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CO-EXPRESSION OF TUMOR SUPPRESSOR P53 (TP53) AND CANCER TESTIS ANTIGENS (CTAS) AS THE POSSIBLE INDICATOR OF "CANCER-FREE" STATUS

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Abstract – *Objective:* *Biomarkers are biological substances that can be measured and objectively evaluated as indicators of concrete processes at different levels. Advances in biomedicine facilitated the use and importance of biomarkers for healthcare purposes. Several biomarkers that are used in the field of oncology are already identified and used in clinical practice, although their sensitivity is not sufficient. To contribute to this issue, we aimed to determine the expression of total cancer-testis antigens (CTAs) in correlation with the expression levels of tumor suppressor proteins p53 (TP53) and p63 (TP63) as well as BRCA1 in a healthy cohort.*

Materials and Methods: *We analyzed samples of 90 blood donors (28, 31.1% – females, 62, 68.9% – males) as they can be considered as an appropriate group for recruiting health cohorts. The age distribution of the subjects was between 20 and 60 years. The enzyme linked immunosorbent assay analysis was used for the determination of CTAs, TP53, TP63, and BRCA1 expression levels.*

Results: *A strong correlation between CTAs and TP53 expression levels has been revealed. The expression variables of targeted biomarkers are not equally distributed. The data specific to CTAs, TP53, and TP63 expression levels are skewed to the left. In the case of BRCA1, the data may indicate the presence of 2 subgroups for study subjects.*

Conclusions: *The co-expression of CTAs and TP53 may be considered as the indicator of "cancer-free" status. This parameter may be piloted for cancer screening and early diagnosis purposes. However, the role of CTAs for cellular process regulation and especially regulation of tumor suppressor gene p53 shall be investigated further.*

KEYWORDS: *Cancer testis antigens, p53, p63, BRCA1, Biomarker, Expression, ELISA.*

INTRODUCTION

Despite technological and diagnostic progress, oncology diseases are still a significant health issue[1]. There were an estimated 18.1 million cancer cases around the world in 2020. Of these, 9.3 million cases were in men and 8.8 million in women. Georgia is not an exception to this unpleasant tendency. In 2020, 9,435 cases (54% of female and 46% of male population) were diagnosed with cancer; 8,024 lethal cancer cases were registered in 2020. 68% of new cases were accounted for 30-70 age groups, 28% - over 70 years[2]. With this growing burden, cancer became and is one of the most significant public health challenges of the 21st century.

Cancer cases are often identified by symptomatic clinical manifestation (i.e., breast lump, rectal bleed-

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ing, persistent cough, lymphadenopathy, weight loss, etc.). In many cases, the patient is asymptomatic; thus, medical treatment is not sought or even performed. Patients rather frequently are unaware of or even ignore the cancer symptoms because of bad health literacy, high costs of hospital visits, cultural and mental attitudes toward seeking medical care, fear of cancer, and other personal reasons. Later symptoms became apparent, although patients seeking medical care may be out of the scope of available clinical treatment. Late diagnosis and detection of cancer make it difficulty to treat because of progressive advancement in disease stage and metastasis[3]. Thus, the early diagnosis of oncologic diseases is among global and national healthcare priorities. This can be achieved through and as an outcome of screening and prevention activities and is highly dependent on the application of effective biomarkers or their combination.

