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Excessive centrosome abnormalities without ongoing numerical chromosome instability in a Burkitt's lymphoma

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Abstract

Numerical and structural centrosome abnormalities are detected in various human malignancies and have been implicated in the formation of multipolar mitoses, chromosome missegregation, and chromosomal instability. Despite this association between centrosome abnormalities and cancerous growth, a causative role of centrosome aberrations in generating chromosomal instability and aneuploidy has not been universally established. We report here excessive numerical and structural centrosome abnormalities in a malignant Burkitt's lymphoma harboring the characteristic t(8;14) chromosomal translocation. Using conventional karyotyping and fluorescence *in situ* hybridization (FISH), we detected no signs of ongoing numerical chromosome instability, although the tumor displayed sporadic multipolar metaphases. These findings demonstrate that centrosome abnormalities are not a universal surrogate marker for chromosomal instability in malignant tumors. Moreover, our results suggest a model in which additional cellular alterations may be required to promote centrosome-related mitotic defects in tumor cells.

Findings

Numerical and structural centrosome abnormalities are hallmarks of various cancers and have been implicated in the generation of multipolar mitoses and chromosomal instability [1,2]. Centrosomes function as major microtubule organizing centers in animal and human cells and thus contribute importantly to proper spindle formation and function during mitosis [3]. Normally, the single centrosome of a G1 cell duplicates precisely once prior to mitosis in a process that is intimately linked to the cell division cycle [4]. Accurate control of centrosome duplication is critical for symmetric mitotic spindle formation and thereby contributes to the maintenance of genome integrity.

Various oncogenic stimuli have been associated with abnormal centrosome numbers [5]. Centrosome alterations in tumor cells are believed to lead to multipolar mitosis, asymmetric chromosome segregation and aneuploidy [2,6]. For most cancers, however, the association between centrosome aberrations and malignant progression is merely based on correlation and a causative role of centrosome abnormalities in establishing and maintaining numerical chromosome instability as defined by dynamic changes in chromosome copy numbers [7] has not been universally established [6].

Here, we studied centrosome abnormalities and chromosomal instability in an abdominal lymph node biopsy sample from a 19-years-old male patient with recurrent Burkitt's lymphoma (the use of patient material in this

