https://doi.org/10.46389/rjd-2022-1099
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2022; volume 29, issue 2, pages 245–252

Original Research

Characteristics of certain genetical and biological properties of carcinogenesis in the development of inflammatory breast cancer with type 2 diabetes mellitus and tumor relapse

Bagmut Irina Yuriivna[1], Movchan Oleksii Volodimirovich[2], Sheremet Michael Ivanovich[3,*], Smolanka Ivan Ivanovich[2], Lyashenko Andriy Oleksandrovich[2], Dosenko Irina Viktorivna[2], Loboda Anton Dmitrovich[2], Kolisnyk Igor Leonidovich[1]

[1] Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine
[2] National Cancer Institute of the Ministry of Health of Ukraine
[3] Surgical Department N°1, Bukovinian State Medical University, Ukraine

*Correspondence to: Michael Ivanivich Sheremet, Surgery Department No1 of Bukovinian State Medical University, Ukraine Address: Holovna str., 191, 58018 Chernivtsi, Ukraine, Phone: 0956064607, E-mail id: mihayl71@gmail.com

Received: 31 March 2022 / Accepted: 16 May 2022

Abstract

Introduction: The aim of the work is the ability to increase the effectiveness of comprehensive treatment of patients with inflammatory breast cancer with type 2 diabetes mellitus by individualization based on the determination of carcinogenesis indicators of the malignant process. **The aim of the study:** The ability to increase the effectiveness of comprehensive treatment of patients with inflammatory breast cancer with type 2 diabetes mellitus by individualization based on the determination of carcinogenesis indicators of the malignant process. **Materials and research methods**: We examined 80 patients with inflammatory breast cancer with type 2 diabetes mellitus, who were determined microsatellite instability (MSI) at the loci of BAT 25; BAT 26, Ki-67 proliferation index, and p53 gene mutation and plasma glucose level control. To assess the effectiveness of treatment depending on the biological properties of the tumor, in tumor tissue studied the expression levels of p53 protein, the index of proliferative activity of Ki-67, determined the presence of MSI at the loci BAT-25 and BAT-26 and their interconnection and dependence on each other. **Results:** It is established in this case; this is realized due to the inability of the mutant form of the p53 gene to synthesize the desired Quaternary configuration of the corresponding protein with the subsequent inability of the latter to stop the process of DNA replication. It was found that the proliferative activity of tumor cells in inflammatory breast cancer had a clear relationship with MSI at both studied chromosome loci. The presence of MSI is somehow associated with hyperglycosemia and is an aggravating factor in the course of inflammatory breast cancer with a poorer response to a specific therapy.

Keywords: inflammatory breast cancer, carcinogenesis pathways, the variability of the occurrence of factors of inflammatory breast cancer, type 2 diabetes mellitus.

Introduction

Inflammatory breast cancer develops as a result of a combination of oncogene activation and inactivation of suppressor genes [1]. Many genetic disorders (amplification and/or hyperexpression of EGFR (HER1) and HER2 (ERBB2) genes, mutations in the p53 gene may be detected in other localizations of cancer [2], i.e., are relatively non-specific for this type of cancer, such as type 2 diabetes mellitus (T2DM) [3].

Breast cancer is one of those cases when HER2 / neu activation involved in cell proliferation, survival, invasion, migration, apoptosis, glucose metabolism, and DNA repair is observed in approximately 20–30% of malignant neoplasms

of this organ and correlates with the aggressive course of the disease [4].

The observation was the basis for clinical trials of specific humanized HER2-specific antibodies, Trastuzumab, which is thought to improve the treatment of patients with HER2 / neu-positive breast cancer [5]. This drug is undoubtedly very relevant in the treatment of the inflammatory form of this disease. But, one of the main criteria was plasma glucose level control, because it can lead to an adverse events like hyperglycemia above 14.0 mmol/l same way hypoglycemia below 4.0 mmol/l [6]. The line is a gene that encodes the protein p53. The p53 gene encodes a nuclear phosphoprotein. p53 is one of the immortalization genes. The product of the p53 gene is involved in the recognition of chemical DNA damage. Protein p53 is involved in the transition of a cell at rest to proliferation, carries out the negative regulation of cell proliferation in the case of disruption of DNA structure. p53 gene abnormalities are one of the factors of continuous tumor progression, which leads to the emergence and selection of increasingly aggressive tumor cell clones [7].

In inflammatory breast carcinomas, the p53 gene is inactivated by micro mutations as well as by deletion of the corresponding locus of chromosome 17 [8].

