SHORT COMMUNICATION

EXPERIMENTAL ONCOLOGY

https://doi.org/10.15407/exp-oncology.2026.01.046

T. Borikun [1, *], Y. Herasymchuk [2]

[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2] Educational and Scientific Centre «Institute of Biology and Medicine» of Taras Shevchenko National University of Kyiv , Kyiv, Ukraine

* Correspondence: E-mail: tborikun@gmail.com

STRESS-INDUCED CHANGES IN THE METHYLATION STATUS OF *Mmp1* AND *Mmp8* GENES IN TUMOR TISSUE OF RATS WITH GUERIN CARCINOMA

Background. Chronic stress is a key determinant of public health, significantly impacting regulatory systems and potentially contributing to carcinogenesis. Chronic stress, through the activation of the hypothalamic-pituitary-adrenal axis and glucocorticoid signaling, can modify the epigenetic landscape of cells, including DNA methylation. This study **aimed** to evaluate whether chronic glucocorticoid-induced stress modulates the methylation status of the promoter regions of the *Mmp1* and *Mmp8* genes in the tumor tissue of rats with Guerin carcinoma. **Materials and Methods.** To simulate chronic stress, dexamethasone (DEX) was administered subcutaneously to laboratory rats with transplanted Guerin carcinoma. Tumor samples were collected on days 7, 14, and 21 of tumor growth. The methylation status of the *Mmp1* and *Mmp8* gene promoters was analyzed using methylation-specific PCR. The level of methylation was quantified as the ratio of methylated to unmethylated PCR products, expressed as a percentage. **Results.** In the control group (Guerin carcinoma without DEX), the *Mmp1* promoter was hypomethylated (38—40% methylation) throughout the observation period. DEX administration led to a further slight decrease in *Mmp1* methylation to 32%, though insignificant. In contrast, the *Mmp8* promoter in tumor tissue showed baseline methylation levels of 55—57%. Under the influence of DEX, *Mmp8* hypermethylation was observed as early as day 7 (61%) and significantly progressed by day 14 (67%) and day 21 (69%). **Conclusion.** Our study demonstrated that chronic glucocorticoid-induced stress altered the epigenetic profile of tumor cells by inducing differential methylation of the matrix metalloproteinase genes. Specifically, it promoted the maintenance of *Mmp1* hypomethylation and significantly increased the *Mmp8* promoter hypermethylation. These findings suggest that chronic stress may contribute to an aggressive tumor phenotype through the epigenetic regulation of extracellular matrix remodeling.

Keywords: DNA methylation, chronic stress, cancer.

Chronic stress poses a major issue in the current population and is acknowledged as a crucial factor in public health. Global epidemiological research indicates that approximately 35% of adults consistently report feeling stressed, and this pattern is steadily rising [1, 2]. Considering the ongoing geopolitical situ-

Citation: Borikun T, Herasymchuk Y. Stress-induced changes in the methylation status of *Mmp1* and *Mmp8* genes in tumor tissue of rats with Guerin carcinoma. *Exp Oncol*. 2026; 48(1): 46-50. https://doi.org/10.15407/exp-oncology.2026.01.046

© PH "Akademperiodyka" of the NAS of Ukraine, 2026. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

ation in Ukraine, this issue impacts the whole population and should be seen as a major factor in public health challenges.

In this regard, chronic stress is considered an important modifier of the functioning of regulatory systems. The prolonged activation of neuroendocrine mechanisms, in particular the hypothalamic-pituitary-adrenal (HPA) axis, is accompanied by increased secretion of glucocorticoids and catecholamines, which can lead to the disruption of cellular homeostasis, changes in the immune response, and the creation of conditions favorable for the development of pathological processes, including carcinogenesis. Glucocorticoid-dependent signaling can affect DNA methyltransferase activity and chromatin remodeling, which leads to stable changes in the epigenetic landscape of cells [3].

The accumulated experimental and clinical data indicate that chronic stress is associated with an increased risk of the occurrence and progression of malignant neoplasms. This is due, in particular, to the influence of stress-induced hormones on the processes of proliferation, apoptosis, angiogenesis, and invasion of tumor cells. The epigenetic mechanisms play an important role in the implementation of these effects, among which DNA methylation is one of the key regulators of gene expression. The changes in methylation of the promoter regions can lead to the activation of oncogenes or suppression of tumor suppressor genes, thereby contributing to malignant transformation of cells [4, 5].