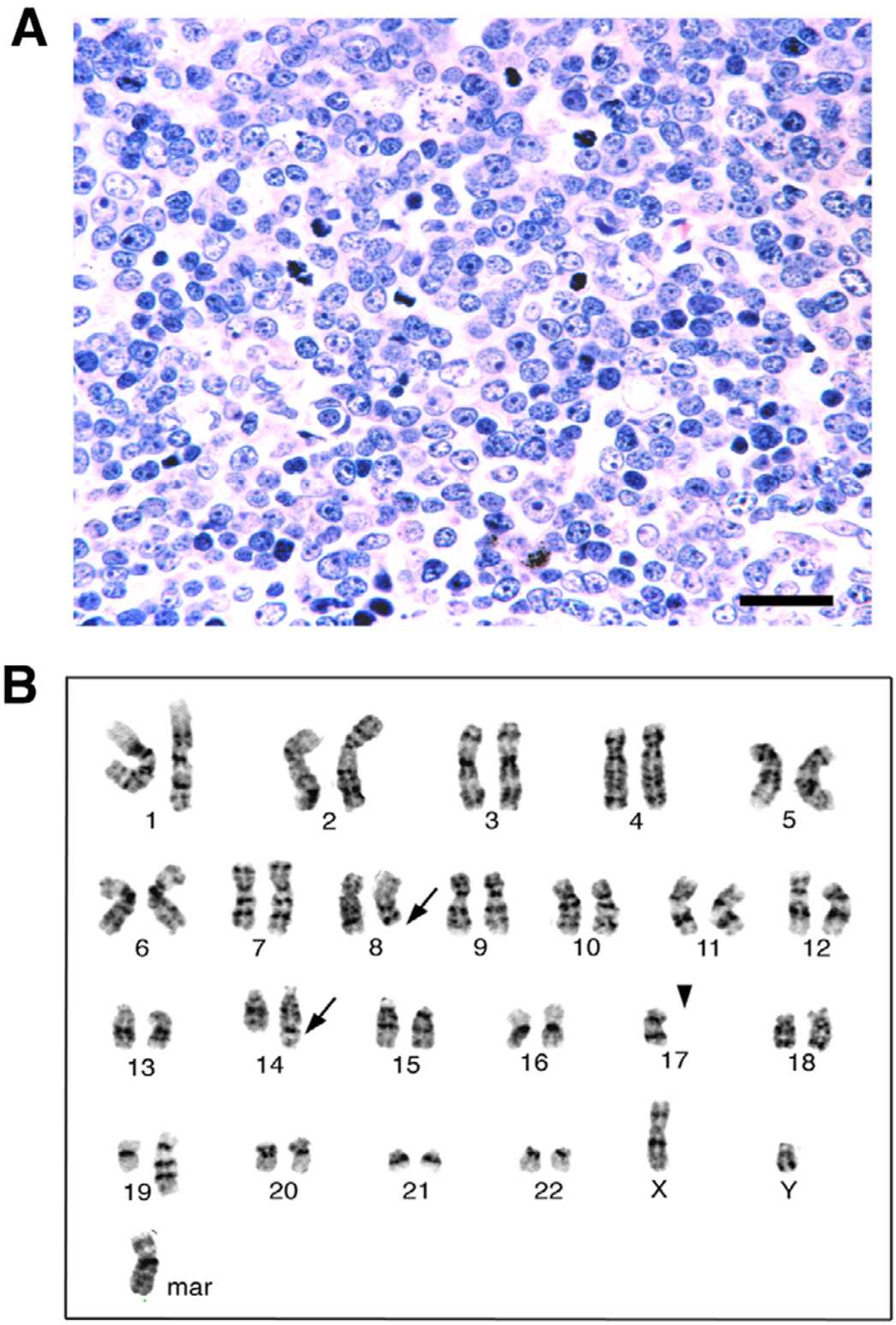

Figure 1
Histology and karyotype of Burkitt's lymphoma. (A) H&E staining of a lymph node biopsy specimen from a patient with Burkitt's lymphoma. Scale bar indicates 50 μm. **(B)** Karyotype analysis. Arrows indicate breakpoints of the diagnostic t(8;14) chromosomal translocation. Arrowhead indicates loss of one copy of chromosome 17 (see text for details).

study was approved by Harvard Medical School Office of Research Subject Protection; HMS IRB docket # E012302-1). Routine hematoxylin & eosin (H&E) staining of formalin-fixed, paraffin-embedded tissue revealed the histomorphology of malignant Burkitt's lymphoma (Fig. 1A). Burkitt's lymphoma is a highly aggressive B cell neoplasia with a characteristic t(8;14) chromosomal translocation involving the c-*MYC* locus on chromosome 8 and typically the immunoglobulin heavy chain locus on chromosome 14 [8]. GTG-banded metaphase cells were obtained from unstimulated 24 hour cultures and the t(8;14) chromosomal translocation was detected in all fifteen metaphases analyzed (Fig. 1B).

Analysis of centrosome abnormalities was performed using immunofluorescence microscopy for the pericentriolar marker γ-tubulin [9]. Briefly, tissue sections were deparaffinized in xylene and dehydrated in ethanol. Slides were then boiled in 10 mM citrate buffer (pH 6.0) for 30 min in a microwave oven followed by digestion with Digest-All-3 pepsin solution (Zymed) for 5 min at 37°C. Slides were blocked with 10% normal donkey serum (Jackson Immunoresearch) for 15 min at room temperature followed by incubation with a mouse monoclonal anti-γ-tubulin antibody (GTU-88; Sigma) at a 1:1000 dilution overnight at 4°C. After washing in PBS, sections were incubated with a donkey anti-mouse rhodamine red conjugated secondary antibody (Jackson Immunoresearch) at a 1:100 dilution in PBS for 2 hours at 37°C. Cells were finally washed in PBS, counterstained with DAPI (Vector), and analyzed by epifluorescence microscopy. Since normal centrosome duplication gives rise to two centrosomes per cell, cells with more than two γ-tubulin dots were considered abnormal. In addition, structural centrosome abnormalities as characterized by deviations from the typical dot-like staining pattern were assessed.