Microsatellite instability (MSI) often leads to additional genetic changes and loss of alleles due to a mutation with a shift in the coding of repetitive gene sequences involved in the regulation of cell growth, apoptosis, glucose metabolism, and DNA repair [9].

These criteria, which were first proposed for the classification of colorectal cancer, are successfully used for breast cancer. The incidence of high levels of MSI in breast cancer averages 2–18%. In Asian countries, the frequency of high levels of MSI reaches 5%, while in West Europe countries from 2% to 15% [10].

There are many ways to visualize and assess the proliferative activity of cells. The most informative is the determination of the index of proliferative activity of cells Ki-67, because it shows not only cells that are in mitosis but also cells that are in the process of preparing for division. Thus, Ki-67 indicates the proliferative potential of the tissue [11].

Thus, the analysis of the current state of the problem of treatment of inflammatory breast cancer with type 2 diabetes mellitus as a complicating factor has shown significant variability of approaches to this issue, which, with moderate success, indicates the relevance of scientific research in this area. In particular, there is an acute shortage of reliable ways to choose an individualized program of complex treatment and prognosis of recurrence in patients with inflammatory breast cancer with type 2 diabetes mellitus in the study of the factors of this disease.

Aim of the work

The ability to increase the effectiveness of comprehensive treatment of patients with inflammatory breast cancer with type 2 diabetes mellitus by individualization based on the determination of carcinogenesis indicators of the malignant process.

Materials and methods

The study is conducted according to the ethical principles of Helsinki Declaration, GCP (Good Clinical Practice), and Law of Ukraine “On medications”. All patients were informed about the research and signed the agreement.

This study was approved by the Institutional Ethics Committee of the National Cancer Institute of Ukraine (Minutes No. 163 of June 23, 2020). The authors declare no conflicts of interest and no funding. The study is conducted according to the ethical principles of Helsinki Declaration, GCP (Good Clinical Practice), and Law of Ukraine “On medications”. All patients were informed about the research and signed the agreement.

Eighty patients with inflammatory breast cancer $T_{4d}N_{0-3}M_0$ with type 2 diabetes mellitus who received treatment at the Department of Breast Tumors and Reconstructive Surgery of the National Cancer Institute of Ukraine were included in the study. In all cases in tumor tissue, MSI at the loci of BAT 25 and BAT 26, Ki-67 proliferation index, and p53 gene mutation, and plasma

https://doi.org/10.46389/rjd-2022-1099

glucose level control were determined. Analysis of dependences in combinations of these biological properties of the tumor was performed. All age groups (18–70 years) and molecular subtypes (LumA, LumB, Her2neu-positive, and triple-negative) were covered.

Evaluation of immunohistochemically staining results was performed using a Carl Zeiss Axio Scope A1 light microscope, manufactured by Carl Zeiss MicroImaging GmbH (Oberhochen, Germany) (magnification × 1250, oil immersion). The results of the immunohistochemically reaction were evaluated by a semi-quantitative method, by counting the percentage of positively stained cells (label index – MI) with different intensities, which was evaluated visually. For p53, the presence of "wild" protein was determined at MI <40.0%, and mutant at MI ≥40.0%.

Proliferative potential (index of proliferative activity) was determined by counting the number of cells expressing Ki-67: Ki-67 <30.0% was considered to be a low proliferative activity, Ki-67 ≥ 30.0% – high proliferative activity.

MSI was assessed by polymerase chain reaction using two quasi-monomorphic mononucleotide markers BAT-25 and BAT-26.

Laboratory assessment

Current laboratory recommendations for plasma glucose measurement are to draw fasting blood samples in the morning rather than later in the day, as glucose levels tend to be higher in the morning than in the afternoon. These samples should be placed on ice to minimize glycolysis and quickly processed (via plasma separation within 60 minutes) as glucose concentrations decrease at a rate of 5–7% per hour. If plasma separation cannot occur within 60 minutes, the lab tech can add a glycolytic inhibitor such as fluoride. Normal range is 4.4–6.6 mmol/l [12].

The behavior and aggressiveness of the tumor were compared and evaluated for different variants of the biological status of the tumors on the background type 2 diabetes mellitus. Conjugation graphs were formed for each variant of biological characteristics.

