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***MYCN* overexpression is linked to significant differences in nuclear DNA organization in neuroblastoma**

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Abstract

Neuroblastomas are the most common extracranial cancers in children. They have a complex morphology and heterogeneous clinical course. Amplification of the *MYCN* oncogene is seen in 50% of high-risk neuroblastomas and is associated with a poor clinical outcome with a higher rate of progression and relapse when compared with tumors without *MYCN* amplification. *MYCN* amplification drives genomic instability, dynamic remodeling of chromosomes and changes in nuclear organization. However, understanding of how the chromatin structure responds to *MYC* overexpression in neuroblastoma is nebulous. In the present study, we examined neuroblastoma samples with and without *MYCN* amplification as well as in the neuroblastoma cell line SH-EP transfected with *MYCN* pUHD. Our neuroblastoma cases segregated into one of three prognostic clusters of increasingly poor prognosis. Clusters I and II encompassed tumors with no *MYCN* amplification, while cluster III included tumors with *MYCN* amplification. Measurements of both the DNA structure ($p < 0.001$) and structure of the DNA-free space ($p < 0.001$) showed significant DNA structural alterations associated with *MYCN* amplification indicating that there is a progressive disruption of nuclear DNA organization with *MYCN* amplification suggesting that *MYCN* may play an functional role in determining the nuclear structure in neuroblastoma.

Keywords: Neuroblastoma, unfavorable prognosis, *MYCN* amplification, genomic instability and 3D-SIM super resolution microscopy.

Introduction

Neuroblastoma is the most common solid extracranial neoplasm in children. The tumor is characterized by morphological, clinical and molecular heterogeneity. Selection of the appropriate treatment and risk calculation must take into consideration all these factors. The tumor is composed of proliferating neuroblasts with variable degrees of differentiation and amounts of Schwannian stroma. A complex morphological classification scheme based on these parameters has been developed and refined over the past several decades [1]. This allows a given tumor to be categorized as having either a favorable or unfavorable histology pattern [1]. Neuroblastoma patients are stratified into low-, intermediate-, and high-risk groups based on stage, age, *MYCN* status, histology, and chromosomal ploidy. High-risk patients survival is about 40–50% even after intensive therapies and immunotherapy [1,2].

Among the most important molecular parameters predictive of an unfavorable prognosis are *MYCN* amplification [2, 3] and *MYCN* protein overexpression [4, 5]. *MYC* dysregulation initiates a dynamic process of genomic instability leading to multiple changes in nuclear organization that alter the position and function of telomeres, such as formation of telomeric aggregates and initiation of breakage-bridge fusion cycles [6,7].

In a previous study three-dimensional telomere profiling was used to differentiate levels of genomic instability in neuroblastoma and to determine whether these parameters might have prognostic significance [8]. Three subgroups could be distinguished based on increasing levels of telomere dysfunction; specifically, a greater number of telomeres, telomer aggregates and short telomeres. Tumors with greater levels of telomere dysfunction were associated with unfavorable prognostic parameters, including greater age at diagnosis, unfavorable histology, higher stage of disease, *MYCN* amplification, and a higher *MYCN* protein expression levels. Tumors with greater levels of telomere dysfunction also had higher levels of intratumoral heterogeneity, a feature also associated more aggressive clinical behavior. This study also demonstrated that *MYCN* overexpression induced in two neuroblastoma cell lines with low constitutive *MYCN* expression resulted in changes in their telomere profiles associated with increased telomere dysfunction and genomic instability.

MYCN overexpression has been linked to chromatin remodeling pointing to a possible role of *MYCN* in histone acetylation and nucleosome remodeling [7]. However, the understanding of how chromatin structure changes in response to *MYC* overexpression in neuroblastoma is still limited. Three-Dimensional Structured Illumination Microscopy (3D-SIM) allows visualization of chromatin distribution within the nucleus through fluorescent DNA staining, capturing the differences in nuclear DNA architecture between normal cells and cancer cells for quantification [9]. The 3D-SIM images of DNA, reveals structural features inside the interphase nucleus and qualitative differences in the DNA structure between cell types that had not been seen with conventional microscopy methods [9]

In the current pilot study, we used quantitative 3D-SIM super resolution microscopy to investigate the nuclear organization of DNA in both a neuroblastoma cell line and in patient tumor samples with and without *MYCN* amplification.

