

The Effect of Magnesium Ions on Chromosome Structure: Insights from G-Banding

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Abstract

Magnesium ion (Mg^{2+}) plays a fundamental role in the chromosome condensation which is important for genetic material segregation. Studies about the effects of Mg^{2+} on the overall chromosome structure have been reported. Nevertheless, its effects on the distribution of heterochromatin and euchromatin region have yet to be investigated. This study was aimed to evaluate the effects of Mg^{2+} on the banding pattern of the chromosome structure. Chromosome analysis was performed using the GTL-banding technique on synchronized HeLa cells. The effect of Mg^{2+} was evaluated by subjecting the chromosomes to three different solutions, namely XBE5 (5 mM Mg^{2+}) as a control, XBE (0 mM Mg^{2+}), and 1 mM EDTA as cations chelator. The results showed a condensed chromosome structure with a clear banding pattern when it was treated with a buffer containing 5 mM Mg^{2+} . In contrast, chromosomes treated with a buffer containing no Mg^{2+} and those treated with an ions chelator showed an expanded and fibrous structure with the lower intensity of the banding pattern. Elongation of the chromosome caused by decondensation resulted in the band splitting. The results of this study further emphasized the role of Mg^{2+} on chromosome structure and gave insights into Mg^{2+} effects on the banding distribution.

Introduction

Chromosome structure is the result of the condensation of chromatin that consists of DNA and histone proteins. The compact structure of the chromosome is required for sister chromatid segregation during the mitotic phase in the cell cycle. One of the essential factors contribute to chromosome condensation is divalent cations, especially Mg^{2+} ¹. Magnesium ions play a role to construct and maintain the structure of the chromatin in a condense state. A previous study showed that Mg^{2+} concentration is high during metaphase and remains high during anaphase in the cell cycle, which promotes sister chromatid condensation². In addition, several studies showed that the chromatin was more condensed when the Mg^{2+} is present²⁻⁵. Inside the cells, concentration of Mg^{2+} ranges from 5 mM until 30 mM³.

The evaluation of the Mg^{2+} effects on the overall chromosome structure has been conducted using several microscopes such as fluorescence microscope (FM), scanning electron microscope (SEM), and scanning transmission electron microscope (STEM). The chromosome showed a less condense structure after treated with buffer lacking Mg^{2+} ⁴. Furthermore, the structure of the chromosome was expanded after treated with a 1 mM ethylenediaminetetraacetic acid (EDTA), an ions chelator⁵. The decondensed chromosomes showed a fibrous structure around the chromatid⁶. All of these results showed the important role of the Mg^{2+} for chromosome condensation.

Chromosome structure consists of heterochromatin and euchromatin regions. These regions reflect the degree of chromatin condensation, that affect the DNA accessibility for gene expression. The heterochromatin region is more condensed compared to the euchromatin region which consists of a transcriptionally active gene⁷. The more loosen structure of euchromatin enabling the transcription factor to access the DNA more easily in an open chromatin fiber. These differences can be visualized by the

banding technique, where G-banding is one of the conventional techniques used in cytogenetics laboratory to identify chromosome structure related to abnormalities. This technique produces G-band that contains a dark band represent heterochromatin and euchromatin as a light band.

A previous study using high resolution scanning ion microprobe (SIM) reported that Mg^{2+} is correlated to the heterochromatin region of the chromosome, where it interacts with DNA and protein that contribute to its condensation⁸. Furthermore, the existence of 5 mM Mg^{2+} makes the heterochromatin more compact⁹. Evaluation of the effects of Mg^{2+} on the overall chromosome structure observed by various microscopes has been reported. However, to date, how the Mg^{2+} affects the chromosome structure correlates to the banding patterns has not been investigated yet. The information about the Mg^{2+} effects on chromosome banding pattern would give better insights into the detailed mechanism of Mg^{2+} roles on chromosome condensation and further related to the gene expression. Thus, in this study, we analyzed the effects of Mg^{2+} on chromosome structure as observed by G-banding.

Results

In this study, we investigated the effects of Mg^{2+} on the morphological changes of chromosome structure and its G-band patterns. Figure 1 displays the spread of the metaphase chromosome under different Mg^{2+} treatments. As a control, the spread metaphase chromosome was obtained by treating the HeLa cell culture with XBE5 buffer which contains 5 mM Mg^{2+} . The 5 mM was used as a representation of the Mg^{2+} concentration in the chromosome during mitosis¹. The 0 mM Mg^{2+} represents the condition of cells where there is no Mg^{2+} in the surrounding and the 1 mM EDTA was used as a chelator cations because Mg^{2+} does not has a specific chelator.

