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THE SYSTEMIC ANTICANCER REPROGRAMMING EFFECT OF VITAMIN E ON DU145 PROSTATE CELL LINE

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ABSTRACT

DU145 is a prostate cancer cell line with an origin epithelial morphology. Therefore, the goal was to show that DU145 cells can be modulated in the degree of malignancy of the corresponding prostate cancer cells. It was revealed that Vitamin E (alpha-tocopherol) observed modulating functions on DU145 cancer prostate cells. By the method of qRT-PCR was detected the targeted vitamin E-dependent decreasing mRNA expression levels of specific regulation genes of cancer-associated oxidative stress as thioredoxin reductase (TXN2) and glutathione peroxidase (GPX4) genes. This is the first modulating as antioxidant vitamin E function. The second modulating function of vitamin E was studied as reversed stemness EMT by repression SNAIL EMT-inducer transcription factor and re-activation of the epithelial marker E-cadherin. The third modulating function of vitamin E was studied as remodeling pluripotency vitamin E function was detected on DU145 cancer prostate cells during vitamin E-dependent repression of Oklt4 pluripotency gene mRNA expression and reactivation of C/EBP alpha differentiation transcription factor. The present research emphasized the systemic anticancer function of Vitamin E (alpha-tocopherol) in Reprogramming of cancer-associated phenotype in DU145 cancer prostate cell line investigation.

INTRODUCTION

DU145 is a prostate cancer cell line with an origin epithelial morphology isolated from the spinal cord of a 69-year-old man with prostate cancer.[1] Thus, DU145 tumor cells have moderate metastatic potential, compared to aggressive metastatic cancer prostate LNCaP cell line.[2] Based on this fact, the DU145 cell line should be a more likely target for the search for therapeutic ways that can modulate the degree of malignancy of the corresponding prostate cancer cells.

The aim of the research was to study the modulating effect of vitamin E (alpha-tocopherol) on the cancer-associated phenotype of DU145 prostate tumor cells based on the study of relative level of mRNA expression of the relevant, regulatory genes, by real-time RT-PCR assay. A cancer-associated, progressive phenotype is usually shaped by three key mechanisms underlying cancer cell progression, namely:

a). Hypoxia and induction of ROS (reactive oxygen species), as a result - disproportionality of redox homeostasis.[2] It has been shown that a violation of the redox balance is one of the most important reasons for the development of cancer against the background of oxidative stress, its progression and metastasis in human cancer cells.[3] The key system of oxidative protection in the cell is thioredoxin reductase-glutathione peroxidase. Therefore, in the study of the relevant regulatory genes of cancer-associated oxidative stress in Du145 prostate cancer cells, the thioredoxin reductase (TXN2) and glutathione peroxidase (GPX4) genes took place (Figure 1).

b). The mechanism of EMT (epithelial-mesenchymal transformation), which is combined with stemness of tumor cells in invasive-metastatic progression.[4] Epithelial-mesenchymal transition (EMT) in carcinogenesis is an important program that is often activated during the process of invasion and metastasis.[5,6] Cancer stem cells (CSCs), as a result of EMT mechanism, initiate and support cancer, participate in cancer invasion and metastasis, including in the development of resistance to cancer therapies. The regulatory genes of the cancer-associated EMT-phenotype for the research conducted on DU145 prostate cancer cell line, we identified two alternative genes of the EMT phenotype: E-cadherin, a key epithelial marker[7], and the transcription factor SNAIL, a key EMT inducer[8], which is a repressor of E-cadherin[9] (Figure 2).

c). Pluripotency as a mechanism of reprogramming of cancer-associated stem cells.[10] Tumor development and the generation of induced pluripotent stem cells are very similar processes with striking similarities.[11]

Cellular plasticity is inherent in tumor evolution, forming cells that acquire a phenotype similar to embryonic pluripotent stem cells with the activation of relevant pluripotent genes, such as Oct4, Sox2, Klf4, Nanog and c-Myc.[12,13] The activation of these genes turned out to be important in the plastic acquisition of the properties of pluripotent stem cells for tumor cells. Understanding the molecular mechanisms underlying the acquisition of cancer cell pluripotency may open new approaches to the development of anticancer strategy treatment. Namely, the terminal differentiation and loss of tumorigenicity of cancer cells through pluripotency-based reprogramming opens an important avenue for cancer stem cell tumor therapy. Based on this, our research investigated the relative levels of mRNA expression of two inversely dependent regulatory genes - C/EBP alpha (CCAAT enhancer binding protein alpha), as transcription factor that induces differentiation, a key regulator of terminally differentiating cells[14] and Okt4, one of 4 pluripotency transcription factors (Oct4, Sox2, Klf4, and c-Myc), a principle marker of pluripotent embryonic stem cells[15] (Figure 3).

