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Epigenetic modulation of three-dimensional telomeric architecture by hypomethylating agent 5-aza-2'-deoxycytidine in luminal and triple-negative breast cancer cells

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Introduction: Genomic instability is a hallmark of cancer and is strongly associated with telomeric dysfunction. Telomeres, structures that protect the ends of chromosomes, when shortened or spatially disorganized, can lead to chromosomal fusion, deoxyribonucleic acid (DNA) breakage, and aberrant chromosome segregation. **Objective:** This study evaluated the impact of the hypomethylating agent 5-aza-2′-deoxycytidine (5-aza-dC) on the three-dimensional reorganization of telomeres in luminal A (MCF7) and triple-negative (DU4475) breast cancer cell lines, focusing on the relationship between telomeric architecture and genomic instability. **Methods:** Cells were treated with 5-aza-dC (10, 20, 30, and 50 µM) for 72 hours. Analysis was performed using Q-FISH and TeloView®, assessing the following parameters: number of telomeres, telomere aggregates, signal intensity (length), spatial distribution, and nuclear volume. Statistical evaluation was performed using analysis of variance (ANOVA) with Bonferroni post-test ($p<0.05$). **Results:** The assays demonstrated that 5-aza-dC induced statistically significant alterations in telomeric parameters, particularly at concentrations of 30 and 50 µM. In MCF7, there was a reduction in the number of telomeres (from 58.4 standard deviation ±6.1 to 42.2±4.8; $p<0.001$) and aggregates (from 8.3±1.2 to 4.1±0.9; $p<0.0001$), indicating reduced genomic instability. In DU4475, nuclear volume decreased (up to 40%; $p<0.0001$), and changes in the spatial distribution ($p<0.01$) suggested nuclear reprogramming and impact on 3D genome organization. **Conclusion:** 5-aza-dC promotes 3D telomeric reorganization associated with the reduction of classical signs of genomic instability. This reinforces the role of telomeres not only as markers of cellular aging but also as epigenetic sensors of chromosomal stability in breast tumors, including luminal and triple-negative cell lines.

Keywords: breast neoplasms; telomeres; genomic instability.