

Cytogenetics in Solid Tumors: Lessons from The Philadelphia Chromosome

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ABSTRACT

Although presently known as an environmentally-related disease and appears mostly sporadic, cancer is regarded as a genetic disease based on the presence of gene mutation as a consistent factor. The “Philadelphia Chromosome” found consistently among chronic myeloid leukemia (CML) patients was the first significant finding of a chromosomal abnormality specifically related to a particular disease. Starting from this point, cytogenetics as the study of chromosomes has become a valuable tool in the assessment of cancer – as an aid in diagnosis, thus guiding therapy, and as a prognostic marker. As is the nature of the proliferating marrow, chromosomal abnormalities were found mostly in hematologic malignancies, and the findings more pathognomonic.

The situation is different in solid tumors, which when visible to the naked eye already will have complex chromosomal changes and thus pose technical difficulties to the cytogeneticist. However, scientists believe that the shift in chromosomal studies from conventional cytogenetics to molecular cytogenetics will provide further information regarding solid tumors.

Key words: solid tumors, cytogenetics.

INTRODUCTION

Remarkable progress has been made in the fields of cancer genetics and cytogenetics since the first report regarding a chromosomal abnormality subsequently known as the “Philadelphia (Ph) chromosome” in a patient with chronic myeloid leukemia (CML) in 1960.^{1,2} This was the first consistent chromosome abnormality in human cancer. Additional chromosomal changes associated with various hematologic malignancies followed in 1970s after the introduction of chromosome banding technique.^{1,2} Besides this conventional cytogenetics, now the field still grows rapidly with the advent of molecular cytogenetics techniques, such as fluorescent in situ hybridization (FISH), multicolour FISH, spectral karyotyping, comparative genomic hybridization, and other molecular techniques.

CYTOGENETICS

Before chromosomal abnormalities in cancer are discussed, we will take a brief overview of human cytogenetics. Cytogenetics is the study of chromosomes and the related disease states caused by abnormal chromosome number and/or structure. Normally, chromosomes cannot be seen with a light microscope, but during metaphase or late prophase they become condensed enough to be analyzed. To ensure that any abnormalities detected represent in vivo conditions, direct preparations and short term cultures are usually preferred.^{1,3}

Human chromosome nomenclature is based on An International System for Human Cytogenetic Nomenclature or ISCN. The normal human somatic cells have 46 chromosomes include 22 pairs of autosomes and two sex chromosomes, XX in female

and XY in male. (**Figure 1**) This is called the diploid number. After the discovery of banding technique, each human chromosome can be precisely identified on the basis of its unique banding pattern.⁴

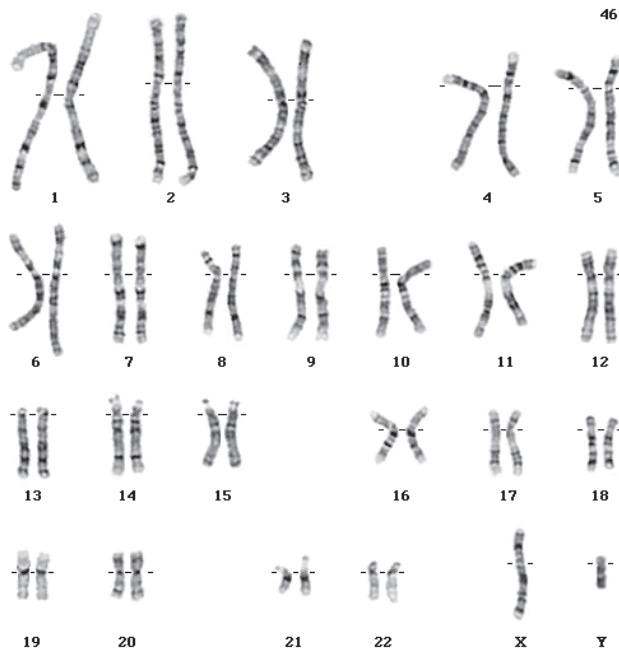


Figure 1. Normal male karyotype, consist of 22 pairs of autosome and 2 sex chromosomes (XY)