Results

The frequency of occurrence of wild and mutant variants of the p53 gene was studied according to the expression level of the corresponding protein depending on the state of the stability genes BAT-25 and BAT-26. Much more often, in 78.18 ± 5.57% of cases with plasma glucose levels above 10.0 mmol/l, cells with a wild variant of the p53 gene were observed, and only in 21.82 ± 5.57%, its mutation was noted. But on the other hand, we have noticed that 92.31 ± 7.39% of p53 mutant variants were found in the presence of MSI (in 12 out of 13 cases) with plasma glucose levels above 10.0 mmol/l. And only in 7.69 ± 1.39% of cases p53 mutation was found when MSI was absent. To compare, the wild variant of p53 gene was found also mostly in MSI positive variant with a much lower difference (64.18 ± 5.86% to 35.82 ±5.86%), Figure 1.

When comparing the differences between the two samples by the four-cell criterion χ^2, it was found that in the presence of MSI by BAT 25, which was observed in 55 patients, the ratio between the absence of p53 gene mutation in 43 patients and its presence in 12, probably ($p<0.05$) differs from the ratio between the absence and presence of p53 mutation (24 cases vs. 1) when MSI was absent.

In the absence of MSI at the specified locus, in all cases, a wild variant of the gene was observed. In its presence, the wild version of p53 was also observed in a significant number of cases, in 77.27 ± 5.16% and the p53 mutation was observed in 22.73 ± 5.16% of samples (15 cases). It should be noted that all these 15 cases of p53 mutation were found only in MSI positive samples (100% with plasma glucose level above 10.0 mmol/l). The wild variant of the gene was also mostly found in MSI positive samples (78.46 ± 5.10% of the total number of cases), but other 21.54 ± 5.10% of cases were observed in MSI negative samples (Figure 2).

To assess the proliferative potential of cells, the expression of Ki-67 in tumor tissue was studied depending on the presence of MSI loci of BAT 25 and BAT 26.

In the presence of MSI at BAT 25, in the vast majority of samples 96.00 ± 3.92% with

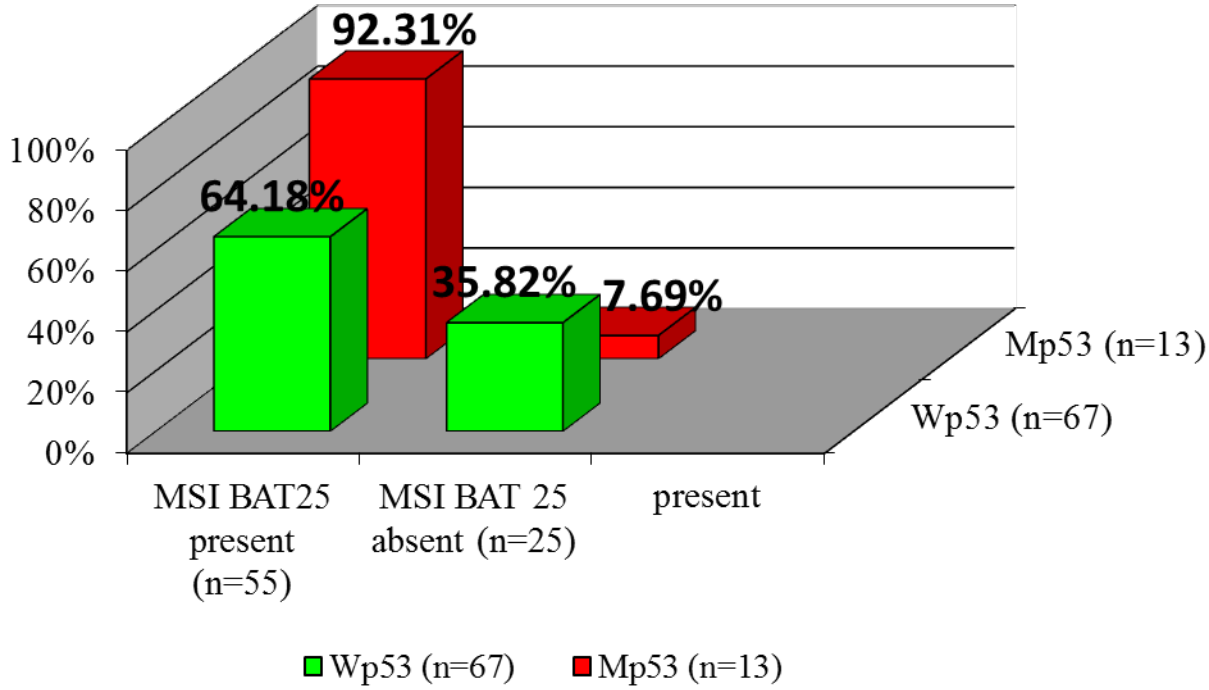

Figure 1: The frequency of the p53 mutation depending on the presence of MSI (BAT 25).