OBTAINED RESEARCH RESULTS

1. The effect of vitamin E (alpha-tocopherol) on the level of expression of the key enzymes of cell protection against oxidative stress — thyroredoxin reductase (TXN2) and glutathione peroxidase (GPX4) — in DU145 prostate cancer cell culture (Fig. 1).

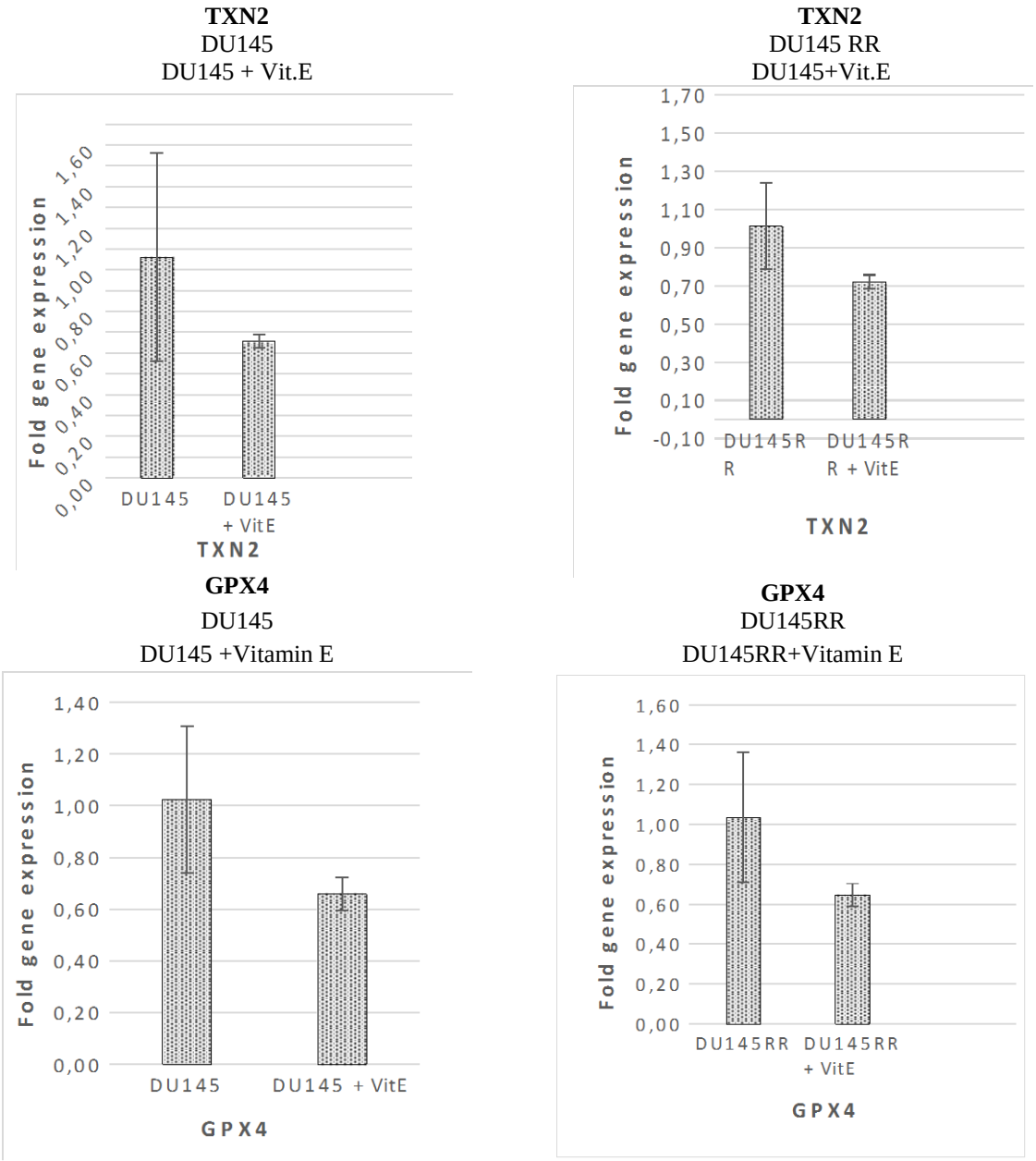

Figure 1: The effect of vitamin E (alpha-tocopherol) on the relative level of expression of the key enzymes of cell protection against oxidative stress — thioredoxin reductase (TXN2) and glutathione peroxidase (GPX4) in DU145 prostate cancer exposed to alpha-tocopherol (100 µM) during 72 h. (according to 2-Δ Δ Ct assay).