Table 1
Evaluation of the effect of Mg^{2+} on chromosome

Parameter	5 mM Mg^{2+} (control)	0 mM Mg^{2+}	1 mM EDTA
Chromosome value	2-3	2-4	3-6
Chromosome structure	Condensed, no fibrous structure	Decondensed and fibrous structure	Decondensed and fibrous structure
Banding pattern	Clear (High intensity)	Less clear (Lower intensity)	Unclear (Lowest intensity)

The results (Table 1) showed that the addition of a buffer containing 5 mM Mg^{2+} resulted in the condensed chromosome structure with a clear banding pattern (Fig. 1a; blue arrow). Meanwhile, the

chromosome treated with 0 mM Mg^{2+} showed a less condensed and more dispersed structure (Fig. 2; green arrow). The intensity of the banding pattern was lower than those in control. The addition of EDTA also resulted in the decondensed chromosome structure with an unclear banding pattern (Fig. 1c; green arrow). The chromosomes were well-spread in 5 mM Mg^{2+} compared to those in 0 mM Mg^{2+} and 1 mM EDTA that had more overlapped chromosomes (Fig. 1b and 1c; red arrow). The chromosome value in 5 mM Mg^{2+} range from 2 until 3, where the 0 mM Mg^{2+} reached the value of 4. Meanwhile, the value of the chromosome in 1 mM EDTA was higher, which range from 3 until 6.

Chromosome structure and banding pattern

To further analyze the effect of the Mg^{2+} , chromosome 1 was chosen as a representative. The results showed that the differences in Mg^{2+} treatment caused changes in the chromosome structure. Chelating Mg^{2+} affected the chromosome structure, length, and banding pattern. As shown in Fig. 2, the chromosome is highly condensed and has a compact structure in 5 mM Mg^{2+} (Fig. 2a). In contrast, chromosomes treated with buffer without Mg^{2+} showed a less condensed structure with the expansion of the chromosome area (Fig. 2b). Similar results were also obtained from the chromosomes treated with 1 mM EDTA (Fig. 2c), which shows that the chromosome area was more expanded than the control. The fibrous structure was observed when the chromosomes were treated with a buffer containing no Mg^{2+} and EDTA (red arrow shown in Fig. 2b' and Fig. 2c').

In regards to the banding pattern, the treatment of Mg^{2+} influenced the quality of the banding pattern's intensity (red box). It is shown that the chromosome in 5 mM Mg^{2+} had a good quality of the banding pattern qualitatively. The dark band of the chromosome has a clear band appearance (Fig. 2a). In contrast, the chromosome in 0 mM Mg^{2+} (Fig. 2b), has a lower band intensity compared to the chromosome in 5 mM Mg^{2+} . The dark band in chromosome lacking Mg^{2+} was more dispersed and it makes the light band was not clear as the one in 5 mM Mg^{2+} . Furthermore, chelating Mg^{2+} with 1 mM EDTA solution resulted in an unclear banding pattern with low intensity (Fig. 2c). The schematic of the chromosome banding pattern under different Mg^{2+} conditions is shown in the blue box. The light grey bands in b" and c" representing the new band formation as a result of the dark band splitting due to Mg^{2+} chelation. The different grey colors in the blue box indicate the different intensity of G-band produced. The lighter of the grey color represents the loosen chromatin structure. The intensity of the dark band where there was no Mg^{2+} , gradually decreased compared to those in the presence of Mg^{2+} .

Chromosome value

In this study, we also analyze the effects of Mg^{2+} on the chromosome value according to the standard of Quality Assessment (QA) from the International System for Human Cytogenetic Nomenclature (ISCN) 2013. Based on this standard, the value of the chromosome is given quantitatively by evaluating the produced number of bands that visible qualitatively and graded by matching those visible bands with the

criteria that matched in the QA descriptions. The value of the chromosome will increase along the number of bands observed.