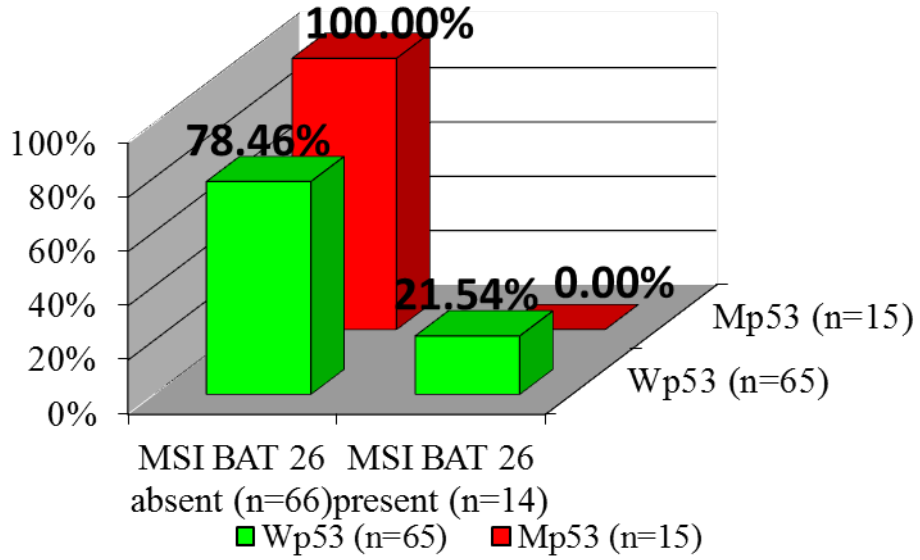

Figure 2: The frequency of the p53 mutation depending on the presence of MSI (BAT 26).

plasma glucose levels above 10.0 mmol/l had low Ki-67 level, and a high level of expression of this marker in the presence of MSI at this locus was observed only in 4.00 ± 3.92% (Figure 3). Among the total number of tumors with no MSI at BAT 25, in 56.36 ± 6.69% cases the expression level of Ki-67 was low, while high level and hyperexpression were determined in 43.64 ± 6.69% of cases.

At the same time among all cases of high Ki-67 levels MSI was negative with plasma glucose levels in the normal range at 96.00 ± 3.92% and positive only in 4.00 ± 3.92% of cases. Low Ki-67 level was found almost equally in MSI negative samples (56.36 ± 6.69%) and MSI positive samples (43.64 ± 6.69%).

Comparison of the frequency and nature of Ki-67 expression in tumors depending on the presence of MSI on BAT 25 by $\chi 2$-test showed that its high level was mainly observed among tumors without MSI on BAT 25: the ratio of the proportion of cases of low and high levels of Ki-67 in MSI positive samples is probably ($p<0.05$) different