Under the microscope chromosomes appear as thin, thread-like structures. They have a short arm designated as **p**, and long arm as **q** which are separated by a primary constriction called the **centromere**. Each chromosome arm consists of one or more regions, and each region is composed of one or more bands. A band is defined as chromosomal area that is distinguishable from adjacent segment by appearing darker or lighter by one or more banding techniques. Thus the term **banding** was conceived. In allocating any particular band, four items are therefore required: the chromosome number, the arm symbol, the region number, and the band number within the region. For example 1q23 means chromosome 1, the long arm, region 2, and band 3.^{3,4}

Although chromosome abnormalities can be very complex, there are two basic types, numerical and structural. Both types can occur concurrently. Numerical abnormalities involve the loss and/or gain of a whole chromosome and can include both autosomes and sex chromosomes.⁵ The abbreviation used are (+) for gained or (-) for loss of chromosome. Cells which have lost a chromosome are categorized

as a monosomy, while those with an extra chromosome are trisomy. There is also a condition called triploidy in which there is an extra copy of every chromosome. Occasionally, an individual carries an extra chromosome which can't be identified by its banding pattern, these are called marker chromosome (mar). The introduction of FISH techniques has been a valuable tool in the identification of marker chromosomes.⁶

Structural abnormalities include translocations, deletions, inversions, and insertions. Translocation (t) occurs when two or more chromosomes exchange material. This exchange may or may not be reciprocal. The Philadelphia (Ph) chromosome, a cytogenetic change seen in chronic myeloid leukemia (CML) provides the classic example of a cancer-associated translocation.^{5,7}

Deletion (del) means loss of chromosomal material. These chromosomal deletions may lead to neoplastic development when a tumor suppressor gene is lost, such as in epithelial adenoma.⁷

Inversion (inv) designates an 180° rotation of a chromosome segment. Insertion (ins) means a chromosomal material moves to a new, interstitial position in the same or another chromosome. These two abnormalities cause an abnormal juxtaposition of genetic materials that can lead to the formation of abnormal proteins that can initiate neoplasia.⁷

Fluorescent In Situ Hybridization (FISH) is a technology using fluorescently labeled DNA probes to detect or confirm gene or chromosome abnormalities that are generally beyond the resolution of conventional cytogenetics.^{6,8} When the mitotic index is low, or the cytogenetics preparation suboptimal, an accurate diagnosis often cannot be achieved using standard banding.^{5,8} In this situation FISH can be useful because FISH methodology allows the detection of specific targets not only in metaphase spreads, but also on nondividing interphase nuclei.^{8,9} This makes FISH a powerful tool for a rapid and sensitive detection of chromosome abnormalities.

CHROMOSOMAL ABNORMALITIES IN CANCER

Cancer, in its various forms is a genetic disease.⁹ This concept comes, among others, from the finding of chromosomal abnormalities. These abnormalities may arise as a consequence of random replication errors; exposure to carcinogens; or damaged DNA repair processes. Three genes that contribute to malignancy are oncogenes, tumor suppressor genes, and DNA repair genes. Genes that promote normal cell growth

(protooncogenes) can convert to oncogens because of point mutation, amplification, or dysregulation. Genes that restrain growth are tumor suppressor genes; therefore unregulated cell growth arises if their function is lost.⁹

Almost all cancers originate from a single progenitor cell, called a clone. It can be concluded as a clonal origin when numbers of cells have the same or closely related abnormal chromosome complements. In the development of cancer, the mutations can occur through multistage process to change a normal cell to malignant. In this process, subclones may have evolved so the clone not necessarily homogenous.⁹

Chromosomal changes in cancer are significant if the changes are non random. When there is an abnormality in chromosome, it has to be determine whether it's accidentally happens (random) or not. Another important thing in examining cancer chromosome changes is to find out whether the abnormalities are primary or secondary. Primary aberrations are frequently found as a solitary abnormality and unique for particular tumor types. This primary aberration is usually already detected at the time a cancer is diagnosed. This fact shows that the changes are related to the carcinogenesis. Conversely, secondary aberrations found in later stage of cancers particularly solid tumors, and therefore do not important in initiation of the disease.^{7,10}

CYTOGENETICS IN HEMATOLOGIC MALIGNANCIES

Relatively ease in obtaining samples and the advances of cytogenetics make chromosome studies in hematological malignancies have grown rapidly. Nowadays cytogenetic analysis has become an integral part of diagnosis, therapy option, and prognostic value in hematologic malignancies.

To detect chromosomal changes associated with hematologic malignancies, bone marrow is the optimal source for affected cells. When necessary, blood cells can be examined, but the success rate for this analysis is low and the information obtained is less than optimal.⁷

Chronic Myeloid Leukemia (CML) was the first hematologic malignancy known related to chromosome abnormalities, a consequence of furious and diligent work by researchers from all over the world and first reported by Janet Rowley from Chicago. The reciprocal translocation t(9;22)(q34;q11) in CML, called Philadelphia chromosome (Ph) became clinically important associated to patient's survival. It is well established that patients with the Ph chromosome have

a much longer median survival than patients who are Ph negative. Subsequent work in molecular genetics has revealed that the Ph translocation causes fusion of gene sites that code for the break cluster region (BCR) and the avian blastic leukemia (ABL) proteins.¹¹ BCR analysis (Southern blotting) indicated in cases such as the Ph chromosome is too small to be identified with conventional cytogenetics, the differentiation of Ph⁺ acute leukemia (particularly acute lymphoblastic leukemia) with CML, and follow-up of CML patients during therapy or after bone marrow transplantation. Moreover, as the disease progresses, additional chromosomal anomalies e.g. +8, +Ph, +19, and i(17q), may precede the development of blast crisis before the manifestations become cytologically or clinically apparent.⁷

In Acute Leukemia, the determination of the chromosomal changes serves as diagnostic tools, clinical and prognostic aspects of a case, development of appropriate therapy, and determination of the presence or absence of a complete remission.⁷

Generally, in Acute Myeloid Leukemia (AML) analysis of chromosome abnormalities allows categorization risk group. Chromosomal abnormalities such as t(8;21), inv(16), and t(15;17) present significantly better prognosis, whereas aberration such as -5/del(5q), -7, abn(3q), and complex karyotype are associated with a high risk for induction failure, relapse, and shortened survival.¹² Cheson et al, (2003) recommended the use of cytogenetic remission (CRc) as a part of the criteria for complete remission (CR). Research by Marcucci et al, (2004) on adult AML patients suggests that conversion to normal karyotype at the time of the first CR is an important prognostic indicator for clinical outcome.¹³

In patients aged 60 years or older, the prognosis of AML is extremely poor. Besides the advance age, another features such increased incidence of primary drug resistance, antecedent myelodysplasia, and karyotypic abnormalities are known to be associated with poor outcome. Despite the generally bad prognosis, a small number of long term survivors exist among patients treated with current standard chemotherapy. Hence it is important to determine whether the patient belongs to this group or not. Observation by Farag et al. (2006) concluded that pretreatment cytogenetics adds to other factors in older AML patient. Patient with at least 5 abnormalities appear to benefit minimally from current treatment and are better suited for investigational therapy or supportive care.¹⁴

Cytogenetic studies in the Myelodysplastic Syndromes (MDS) may have pathogenetic, diagnostic, and prognostic implications. The most frequent abnormalities found in MDS are del(5q), monosomy 7, trisomy 8, and complex karyotype.¹⁵ Conventional cytogenetics methods are routinely used to detect karyotypic abnormalities in bone marrow cells of MDS patient. Identification of the origin of aberrant or marker chromosome could be done by combining FISH and immunophenotypic or cytochemistry analyses.¹⁶