Biomarkers can be defined as biological substances that can be measured and objectively evaluated as indicators of concrete processes at the cellular, tissue, organ or whole body level. Advances in cell biology and genetics, molecular pathology, and precision medicine facilitated the use and importance of biomarkers for clinical trials, analytic epidemiology, and disease management[3]. Several biomarkers that are used for oncology disease prognosis, cancer cases follow up, and monitoring issues are already identified and used in clinical practice. Although the sensitivity of available biomarkers is not sufficient[4,5]. The first successful cloning of a human tumor antigen – melanoma antigen-1 (MAGE-1) that was elucidated as autologous cytotoxic T-lymphocyte response in a melanoma patient was reported in the 1990s. The expression of MAGE-1 (later it has been named MAGE-A1) has been determined in different cancer of various histological origin. In norm the expression of this protein has been reported only in testis and placenta. Later on, other tumor antigens were identified, including MAGE-A2, MAGE-A3, BAGE, GAGE-1, and New York esophageal squamous cell carcinoma 1 (NY-ESO-1). To encompass the list of proteins expressed in different tumors but not in normal tissues except testis and placenta, the term cancer testis antigen – CTA, has been introduced. At least 70 families of CTAs with over 140 members have been identified and listed in a database established by the Ludwig Institute for Cancer Research[6]. Most CTAs genes are located in clusters at chromosome X, whereas others exist as autosomes allocated single genes. The X and autosomal chromosomes located genes encoding CTAs, are expressed at discrete stages of spermatogenesis and regulated different cellular processes[7]. The aberrant expression of CTA genes in cancer and the correlation of CTAs expression with higher rates of structural chromosomal abnormalities and chromosomal instability has been revealed[8]. Although, the biology of CTAs, their expression profiles, and their role in cellular processes regulation need further investigation. To contribute to this issue, we aimed to determine the expression of total CTAs in correlation with the expression levels of tumor suppressor proteins p53 (TP53) and p63 (TP63) as well as BRCA1 in a healthy cohort.

MATERIALS AND METHODS

In our study, we analyzed samples of 90 blood donors (28, 31.1% – females, 62, 68.9% – males) as they can be considered as an appropriate group for recruiting health cohorts[9]. The age distribution of the subjects was between 20 and 60 years. The present study has been approved by the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy. All procedures performed in the present study were in accordance with the Helsinki Declaration (as revised in 2013). The participants were informed about the study design and objectives. All participants provided written informed consent for inclusion and for anonymous data publication before they participated in the study. We applied enzyme-linked immunosorbent assay (ELISA) method-based kits for research usage (Wuhan Fine Biotech Co. Ltd, Wuhan, Hubei, China) to determine CTAs, TP53, TP63, and BRCA1 expression levels. We performed the study according to the ELISA-specific protocol; the absorbance was measured at 450 nm. For the calculation of results, we used the manufacturer's guidelines: the target concentration of the samples has been interpolated from standard curve. For this calculation, as recommended by the manufacturer the professional software CurveExpert Basic (Hyams Development, https://www.curveexpert.net), was used. The range and sensitivity data per biomarker specific ELISA kit are given below (Table 1).

The data were statistically analyzed for the presentation of descriptive statistics and differences by sex, correlation, and distribution specific to the expression levels of targeted biomarkers.

RESULTS

The data characterizing the expression levels of CTAs, TP53, and BRCA1 specific to sex are presented in Table 2.

TABLE 1. Range and sensitivity data of ELISA kits applied for detection of CTAs, TP53, TP63 and BRCA1 expression levels.

ELISA kit Parameter	*CTAs*	*TP53*	*TP63*	*BRCA1*
Range	0.156-10 ng/ml	0.078-5 ng/ml	0.156-10 ng/ml	0.313-20 ng/ml
Sensitivity	0.094 ng/ml	0.047 ng/ml	0.094 ng/ml	0.188 ng/ml

TABLE 2. Descriptive statistics of CTAs, TP53, and BRCA1 expression level specific to subjects of both sexes.