https://doi.org/10.46389/rjd-2022-1099

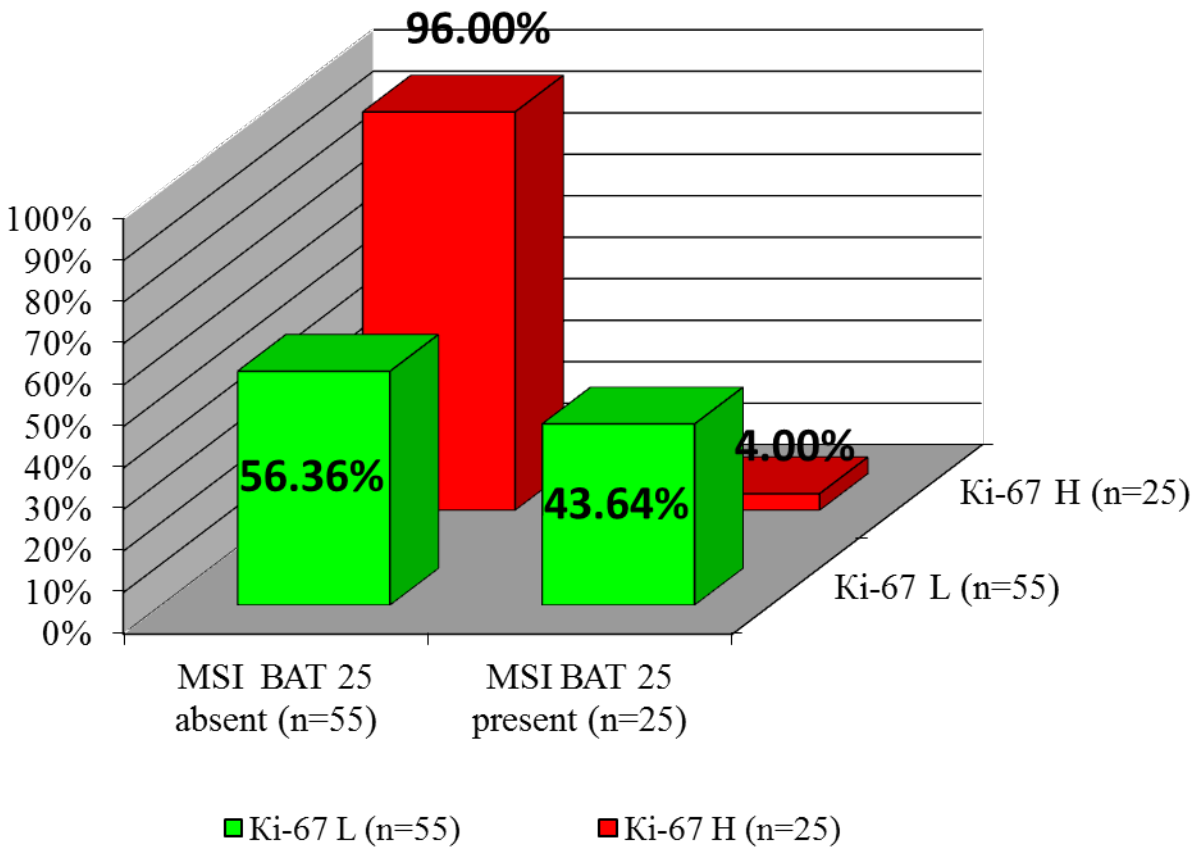

Figure 3: The frequency of different variants of expression of the marker of proliferative activity of Ki-67 depending on the presence in tumor cells of MSI at BAT 25.

from the specified ratio in MSI negative cases at BAT 25 locus.

The study of the dependence of Ki-67 expression on the state of MSI at BAT 26 showed that in the presence of MSI in 92.86 ± 6.88% cases, the expression of Ki-67 was low with plasma glucose levels above 10.0 mmol/l. Ki-67 high level was observed under the above conditions only in 7.14 ± 6.88% of cases. In the absence of MSI at BAT 26, a low level of expression of the proliferative activity index was observed in 63.64 ± 5.92% of the studied samples with plasma glucose levels in the normal range. Under the same conditions, a high level of Ki-67 or its hyperexpression was observed in 36.36 ± 5.92% of cases. Although, regardless of the MSI status in all cases low cell proliferation dominated, when comparing the ratios of proliferative activity depending on the presence of MSI, clear relation is visible. $\chi 2$-test showed that its high level was mainly observed among tumors without MSI at BAT 26: the ratio of the proportion of cases of low and high levels of Ki-67 in MSI positive samples is probably ($p < 0.05$) different from the same ratio in MSI negative cases (Figure 4).

The level of the proliferative activity in inflammatory breast cancer was also assessed depending on the presence of the p53 mutation (Figure 5).

In all 13 cases of the existing p53 mutation, the proliferative activity of the cells was low. Regarding the wild variant of the gene, in 62.69 ± 5.91% of tumor tissue samples, the level of Ki-67 was also low, and in 37.31 ± 5.91%, it was high. At the same time, a high level of Ki-67 was observed in 100% of cases in samples with no p53 mutation. Low rates of Ki-67 were also mostly observed in p53 negative samples (76.36 ± 5.72%), but in 23.63 ± 5.72% of cases, p53 mutation was identified. $\chi 2$ shows us that the ratio of Ki-67 low to high levels in p53-positive samples reliably ($p<0.05$) differs from the same ratio in p53-negative samples. And at the same time, $\chi 2$ shows us that the ratio of p53 negative to positive samples in Ki-67 high-level cases reliably ($p < 0.05$) differs from the same ratio in Ki-67 low-level cases.