Numerical and/or structural centrosome abnormalities were detected in approximately 30% to 50% of cells (Fig. 2A). Accurate detection of mitotic spindle poles, however, was obscured due to tissue sectioning which precluded a quantitative evaluation of centrosome-associated mitotic abnormalities. However, some abnormal mitotic figures were observed in H&E stained sections (Fig. 2B,2C). Quantitation of more than one-hundred mitoses revealed a normal configuration in 95% of cells (76% normal metaphases and 19% normal anaphases – Fig. 2B,2D). We found a multipolar metaphase arrangement in 5% of cells (Fig. 2C,2D), however, multipolar anaphases were absent.

To investigate whether the observed centrosome abnormalities were associated with ongoing numerical chromosome instability, chromosome 11 copy numbers were analyzed by fluorescence *in situ* hybridization (FISH). Chromosome 11 copy number changes are among the five most frequently found numerical chromosome abnormalities in Burkitt's lymphoma karyotypes retrieved from the CGAP database [10]. For interphase FISH, a Spectrum Green-labeled chromosome 11 α-satellite probe (D11Z1) was used according to the manufacturer's protocol (Vysis). None out of more than one-thousand cells analyzed showed more than two copies of chromosome 11 (Fig. 2E). Consistent with this observation, cytogenetic analysis revealed a single predominant pseudo-diploid karyotype: 46, XY, t(8;14)(q24;q32),-17, der(19)t(7;19)(q11.2;q13), +mar (Fig. 1B). This karyotype is in line with previous studies that showed no evidence for gross aneuploidy in most Burkitt's lymphomas [10]. The absence of ongoing numerical chromosome instability was not due to impaired proliferative activity as evidenced by the high mitotic index (Fig. 1A).

The tumor suppressor protein p53 is inactivated in up to 40% of Burkitt's lymphoma [11] and loss of one copy of chromosome 17, which harbors the p53 gene, is found in the case presented here. In order to test whether the remaining p53 allele might be mutated and since most mutant p53 proteins are stabilized [12], we performed p53 immunofluorescence staining of the Burkitt's lymphoma in comparison to normal lymph node tissue (Fig. 3). Tissue sections were processed as described above and incubated with a mouse monoclonal anti-p53 antibody (DO-7, Novocastra) at a 1:100 dilution overnight at 4°C followed by a rhodamine red-conjugated secondary donkey anti-mouse antibody at a 1:100 dilution for 2 hours at 37°C. In contrast to normal tissue, immunofluorescence analysis of the Burkitt's lymphoma revealed strong p53 positivity suggesting that the remaining p53 allele has undergone mutation (Fig. 3).

Taken together, our results show that centrosome abnormalities do not necessarily correlate with ongoing numerical chromosome instability and that even the presence of multipolar metaphases is not sufficient to predict whether a cell or a tumor is chromosomally unstable. This indicates that numerical chromosome stability can be maintained in a tumor despite high-level centrosome abnormalities. In conclusion, the impact of centrosome abnormalities on genomic destabilization and malignant progression needs to be analyzed in the context of the cellular background in which these alterations occur.

The question why a tumor cell with centrosome abnormalities does not necessarily acquire numerical chromosome imbalances may have several answers. In general, two models that lead to centrosome abnormalities with distinct functional consequences can be envisioned [13]. According to the first model, numerical centrosome abnormalities can arise as a consequence of abortive mitotic events and impaired cytokinesis [14–16]. Under