Thirty spread of metaphase chromosomes (triplicate) were assessed for each treatment. According to the obtained results, we found that different concentrations of Mg^{2+} alter the chromosome value. In Fig. 3, the highest value of the chromosome treated with 5 mM Mg^{2+} was 3. Meanwhile, the chromosomes treated with 0 mM Mg^{2+} could reach the value of 4. Moreover, the value of EDTA-treated chromosomes achieved the optimum value until 6, even though the bands were poorly resolved. Based on Fig. 2, a new band at the end of the q arm of chromosome 1 (black arrows) was produced in 0 mM Mg^{2+} and 1 mM EDTA. This band was not visible at the chromosome treated with 5 mM Mg^{2+} . The appearance of this new band generated due to Mg^{2+} depletion increased the chromosome value which represents the chromosome length based on ISCN.

Discussion

In this study, we evaluate the effects of Mg^{2+} on chromosome structure by subjecting the chromosomes into three different treatments, i.e. buffer containing 5 mM Mg^{2+} as the control, buffer containing no Mg^{2+} , and EDTA as an ions chelator according to the previous report^{4,5}. The results of this study demonstrated the changes in chromosome structure with the appearance of fibrous chromatin and disrupted G-band as a consequence of lacking Mg^{2+} . The more decondensed structure, longer arms, and fibrous structure of the chromosomes resulted from the treatment of 0 mM Mg^{2+} and EDTA were also established from the ultrastructure visualization using electron microscopy^{2,5} which showed the obvious structural alteration of the chromosomes upon Mg^{2+} concentration chelation. The chromosomes were less condensed and showed the fibrous structure once they were treated with buffer without Mg^{2+} . Furthermore, when the cations were removed by using EDTA, the large expansion of the chromosome structure was evident^{2,5}.

Chelating Mg^{2+} from the chromosome, followed by enzymatic treatment, resulted in the structural collapse of chromatin¹⁰. In contrast, the chromosome in XBE5 which contained 5 mM Mg^{2+} was remained condensed. Magnesium ions maintain the structure of the chromosome more condensed. There was no fibrous structure that appeared in the edges of the chromosome treated with 5 mM Mg^{2+} . This observation exhibit that lacking Mg^{2+} makes the structure of the chromosome undergo decondensation. This result implies that Mg^{2+} has an important role in the structural maintenance of the chromosome. Magnesium ions stabilize the charge of DNA and proteins in the nucleosome, inducing chromatin folding¹¹. The depletion of the Mg^{2+} caused instability of the nucleosome interaction and unraveling chromatin which further resulted in the structural alteration. The fibrous structure in this research was shown along the chromosome arm, indicating the chromatin fibers radiated out of the decondensed chromosome¹². Besides, divalent cation used to protect nucleosome by forming a layer outside the nucleosome¹³. Consequently, chelating Mg^{2+} using EDTA as a cation chelator caused

chromosome decondensation, yield a fibrous structure. Therefore, chelating cation using EDTA indicates that divalent cation plays an important role to serve the chromatin in its condense state. This change suggested the importance of these divalent cations for compaction of chromatin and maintenance of the chromosome structure.

The changes in chromosome length are consistent with the study reported by Martonfiova¹⁴, which stated that chromosome length was affected by the condensation of the chromosome. The condensation resulted in a shorter chromosome, as shown in the results of chromosomes treated with 5 mM Mg²⁺ because Mg²⁺ induced nucleosome compaction^{15,16}. Lacking Mg²⁺ allows chromosome structure to be stretched and induced the changes of its length, as depicted by the results of chromosomes treated with buffer containing 0 mM Mg²⁺ and those treated with EDTA. The elongation indicated the unwinding chromatid that reflected different condensation of chromatin¹⁷.