Methods

Patient Samples

Five primary treatment neuroblastoma tissue samples, 5 μ m thickness, were obtained from the Health Sciences Centre (Winnipeg, Manitoba, Canada) and examined in the lab in a blinded manner. The clinical data for the five patients is shown in Table 1. Two patients had tumor with *MYCN* amplification. Three had tumors that were negative for *MYCN* amplification, but all three of these showed *MYCN* protein expression. The clinical stage of each patient is also shown in Table 1. Hematoxylin and eosin–stained sections were used to identify areas of the tumor for analysis. Patients were classified according to the International Neuroblastoma Staging System (INSS) [10] and divided into clinical-genetic risk groups using the COG risk scoring system [11]. The *MYCN* amplification status was determined by FISH. This study was approved by Pathology Access Committee for Tissue (12-0048), Health Science Centre Research Impact committee (2012:187), CancerCare Manitoba Research Resource Impact Committee (92-2012), and Research Ethics Board (H2012:391)

Table 1: The clinical data for the five neuroblastoma patients

Groups	Case Number	Age	Gender	Stage*	Histology	MYCN protein
A. MYCN amplified	A1	2	M	L2(4)	Unfavourable	ND
	A2	2	M	L1(1)	Unfavourable	ND
B. MYCN not amplified	B1	1	F	L2(4)	Unfavourable	Yes
	B2	2	F	L2(4)	Unfavourable	Yes
	B3	8 mo	M	Ms (4s)	Favourable	No

*International neuroblastoma risk group staging system - INRGSS (International neuroblastoma staging system INSS). ND – not done.

Cell Line and *MYCN* Transfection

The established neuroblastoma cell line SH-EP was cultured and transfected as previously described [8]. Briefly, SH-EP cells were transfected with *MYCN* pUHD 10-3, which contains the entire *MYCN* coding sequence under the control of an hCMV minimal promoter. The transfection efficiency was 40% and after sterile sorting by GFP expression the transfected cells were cultured for 120h before slide preparation for 3D structured illumination microscopy [12]. Mock-transfected cells were used as a control.

3D Telomere profile and 3D structured illumination microscopy (SIM)

Nuclei from five 5- μ m tissue sections underwent 3D telomere profiling with a peptide nucleic acid telomere probe (DAKO, Glostrup, Denmark). The hybridization and image acquisition were performed according to previously published protocols [13,14]. The slides were imaged using an

Axiolmager Z2 microscope (Carl Zeiss) with a 63×/1.4 oil objective lens (Carl Zeiss), and DAPI and Cy3 filters for detection of nuclear DNA staining and telomere probe signals, respectively (Carl Zeiss). One hundred cells were analyzed per tumor sample. The 3D interphase telomere signals were quantified with TeloView software [15] and the samples were classified based on their 3D telomere organization using hierarchical cluster analysis as previously described [8].

The slides for 3D structured illumination microscopy (3D-SIM) were prepared according to the protocol published by Righolt et al. [4]. One hundred cells were imaged with a Zeiss Elyra PS1 SIM equipped with a Plan-Apochromat 63×/1.40 oil immersion objective using an Andor EM-CCD iXon 885 camera and a 1.6× tube lens. The DAPI channel was obtained with 405nm laser excitation. The 3D-SIM images were reconstructed with ZEN 2012 black edition (Carl Zeiss, Jena, Germany).

Statistical Analysis

Statistical analysis for 3D telomere profile was conducted using SAS 9.3 (SAS Institute Inc., Cary, NC) software. Hierarchical clustering analysis was used to subgroup the neuroblastoma tissue samples based on the following telomere parameters: number of telomeres per cell, number of telomeric aggregates per cell, percentage of cells per patient with aggregates, number of telomeres per nuclear volume, and *a/c* ratio. The nested factorial analysis of variance with Tukey's multiple-comparisons test was used to compare the telomere parameters between the subgroups [8].

The image processing and measurement steps for 3D structured illumination microscopy were performed in a granulometry program [12] that measured the DNA structure and DNA-free space [12]. To assess the significance of the measured difference, we used two-sided, two-sample Kolmogorov–Smirnov (KS) tests and the significance of differences in the classification error was assessed with the McNemar test [12]. A *p*-value < 0.05 was considered significant. The “light granulometry” analysis quantifies the DNA structure, and the “dark granulometry” analysis quantifies the spaces void of DNA structure [12].