CONCLUSION. On the results obtained we have revealed the protective antioxidant effect of vitamin E (alpha-tocopherol) against cancer-associated oxidative stress of DU 145 prostate cancer cells was shown due to the vitamin E-dependent targeted inhibition of the gene mRNA expression level of thioredoxin reductase (TXN2) and glutathione peroxidase (GPX4), compared to the control (untreated DU 145 prostate cancer cells).

2. Modulating effect of vitamin E on EMT phenotype change in DU145 prostate cancer cells exposed to vitamin E (alpha-tocopherol) (100 µM) for 72 hours. The relative level of gene expression of the corresponding genes was determined by the 2-ΔCt method (Figure 2).

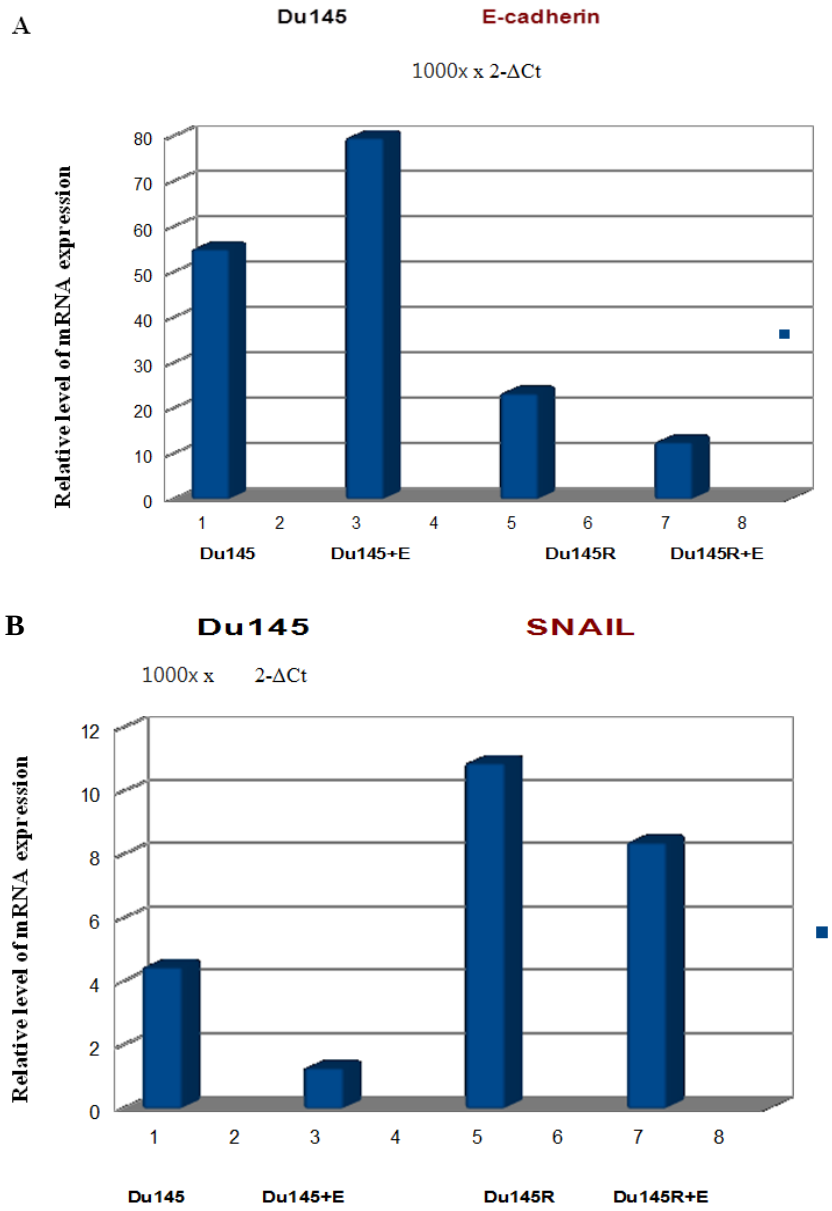

Figure 2: The relative levels of mRNA expression of E-cadherin (A) and SNAIL (B) genes in DU145 prostate cancer cells exposed to vitamin E (alpha-tocopherol) (100 µM) during 72 hours.

