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Cancer Letters xxx (2006) xxx–xxx
CANCER Letters
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SKY analysis of childhood neural tumors and cell lines demonstrates a susceptibility of aberrant chromosomes to further rearrangements

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Received 8 June 2006; received in revised form 23 August 2006; accepted 15 September 2006

Abstract

Malignant solid tumors are commonly characterized by a large number of complex structural and numerical chromosomal alterations, which often reflect the level of genomic instability and can be associated with disease progression. The aim of this study was to evaluate whether chromosomes that harbor primary aberrations have a higher susceptibility to accumulate further alterations. We used spectral karyotyping (SKY), to compare the individual chromosomal instability of two chromosome types: chromosomes that have a primary aberration and chromosomes without an aberration, in 13 primary childhood neural tumors and seven cell lines. We found that chromosomes that contain a primary aberration are significantly (p-value < 0.001) more likely to gain further structural rearrangements or to undergo numerical changes (22.6%, 36 of 159 chromosomes) than chromosomes with no initial aberration (4.9%, 54 of 1099 chromosomes). These results are highly suggestive that aberrant chromosomes in solid tumors have a higher susceptibility to accumulate further rearrangements than "normal" chromosomes.

Keywords: Spectral karyotyping; Additional rearrangements; Neural tumors

1. Introduction

Many malignant neoplasms acquire multiple chromosomal changes with time and the patterns of the karyotype alterations may shed light on the genetic pathways involved in disease progression. In a large subset of malignant solid tumors complex karyotypes, with a high degree of aneuploidy and complicated structural rearrangements that often reflect the level of genomic instability, can be already found at the time of diagnosis. Therefore, being able to determine which changes are of pri-

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doi:10.1016/j.canlet.2006.09.015

mary pathogenetic importance and which are secondary events is more difficult than in hematological malignancies. Several statistical models were applied to describe the development of chromosomal changes in cancer cells and several fundamental features of the karyotypic evolution have been revealed by the investigation of data obtained from cytogenetic banding analysis [1–6].

Spectral karyotyping (SKY) technique based on the simultaneous hybridization of 24 fluorescently labeled chromosome painting probes provides a valuable addition to the investigation of chromosomal alterations that can be difficult to define by conventional banding analysis. It has been shown in a number of studies that SKY has enhanced the characterization of highly complex karyotypes with unidentified marker chromosomes [7–10]. In childhood neural tumors, which are frequently characterized by poor chromosome morphology and complex karyotypes, the SKY technique allows definition of aberrant chromosomes that are indefinable by G-banding alone and subsequently a better characterization of the sub-clones present, hence, revealing different evolution pathways of chromosome rearrangements [11–13]. We hypothesize that statistical investigation of data obtained from combined SKY and conventional banding analysis of complex cancer karyotypes can reveal new features of karyotypic evolution. It is well known that the SKY analyses of solid tumors show that marker chromosomes often accumulate additional structural rearrangements and duplications. In this study, we used this advantage of SKY to estimate the likelihood of rearranged aberrant chromosomes to accumulate further alterations by analyses of 13 primary childhood neural tumors and seven cell lines. We used SKY to compare the rates of instability of two chromosome groups: one group of chromosomes that harbored a primary aberration and one group of chromosomes that did not have primary aberration. The null hypothesis, tested by the Mantel–Haenzel test, was independence between the primary and the subsequent aberrations in each of the 20 samples.

2. Materials and methods

2.1. Tumor specimens

All cases were studied using G-banding and SKY according to standard procedures [12,14]. Except for case 20, all other cases were previously published: cases 1–10 in [12], cases 11–17 in [13] and cases 18–19 in [15]. Case 20 is the cell line STA-BT-1 that was generated from glioblastoma multiform cells of a patient who was born in November 1972 and suffered from neurofibromatosis type 1, non-Hodgkin lymphoma, (chemo- and radiation therapy of the brain), and glioblastoma grade IV, (radio therapy 40 Gy), and who died in April 1990.