Results

3D Telomere profiling: Our previous paper showed that neuroblastoma patient samples have unique levels of genomic instability and higher levels of telomere dysfunction, allowing 3D telomere profiles to be used as a tool that can stratify neuroblastoma patient into subgroups [8]. The hierarchical cluster analysis based on the measured telomere parameters revealed the presence of three unique subgroups [8]. We classified our five neuroblastoma patients using the same criteria and cluster analysis (Figure 1). In accordance with the previously results, the subgroups I and II consisted of tumors with no *MYCN* amplification. Tumors with *MYCN* amplification segregated to subgroup III.

Figure 1: Hierarchical cluster analysis based on the measured telomere parameters (A) and 3D telomere profile (B). One patient was classified as subgroup I (purple), two in subgroup II (red and green) and patients with *MYCN*-amplification in subgroup III (both in blue) and 3D telomere profile matched with previous results.

Super-resolution microscopy: Super-resolution microscopy is a technology that has been used to study the cancer cell genome, specifically the nuclear DNA organization of normal and malignant cells. In general, nuclei of cancer cells show more DNA-free and DNA-poor spaces compared to normal cells [12,16,17]. In order to investigate differences in the DNA structure and DNA poor spaces produced by *MYCN* amplification, we compared the neuroblastoma patient samples (with and without *MYCN* amplification) and the SH-EP cell line transfected with *MYCN* with control (mock cells) using 3D-SIM (Figure 2).

Figure 2: Representative slices from DAPI-stained cell nuclei from patient samples with (A and B) and lacking (C and D) *MYCN* amplification. The scale bars are 10µm for each figure.

We used 3D structured illumination microscopy (SIM) and a granulometry-based measurement technique to quantify the DNA structure and structure of DNA-free space within interphase nuclei. The cumulative distributions of structure sizes are presented in Figure 3 and 4. The size distribution of both the DNA structure ($p < 0.001$; two-sided, two-sample Kolmogorov–Smirnov test) and DNA-free space ($p < 0.001$) was significantly different between *MYCN*-amplified and tumors without *MYCN* amplification. These differences were also seen in our study of *MYCN*-transfected cell lines. Both the DNA structure ($p < 0.001$) and structure of the DNA-free space ($p < 0.001$) contained more structure for *MYCN* amplified samples. *MYCN* amplified patient samples and *MYCN*-transfected SH-EP cells contained more DNA-free space; this is also visible in the cell images (Figure 2) $p < 0.001$. This exemplifies the progressive disruption of nuclear DNA organization in *MYCN* amplified samples when compared with those without *MYCN* amplification, suggesting that *MYCN* may play an important role in determining the nuclear structure in neuroblastoma.

Figure 3: Comparison between the neuroblastoma cell line SH-EP (I) in red and SH-EP cell line transfected with *MYCN* pUHD 10-3 after 120h (II) in blue, which contains the entire *MYCN* coding sequence under the control of an hCMV minimal promoter. The granule size distribution of the DNA is bigger for wild-type SH-EP cells. SH-EP transfected cells contain more DNA structures with lower size ($p<0.001$). In addition, SH-EP transfected cells have more DNA-free space when compared with wild-type SH-EP cells ($p<0.001$).

Figure 4: Comparison between neuroblastoma patients samples with *MYCN* amplification (I) in red and without *MYCN*-amplification (II) in blue. The *MYCN*-amplified cells contain more DNA structures than those without *MYCN*-amplification and the size of the structures are smaller in *MYCN*-amp than in without *MYCN*-amp ($p<0.001$). *MYCN*-amp cells also have more DNA-free space when compared to cells without *MYCN*-amp ($p<0.001$).

Discussion

The three-dimensional nanometer-scale resolution enabled by 3D SIM has transformed our understanding of DNA structure, allowing visualization of chromatin distribution within the cell nucleus inaccessible to conventional light optical techniques [18-22]. Super-resolution (SR) imaging has evolved from a tool built and used by physicists, to a commercially available imaging system that is now more broadly applied to multiple fields, especially in the past decade when it became obvious that sub-Abbe resolution of cellular structures became feasible and enhanced our knowledge of structures in cells dramatically [23-25]. For example, recent work used SR imaging to visualize CD13 for drug targeting in acute myeloid leukemia [26], hepatitis C infection [27], and organelle-cytoskeleton interactions [28]; neuroscience [29], plant cell biology [30], bacterial enzymatics [31], and cell biology [32,33] have all taken off since the development of SR imaging. Chromatin interactions and chromatin domains have been mapped using SR [34-36] and linked to epigenetic states [37]. SR has also been used to examine the plasticity of the genome and reversible changes it may undergo after ischemic stress [38] and to map double stranded DNA breaks [39].

