



## A Broader View of Cancer Cytogenetics: From Nuclear Aberrations to Cytogenomic Abnormalities

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### Description

Cancer Cytogenetics has been focused on the study of acquired chromosomal abnormalities causing the cellular transition from normal to malignant proliferation. Since the discovery of the Philadelphia chromosome as associated with chronic myelogenous leukemia in 1960, recurrent clonal chromosomal abnormalities have been detected in various types of cancers and cytogenetic testing has been one of the fastest-growing areas in cancer diagnosis [1]. Recently cancer cytogenetic guidelines has integrated DNA-based oligonucleotide array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) array into cell-based karyotyping and fluorescent in situ hybridization (FISH) testing [2]. Karyotype and FISH analyses can detect recurrent chromosomal abnormalities and clonal evolution in a cellular level, while aCGH can delineate the genomic coordinates and gene content for cancer-related copy number changes. This integrated cytogenomic approach has improved the analytic resolution and diagnostic yield significantly and provided evidence-based gene-centric interpretation of clonal abnormalities [3]. However, all these methods overlooked non-clonal nuclear (also termed karyotypic or chromosome) aberrations likely related to genome instability and somatic cell evolution. These largely ignored and unclassified nuclear- and chromosome-level morphologic aberrations may represent cellular phenotypes during the transition from normal to malignant conditions [4]. A broader view from unclassified non-clonal nuclear aberrations to well-defined clonal cytogenomic abnormalities could provide a new framework for tracing nuclear and chromosomal alterations across the genome and within a cell population.

### Heterogeneity of Ignored and Unclassified Nuclear Aberrations

The diagnostic focus on clonal chromosomal abnormalities has resulted in the ignorance of many types of nuclear aberrations which are detectable in the interphase nuclei and metaphase chromosomes under a microscope. The following are some typical ignored and unclassified nuclear aberrations on slides of conventional cytogenetic preparations [4]. Free chromatin (FC) is spindle- or rope-shaped chromatin released from interphase nuclei. Originally, this structure was thought to be slide preparation artifacts from hypotonic treatment. However, the detection of a clear dose-response relationship between FC and many chemotherapeutics suggested that the frequencies of FC are related to nuclear envelop instability and could be used to monitor toxicity. Defective mitotic figures (DMFs) were initially described as 'uncompleted-packing-mitotic-figures' based on the co-existence of condensed chromosomes and under condensed chromatin fibers

within one mitotic figure. DMFs may be caused by defects in chromosome condensation and G2-M check points and are detected in various cancer cell lines and patient samples. Sticky chromosomes (SCs) refer to chromosomes sticking to each other and being tangled by chromatin fibers. There seems to be a correlation between SCs and difficulties in preparing mitotic spreads from cancer cells. It has been suggested that SCs may be related to either methylation and condensation status or a structure connecting all chromosomes within a nucleus. Unit fibers (UFs) were described as a substructure of metaphase chromosomes with a constant diameter of about 0.4  $\mu$ m, which is approximately 5-fold less than the final condensed chromosomes and likely represents the last level of chromatin packaging. UFs can be induced by topoisomerase II inhibition in short-term lymphocyte cultures. Chromosome fragmentation (C-Frag) is a form of mitotic cell death where condensed chromosomes are progressively degraded. C-Frag represents a general response to system stress including gene mutations, infection, drug treatment, and centrosome dysfunction. C-Frag can lead to aneuploidy and genome chaos and thus contribute to karyotype abnormalities. Genome chaos (GCs) refers to massive and rapidly acquired changes including polyploidies, multiple translocations, long fused chromosomes, single sister chromatids, ring chromosomes and C-Frag. The majority of GCs will eventually be eliminated but the self-repaired and evolutionally-selected GCs can be seen in clonal complex chromosomal abnormalities from various cancer cell lines and tumor samples. Micronuclei (MN) are small bud-off nuclei containing whole chromosome, fragment of chromosomes or combinations. Mechanisms for MN formation are related with misplacement and mal-segregation of chromosomes during mitosis. MN frequency in peripheral lymphocytes is increased with age (especially after 30 years) and is elevated upon exposures to harmful chemicals and radiation that cause DNA damage. MN is indicative of overall genome instability and can be associated with various types of cell death.

In addition to FC, DMFs, SCs, UFs, C-Frag, GCs and MN, other unclassified nuclear aberrations include nuclei with a small hole (donut shaped) in bone marrow cells treated with pingyangmycin or BrdU, abnormal nuclear morphology and giant nuclei induced by chemotherapeutics such as doxorubicin. All these nuclear aberrations could be viewed as unique cellular phenotypes. Their relations to nuclear integrity, genome stability, and chromosomal abnormalities and clinical implications for cancer progression and treatment response need to be systematically analyzed [4].