Data Biomarker	*Sample number*	*Missing data*	*Mean, ng/ml*	*Median, ng/ml*	*Variance*
CTAs, females	20 (71.4%)	8 (28.6%)	0.35	0.236	0.26
CTAs, males	51 (82.3%)	11 (17.7%)	0.38	0.255	0.26
TP53, females	18 (64.3%)	10 (35.7%)	0.13	0.07	0.04
TP53, males	51 (82.3%)	11 (17.7%)	0.13	0.07	0.04
TP63, females	20 (71.4%)	8 (28.6%)	0.2	0.14	0.15
TP63, males	37 (59.7%)	25 (40.3%)	0.18	0.14	0.12
BRCA1, females	28 (100%)	0 (0%)	12.28	11.25	15.79
BRCA1, males	62 (100%)	0 (%)	11.92	11.1	15.13

The difference by sex of target biomarkers expression levels has not been revealed (Figure 1). Although the strong correlation between CTAs and TP53 expression levels has been demonstrated for both sexes. The expression variables of targeted biomarkers for both sexes are not equally distributed. The data specific to CTAs, TP53, and TP63 expression levels are skewed to left. The data of BRCA1 expression may indicate the presence of 2 subgroups for study subjects (Figure 2).

DISCUSSION

TP53 is a key transcription factor that controls the basic cellular processes in response to different stimuli and cellular stresses. The inactivating mutations of TP53 that confer neoplastic growth and resistance to treatment were reported in several cancers, although mutations in this transcriptional factor encoding gene cannot be observed in bone, testis, or skin tumors. As an alternative to TP53 mutation a range of proteins have been described; they are targeted to wild type transcriptional factor function through different mechanisms[10]. From this perspective is important the concrete class of CTAs - MAGE-A (melanoma antigen) proteins. They form a 12-member subgroup of the CTAs that show striking identity with each other and are encoded as a cluster at the Xq28 region[11]. The downregulation of TP53 by this class of CTAs - MAGE-A proteins, is already reported[10-12]. However, our findings suggest a strong correlation between CTAs and TP53 expression levels for study subjects. By consideration of experimentally determined function of CTAs group belonging protein – human neurotrophin receptor interacting MAGE homologous protein (hNRAGE)[13] reported data that

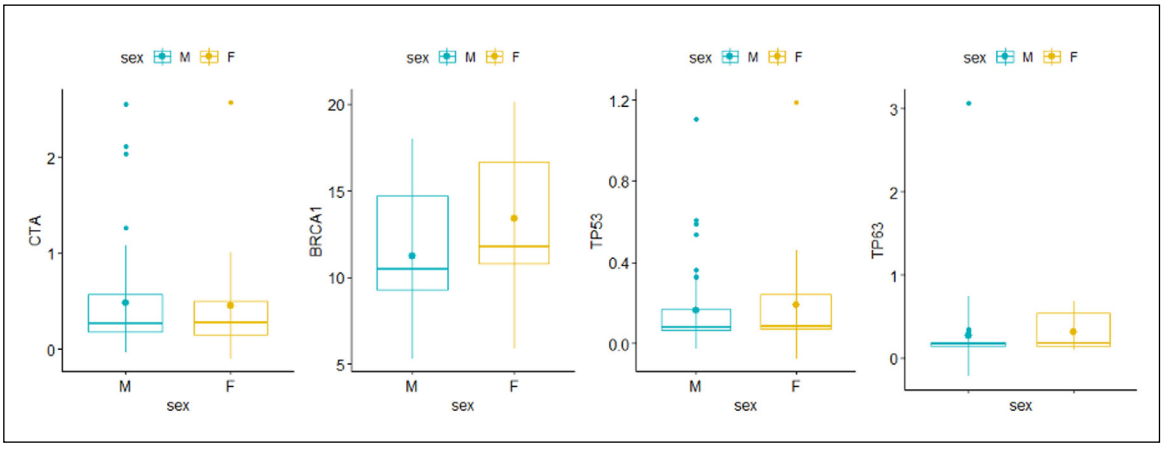

Fig. 1. CTAs, TP53, BRCA1 expression levels difference by sex.