Analysis of nuclear chromatin distribution has been proven to be a highly effective tool to quantify changes in chromatin architecture associated with malignancy and disease progression [20-22]. The value of super-resolution microscopy technique in assessing and quantifying differences in nuclear structure between normal and disease cells has already been demonstrated in Hodgkin's lymphoma, multiple myeloma and in Alzheimer's disease [12,18-19, 21-22]. The first comprehensive 3D SIM studies investigating the 3D nuclear DNA organization in cancer and addressing the changes in the organization were performed by our group [12, 16, 17]. We found that cancer-associated alterations to DNA organization in Hodgkin's lymphoma and multiple myeloma respectively. Using quantitative measurements of nuclear DNA structure and of DNA-poor/free spaces, this difference became important in distinguishing normal DNA organization from that of early lesions and cancer in myeloma patients [17] and to mapping the progression from mono-nucleated Hodgkin's to multi-nucleated Reed Sternberg cells in Hodgkin's Lymphoma [12,16].

The current study on neuroblastomas with *MYCN* amplification continues this line of investigation. Here, we are able to demonstrate the role of *MYCN* in nuclear remodeling of the neuroblastoma cell line SH-EP, transfected with *MYCN*. *MYCN* overexpression altered nuclear location and unbalanced gain of 17q in neuroblastoma cell line SH-EP suggesting a functional relationship between *MYCN* and chromosome 17 copy number status in neuroblastoma [7]. The unbalanced gain of 17q is the most frequent chromosome aberration in neuroblastoma and is an independent prognostic factor of poor outcome [8]. This functional role of *MYCN* in nuclear remodeling is reinforced by primary tumor sections that confirm the same association of nuclear remodeling and *MYCN* amplification status. Our results highlighted the increase in DNA-free/poor space in the nuclei of samples from *MYCN*-amplified patients. Similar findings were described in other diseases, where disease aggressiveness was correlated with an increase in DNA-poor space [12,16,17].

There are quite few studies addressing the nuclear organization of the genome using 3D SIM. Pioneering studies into the 3D spatial genome arrangement indicate that nuclei of normal cells

show a network of channels and wider lacunas, named interchromatin compartment [40]. Interchromatin compartments or DNA poor spaces can expand upon ischemic stress and revert to normal conditions [40]. The 3D SIM data suggest that the DNA poor spaces are indeed spaces in between chromosomes. It seems that chromosome territories (CT) can mix and form contacts with each other in the absence of DNA poor spaces [41]. CT arrangements are not random, and transcriptionally inactive heterochromatin in the nucleus is preferentially localized along the nuclear periphery [42]. In addition, individual genes that are expressed are typically found more interiorly in the nuclei than those that are not expressed. The nonrandom radial positioning of CT suggests possible interaction patterns among the CT population [42]. The DNA poor spaces formation can disturb CT location and interaction, leading to a disease aggressiveness phenotype.

Although *MYCN* amplification is considered a strong marker of poor outcome in clinical and treatment implications, some patients present with intratumoural heterogeneity in *MYCN* status, with coexistence of clusters of *MYCN* amplified tumor cells with non-*MYCN*-amplified cells [43]. Patients with heterogeneous *MYCN* amplification are difficult to diagnosis, leading to undetected cases and wrong treatment decision. No specific therapeutic strategies exist for those patients, due to the unclear biological and clinical impact of this heterogeneity, highlighting the necessity to combine investigational tools [43]. These findings from this pilot study support the regular use of super-resolution technologies to detect genomic instability and define structural biomarkers of disease for diagnosis and disease-monitoring purpose. To achieve this goal, larger cohort studies are required.

Conclusions

The data of this pilot study indicate the role of *MYCN* in nuclear remodeling of genome in neuroblastoma and highlight future possible applications of 3D – SIM as combine investigational tool in neuroblastoma diagnosis and management.

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References

1. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, et al. The International Neuroblastoma Pathology Classification (the Shimada system). *Cancer*. 1999;86(2):364-72.
2. Schwab M. Amplification of N-myc as a prognostic marker for patients with neuroblastoma. *Semin Cancer Biol*. 1993;4(1):13-8.
3. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med*. 1985;313(18):1111-6.
4. Hiyama E, Hiyama K, Yokoyama T, Ishii T. Immunohistochemical analysis of N-myc protein expression in neuroblastoma: correlation with prognosis of patients. *J Pediatr Surg*. 1991;26(7):838-43.

