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Effect of Cytotoxic Therapy on ALU Expression is Modulated by Hormonal Status in Patients with Breast Cancer

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To the Editor,

Breast cancer (BC) is the most commonly diagnosed cancer in women globally. The incidence and mortality rates of BC have been increasing mostly in developing countries including Turkey.[1] The use of cytotoxic chemotherapy in both early-stage and advanced BC has made significant progress with clear survival benefits.More than 50% of the human genome is composed of repeat DNA. These elements are repressed in human cells under normal conditions, predominantly via silencing by DNA methylation.[2] However, in response to cellular stress, repetitive elements are transcribed through the loss of epigenetic silencing. However, the effect of cytotoxic therapy as a stress source on the expression of repetitive elements is unknown. In this study, we aimed to assess the effect of cytotoxic therapy on the plasma RNA expression of the ALU repeat in BC.The ALU element, one of most abundant repeats in the human genome, was utilized for the analysis. Archived blood samples from patients (n=40) with locally advanced BC who had received chemotherapy in the neoadjuvant setting were used. Neoadjuvant chemotherapy (NAC) consisted of four cycles of adriamycin and cyclophosphamide and 12 cycles of paclitaxel therapies. Plasma ALU-RNA levels were measured by quantitative reverse-transcriptase polymerase chain reaction in plasma samples obtained at baseline and end of the fourth NAC cycle. We used MALAT1, a non-repeat non-coding RNA, as the control molecule. The plasma levels of ALU-RNA and MALAT1 were semiquantitatively determined with the *glyceraldehyde-3-phosphate dehydrogenase* gene as the internal control.The assessment of the association of the pretherapeutic plasma ALU expression with clinicopathological variables such as age, menopausal status, hormone receptor status, HER2 expression, tumor size, and disease stage revealed a link only for menopausal status. Pretherapeutic plasma ALU-RNA levels were significantly lower in premenopausal women than in postmenopausal women (relative expression values, 1703 vs. 2853, respectively; $p = 0.036$ according to the Mann-Whitney U test, Figure 1a) indicating the effect of estrogens on the regulation of repeat element expression. Pretherapeutic plasma levels of MALAT1 were comparable in pre- and postmenopausal women (relative expression values of 0.13 and 0.16, respectively; $p = 0.6$, Figure 1b).

In contrast to other molecular markers such as ALU-DNA or non-coding RNAs whose plasma levels generally decline during chemotherapy,[3,4] we unexpectedly found that plasma ALU-RNA levels increased from baseline to the end of the fourth NAC cycle in the whole group (from 1,866 to 3,368, $p = 0.03$, Wilcoxon signed-rank test, Figure 1c). By contrast, MALAT1 levels decreased during chemotherapy (from 0.14 to 0.09, Figure 1d), indicating the specificity of the observed effect on the ALU expression. The ALU-RNA levels increased from baseline to the end of the fourth cycle because the samples were mainly from premenopausal women (Figure 1e-g). Thus, we speculate that cytotoxic therapy induces ALU expression by altering its epigenetic silencing in premenopausal status, whereas this effect was absent in postmenopausal women as the ALU element is epigenetically not silenced in them as a consequence of the changed hormonal status.

In conclusion, this is the first study on the ALU expression in blood and provides evidence that ALU expression and kinetics of blood ALU-RNA levels during cytotoxic therapy are modulated by the menopausal status of patients with BC. The absence of the observed effects on MALAT1 validates our findings. Our findings warrant further research to explore the mechanisms underlying the link between hormonal status and ALU expression and the effect of cytotoxic therapy on ALU expression status.