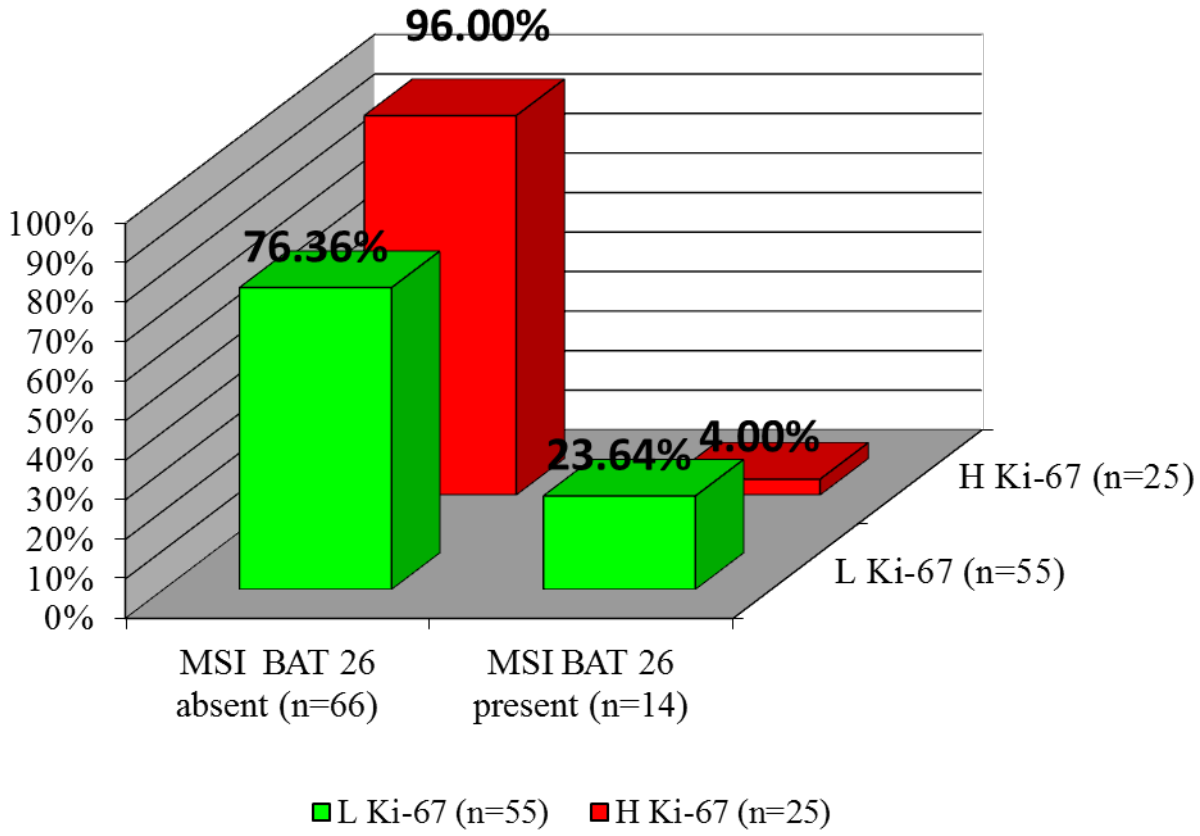

Figure 4: The frequency of different variants of expression of the marker of proliferative activity of Ki-67 depending on the presence in tumor cells of MSI at BAT 26.