Chromosome banding has been used extensively to delineates euchromatin from heterochromatin visually, which can be seen from the appearance of G-bands. In the chromosome banding, the level of condensation is reflected by the band that was produced and the intensity of the band correlated with GC content¹⁸. Heterochromatin has a low GC content with a more condensed structure compared to the euchromatin. The less condense region, euchromatin, stained less intensely. The produced bands were a result of the formation of complex dye that interacts with the DNA in the chromosome¹⁹. The condensed structure which was maintained by the presence of Mg²⁺ produced a clear and high intensity of the dark band. Furthermore, the G-bands produced in the compact chromosome are easily distinguishable. Previous studies reported that a high concentration of Mg²⁺ (≥ 2 mM) reserved heterochromatin in a condense state⁹. The presence of Mg²⁺ leads the DNA to maintain their position link to each other that facilitates their interaction with the dye, mediates the construction of the complex²⁰. It indicates that the presence of Mg²⁺ maintains the heterochromatin in its condense state. Another study demonstrated that the presence of Mg²⁺ modulated SIR heterochromatin folding became compact^{21,22}. The more loosen structure as a result of the absence of Mg²⁺ resulted in the low intensity of the dark band, it showed that the dye could not intercalate well which prohibit the dye interaction with the DNA, so the complex dye would not be achieved in adjacent conformation^{19,23}. The dispersed band in 0 mM Mg²⁺ showed that the heterochromatin of the chromosome underwent the decondensation causing decreases width among the DNA that allowed the dye to bond to the enable DNA to promote the complex formation. Moreover, the bands in the chromosome treated with EDTA were still visible, although the produced G-bands were unclear with low intensity. As a consequence of chromosome decondensation, the complex dye was not adequately formed because the dye could not intercalate into the chromosome structure which prevents the binding of the dye, where this condition led to poorly stained bands²⁴. These results implied that different degrees of chromatin condensation affected the accessibility of DNA to the binding of the dye²⁵.

The collapse of the chromosome structure increases the chromosome length, and in turn, increases their value according to ISCN¹⁰. The band resolution that was produced depends on the chromosome

condensation²⁶. Elongation of the chromosome due to the decondensation affects the reproducibility of the band number. The chromosome elongation induced the band splitting, where this phenomenon enhanced the observable number of the band. Band splitting occurred of the dark band into subband²⁶⁻²⁸. As chromosome size decreased, a lower number of the band was visualized. In chromosomes treated with 0 mM Mg²⁺ and 1 mM EDTA, new bands were formed as compared to those treated with 5 mM Mg²⁺. The qualitative evaluation of the band level was done by identifying the landmark of the chromosome that can be seen, later it was converted to the estimation of quantitative scale classification based on the assessment criteria²⁹. The identification of the optimum band number in chromosomes treated with 1 mM EDTA was relatively more difficult due to the poor banding.

In conclusion, the condensation of chromatin forming chromosome structure was affected by Mg²⁺. This is the first report revealing the effects of Mg²⁺ on the chromosome banding pattern. Magnesium ions are important for the maintenance of the chromosome structure. In addition, this study also provides new insight into the correlation of chromosome condensation with the production of G-bands.

Methods

Cell culture and chromosome harvest. The HeLa wildtype cells were cultured on a coverslip in a petri dish. The culture medium consisted of Rosewell Park Memorial Institute (RPMI) 1640 (Gibco), 10% Fetal Bovine Serum (FBS) (Gibco), and 1,000 U/mL Penicillin-Streptomycin (Gibco). The culture was incubated at 37 °C with 5% CO₂ for 72 hours. The cell culture was synchronized using bromodeoxyuridine (BrdU) followed by incubation for 14–16 hours at 37 °C with 5% CO₂. After that, the medium was changed by the new culture medium, following the addition of colchicine. The hypotonic solution (RPMI: ddH₂O) was added followed by 20 minutes incubation. The treatment for evaluating the effects of Mg²⁺ was carried out after the hypotonic solution, using three different buffer solutions according to Dwiranti et al.⁵, namely XBE5 (10 mM HEPES pH 7.7, 100 mM KCl, 5 mM EGTA, and 5 mM MgCl₂) as the control, XBE (10 mM HEPES pH 7.7, 100 mM KCl, 5 mM EGTA) which contains no Mg²⁺, and 1 mM ethylenediaminetetraacetic acid (EDTA) as an ions chelator. Cells were subjected to these three different treatments for 30 minutes. After the treatment, the chromosome was fixed in methanol: acetic acid (3:1).

Chromosome Banding. GTL banding (G bands by trypsin using Leishman) was performed after the aging process. The chromosomes were banded using 0.25% trypsin (Gibco) for 16 seconds, washed in Phosphate Buffer Solution (PBS), and then followed with staining using Leishman solution (Sigma) for 4 minutes.

Chromosome Imaging. The slides were observed using a light microscope [Nikon] with 1000x magnification. The spreads of the metaphase chromosome were captured using VideoTestKaryo 3.1 System software (VideoTest Ltd.).