## Spectrum of Delineated Cytogenomic Abnormalities in Cancers

Karyotyping and FISH testing have been routinely used to detect clonal chromosomal abnormalities for patients with various types of hematopoietic malignancies and solid tumors; these abnormalities including numerical abnormalities from simple aneuploidy to polyploidy, structural abnormalities from intra-chromosomal deletions, duplications, inversions and ring to complex inter-chromosomal rearrangements, and a combination of both [1]. Recently integrated aCGH and SNP array analyses, with increased analytical resolution to kilobase (Kb) range, can delineate the genomic coordinates and gene contents for not only all chromosomally visible numerical and structural imbalances but also submicroscopic copy number changes. This genomic information facilitates fine mapping of critical regions containing candidate tumor suppressor genes and oncogenes. The application of this cytogenomic approach characterized chromosomal and cryptic imbalances in patients with myelodysplastic syndrome and acute myeloid leukemia; clinically significant recurrent or cryptic deletions of 5q (involving the RPS14 gene), 9p21.3 (CDKN2A/CDKN2B genes), 12p12.3 (ETV6 gene), 13q14.3 (RB1 gene), 17p13 (TP53 gene), 17q11.2 (NF1 gene) and 20q, double minutes containing the MYC gene, and segmental amplification involving the MLL gene were delineated [3,5,6]. Another benefit from DNA-based aCGH analysis is the detection of recurrent chromosomal abnormalities from solid tumors frequently failed in cell culture or stored as formalin-fixed paraffin-embedded tissue blocks. For example, aCGH on DNA extracted from paraffin-embedded tissues of intravenous leiomyomatosis of the uterus detected most frequent recurrent losses and gains and defined gene contents within these regions [7]. Furthermore, clinical application of next-generation sequencing could further characterize complex chromosomal rearrangements and detect point mutations. Combined data from SNP array analysis, FISH testing and transcriptome sequencing (RNA-Seq) were used to uncover subclone markers in heterogeneous melanoma biopsies [8].

Using next-generation sequencing on complex chromosomal rearrangements detected from patients with chronic B cell lymphocytic leukemia, osteosarcoma and chordoma, a new type of chromosomal abnormality termed chromothripsis was identified. This abnormality involves tens to hundreds of genomic rearrangements crisscross back and forth in one or a few chromosomes from a one-off cellular crisis [9]. The nuclear aberrations of GCs has been linked to chromothripsis [4]. Interestingly, leukemic clonal abnormalities detected from cultured leukocytes and bone marrow cells could be revealed by next-generation sequencing on cell-free DNA from blood plasma and bone marrow fluid. This finding indicated that cell-free DNA can be used to detect degraded or apoptotic abnormal clones for evaluating the cellular dynamics and the efficacy of treatment [10]. Taking together, current cytogenomic approach could effectively detect a spectrum of clonal chromosomal and genomic abnormalities for various types of cancers. This integrated genetic analysis provides crucial information for classifying various types of tumors, selecting proper treatment protocols, and monitoring clinical remission or relapse.

## Toward Molecular Karyotyping and Cellular Phenotyping in Cancer Cytogenetics

The application of genomic technologies such as aCGH, SNP array and next-generation sequencing has enabled genome-wide

characterization of clonal chromosomal abnormalities, copy number changes and gene mutations for various cancers. Clonal complex chromosomal rearrangements and hidden cryptic genomic aberrations can be delineated in a single nucleotide level to achieve a molecular karyotype [9]. Classic cancer cytogenetics has been changed to genome-based integrated analysis to reveal tumor heterogeneity, clonal evolution and cellular dynamics [8-11]. An evolutionary model unifying the hallmarks of cancer through genome reorganization has been proposed [12,13]. This model divided cancer evolution into a stochastic punctuated phase featuring non-clonal nuclear aberrations and a stepwise phase presenting clonal expansion of re-organized and selected genome. Nuclear aberrations have been suggested as cellular phenotypes for nuclear defects (FC and MN), chromatin condensation errors (DMF, SC and UF), genome instabilities (C-Frag, GC and MN), and cell death (C-Frag, GC and MN) [4]. However, the underlying molecular mechanisms causing these aberrations and cellular processes from non-clonal aberrations to clonal abnormalities remain largely unknown. Because of the heterogeneity and low frequency (less than 4%) of these non-clonal nuclear aberrations in a cell population, microscopic analysis for these aberrations could be labor-intensive, time-consuming, and error-prone.

Currently, most cytogenetic laboratories have adopted an automatic scanning system to capture metaphases. Modification to this system for measuring cellular metrics including nuclear aberrations and mitotic index should be technically feasible and reliable. The potential application of this modified automation system could establish baseline levels of nuclear aberrations for normal controls and reveal clinical implications in cellular dynamics and treatment responses for cancer cells from patient samples. A perspective framework combined molecular karyotyping with cellular phenotyping could lead to a better understanding of tumor heterogeneity through genome stability/instability status and evolutionary potential and a systematic monitoring of cellular transition, clonal evolution, treatment responses, and disease progression.

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