CONCLUSION. On the results obtained we have revealed the modulatory effect of vitamin E on the activation of E-cadherin expression and the repression of the EMT-inducer SNAIL transcription factor was shown, indicating vitamin E-dependent inhibition of the EMT phenotype of prostate cancer cells DU145 and its resistant culture DU145 under exposure to vitamin E (100 µM) during 72 hours. It was shown that the resistant Du145 cancer prostate cell line (Du145RR) observed a significantly lower level of E-cadherin and a correspondingly higher level of the EMT-inducer SNAIL in comparison with non-resistant Du145 cancer prostate cells, but the vitamin E-dependent effect of was less pronounced respectively.

3. Vitamin E-dependent suppression of the expression of the pluripotent transcription factor Okt4 and activation of the differentiation factor C/EBPalpha in DU145 prostate cancer cell culture under exposure to vitamin E (alpha-tocopherol) (100 µM) during 72 hours. (Figure 3).

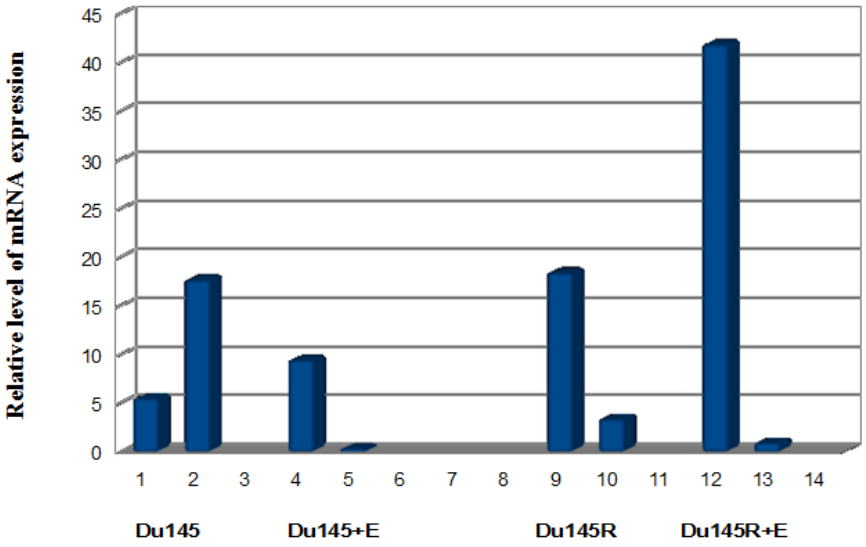

Figure 3: The relative levels of mRNA expression of the pluripotent transcription factor Okt4 gene and activation of the C/EBPalpha gene differentiation factor in DU145 prostate cancer cell culture under exposure to vitamin E (100 µM) during 72 hours.

The Table. The **2-ΔCt** digital values (**SD**)of the relative levels of mRNA expression of the pluripotent transcription factor Okt4 gene and activation of the C/EBP alpha gene differentiation factor in DU145 prostate cancer cell culture under exposure to vitamin E (100 µM) during 72 hours.

Gene	Du145	Du145+vit.E	Du145R	Du145R+ vit. E
Okt4	17,675	0,317	3,359	0,91
C/EBP alpha	5,478	9,422	18,401	41,850

CONCLUSION

On the results obtained we have revealed the cancer pluripotency reprogramming function of vitamin E on DU145 prostate cancer cell culture under exposure to vitamin E (100 µM) during 72 hours. It was shown the vitamin E-dependent targeted repression of Okt4 transcription factor of pluripotency and activation of C/EBP alpha transcription factor of differentiation respectively. DU145R (resistance) prostate cancer cell culture detected somewhat higher C/EBP alpha differentiation transcription factor mRNA expression and lower mRNA expression level of Okt4 gene of pluripotency in comparison with non-resistance DU145 prostate cancer cells. However, remodelling effect of vitamion E also was confirmed (Figure 3, the Table).

The Resulting Conclusion. On the date obtained in the present research we first emphasized the systemic anticancer function of Vitamin E (alpha-tocopherol) in Reprogramming of cancer-associated phenotype on the three cancer-associated levels:

- On the targeted Vitamin E-dependent anticancer oxidative stress level;
- On the targeted Vitamin E-dependent anticancer EMT level;
- On the targeted Vitamin E-dependent anticancer pluripotency level;

Conflict of interest statement

The authors declare that they have no conflict of interest.

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