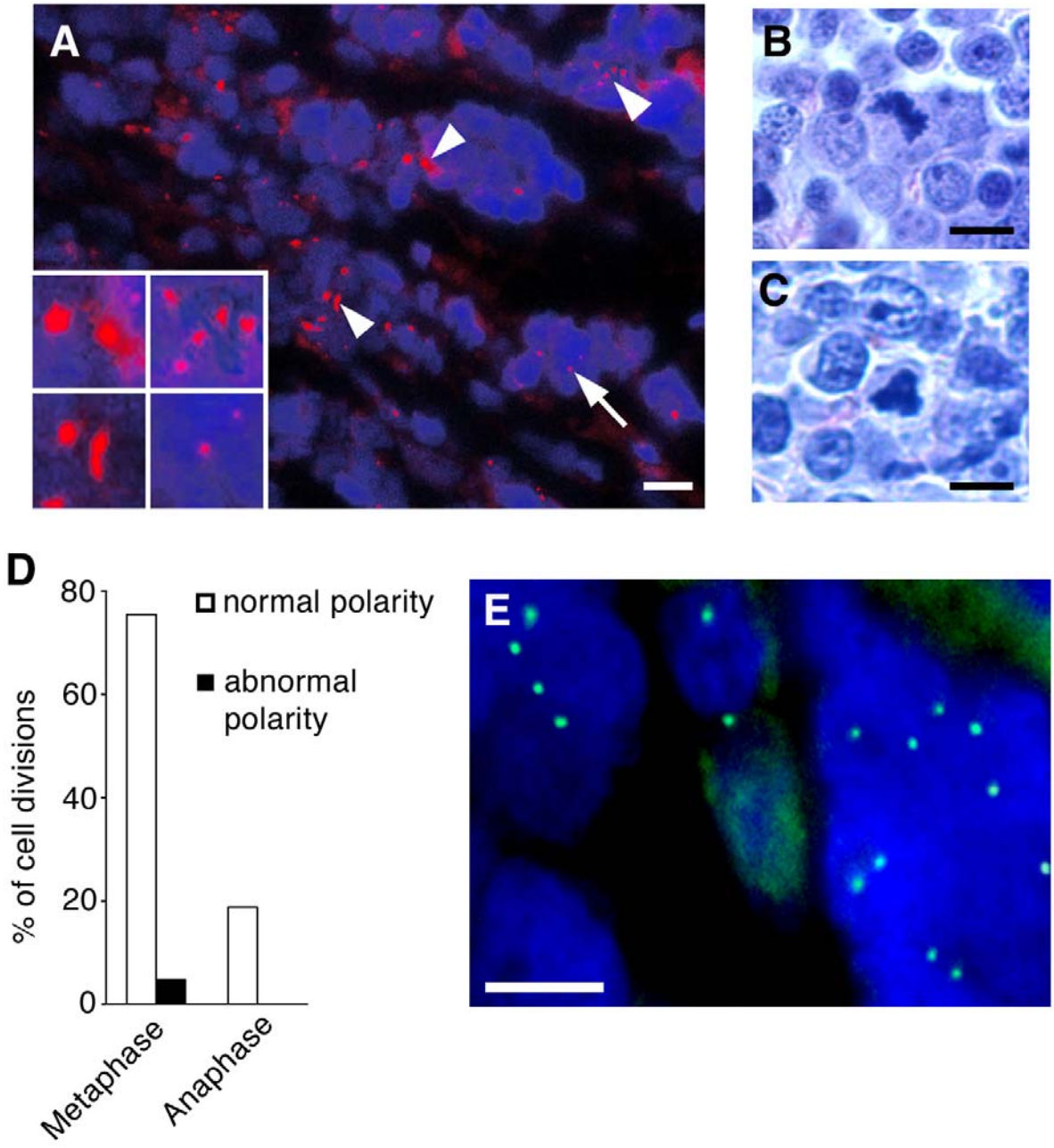

Figure 2

Excessive centrosome abnormalities without ongoing numerical chromosome instability. (A) Centrosome abnormalities as detected by immunofluorescence microscopy for γ-tubulin. Arrow indicates a normal centrosome (corresponding to lower right insert). Arrowheads indicate abnormal centrosomes (arrowheads and inserts correspond counter-clockwise). Both numerical abnormalities (top right insert) and structural abnormalities with excessive γ-tubulin positive material are found (top left and lower left inserts). Nuclei stained with DAPI. Scale bar indicates 10 μm. **(B)** Normal mitotic figure detected in H&E stained section. Scale bar indicates 10 μm. **(C)** Abnormal multipolar mitotic figure in H&E stained section. Scale bar indicates 10 μm. **(D)** Quantitation of lymphoma cells undergoing metaphase or anaphase with a normal polarity (open bars) as opposed to an abnormal polarity (black bars) detected in H&E stained sections. More than one-hundred cell division figures were analyzed. **(E)** Fluorescence *in situ* hybridization (FISH) analysis for chromosome 11 confirms diploidy of the malignant lymphoma. Nuclei stained with DAPI. Scale bar indicates 10 μm.