2.2. Karyotype analysis

The karyotype of each case was analyzed using five parameters (Table 1): maximum number of chromosomes per cell in all analyzed mitoses (1); number of chromosomes in which there was a primary aberration. i.e., chromosomes that harbor alterations in the original karyotypically described clone (2); number of chromosomes in which there were both primary and subsequent alterations (3); number of chromosomes in which there was no primary aberration (4); number of chromosomes in which there was no primary aberration but that had subsequent alterations (5).

We studied two types of subsequent alterations: duplications of whole chromosomes or derivatives and additional rearrangements. It is necessary to emphasize that the number of chromosomes were counted and not the

Table 1
The assessment of chromosome rearrangement according to 5 parameters in 13 primary tumors and 7 cell lines

Case No.	Type of tumor[a]	Parameters[b]				
		1	2	3	4	5
1	PT	47	2	1	45	1
2	PT	46	1	0	45	0
3	PT	69	8	2	61	1
4	PT	61	4	0	57	4
5	PT	46	6	0	40	2
6	PT	46	5	1	41	0
7	PT	72	11	1	61	6
8	PT	89	3	0	86	0
9	PT	89	9	1	80	0
10	PT	47	4	1	43	1
11	PT	42	11	2	31	1
12	PT	90	10	0	80	2
13	CL	94	21	2	73	5
14	PT	47	2	1	45	3
15	CL	47	3	0	44	1
16	CL	76	18	4	58	3
17	CL	75	7	0	68	3
18	CL	49	7	3	42	3
19	CL	47	9	3	38	0
20	CL	79	18	14	61	18

[a] PT, primary tumor; CL, cell line.

[b] Parameters: 1, maximum number of chromosomes per cell in all analyzed mitoses; 2, number of chromosomes in which there was a primary aberration; 3, number of chromosomes in which there were both primary aberration and subsequent alterations; 4, number of chromosomes in which there was no primary aberration; 5, number of chromosomes in which there was no primary aberration but which had subsequent alterations.

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number of changes. Hence, in the case of more than one subsequent alteration of the same chromosome it was counted only once. For example, the marker der(9)t(7;9;9;7) (Table 1, case #13) was involved in four additional alterations, but was counted only once [13]. Fig. 1 illustrates the determination of these parameters for a neuroblastoma primary tumor (Table 1, case #10).

For all 20 cases we applied the following classification assessments:

1. Subsequent alterations included both clonal and non-clonal alterations.
2. The analysis did not include losses of whole chromosomes.
3. The analysis included only the aberrant clone(s) within a case.
4. The analysis was based only on whole chromosomes and chromosome derivates, not on segments without centromeres. For example, der(2)t(2;9) was counted as a single aberrant chromosome. Whereas balanced translocations such as t(2;9) were counted as two aberrant chromosomes. The same rule was applied to the counting of duplications. For example: der(1)t(1;12) that was duplicated to der(1)t(1;12) × 2 was counted as duplication of one chromosome.
5. In triploid and tetraploid karyotypes, specific rules were applied for the counting of chromosome gain. In a tetraploid karyotype, a duplication of aberrant chromosome was counted only when this chromosome appeared at least three times. In a triploid karyotype, a duplication of the aberrant chromosome was counted only when it was detected in some of the mitoses once and appeared twice in others.