5. Bordow SB, Norris MD, Haber PS, Marshall GM, Haber M. Prognostic significance of *MYCN* oncogene expression in childhood neuroblastoma. *J Clin Oncol*. 1998;16(10):3286-94.
6. Mai S. Initiation of telomere-mediated chromosomal rearrangements in cancer. *J Cell Biochem*. 2010;109:1095-1102
7. Kuzyk A, Mai S. c-MYC-induced genomic instability. *Cold Spring Harb Perspect Med*. 2014 Apr 1;4(4):a014373.
8. Kuzyk A, Gartner J, Mai S. Identification of Neuroblastoma Subgroups Based on Three-Dimensional Telomere Organization. *Transl Oncol*. 2016;9(4):348-56.
9. Szczurek A, Birk U, Knecht H, Dobrucki J, Mai S, Cremer C. Super-resolution binding activated localization microscopy through reversible change of DNA conformation. *Nucleus*. 2018 Jan 1;9(1):182-189.
10. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol*. 1993;11:1466–1477.
11. Maris JM. The biologic basis for neuroblastoma heterogeneity and risk stratification. *Curr Opin Pediatr*. 2005;17:7–13
12. Righolt CH, Guffei A, Knecht H, Young IT, Stallinga S, van Vliet LJ, et al. Differences in nuclear DNA organization between lymphocytes, Hodgkin and Reed-Sternberg cells revealed by structured illumination microscopy. *Journal of Cellular Biochemistry*. 2014;115, 1441–1448.
13. Knecht H, Kongruttanachok N, Sawan B, Brossard J, Prevost S, Turcotte E. Three-dimensional telomere signatures of Hodgkin- and Reed-Sternberg cells at diagnosis identify patients with poor response to conventional chemotherapy. *Transl Oncol*. 2012;5:269–277.
14. Kuzyk A, Mai S. Selected telomere length changes and aberrant three-dimensional nuclear telomere organization during fast-onset mouse plasmacytomas. *Neoplasia* 2012;14:344–351.
15. Vermolen BJ, Garini Y, Mai S, Mougey V, Fest T, Chuang TC. Characterizing the three-dimensional organization of telomeres. *Cytometry A*. 2005;67:144–150
16. Righolt CH, Knecht H, Mai S. DNA superresolution structure of reed-sternberg cells differs between long-lasting remission versus relapsing Hodgkin's lymphoma patients. *Journal of Cellular Biochemistry*. 2016; 117, 1633–1637.
17. Sathitruangsak C, Righolt CH, Klewes L, Tammur P, Ilus T, Tamm A, et al. Quantitative superresolution microscopy reveals differences in nuclear DNA organization of multiple myeloma and monoclonal gammopathy of undetermined significance. *Journal of Cellular Biochemistry* 2015;116, 704–710.
18. Birk UJ. *Super-Resolution Microscopy: A Practical Guide*. Weinheim (Berlin): Wiley-VCH Verlag GmbH & Co. KGaA; 2017
19. Cremer C, Masters BR. Resolution enhancement techniques in microscopy. *Eur Phys J H*. 2013;38:281–344.
20. Galbraith CG, Galbraith JA. Super-resolution microscopy at a glance. *J Cell Sci*. 2011;124:1607–1611.
21. Sathitruangsak C, Righolt CH, Klewes L, Chang DT, Kotb R, Mai S. Distinct and shared three-dimensional chromosome organization patterns in lymphocytes, monoclonal gammopathy of undetermined significance and multiple myeloma. *Int J Cancer*. 2017;140:400–410.
22. Garcia A, Huang D, Righolt A, Righolt C, Kalaw MC, Mathur S, et al. Super-resolution structure of DNA significantly differs in buccal cells of controls and Alzheimer's patients. *J Cell Physiol*. 2017;232:2387–2395.