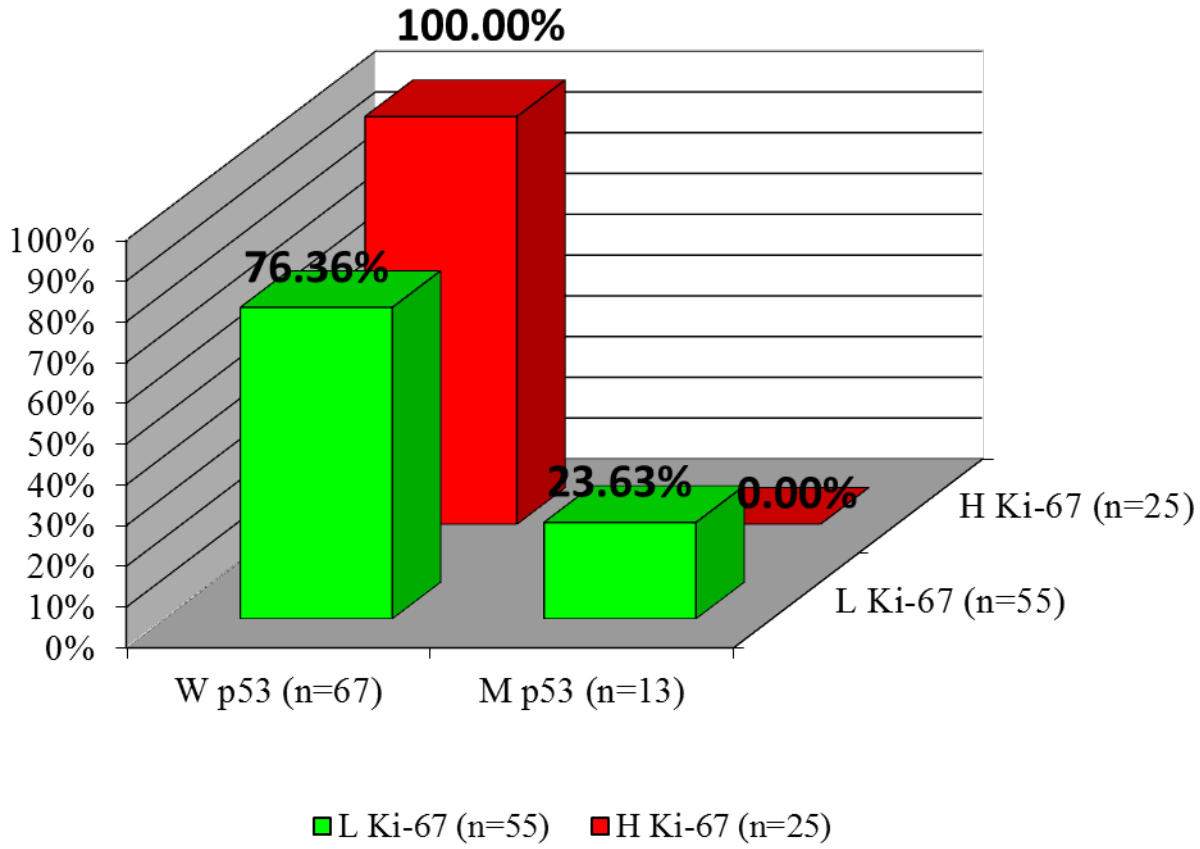

Figure 5: The frequency of different variants of expression of the marker of proliferative activity of Ki-67 depending on the presence in tumor cells of the p53 mutation.

https://doi.org/10.46389/rjd-2022-1099

Discussion

MSI absence in tumor cells at the BAT 25 locus, a wild variant of the p53 gene was observed in most cases, in 96.00 ± 3.92% with plasma glucose level above 10.0 mmol/l against 4.00 ± 3.92% with plasma glucose level in the normal range, when its mutation was noted.

In inflammatory breast cancer samples, mutation of the p53 gene is observed mainly in tumors with MSI at the locus of BAT 25, and rarely found if MSI is negative. A similar situation arose in the study of the relationship between the presence of MSI at BAT 26 locus and a mutation in the p53 gene.

When comparing the differences between the two samples by the four-cell criterion $\chi 2$, it was found that in the presence of MSI at BAT 26, which was observed in 66 patients, the ratio between the absence of mutation of the p53 gene in 51 patients and its presence in 15, probably ($p < 0.05$) differs from the ratio between the absence and presence of p53 mutation when MSI at BAT 26 was negative (14–0).

Therefore, it can be argued that in inflammatory breast cancer, the p53 gene mutation is observed mainly in tumors with MSI at the BAT 26 locus with plasma glucose levels above 10.0 mmol/l. The data obtained indicate that mutations in the p53 gene are observed with a significantly higher frequency in the presence of MSI in the loci of chromosomes responsible for post-replication repair. In these tumors, despite the preserved ability to restore chromosomal breakdowns, the emergence and further development of the tumor are associated with another mechanism of carcinogenesis – mutations in the "genome guard" p53 gene – with a subsequent conformational change in the quaternary structure of the protein and loss of its control function, and subsequent violation of apoptosis. It is known that the rate of tumor growth is one of the most important integral indicators of the features of its clinical course. It should be understood that the growth rate is determined by the balance between the activity of proliferative processes on the one hand, and apoptosis on the other.

There is an interesting interconnection between MSI and the activity of Ki-67. We are dealing with various ways of the progressive development of breast cancer; each of the above markers (when enhanced) indicates an increase in the aggressiveness and the speed of development of the tumor process. However, as we see from the results we obtained, the presence of MSI and the increased proliferative activity of the Ki-67 index are mutually exclusive. These findings may influence special treatment corrections.