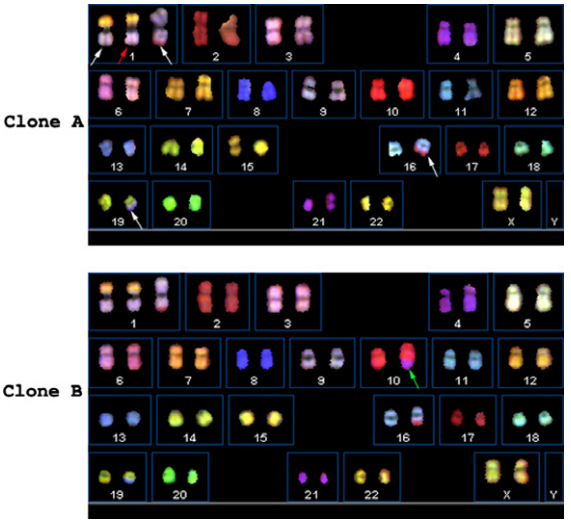

Fig. 1. Determination of the five parameters for the karyotypes of two clones of primary neuroblastoma (Table 1, case #10): parameter 1 is equal to 47. In clone A, there are four chromosomes with a primary aberrations (marked by white arrows) thus parameter 2 will be equal to 4. One of these chromosomes is duplicated (marked by red arrow) thus, parameter 3 will be equal to 1. In contrast, there are 43 chromosomes without any primary change; it means that parameter 4 will be equal to 43. Clone B is different from clone A by harboring a subsequent alteration of chromosome 10 (marked by green arrow) and thus parameter 5 is equal to 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

2.3. Statistical analysis

The goal of the statistical analysis was to compare the probability of subsequent aberrations between chromosomes with primary aberrations and chromosomes with no primary aberrations. For each of the 20 assays we constructed a 2×2 contingency table; the rows of the contingency tables correspond to the number of chromosomes with and without primary aberrations; the columns of the table correspond to the number of chromosomes with and without subsequent aberrations. The null hypothesis tested is independence between the row and the column variables in all the contingency tables – in each assay all chromosomes share a common characteristic probability of undergoing subsequent aberrations. To test the null hypotheses, we applied the Mantel–Haenzel χ^2 test (Mantel–Haenzel test function in S-PLUS 6.2, Copyright Insightful Corp) with the assay acting as the stratum variable. The Mantel–Haenzel indicates whether the row and column variable are independent but cannot reveal the form of their dependency. Lastly, we computed the Z-score statistic corresponding to the Mantel–Haenzel one degree of freedom χ^2 statistic to verify that a primary aberration in a chromosome raises the probability of subsequent aberrations.

3. Results

We found that the proportion of further numerical and structural rearrangements in chromosomes in which there was a primary aberration (22.6%, 36 of 159 chromosomes) was higher than in chromosomes in which there was no primary aberration (4.9%, 54 of 1099 chromosomes). Moreover, stratifying the 20 tumors, the Mantel–Haenzel test rejects the null hypothesis: i.e., primary and subsequent chromosome deformations are statistically dependent (p-value < 0.001). The Z-score corresponding to the Mantel–Haenzel test reveals that chromosomes with primary aberration are more likely to undergo further aberrations than unchanged chromosomes.

It was particularly noticeable that often the same chromosome was affected by more than one subsequent rearrangement or duplication (cases #9, #13, #19, and #20). An example of subsequent rearrangements is shown in

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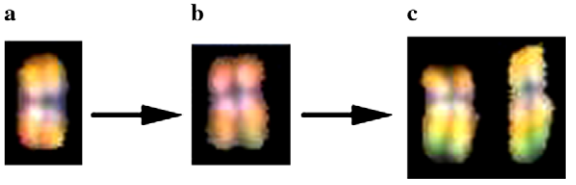

Fig. 2. Subsequent alterations of the rearranged chromosome: (a) der(11)(7qter->7q21::11p15->11q22::7q21->7qter), (b) der(11)(18qter->18q11.2::7q3?->7q21::11p15->11q22::7q21->7qter), (c) a further duplication (Table 1, case 9).

Fig. 2 (Table 1, case #9). Moreover, subsequent rearrangement usually occurred in the same arm that was affected primarily (cases #3, #6, #9 ,#19, and #20).