Declarations

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Author contribution

AD contributed to the study conception and design. Material preparation, data collection, and analysis were performed by M and HK. The first draft of the manuscript was written by M and all the authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Figures

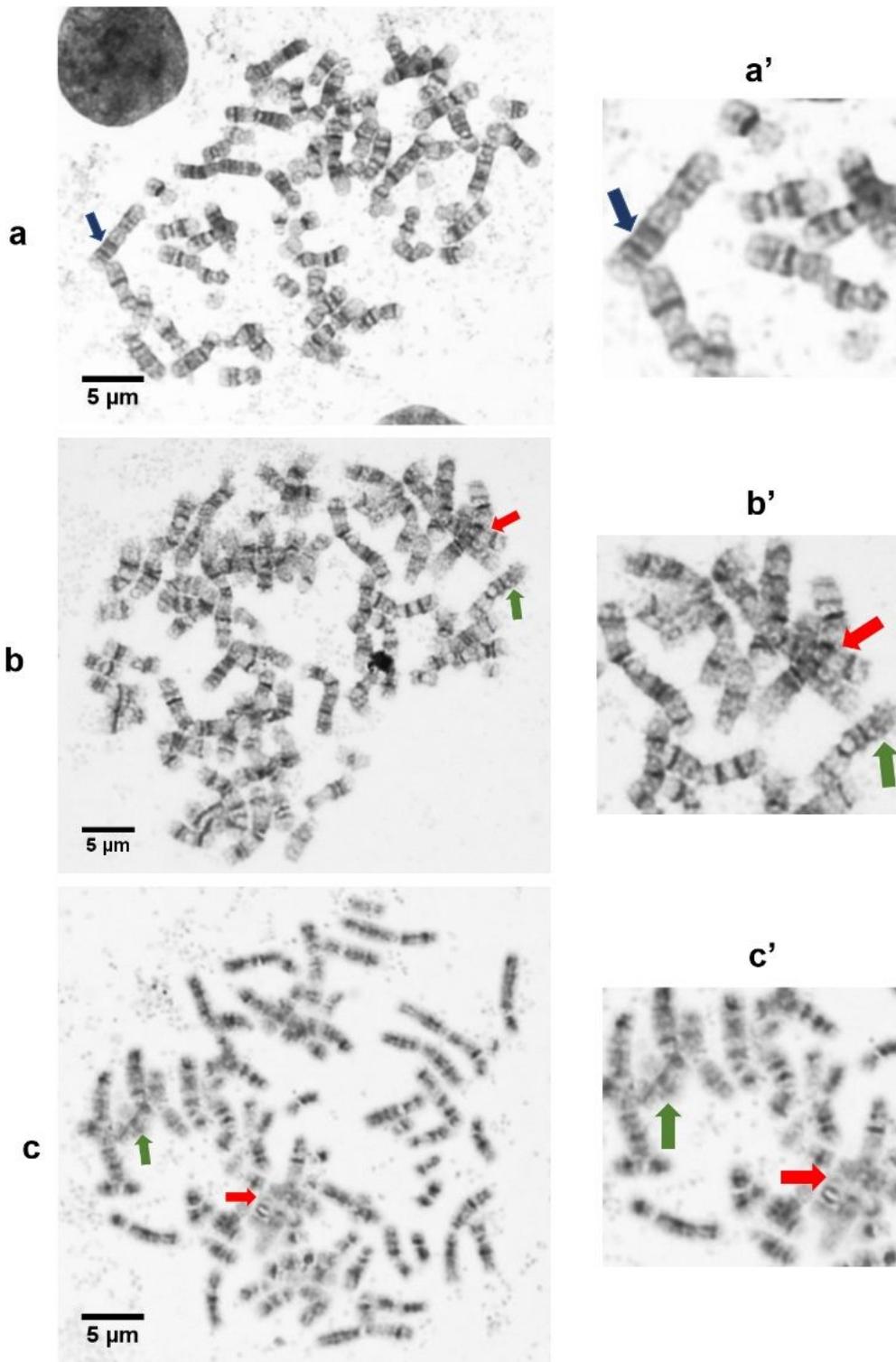


Figure 1

Spread metaphase chromosomes depicting the effect of Mg^{2+} . Chromosomes prepared with the buffer containing 5 mM Mg^{2+} as a control (a), treated with the buffer without Mg^{2+} (b), and 1 mM EDTA (c); blue arrow showed condense structure and clear banding pattern (magnified a'); green arrow showed uncondensed structure and low intensity of banding pattern (magnified b', c'); red arrow showed overlapped chromosome (magnified b', c').