Conclusions and perspectives

1. This data can be considered as confirmation of the versatility of genetic disorders in the carcinogenesis of breast cancer. In tumors with the preserved function of stability genes, i.e., with the possibility of restoration of chromosomal breakdowns, the emergence and proliferation of malignant cells are associated with impaired apoptosis at critical points of cell division. In this case, this is realized due to the inability of the mutant form of the p53 gene to synthesize the desired quaternary configuration of the corresponding protein with the subsequent inability of the latter to stop the process of DNA replication.
2. It has been shown that, apparently, a mutation in the basic control gene leads to a loss of control over DNA and can only contribute to the emergence of a malignant cell. And the further loss of the level of cell differentiation is probably due to other factors.
3. It was found that the proliferative activity of tumor cells in inflammatory breast cancer had a clear relationship with MSI at both studied chromosome loci.
4. Presence of MSI and mutant form of the p53 gene vs. the increased proliferative activity of the Ki-67 index are mutually exclusive, which also gives us the opportunity to correct special treatment options in order to reduce toxic action and improve the quality of life of patients during therapy.
5. The presence of MSI is somehow associated with hyperglucosemia and is an aggravating factor in the course of inflammatory breast cancer with a poorer response to a specific therapy.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Colaprico, A., Olsen, C., Bailey, M. H., et al. (2020). Interpreting pathways to discover cancer driver genes with Moonlight. *Nat Commun*. 11:65–69. https://doi.org/10.1038/s41467-019-13803-0.
2. Doyle, H. A., Gee, R. J., Masters, T. D., Gee, C. R., Booth, C. J., Peterson-Roth, E., Koski, R. A., Helfand, S. C., Price, L., Bascombe, D., Jackson, D., Ho, R., Post, G. R., Mamula, M. J. (2021). Vaccine-induced ErbB (EGFR/HER2)-specific immunity in spontaneous canine cancer. *Transl Oncol*. 14(11):101–205. doi: 10.1016/j.tranon.2021.101205. Epub 2021 Aug 19. PMID: 34419682; PMCID: PMC8379704.
3. Lee, K. N., Torres, M. A., Troeschel, A. N., He, J., Gogineni, K., McCullough, L. E. (2020). Type 2 diabetes, breast cancer specific and overall mortality: Associations by metformin use and modification by race, body mass, and estrogen receptor status. *PLoS One*. 15(5):e0232581. https://doi:10.1371/journal.pone.0232581.
4. Miricescu, D., Totan, A., Stanescu-Spinu, II, Badoiu, S. C., Stefani, C., Greabu, M. (2020). PI3K/AKT/mTOR signaling pathway in breast cancer: From molecular landscape to clinical aspects. *Int J Mol Sci*. 22(1):168–173. doi: 10.3390/ijms22010173. PMID: 33375317; PMCID: PMC7796017.
5. Wang, J., Xu, B. (2019). Targeted therapeutic options and future perspectives for HER2-positive breast cancer. *Sig Transduct Target Therapy*. 4:29–34. https://doi.org/10.1038/s41392-019-0069-2.
6. Amrith, B. P., Jain, P., Chaudhari, K. A., Sharma, M., Batra, U. (2019). Severe hypoglycemia with trastuzumab: An unseen adverse event. *South Asian J Cancer*. 8(3):140–149. https://doi:10.4103/sajc.sajc_400_18.
7. Marei, H. E., Althani, A., Afifi, N., et al. (2021). p53 signaling in cancer progression and therapy. *Cancer Cell Int*. 21:667–703. https://doi.org/10.1186/s12935-021-02396-8.
8. Maurizio Di, B., Monica, C., Gerardo, B. (2018). Pathological and molecular characteristics of inflammatory breast cancer. *Translational Cancer Res*. (*Rare Tumors of the Breast*). 8:109–121. https://doi: 10.21037/tcr.2019.03.24.
9. Smith, M. T., Guyton, K. Z., Kleinstreuer, N., et al. (2020). The key characteristics of carcinogens: Relationship to the hallmarks of cancer, relevant biomarkers, and assays to measure them. *Cancer Epidemiol Biomarkers Prev*. 29(10):1887–1903. doi: 10.1158/1055-9965.EPI-19-1346. Epub 2020 Mar 9. PMID: 32152214; PMCID: PMC7483401.
10. Qian, S., Golubnitschaja, O., Zhan, X. (2019). Chronic inflammation: key player and biomarker-set to predict and prevent cancer development and progression based on individualized patient profiles. *EPMA J*. 10:365–381. https://doi.org/10.1007/s13167-019-00194-x.
11. Xiaoding, H., Emilly, S. V., Richard, L., (2021). Decorin-mediated suppression of tumorigenesis, invasion, and metastasis in inflammatory breast cancer. *Commun Biol*. 4:67–72. https://doi.org/10.1038/s42003-020-01590-0.
12. Gurung, P., Jialal, I. (2021). Plasma glucose. [Updated 2021 Aug 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541081.

https://doi.org/10.46389/rjd-2022-1099