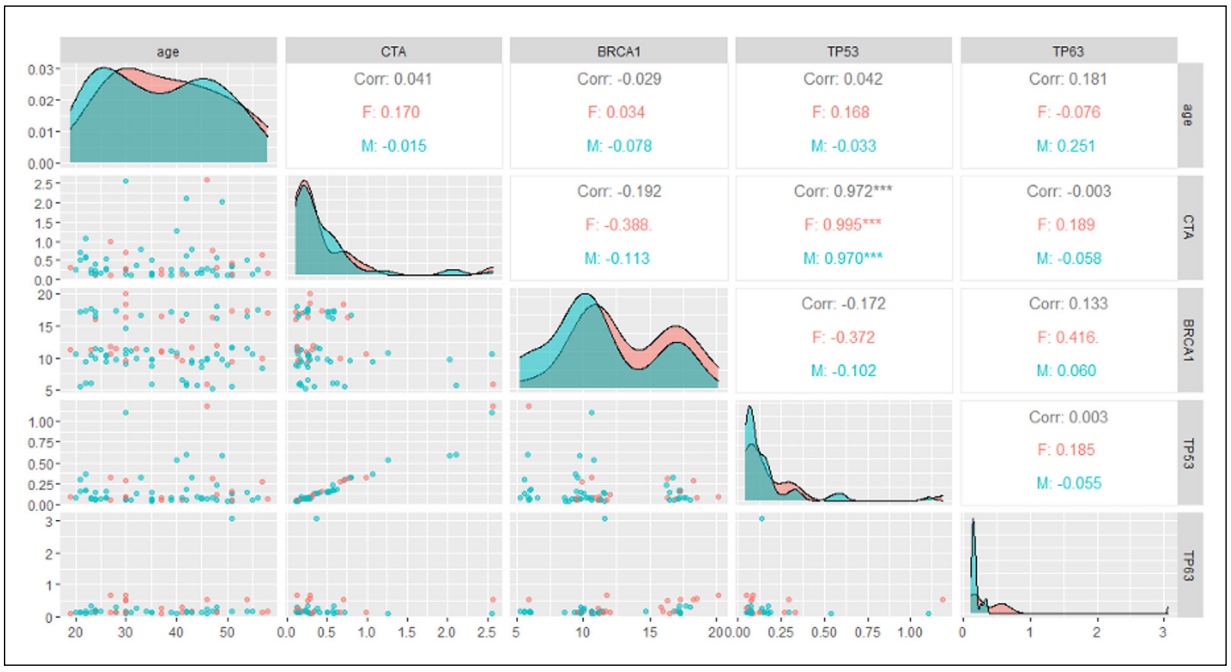

Fig. 2. Correlation and distribution of CTAs, TP53, TP63 and BRCA1 expression data for both sexes.

MAGE-A2 may contribute to the stability of TP53 transcriptional activity in p53-mutated lung cancer cells[14], we expect that co-expression of CTAs and TP53 may indicate upregulated activity of tumor suppressor protein p53 through its CTAs enhanced phosphorylation and accumulation. Moreover, the upregulation of p21 expression by hNRAGE dependent on TP53 activity and hNRAGE enhanced phosphorylation and accumulation of TP53 without chaning the mRNA level of this transcriptional factor has been reported[13]. We assume, that CTAs can upregulate TP53 activity through post-transcriptional modification of the protein.

CONCLUSIONS

The co-expression of CTAs and TP53 may be considered the indicator of "cancer-free" status. This parameter may be piloted for cancer screening and early diagnosis purposes. However, the role of CTAs for cellular process regulation and especially the regulation of tumor suppressor gene p53 shall be investigated further.

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Ethics approval and consent to participate:

The present study has been approved by the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy. All procedures performed in the present study were in accordance with the Helsinki Declaration (as revised in 2013). The participants were informed about the study design and objectives.

Informed Consent:

All participants provided informed consent for inclusion and for anonymous data publication before they participated in the study.