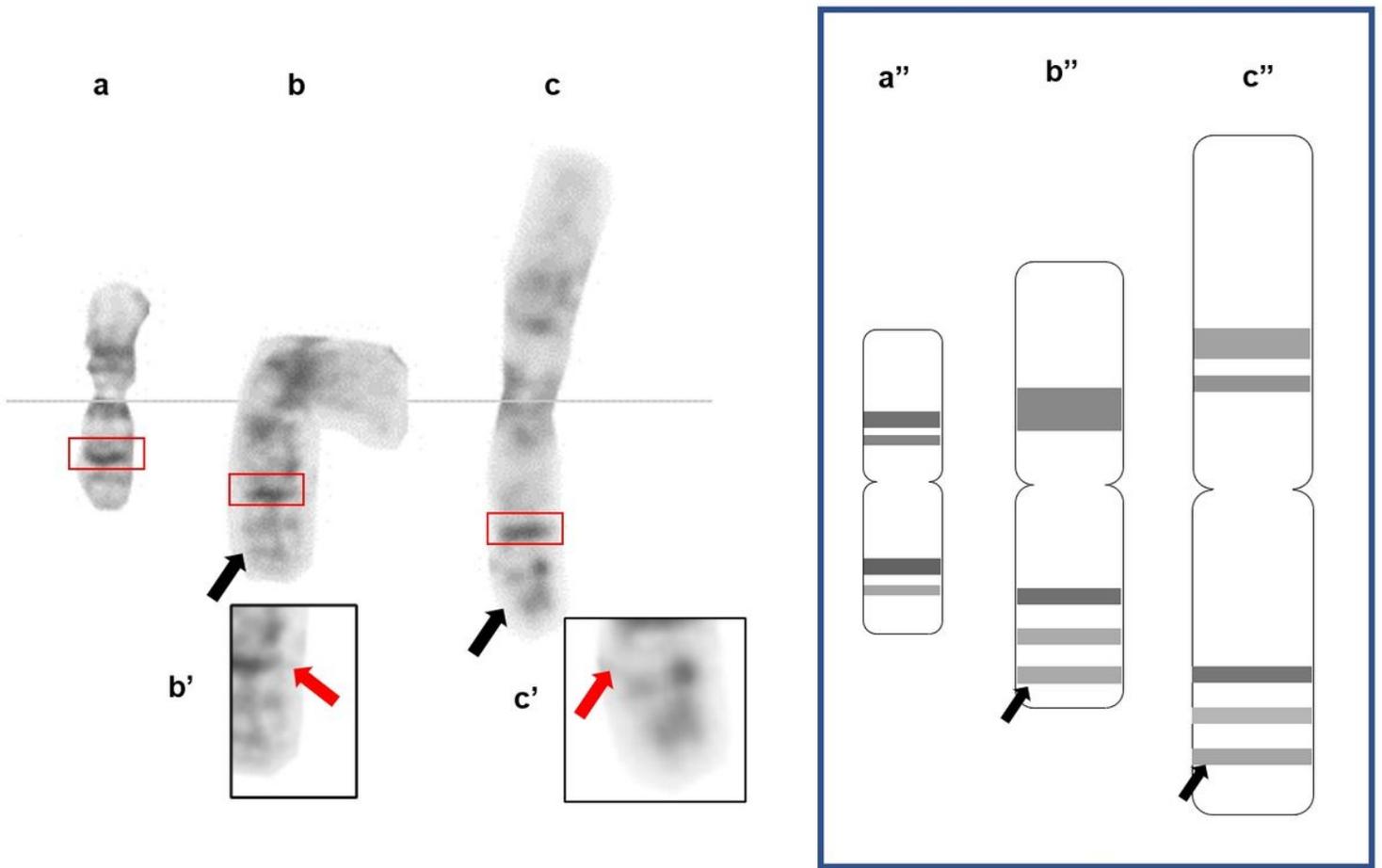


Figure 2

Magnification of chromosome 1 from the spread metaphase chromosomes depicting the effect of Mg^{2+} on chromosome structure. Chromosomes prepared with the buffer containing 5 mM Mg^{2+} as a control (a) is more condense compared with those treated with the buffer without Mg^{2+} (b) and treated with 1 mM EDTA (c) which caused greater expansion of the chromosome (magnified b', c'); red arrow showed the fibrous structure of the chromosome; red box showed the different intensity of the band; black arrow showed the new band. The schematic of band differences under different Mg^{2+} conditions (5 mM Mg^{2+} , 0 mM Mg^{2+} , EDTA are labelled as a'', b'', and c'', respectively) is shown in the blue box.