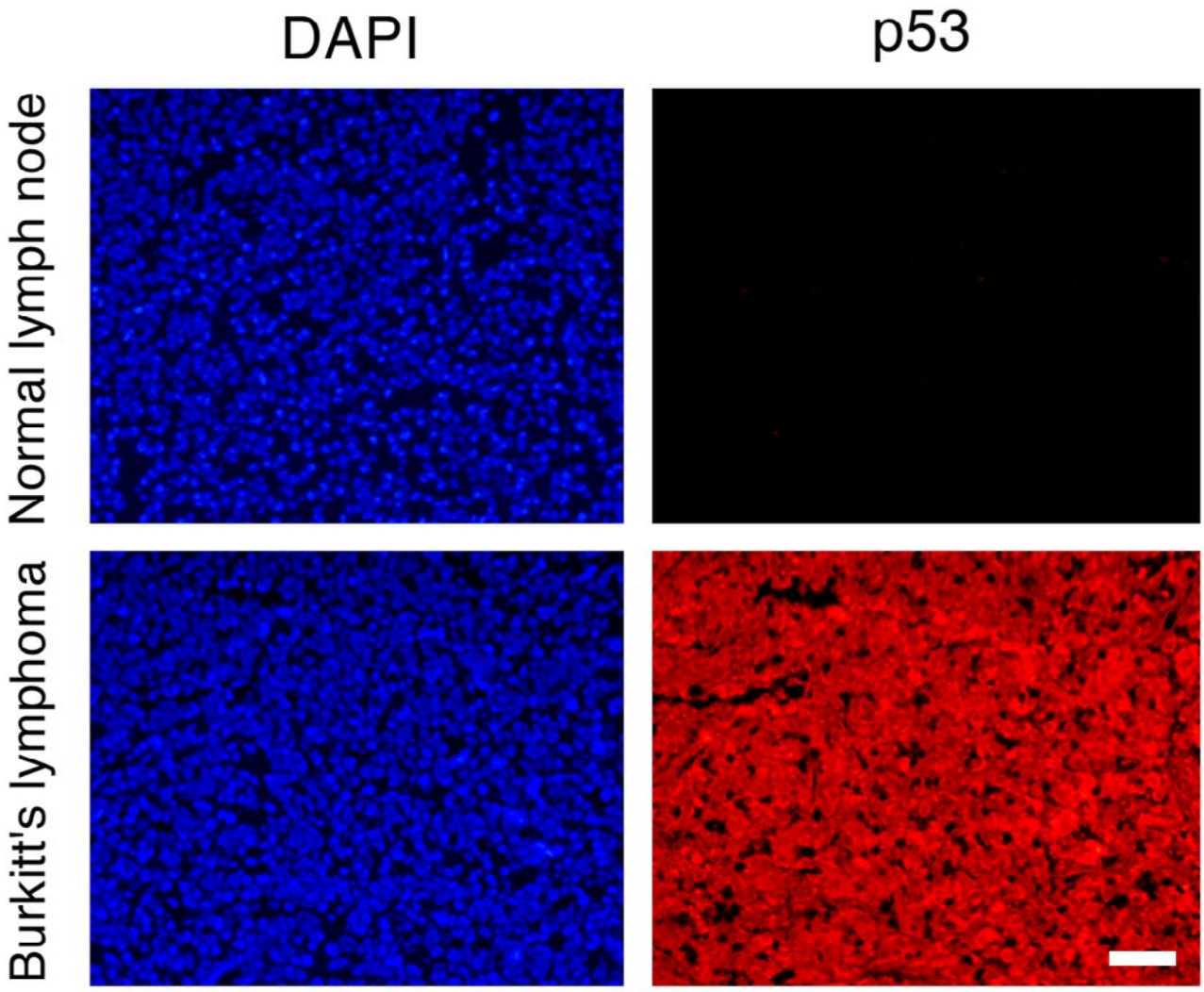

Figure 3
Accumulation of p53 in Burkitt's lymphoma. Expression of p53 was assessed by immunofluorescence staining (DO-7 monoclonal antibody) using sections from a normal lymph node (upper panels) as negative control. The Burkitt's lymphoma (lower panels) shows strong immunoreactivity for p53. Scale bar indicates 50 μm.