Of particular interest are the genes of the matrix metalloproteinase family, *MMP1* and *MMP8*, whose products are involved in the degradation of extracellular matrix components and tumor cell invasion [6]. It was shown that the increased expression of *MMP1* is associated with tumor progression and decreased survival in xenograft animal models [7]. The role of *MMP8* in cancer is more complex and context-dependent, since, along with participation in matrix degradation, this enzyme can exhibit both pro- and antitumor properties [8].

The *MMP* family gene expression is regulated at various levels, including epigenetic mechanisms, among which DNA methylation plays an important role. It has been shown that the level of methylation of the *MMP* promoter regions is inversely related to MMP expression and invasive potential of cells [9].

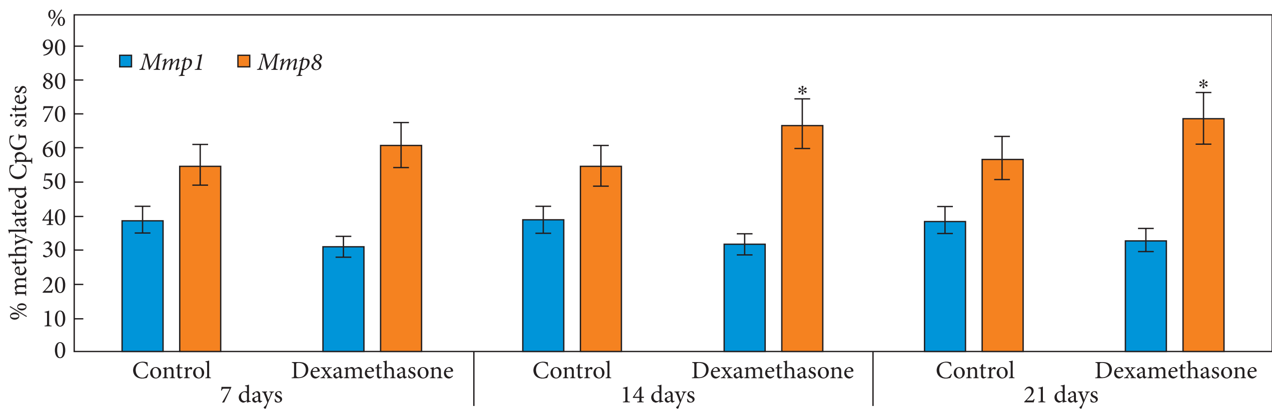
Despite the available data on the role of stress and epigenetic changes in carcinogenesis, it remains unclear how chronic stress modulates the methylation of specific genes involved in extracellular matrix degradation, in particular *MMP1* and *MMP8*. Thus, we aimed to evaluate whether chronic glucocorticoid-induced stress modulates the methylation status of the promoter regions of the *Mmp1* and *Mmp8* genes in the tumor tissue of rats with Guerin carcinoma.

Materials and Methods

Animal model. The study was conducted on male Wistar rats aged 2.5 months and weighing 180–200 g. The animals were provided by the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. During the study, the animals were kept in standard vivarium conditions with natural lighting, on a full diet with free access to food and water. The study was conducted in accordance with the standard international rules on biological ethics and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. All animals underwent a 10-day quarantine before being included in the study. After the adaptation period, the animals were weighed, divided into groups, and marked by serial number. As a model of tumor growth, Guerin carcinoma was used, obtained from the Bank of Cell Lines from Human and Animal Tissues of the IEPOR NASU.

A suspension of Guerin carcinoma cells in saline was transplanted (10^6 cells per animal) subcutaneously to the right side, closer to the back. The animals were divided into groups of 5 animals each. The animals of the experimental group were injected with 0.5 mL of dexamethasone (dexamethasone solution for intravenous use 4 mg/mL, PJSC «Lekhim-Kharkiv», Kharkiv, Ukraine) at a concentration of 0.5 mg/kg, subcutaneously starting from day 2 after Guerin carcinoma inoculation every other day for 12 days. The solutions were administered using a U-40 BD Micro-Fine Plus 30G microinjection insulin syringe (Becton, Dickinson and Company, USA). The animals were slaughtered on days 7, 14, and 21 after the start of the experiment.

Methylation-specific real-time PCR. Genomic DNA from frozen tumor tissue was isolated using a commercial DNA/RNA-Mag reagent kit (Xema, Ukraine) and treated with sodium bisulfite according to the manufacturer's instructions.



The degree of methylation of the promoter regions of the *Mmp1* and *Mmp8* genes in tumor tissue of rats with Guerin carcinoma. * $p < 0.05$ compared to control

The amount of isolated DNA was determined using a NanoDrop 2000c Spectrophotometer (ThermoScientific, USA). The purity of the isolated DNA was monitored by the ratio of optical absorption values at wavelengths of 260 and 280 nm. The DNA was stored at −20 °C before use.

For real-time PCR, the sequences of the primers were obtained from the resource https://www.ncbi.nlm.nih.gov/tools/primer-blast and synthesized by Metabion, Germany (Table) using the commercial EpiTect MethyLight PCR +ROX Vial Kit (QIAGEN GmbH, Germany). They were used according to the manufacturer's protocol.

After standardization of methylated (M) and unmethylated (U) PCR products by the $2^{-\Delta Ct}$ method, methylation percent was calculated as M/(M + U), which indicated the levels of gene methylation.

Statistical methods. Statistical processing of the obtained results was carried out using the STATISTICA 6.0 program (Statistica Inc., USA). The standard descriptive, parametric, and non-parametric statistical methods were used. The significance of the differences between the mean values was assessed using Student's *t*-test (for a parametric distribution) or the Mann — Whitney U-test (for a non-parametric distribution). Differences at $p \leq$ ≤ 0.05 were considered significant.

Results and Discussion

The features of methylation of the promoter region of the *Mmp1* and *Mmp8* genes in tumor tissue of rats with Guerin carcinoma were studied. It was found that the *MMP1* gene promoter was hypomethylated on day 7 after transplantation (the ratio of methylated PCR products to unmethylated ones was 39%). From day 7 to day 21 of tumor growth, this ratio did not significantly change and fluctuated within 38—40% (Figure).

Dexamethasone (DEX) also caused a slight decrease in the level of methylation of the *MMP1* gene promoter (up to 32%); however, insignificant compared to the control. Genevay et al. [10] mentioned in their study that DEX can inhibit MMP1

Primers used in the study

Gene	Methylation status	Primer	
Mmp1	Methylated	Forward	5′-ATTTTAAA TAAGATGTGTGCG-3′
	Methylated	Reverse	5′-AACCATCA AAACCAATCTTTT-3′
	Unmethylated	Forward	5′-ATTTTAAA TAAGATGTGTGTG-3′
	Unmethylated	Reverse	5′-AACCATCA AAACCAATCTTTT-3′
Mmp8	Methylated	Forward	5′-TTGTAGAC GTGAGTTATCGTATTCG-3′
	Methylated	Reverse	5′-AATCGTAA AAACCTAACCCTAACG-3′
	Unmethylated	Forward	5′-TGTAGATG TGAGTTATTGTATTTGG-3′
	Unmethylated	Reverse	5′-ATCATAAAA ACCTAACCCTAACAA-3′

activity, which is probably due to its effect on the glucocorticoid-dependent signaling pathways.

When studying the methylation status of the *Mmp8* gene promoter, we found that in Guerin carcinoma tissue, it was slightly hypermethylated (55% of methylated sites on days 7 and 14 of tumor growth and 57% on day 21). In the tumor tissue of DEX-treated rats, the *Mmp8* gene promoter showed increased hypermethylation above control levels by day 7 (61% of methylated sites), with this trend persisting over time. Our data were consistent with the results of current molecular genetic studies, which identify *MMP8* as one of the most stress-sensitive genes. In particular, Daskalakis et al. [11] have shown that *MMP8* is among the key genes whose expression is significantly altered by exposure to traumatic events and is regulated by glucocorticoids. The progressive hypermethylation of the promoter under the action of DEX, which we have identified, may be an epigenetic mechanism leading to reduced expression of this gene, similar to what is observed in stress-induced conditions in other models.

To simulate chronic stress conditions in laboratory animals, we used DEX administered subcutaneously. DEX, a synthetic glucocorticoid receptor agonist, is widely used as a pharmacological model of stress, and its long-term administration mimics the activation of the HPA axis [12].

Glucocorticoids, in particular DEX, are able to modulate the epigenetic state of cells, namely DNA methylation, which is a key mechanism for the long-term regulation of gene expression. Activation of glucocorticoid receptors causes interaction with regulatory regions of the genome and the recruitment of epigenetic modification enzymes, such as DNA methyltransferases, which leads to chromatin remodeling and changes in DNA accessibility to transcription factors. Experimental studies show that administration of DEX can induce both local and genome-wide methylation changes, including differential methylation of CpG sites in genes involved in metabolic and signaling pathways, mostly hypomethylation [13—15].

Methylation of the promoter regions of the *MMP1* and *MMP8* genes is one of the key mechanisms of epigenetic regulation of their expression, which determines the level of transcription and functional activity of these enzymes in cells. For *MMP1*, experimental data show that hypomethylation of the promoter region is associated with a significant increase in the expression of the enzyme, which contributes to the active degradation of collagen types I and III, increased invasiveness, and metastatic potential of tumor cells [16].

In the case of *MMP8*, methylation changes are more differentiated: in some tumor contexts, hypomethylation is observed, which increases the expression of the enzyme, enhancing inflammatory and remodeling processes, while in other cases, hypermethylation occurs, which reduces the antitumor activity of MMP8 and may contribute to the loss of its protective function [9].

To sum up, our study confirmed the effectiveness of using DEX as a relevant pharmacological model of chronic stress for studying epigenetic modifications in tumor tissue. The key result of the work is the identification of a differential effect of stress on the methylation of the *Mmp1* and *Mmp8* genes. The simulated chronic stress contributed to the formation of an aggressive epigenetic profile of the tumor, enhancing the imbalance in the metalloproteinase system.

Funding

This study was supported by the Research Program "Development of Technology for Identifying Stress-Induced Factors of Initiation of Metastatic Bone Lesions" (No. 0125U000655) funded by the National Academy of Sciences of Ukraine.

REFERENCES

1. Smith MD, Wesselbaum D. Global evidence on the prevalence of and risk factors associated with stress. *J Affect Disord*. 2025;374:179-183. https://doi.org/10.1016/j.jad.2025.01.053
2. Piao X, Xie J, Managi S. Continuous worsening of population emotional stress globally: universality and variations. *BMC Public Health*. 2024;24(1):3576. https://doi.org/10.1186/s12889-024-20961-4
3. Yan J, Chen Y, Luo M, et al. Chronic stress in solid tumor development: from mechanisms to interventions. *J Biomed Sci*. 2023;30(1):8. https://doi.org/10.1186/s12929-023-00903-9
4. Lempesis IG, Georgakopoulou VE, Papalexis P, et al. Role of stress in the pathogenesis of cancer (Review). *Int J Oncol*. 2023;63(5):124. https://doi.org/10.3892/ijo.2023.5572
5. Zannas AS. Emerging evidence linking stress and glucocorticoid signaling with cancer phenotypes. *J Transl Med*. 2024;22(1):147. https://doi.org/10.1186/s12967-024-04962-w

6. Das A, Monteiro M, Barai A, et al. MMP proteolytic activity regulates cancer invasiveness by modulating integrins. *Sci Rep*. 2017;7(1):14219. https://doi.org/10.1038/s41598-017-14340-w
7. Liu H, Kato Y, Erzinger SA, et al. The role of MMP-1 in breast cancer growth and metastasis to the brain in a xenograft model. *BMC Cancer*. 2012;12(1):583. https://doi.org/10.1186/1471-2407-12-583
8. Juurikka K, Butler GS, Salo T, et al. The role of MMP8 in cancer: a systematic review. *Int J Mol Sci*. 2019;20(18):4506. https://doi.org/10.3390/ijms20184506
9. He J, Qin M, Chen Y, et al. Epigenetic regulation of matrix metalloproteinases in inflammatory diseases: a narrative review. *Cell Biosci*. 2020;10(1):86. https://doi.org/10.1186/s13578-020-00451-x
10. Genevay S, Finckh A, Mezin F, et al. Influence of cytokine inhibitors on concentration and activity of MMP-1 and MMP-3 in disc herniation. *Arthritis Res Ther*. 2009;11(6):R169. https://doi.org/10.1186/ar2858
11. Daskalakis NP, Xu C, Bader HN, et al. Intergenerational trauma is associated with expression alterations in glucocorticoid- and immune-related genes. *Neuropsychopharmacol*. 2021;46:763-773. https://doi.org/10.1038/s41386-020-00900-8
12. Bassil K, De Nijs L, Rutten BPF, et al. In vitro modeling of glucocorticoid mechanisms in stress-related mental disorders: current challenges and future perspectives. *Front Cell Dev Biol*. 2022;10:1046357. https://doi.org/10.3389/fcell.2022.1046357
13. Li Y, Cai HY, Liu GH, et al. Effects of stress simulated by dexamethasone on jejunal glucose transport in broilers. *Poult Sci*. 2009;88(2):330-337. https://doi.org/10.3382/ps.2008-00257
14. Braun PR, Tanaka-Sahker M, Chan AC, et al. Genome-wide DNA methylation investigation of glucocorticoid exposure within buccal samples. *Psychiatry Clin Neurosci*. 2019;73:323-330. https://doi.org/10.1111/pcn.12835
15. Karlsson L, Barbaro M, Ewing E, et al. Epigenetic alterations associated with early prenatal dexamethasone treatment. *J Endocr Soc*. 2019;3(1):250-263. https://doi.org/10.1210/js.2018-00377
16. Kim HW, Park JE, Baek M, et al. Matrix metalloproteinase-1 (MMP1) upregulation through promoter hypomethylation enhances tamoxifen resistance in breast cancer. *Cancers*. 2022;14(5):1232. https://doi.org/10.3390/cancers14051232

Submitted: March 01, 2026

Т. Борікун [1], *Є. Герасимчук* [2]

[1] Інститут експериментальної патології, онкології та радіобіології
ім. Р.Є. Кавецького Національної академії наук України, Київ, Україна
[2] Навчально-науковий центр «Інститут біології та медицини»
Київського національного університету імені Тараса Шевченка, Київ, Україна

СТРЕС-ІНДУКОВАНІ ЗМІНИ СТАТУСУ МЕТИЛЮВАННЯ ГЕНІВ *Mmp1* ТА *Mmp8* У ПУХЛИННІЙ ТКАНИНІ ЩУРІВ З КАРЦИНОМОЮ ГЕРЕНА

Стан питання. Хронічний стрес є ключовим фактором громадського здоров›я, який суттєво впливає на регуляторні системи та потенційно сприяє канцерогенезу. Хронічний стрес, через активацію гіпоталамо-гіпофізарно-надниркової осі та глюкокортикоїдної сигналізації, може змінювати епігенетичний ландшафт клітин, включаючи метилювання ДНК. **Метою** даного дослідження було оцінити, чи хронічний стрес, викликаний глюкокортикоїдами, модулює метилювання промоторних ділянок генів *Mmp1* та *Mmp8* у пухлинній тканині щурів з карциномою Герена. **Матеріали та методи.** Для моделювання хронічного стресу лабораторним щурам з трансплантованою карциномою Герена вводили дексаметазон підшкірно. Зразки пухлини збирали на 7, 14 та 21 день росту пухлини. Статус метилювання промоторів генів *Mmp1* та *Mmp8* аналізували за допомогою метил-специфічної ПЛР. Рівень метилювання кількісно визначали як співвідношення метильованих до неметильованих продуктів ПЛР, виражене у відсотках. **Результати**. У контрольній групі (карцинома Герена без дексаметазону) промотор *Mmp1* був гіпометильований (38—40% метилювання) протягом усього періоду спостереження. Введення дексаметазону призвело до подальшого незначного зниження метилювання *Mmp1* до 32%, хоча й не статистично значущого. Натомість, промотор *Mmp8* у пухлинній тканині показав базовий рівень метилювання 55—57%. Під впливом дексаметазону гіперметилювання *Mmp8* спостерігалося вже на 7-й день (61%) і значно прогресувало до 14-го (67%) та 21-го (69%) дня. **Висновок.** Наше дослідження демонструє, що хронічний стрес, індукований глюкокортикоїдами, змінює епігенетичний профіль пухлинних клітин, індукуючи диференціальне метилювання генів матриксних металопротеїназ. Зокрема, стрес сприяє підтримці гіпометилювання *MMP1* та значно збільшує гіперметилювання промотора *MMP8*. Ці результати свідчать про те, що хронічний стрес може сприяти агресивному фенотипу пухлини через епігенетичну регуляцію ремоделювання позаклітинного матриксу.

Ключові слова: метилювання ДНК, хронічний стрес, рак.