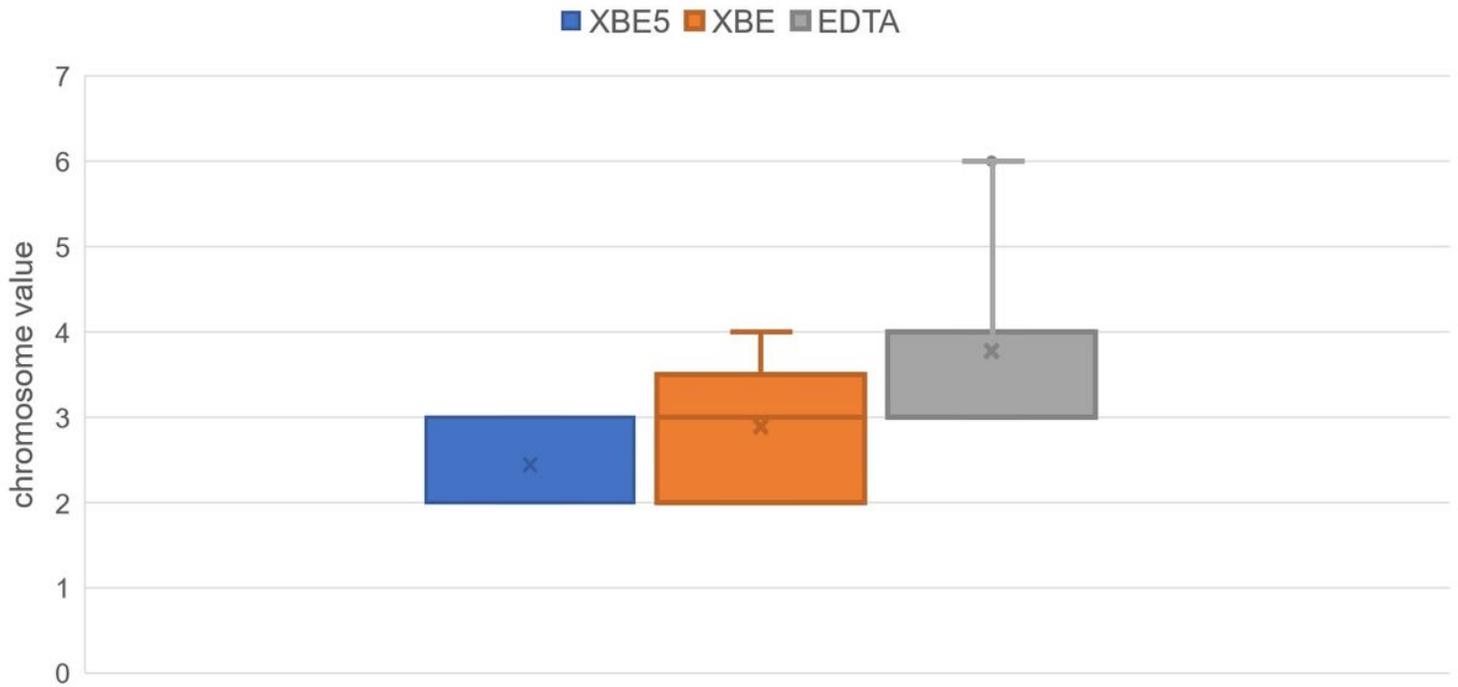


Figure 3

Chromosome value of chromosomes treated with XBE, EDTA, and control (30 chromosomes of each treatment). In XBE5 (5 mM Mg²⁺), the value of the chromosome range from 2 to 3. Meanwhile, XBE solution (0 mM Mg²⁺) the higher value was obtained until 4. Besides that, the chromosome treated with 1 mM EDTA undergoes elongation resulting in higher value which was reached the value of 6 and had the minimum value of 3.