4. Discussion

The present study demonstrated the importance of utilizing a combination of G-banding and SKY techniques for the characterization of cytogenetic heterogeneity of childhood neural tumors. We could evaluate whether chromosomes that harbor primary aberrations in childhood neural tumors have a higher susceptibility to accumulate further alterations. We found that chromosomes that contain a primary aberration are significantly (p-value < 0.001) more likely to gain further structural rearrangements or to undergo numerical changes (22.6%, 36 of 159 chromosomes) than chromosomes with no initial aberration (4.9%, 54 of 1099 chromosomes). Moreover, we found that often the same chromosome can be affected by more than one subsequent rearrangement or duplication. Therefore, the results supported the notion that primary chromosomal aberrations may accelerate the appearance of additional changes in the affected chromosome. Further, SKY enabled the analysis of more metaphases and characterized numerous aberrant chromosomes, particularly markers of unknown origin that remained undefined by G-banding alone. This led to the uncovering of a larger number of subclones, revealing different evolution pathways of chromosome rearrangements.

We suggest that these findings should be taken into account when mathematical models to determine karyotypic evolution in cancer are constructed. In existing statistical models of data obtained from conventional banding analysis of complex cancer karyotypes, all the chromosomal rearrangements including ''markers'' are counted as single event [1–6]. But cytogenetic banding analysis can underestimate the true prevalence of chromosome abnormalities because in a single marker the presence of several abnormalities of different chromosomes can be masked. Thus, the precise definition of markers by the SKY technique may lead to the determination of an increased number of aberrations per tumor and identification of more spectrums of chromosomes regions involved in the karyotype evolution. Recently, it was shown that tumors frequently progress through at least two karyotypic phases: during Phase I the karyotypes exhibited a power law distribution with an exponent close to unity but when the later transition from Phase I to Phase II/III occurs power law distribution is lost. This transformation indicates a transition from an ordered and highly structured process to chaotic disorder [2,5]. It is possible to speculate that the same transition, from the single rearrangement to multiple alterations, may also occur at the single chromosome level.

Several possible explanations for the phenomena described in our study can be imagined:

1. The organization of rearranged chromosomes may show distinct differences from normal chromosomes (e.g. protein structure, methylation, telomere length) that as a consequence makes them more prone to further rearrangements.

2. Chromosomes are organized into discrete territories and occupy defined positions in three-dimensional space of the interphase nuclei [16]. This nuclear order is essential for the integration of complex biological processes such as DNA replication, RNA processing and transcription and should have a strong influence on interactions between chromosomes such as recombination and double strand DNA repair. Certain chromosomes will be more likely to interact because they are non-randomly close together, and some chromosomes would never be able to come into contact because they occupy very distant positions. An alteration involving a chromosome could not only change its territory position but also boundary or insulator elements of this chromosome and its neighbors and thus increase the possibility to interact with chromosomes that were previously distant [17–20]. In addition, the relocalization of chromosomes can dramatically change their specific replication timing and thus promote their duplication.

In addition, if we assume that a rearranged chromosome results in a gene expression change that is advantageous for the tumor cell then it is realistic to expect that a further gain that results in further

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over or under expression may provide the cell with a further competitive advantage [21].

Thus, we suggest that individual aberrant chromosomes containing structural rearrangements have a higher susceptibility to accumulate further changes. This may imply an associated mechanism both for structural and numerical chromosome instability. Furthermore, unstable chromosomes with an elevated rate of alterations may not only be a key generator in the formation of genetic variability in cancer cells, but might also harbor crucial events involved in tumor development. It is also of note that subsequent alterations and duplications of aberrant chromosomes have been frequently reported in hematological malignancies [22–24]. We postulate that the phenomenon described in the present report represents a common pattern of karyotypic evolution in many cancer cells and the mechanisms involved warrant further investigation.

Acknowledgements

G.R. holds the Djerassi Chair in Oncology at the Sackler School of Medicine, Tel-Aviv University, Israel. This work was performed in partial fulfillment of R.S.'s requirements for a M.Sc. at the Sackler School of Medicine, Tel-Aviv University.

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