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All authors contributed equally to this article. DM, EG, SG, AS under supervision performed experimental activities; SI performed data analysis; VD organized and performed samples collection; EK contributed to data interpretation and manuscript preparation; CS contributed to article preparation and final review. All authors read and approved the final manuscript.

Conflict of interest:

The authors declare that they have no conflict of interest to disclose.

Data availability statement:

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

1. The Cancer Atlas - https://canceratlas.cancer.org/the-burden/the-burden-of-cancer/ (accessed August 7, 2022).
2. NCDC - https://www.ncdc.ge/#/pages/file/ebe72ea5-5087-4dc3-aaf1-c94cda232ad2 (accessed August 7, 2022).
3. Tobore TO. On the need for the development of a cancer early detection, diagnostic, prognosis, and treatment response system. Future Sci OA 2019; 6: FSO439.
4. Maruvada P, Wang W, Wagne PD, Srivastava S. Biomarkers in molecular medicine: cancer detection and diagnosis. Biotechniques 2018; 38: 4S.
5. Mukama T, Fortner RT, Katzke V, Hynes LC, Petrera A, Hauck SM, Johnson T, Schulze M, Schiborn C, Rostgaard-Hansen AL, Tjonneland A, Overvad K, Sanchez Perez M, Crous-Bou M, Chirlague MD, Amiano P, Ardanaz E, Watts EL, Travis RC, Sacerdote C, Grioni S, Masala G, Signoriello S, Tumino R, Gram IT, Sandanger TM, Sartor H, Lundin E, Idahl A, Heath AK, Dossus L, Weiderpass E, Kaaks R. Prospective evaluation of 92 serum protein biomarkers for early detection of ovarian cancer. Br J Cancer 2022; 126: 1301-1309.
6. Fratta E, Coral S, Covre A, Parisi G, Colizzi F, Danielli R, Nicolay HJM, Sigalotti L, Maio M. The biology of cancer testis antigens: Putative function, regulation and therapeutic potential. Mol Oncol 2011; 5: 164-182.
7. Li XF, Rein P, Shen WZ, Jin X, Zhang J. The expression, modulation and use of cancer-testis antigens as potential biomarkers for cancer immunotherapy. Am J Transi Res 2020; 12: 7002-7019.
8. Jay A, Reitz D, Namekawa SH, Heyer WD. Cancer testis antigens and genomic instability: More than immunology. DNA Repair 2021; 108.
9. Raivola V, Snell K, Helén I, Partanen J. Attitudes of blood donors to their sample and data donation for biobanking. Eur J Human Genetics 2019; 27: 1659-1667.
10. Monte M, Simonatto M, Peche LY, Rublik DR, Gobessi S, Pierotti MA, Rodolfo M, Schneider C. MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer. PNAS 2006; 103: 11160-11165.
11. Marcar L., MacLaine N.J., Hupp T.R., Meek D.W. Mage-A cancer/testis antigens inhibit p53 function by blocking its interaction with chromatin. Cancer Res 2010; 70: 10362-10370.
12. Pascucci FA, Ladelfa MF, Toledo MF, Escalada M, Suberbordes M, Monte M. MageC2 protein is upregulated by oncogenic activation of MAPK pathway and causes impairment of the p53 transactivation function. Biochemica et Biophysica Acta (BBA) – Mol Cell Res 2021; 1868: 118918.
13. Wen CJ, Xue B, Qin WX, Yu M, Zhang MY, Zhao DH, Gao X, Gu JR, Li CJ. hNRAGE, a human neutrophin receptor interacting MAGE homologue, regulates p53 transcriptional activity and inhibits cell proliferation. FEBS Letters 2004; 564: 171-176.
14. Ujiiie H, Kato T, Lee D, Hu HP, Fujino K, Kaji M, Kaga K, Matsui Y, Yasufuku K. Overexpression of MAGEA2 has a prognostic significance and is a potential therapeutic target for patients with lung cancer. Int J Oncol 2017; 50: 2154-2170.