Secondary acute leukemia is often preceded by a MDS. These conditions are often associated with specific chromosomal changes, the most common are 5q-/5-, 7q-/7-, and del(20q). Since the cytogenetic findings in these conditions are generally diagnostic and prognostic (e.g., 5q- is associated with a relatively good prognosis, and -7 with poor prognosis), cytogenetics examination of the bone marrow is an important step in the management of these states.⁷

CYTOGENETICS IN SOLID TUMORS

Although solid tumors, particularly carcinoma, play a larger part in human morbidity and mortality, the developments of cytogenetics in solid tumors developed more slowly than in hematologic malignancies. This situation happens because of several technical limitations. Firstly, the chromosome quality in solid tumors is often suboptimal regarding to necrotic samples that result in destruction of cancer cells before culturing. Second, in contrast to

hematological malignancies, which often contain few cytogenetic changes, most solid tumors have multiple and complex chromosomal changes acquired during tumor progression which cause difficulties in identifying the primary chromosome changes associated with specific tumor type. Third, low mitotic index that necessitates short term culture of cancer cells to avoid overgrowth by normal stromal or supporting cells.^{10,17} In recent years, the development of cytogenetic techniques for example FISH has led to the description of specific chromosome abnormalities in solid tumors because it can be performed on fresh tumor tissue, exfoliative cells, and embedded specimens.¹⁸⁻²⁰

The complexity of chromosomal changes in solid tumors can be associated with a multistage cascade of genetic changes. According to this concept, an initial genetic change leads to an increase in cell growth, followed by a series of changes that consequently result in malignant transformation and metastatic spread. An example for this condition is epithelial origin tumors, including breast cancer, colon cancer, bladder cancer (Figure 2).^{7,10,17}

In clinical oncology, cytogenetics for solid tumors is used in diagnosis, evaluating treatment response of metastatic cancer, marker for prognosis and targeted therapy.²⁰⁻²² In tumors where histologic features overlap, cytogenetics plays an important role for diagnosis, for example the t(12;16) in myxoid liposarcoma, the t(2;13) in alveolar rhabdomyosarcoma, and the t(X;18) in synovial sarcoma. These are also related to