such conditions, centrosome abnormalities are unlikely to cause expansion of a population of genomically unstable tumor cells because such cells are unable to complete mitosis and to give rise to viable progeny. In striking contrast, studies in breast cancer and primary human cell populations expressing the human papillomavirus type 16 (HPV-16) E7 oncoprotein provided evidence that abnormal centrosome duplication can precede genomic destabilization and arise in morphologically normal cells with functional p53 [15,17]. Such cells have no inherent cell division defects, and thus, they are more likely to undergo abnormal mitosis even though additional checkpoint mechanisms may need to be subverted in order to give rise to chromosomally unstable daughter cells. In support of the notion that abnormal centrosome numbers and multipolar mitoses do not necessarily cause genomic instability, it has been shown that in HPV E7-expressing cells there are dramatic quantitative differences between multipolar metaphase cells and cells with a multipolar ana- or telophase configuration [18,19]. A similar finding is presented here where multipolar anaphases were undetectable despite approximately 5% of multipolar metaphase cells (Fig. 2B,2C,2D). The molecular basis for a hypothetical checkpoint that halts mitotic progression in

the presence of multiple spindle poles has not yet been established [20]. Although p53 has been implicated in a post-mitotic checkpoint following spindle disruption [21], results presented here and reports by others [22,23] suggest that checkpoint mechanisms other than p53 are involved in suppressing aneuploidy. This does not rule out that p53 inactivation contributes indirectly to genomic destabilization [24] by interfering with apoptotic cell death and thus allowing chromosomally unstable cells to remain in the proliferative pool. Another mechanism that may limit chromosome missegregation in the presence of multiple spindle poles involves coalescence of centrosomes whereby a bipolar spindle is formed by clusters of multiple centrosomes [25].

Overexpression of c-MYC *in vitro* has been shown to result in structural chromosomal aberrations [26] and DNA damage [27]. Structural chromosome abnormalities in addition to the t(8;14) translocation are frequently detected in Burkitt's lymphoma including the case presented here. These findings, together with the observed loss of one copy of chromosome 17, may indicate that chromosomal instability might have been present at earlier stages of lymphomagenesis.

Since overexpression of c-MYC can cause a G2/M arrest and polyploidization [28,29], accumulation of centrosomes in cells with impaired mitotic progression likely accounts for the majority of cells with centrosome abnormalities. Whether the c-*MYC* oncogene can directly contribute to the formation of multiple centrosomes in otherwise normal cells as it has been shown for the HPV-16 E7 oncoprotein [15] awaits further clarification.

In summary, our results show that numerical and/or structural centrosome abnormalities are not a universal surrogate marker for chromosomal instability. Further studies are warranted to investigate whether other cancers show a similar lack of correlation between centrosome abnormalities and numerical chromosome instability. Moreover, our study suggests that cellular mechanisms other than p53 inactivation may interfere with progression through mitosis in the presence of centrosome-related cell division defects. Further investigations addressing these problems will help to lead to a more definitive model of the role of centrosome abnormalities in cancer development and progression.

Abbreviations

DAPI, 4',6'-diamidino-2-phenylindole; EDTA, ethylenediaminetetraacetate; GTG, G-bands obtained with trypsin and Giemsa; H&E, hematoxylin & eosin; PBS, phosphate-buffered saline.

Authors Contributions

Author 1 (S. D.) coordinated the study and performed immunofluorescence analysis of centrosomes and FISH. Author 2 (B. H. L.) participated in the histological diagnosis of the lymphoma and also in the collection of tumor material and clinical information, and provided the hematoxylin & eosin image. Author 3 (P. D. C.) performed the cytogenetic analysis and diagnosis of the malignant lymphoma and provided the image of the karyotype. Author 4 (K. M.) participated in the design and interpretation of the study. All authors read and approved the final manuscript.

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