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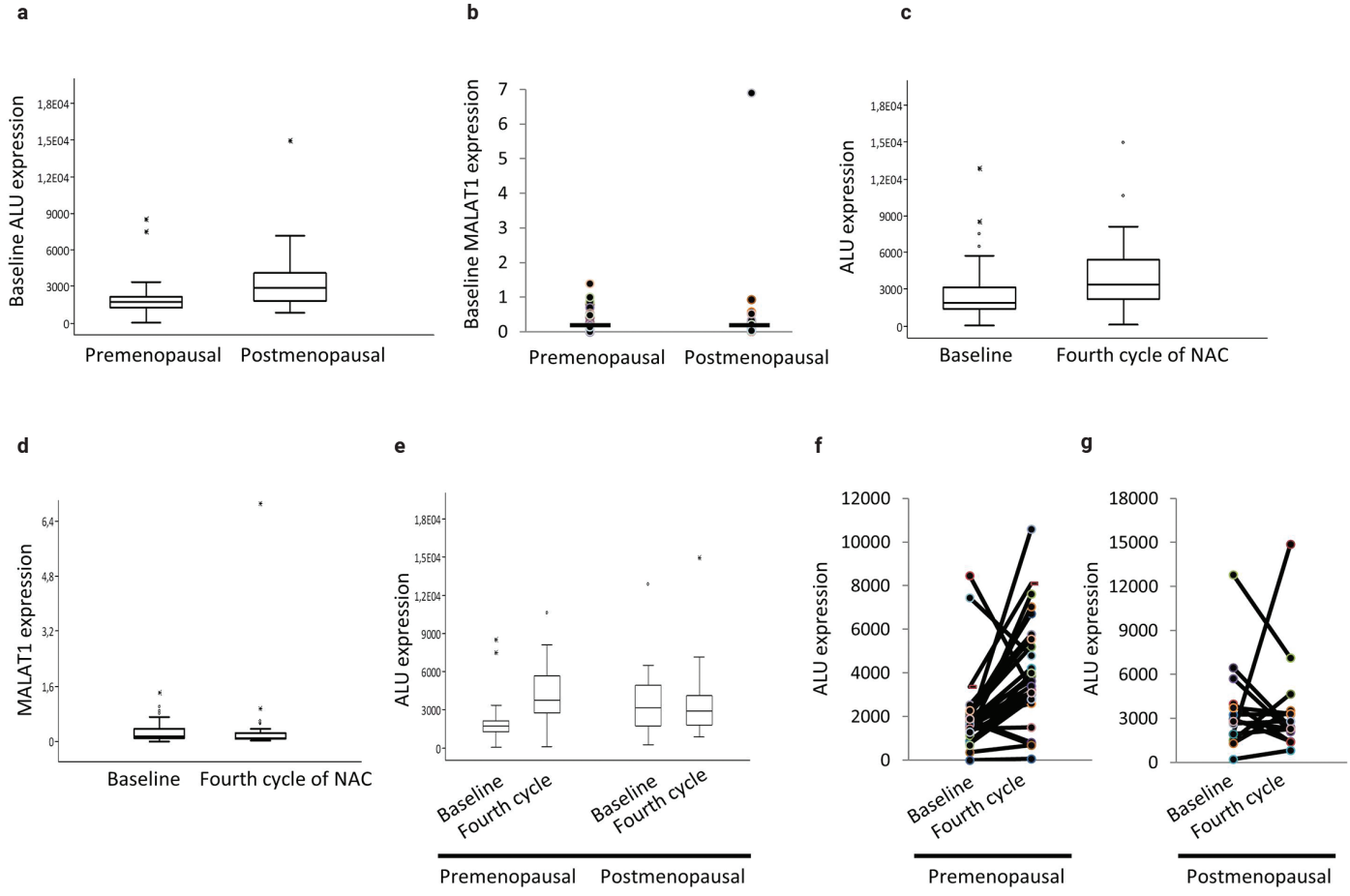

FIG. 1. ALU expression in patients with breast cancer during neoadjuvant chemotherapy. Plasma levels of ALU-RNA were relatively determined by quantitative reverse-transcriptase polymerase chain reaction with the *GAPDH* gene as reference molecule. **a)** Pretherapeutic ALU expression in pre- and postmenopausal groups. **b)** Pretherapeutic MALAT1 expression in the plasma in the pre- and postmenopausal groups where (-) indicates median levels. **c)** ALU expression at baseline and end of the fourth cycle of chemotherapy in the whole cohort. **d)** MALAt1 expression at baseline and end of the fourth cycle of chemotherapy in the whole cohort. **e)** ALU expression at baseline and end of the fourth cycle of chemotherapy in patients at the pre- or postmenopausal phase. **f)** Change in ALU expression from baseline to the end of the fourth cycle of chemotherapy in each premenopausal patient. **g)** Switch of ALU expression from baseline to the end of the fourth cycle of chemotherapy in each postmenopausal patient. The box plots show the minimum and maximum levels of ALU and MALAT1 and median levels along with 25th and 75th percentiles.
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

Ethics Committee Approval: This study was approved by the İstanbul Medical Faculty Ethics Committee (2022/148).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer.* 2019;144:1941-53. [CrossRef]
2. Cordaux R, Batzer MA: The impact of retrotransposons on human genome evolution. *Nat Rev Genet.* 2009;10:691-703. [CrossRef]
3. Lehner J, Stötzer OJ, Fersching D, Nagel D, Holdenrieder S. Circulating plasma DNA and DNA integrity in breast cancer patients undergoing neoadjuvant chemotherapy. *Clin Chim Acta.* 2013;425:206-11. [CrossRef]
4. Özgür E, Ferhatoğlu F, Şen F, Saip P, Gezer U. Circulating lncRNA H19 may be a useful marker of response to neoadjuvant chemotherapy in breast cancer. *Cancer Biomark.* 2020;27:11-7. [CrossRef]