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Relationship between chromatin complexity and nuclear envelope circularity in hippocampal pyramidal neurons ☆

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ABSTRACT

In this study we tested the existence and strength of the relationship between circularity of nuclear envelope and mathematical parameters of chromatin structure. Coronal sections of the brain were made in 10 male albino mice. The brain tissue was stained using a modification of Feulgen method for DNA visualization. A total of 200 hippocampal pyramidal neurons (20 per animal) were visualized using DEM 200 High-Speed Color CMOS Chip and Olympus CX21FS1 microscope. Circularity of the nuclear membrane was calculated in ImageJ (NIH, USA) after the nuclear segmentation, based on the freehand selection of the nuclear regions of interest. Circularity was determined from the values of area and perimeter. For each chromatin structure, using fractal and grey level co-occurrence matrix (GLCM) algorithms, we determined the values of fractal dimension, lacunarity, angular second moment, GLCM entropy, inverse difference moment, GLCM correlation, and GLCM contrast. It was found that circularity is in a significant correlation ($p < 0.05$) with fractal dimension as the main parameter of fractal complexity analysis. Also, circularity was in a very strong relationship ($p < 0.001$) with certain parameters of grey level co-occurrence matrix such as the angular second moment and GLCM correlation. This is the first study to indicate that nuclear shape is significantly related to mathematical parameters of higher chromatin organization. Also, it seems that circularity of the nuclear envelope is a good predictor of certain features of chromatin structure. Our study suggests that GLCM algorithm, as well as circularity analysis, have potentially substantial application in future cellular physiology research.

1. Introduction

Nuclear envelope (nuclear membrane) encloses the nucleoplasm and has an important role in the regulation of gene expression and chromatin structural organization. It interacts with proteins responsible for modulation of euchromatin and heterochromatin distribution during all phases of the cell cycle. Nuclear envelope differs in shape, depending on the cell type, mitotic activity, cell movement in the tissue, and relationship with other biological structures. Despite extensive research on the topic of envelope-chromatin interaction, the exact relationship between parameters of envelope shape and many chromatin structural features remain unknown.

The periphery of the nucleus is known to be relatively transcriptionally silent, compared to the nucleus center. Also, heterochromatin is more abundant near the nuclear

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envelope, while more active euchromatin is located in the central part of the nucleus. It is thought that individual genes can change their location from the center to the periphery and vice versa depending on the need for messenger RNA and protein synthesis. One of the important factors influencing this relocation may be specific pores in the envelope (nuclear pore complexes), and a dense fibrillar layer in the vicinity of inner nuclear membrane, called nuclear lamina. Nuclear lamina consists mostly of intermediate filaments and is in close contact with the envelope through membrane-associated proteins. This fibrillar network participates in regulation of nuclear shape, chromatin architecture, gene expression, as well as processes that occur before and during DNA replication and mitosis [1–3].

So far, most of the research has been focused on molecular events taking place in nuclear lamina, regarding their impact on DNA/chromatin structure and distribution. However, very few data exist on the possible influence of nuclear envelope shape on higher levels of chromatin organization. Although there have been some studies to relate nuclear circularity with fractal features of the nucleus [4,5], generally, quantitative features, especially textural characteristics of overall chromatin architecture obtained through the methods of image analysis are underexplored.

In this article, we present results indicating that certain parameters of nuclear envelope shape, such as circularity are in significant correlation to chromatin complexity quantified using contemporary image analysis methods. The study was performed on nuclei of pyramidal hippocampal neurons having in mind their relatively large nuclear size, high nuclear roundness, low mitotic potential, and lack of DNA replication as potential confounding factors during correlation analysis. This is the first study to indicate that chromatin angular second moment and textural correlation are in significant relationship with nuclear envelope shape. To our knowledge, this research is also the first to analyze features of grey level co-occurrence matrix analysis in neuron chromatin structures stained with DNA-specific Feulgen dye.

2. Materials and methods

2.1. Experimental protocol and Feulgen staining

Coronal sections of the brain, approximately 2 mm posterior to the bregma, were made in 10 1-month-old male Swiss albino mice. The tissue was stained using a modification of Feulgen method for DNA visualization [6]. The experimental protocol included methanol fixation, treatment with 5 N hydrochloric acid (120 min) and 0.1 N hydrochloric acid. Schiff's reagent, made with basic fuchsin and sodium metabisulfite was used for staining of the tissue in the dark for 120 min. The sections were rinsed with bisulfite (3 × 5 min), and washed with distilled water.

For the details about the Feulgen and its applications in cisualiyation of DNA and chromatin, the reader is referred to the previously published data [6–8]. In brain tissue sections, nuclei and DNA material are stained in red color,

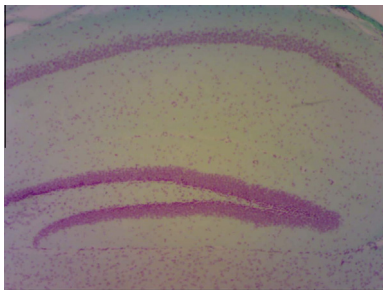

Fig. 1. Example of Feulgen-stained mouse hippocampus with visible layer of pyramidal neuron nuclei.

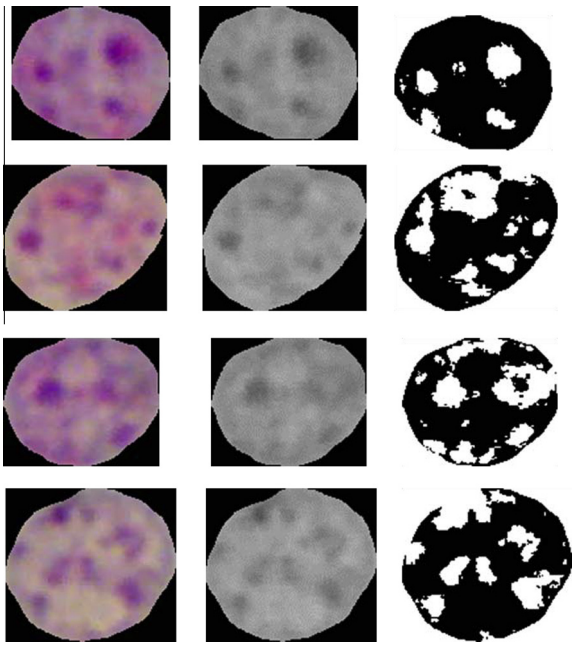

Fig. 2. Segmented Feulgen-stained (red/violet) nuclei of pyramidal neurons. The same nuclei were converted to 8-bit (greyscale) format for GLCM analysis and binary format for fractal analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

while other parts of neurons are stained in green (Figs. 1 and 2).

A sample of 200 hippocampal pyramidal neurons (20 per animal) were visualized using DEM 200 High-Speed Color CMOS Chip DC 5 V/250 mA (Oplenic Optronics, Hangzhou, CN) and Olympus CX21FS1 microscope (1000× magnification). The chromatin structures were segmented, and separate micrographs in TIFF format for each nucleus were created in National Institutes of Health ImageJ software (Bethesda, MD, USA).

2.2. Circularity assessment

Circularity (f) of the nuclear membrane was calculated in ImageJ after the nuclear segmentation, based on the freehand selection of the nuclear regions of interest (ROIs). Circularity is determined from the values of area (A) and perimeter (P), measured in resolution units: