Unbalanced translocations	Chromosome 8 is the most commonly affected
Burkitt's lymphoma-derived B cell line (56)	3,8,13,14,17	Translocations	Novel rearrangements discovered besides t(8;14)
Oral squamous cell carcinoma cell lines (57)	1,3,5,8,10,11,16,X	Isochromosomes and derivative chromosomes	11p13 was the most commonly affected site
Virus infected cells (58)	All	Structural and numerical aberrations	Evidence that viruses causes cancer by inducing massive chromosomal instability
Colorectal cancer cell lines (59)	All	Translocation, deletion and uniparental disomy	Uniparental disomy identified as an early mutational event
Lung cancer (60)	1,2,3,5,6,9,11,12,13	Numeral and structural abnormalities	Identifying chromosomal aberrations leading to lung carcinogenesis by studying smokers with and without lung cancer
Small cell lung cancer cell lines (61)	3,5,16,18	Derivative chromosomes	Long arm of chromosome 18 shows potential for a marker of SCLC
Various cancer cell lines (62)	The whole genome	Multiple catastrophic breakpoints with several structural aberrations	The term "chromothripsis" was coined to describe the massive breakpoints and its rearrangement in cancer development
Urothelial cancer (63)	5,9	Deletions and rearrangements	Detection of p53 isoforms
Colon cancer (64)	8	Structural abnormalities	Discovery of CCAT2, a novel noncoding RNA mapping to 8q24
Gastric cancer (65)	10,12,17	Structural rearrangements especially amplification of some genes	Identification of mutually amplified FGFR2, HER2, and KRAS
Lung cancer (66)	1,3,5,8,11	Numerous structural abnormalities	Chromosome 8 had the most frequent alterations.
Pancreatic cancer cell Lines (67)	1,2,3,4,6,7,8,12,16,17,18	Structural abnormalities	Discovery of novel PLXNA1 mutation in chromosome 3 and development of 3 novel cell lines
Head and neck squamous cell carcinoma cell lines (68)	1,2,3,7,8,11,14,	Complex structural amplifications	Chromosomes 1, 7 and 11 were the most frequently affected
Colorectal cancer (69)	All	Structural abnormalities	Identification of MIIIP gene in Chromosome 1 as a novel potential tumor suppressor
Small cell lung cancer (70)	5,18,20,22	Structural abnormalities	Identification of unique genomic regions
Colorectal cancer cell lines (71)	All	Structural abnormalities	Profound differences in genes of various cell lines demonstrating the diversity of clonal evolution
HepG2 cell lines (72)	2,6,16,17	Structural abnormalities	Identification of the genomic complexity of the cancer cell line
Acute myeloid leukemia (73)	5,7,17	Structural abnormalities	Identification of different complex genomes in typical and atypical subtypes

RhoGDI2: Rho GDP Dissociation Inhibitor 2, HPV: Human papilloma virus, SCLC: Small cell lung cancer, CCAT2: Colon cancer associated transcript 2, FGFR2: Fibroblast growth factor receptor 2, HER2: Human epidermal growth factor receptor 2, PLXNA1: Gene that codes for plexin-A1, MIIP: Migration and invasion inhibitory protein.

CONCLUSION

Since the discovery of SKY by Schrock et al. in 1996, the technique has been extensively used to highlight chromosomal aberrations in both hematological and solid malignancies. The introduction of this technique to clinical research of malignancies has led to the identification of novel recurrent aberrations and the characterization of highly unstable chromosomes that were otherwise difficult to visualize. These findings are of vital influence to diagnosis, therapy and further research.

Perhaps, one of the challenges of SKY – detection of intrachromosomal arrangements, has been remedied with the introduction of

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mCMB and SCAN. These techniques follow the principle of SKY and analyze every single chromosome in a genome, thus allowing simultaneous visualization of chromosome bands in different colors in a single hybridization. Therefore, if SKY analyzes a whole karyotype in different colors, SCAN and mCMB with SKY analyze each single chromosome in a genome highlighting the bands in different colors. A combination of SKY and SCAN or mCMB can serve as a very powerful cytogenetic tool.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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