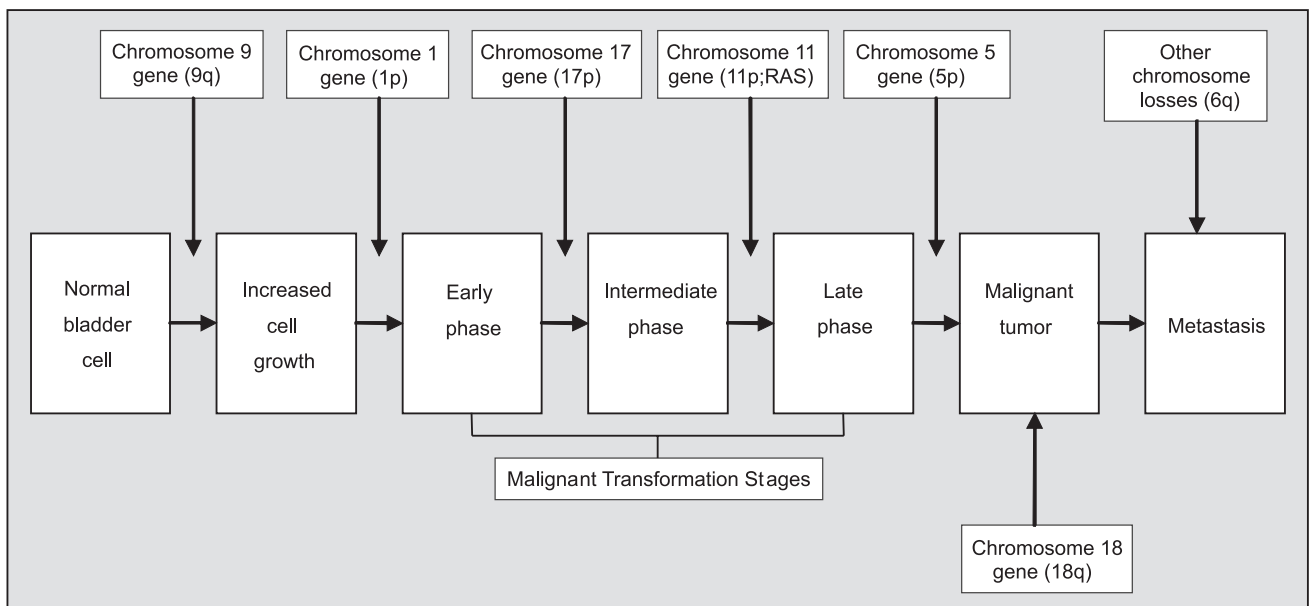


Figure 2. Suggested pathway for the development of bladder cancer through a series of genetic events (Sandberg, 1994)

a rational approach of therapy. In surgical cases, cytogenetics has been used to monitor the surgical margin in head and neck carcinoma that identified subclinical tumorigenesis.¹⁹

In metastatic cancer, molecular cytogenetics can accurately monitor tumor response to therapy by detection of chromosomal aneusomy in cerebrospinal fluid, so the clinician can change ineffective therapy at an earlier stage in the course of treatment.¹⁹

Molecular cytogenetics, particularly dual color FISH assay has proven to be as effective as a prognostic marker and predictor for response to therapy in breast cancer patient by detection of HER-2 gene amplification in tumor cells. Overexpression of HER-2 has been found in 20-30% of breast cancers and has been associated with a poor overall survival.¹⁹

Detection of residual disease in patient with solid malignancies is essential, and FISH technology answers this aim. Interphase FISH application can detect tumor cells in body fluids through non-invasive procedures. Urinary cytology, for illustration, has been the reference test for the evaluation of symptomatic patients, detection of lesions in high-risk patients, and follow-up the patient with prior history of transitional cell carcinoma.^{19,21}

Interphase cytogenetics by fluorescence in situ hybridization (FISH) is one method, demonstrated to be a valuable diagnostic tool in effusions from patients with solid tumors. Patterns of numeric aberrations in malignant effusion cells can supply prognostic information. An example can be made from the study by Massoner in which 55 effusions from breast cancer and 39 effusions from non-small cell lung cancer (NSCLC) were classified as malignant by cytology or FISH and Predominant cytogenetic anomalies and patterns of intratumor cytogenetic heterogeneity were brought in relation to overall survival rate. The study shows that simple chromosomal changes as determined by FISH, such as gain of chromosome 11 copy numbers in breast cancer, may be prognostic.²²

Conventional cytogenetics and spectral karyotype (SKY) were used in a study on ovarian cancer cell lines and, combined with molecular techniques on p53, keratin and her-2 expression, resulted in the elucidation of the tumor's characteristics [potentially useful for decision making in treatment].²³

CONCLUSION

Non random chromosomal abnormalities are found in various types of cancer. These conditions brought

cytogenetics as a tool for diagnostic, therapy, and prognostic marker in the field of oncology. The development from conventional technology to molecular cytology has led to prompt development of cancer cytogenetics. The Philadelphia Chromosome in CML was the first significant cytogenetics finding specifically related to a particular disease. In retrospect, the "discovery" of chromosomal abnormalities in CML was relatively "simple" in the sense that it occurs very commonly, is reproducible, and reflected changes occurring early in the disease development. On the other hand, solid tumors are already advanced when they are visible, thus the complex chromosomal changes.

However, there are several lessons learned from the first endeavour with the Philadelphia chromosome. Firstly, that the seemingly-simple discovery was the result of years of research, a fact that translates into the present research with solid tumors. And that further elucidation on the cancer process with more advanced methods of chromosomal studies such as molecular cytogenetics will further shed light on solid tumors. Secondly, that most probably it is just a matter of time that translational research on solid tumor cytogenetics – though not always using the "conventional" cytogenetics - will provide scientists and clinicians with answers.

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