23. Schermelleh L, Carlton PM, Haase S, Shao L, Winoto L, Kner P, et al.. Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. *Science*. 2008 Jun 6;320(5881):1332-6..
24. Kner P, Chhun BB, Griffis ER, Winoto L, Gustafsson MG. Super-resolution video microscopy of live cells by structured illumination. *Nat Methods*. 2009 May;6(5):339-42.
25. Shao L, Kner P, Rego EH, Gustafsson MG. Super-resolution 3D microscopy of live whole cells using structured illumination. *Nat Methods*. 2011 Oct 16;8(12):1044-6.
26. Xi Y, Wang D, Wang T, Huang L, Zhang XE. Quantitative profiling of CD13 on single acute myeloid leukemia cells by super-resolution imaging and its implication in targeted drug susceptibility assessment. *Nanoscale*. 2019 Jan 23;11(4):1737-1744.
27. Pereira PM, Jacobs C, Grove J. Investigating Hepatitis C Virus Infection Using Super-Resolution Microscopy. *Methods Mol Biol*. 2019;1911:247-261.
28. Guo Y, Li D, Zhang S, Yang Y, Liu JJ, Wang X, et al. Visualizing Intracellular Organelle and Cytoskeletal Interactions at Nanoscale Resolution on Millisecond Timescales. *Cell*. 2018 Nov 15;175(5):1430-1442.e17.
29. Igarashi M, Nozumi M, Wu LG, Cella Zanacchi F, Katona I, Barna L, et al. New observations in neuroscience using superresolution microscopy. *J Neurosci*. 2018 Oct 31;38(44):9459-9467.
30. Shaw SL, Thoms D, Powers J. Structured Illumination Approaches for Super-Resolution in Plant Cells. *Microscopy (Oxf)*. 2018 Oct 8.
31. Söderström B, Chan H, Daley DO. Super-resolution images of peptidoglycan remodelling enzymes at the division site of *Escherichia coli*. *Curr Genet*. 2019 Feb;65(1):99-101.
32. Walther N, Ellenberg J. Quantitative live and super-resolution microscopy of mitotic chromosomes. *Methods Cell Biol*. 2018;145:65-90.
33. Xiang W, Roberti MJ, Hériché JK, Huet S, Alexander S, Ellenberg J. Correlative live and super-resolution imaging reveals the dynamic structure of replication domains. *J Cell Biol*. 2018 Jun 4;217(6):1973-1984.
34. Schmid VJ, Cremer M, Cremer T. Quantitative analyses of the 3D nuclear landscape recorded with super-resolved fluorescence microscopy. *Methods*. 2017 Jul 1;123:33-46.
35. Bintu B, Mateo LJ, Su JH, Sinnott-Armstrong NA, Parker M, Kinrot S, et al. Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science*. 2018 Oct 26;362(6413).
36. Nozaki T, Imai R, Tanbo M, Nagashima R, Tamura S, Tani T, et al. Dynamic Organization of Chromatin Domains Revealed by Super-Resolution Live-Cell Imaging. *Mol Cell*. 2017 Jul 20;67(2):282-293.e7.
37. Xu J, Ma H, Jin J, Uttam S, Fu R, Huang Y, Liu Y. Super-Resolution Imaging of Higher-Order Chromatin Structures at Different Epigenomic States in Single Mammalian Cells. *Cell Rep*. 2018 Jul 24;24(4):873-882.
38. Kirmes I, Szczurek A, Prakash K, Charapitsa I, Heiser C, Musheev M, et al. A transient ischemic environment induces reversible compaction of chromatin. *Genome Biol*. 2015 Nov 5;16:246.
39. D'Abrantes S, Gratton S, Reynolds P, Kriechbaumer V, McKenna J, Barnard S, et al. Super-Resolution Nanoscopy Imaging Applied to DNA Double-Strand Breaks. *Radiat Res*. 2018 Jan;189(1):19-31.
40. Markaki Y, Gunkel M, Schermelleh L, Beichmanis S, Neumann J, Heidemann M, et al. Cold Spring Harb Symp Quant Biol. 2010;75:475-92.

41. Cremer T, Cremer M, Hübner B, Strickfaden H, Smeets D, Popken J, et al. The 4D nucleome: evidence for a dynamic nuclear landscape based on co-aligned active and inactive nuclear compartments. *FEBS Letters* 2010, 589, 2931–2943.
42. Fritz AJ, Sehgal N, Pliss A, Xu J, Berezney R. Chromosome territories and the global regulation of the genome. *Genes Chromosomes Cancer*. 2019 Jan 21.
43. Berbegall AP, Bogen D, Pötschger U, Beiske K, Bown N, Combaret V, et al. Heterogeneous *MYCN* amplification in neuroblastoma: a SIOP Europe Neuroblastoma Study. *Br J Cancer*. 2018 May;118(11):1502-1512.