Healthcare in Low-resource Settings

eISSN: 2281-7824

https://www.pagepressjournals.org/index.php/hls/index

Publisher's Disclaimer. E-publishing ahead of print is increasingly important for the rapid dissemination of science. The ***Early Access*** service lets users access peer-reviewed articles well before print / regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.

These articles are searchable and citable by their DOI (Digital Object Identifier).

The **Healthcare in Low-resource Settings** is, therefore, e-publishing PDF files of an early version of manuscripts that undergone a regular peer review and have been accepted for publication, but have not been through the typesetting, pagination and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear on a regular issue of the journal.

E-publishing of this PDF file has been approved by the authors.

Healthc Low-resour S 2024 [Online ahead of print]

To cite this Article:

Herasymenko O, Gerasymenko K, Herasymenko O. **The amount of DNA and RNA in primary cancer cells and their metastases.** *Healthc Low-resour S* doi: 10.4081/hls.2024.12456

The amount of DNA and RNA in primary cancer cells and their metastases

Oleksandr Herasymenko,[1] Kostyantyn Gerasymenko,[1] Olha Herasymenko[2]

[1]Department of Pathological Anatomy, Forensic Medicine, Histology, Donetsk National Medical University, Kropyvnytskyi; [2]Feofaniya Clinical Hospital, Kyiv, Ukraine

Correspondence: Oleksandr Herasymenko, Department of Pathological Anatomy, Forensic Medicine, Histology, Donetsk National Medical University, Kropyvnytskyi, Ukraine.

E-mail: herasymenkool@ukr.net

Key words: carcinogenesis, advanced molecular genetic analysis, microspectrophotometry, nucleic acids, oncodiagnosis, non-cell autonomous effectors.

Contributions: OH and KG, conceptualization, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review and editing; OH, conceptualization, investigation, methodology, validation, and writing – original draft, review and editing. All the authors have read and approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: None.

Ethics approval and consent to participate: all procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Patient consent for publication: informed consent was obtained from all individuals included in this study.

Availability of data and materials: the data that support the findings of this study are available on request from the corresponding author.

Abstract

Considering the significant impact of oncopathology on global morbidity and mortality, as well as changes in its prevalence and typology, research into the molecular-genetic mechanisms of oncogenesis and metastasis, particularly the role of nucleic acids, becomes crucial. The aim of this study is to determine the content of DNA and RNA in cells of primary cancer and its metastases through microspectrophotometric analysis of malignant cells collected from 30 patients with primary

and metastatic oncological processes. The following methods were employed: microspectrophotometric analysis using the automated complex “Protva-PM11-DVK-3m,” staining of histological specimens with halo cyanin-chrome alum, the discrete statistics method, frequency and cluster analysis, and the bibliographic method. Changes in the concentration of nucleic acids in the cells of primary and metastatic tumors were identified during the study. The changes did not always correlate with each other, indicating morphofunctional polymorphism in tumor cell populations. A trend towards a decrease in nuclear RNA concentration and an increase in cytoplasmic RNA concentration in metastases were observed, suggesting alterations in metabolic and synthetic processes in cells and a decrease in their differentiation degree.

Introduction

Molecular studies play a key role in the development of modern oncology as a clinical discipline, as they allow for the uncovering of fundamental mechanisms of the initiation and progression of cancer at the cellular and molecular levels. This knowledge is crucial for developing new, more effective treatment methods and a personalized approach to each type of oncopathology, significantly increasing the chances of successful treatment and improving the quality of life for patients. RNA, regardless of the state of DNA, can independently influence the synthesis and structure of protein compounds. In the case of oncological diseases, such changes often lead to a decrease in the level of proteins responsible for destroying abnormal cells in tumors. At the same time, an increase in the concentration of proteins that stimulate the division of malignant cells is observed. This issue emphasizes the need to study the interaction between DNA and RNA and their role in the process of oncogenesis.

In their research, Sulaieva *et al.*[1] focused significantly on the perspective of genetic studies in modern healthcare. They examined the role of genetic factors in the diagnosis and treatment of various

diseases, emphasizing the importance of molecular-biological methods in understanding the etiology and pathogenesis of diseases. The authors also emphasized the integration of genetic data into clinical practice, opening new possibilities for developing personalized approaches to treatment and disease prevention. However, while this study covered this important topic, the focus was shifted towards biobanking, leading to insufficient coverage of the molecular mechanisms of oncogenesis. As highlighted in the work of Bondarenko *et al.*[2], recent research has revealed that RNA-binding proteins play a crucial role in the spread of cancer. These proteins, present in all types of cells, exhibit particular activity in malignantly transformed cell populations, where they bind to RNA molecules and contribute to the acceleration of tumor growth. Despite this, none of the existing cancer treatment methods currently target these proteins, underscoring the need for further research in this direction for the development of more effective treatments. The study did not adequately address the issues of oncodiagnosis and oncoprevention.

In their exploration of this topic, Kirkilevskyi *et al.*[3] elucidated the role of RNA in the formation of cancerous tumors and explained the reasons for their insensitivity to therapy. To comprehend the causes of neoplasia and optimize the process of its treatment, patients must undergo a series of investigations. The most effective method turned out to be an extended molecular-genetic analysis of tumors. The application of this method allows for the construction of the most optimal treatment schemes for cancer patients, achieving maximum efficacy with minimal chemotherapy toxicity. Despite the comprehensive data on gastrointestinal oncopathology, this study requires additional clarification regarding tumors in other organs and systems. In their study, Dumanskyi *et al*.[4] identified that the molecular phenotype of a tumor population largely determines its sensitivity to radiation therapy. The researchers emphasized the prospect of molecular-genetic analysis as a leading method for optimizing treatment planning. However, the theme of metastasis and its prevention was insufficiently addressed, requiring further clarification. Kulak[5] discussed the prospect of influencing molecular-genetic mechanisms during the treatment of oncological diseases. The author emphasized the therapeutic modality of mRNA as a target for developing chemotherapeutic agents. Despite the

significant potential in this field, developments within it face considerable challenges, leading to only a few projects reaching the stage of clinical trials. Despite a detailed examination of the application of RNA interference methods, the issue of diagnosis and prevention at the molecular-genetic level was not adequately addressed in this study.

Thus, considering the increase in atypia, polymorphism, and changes in nuclear-cytoplasmic ratio, alongside the significant feature of tumor growth being the alteration of DNA and RNA levels, the goal of the research is to determine DNA and RNA in cells of the primary tumor and its metastases. This will help understand the mechanism of metastasis development and develop measures to prevent metastasis.

Materials and Methods

During the research, the microspectrophotometric analysis method was primarily utilized. This method was employed to determine the levels of DNA and RNA in colonies of primary malignant cells and metastatic tumor nodes. The analysis included 30 cases of primary cancer and 30 cases of metastatic foci. Gallocyanin-chromium gallate staining with BaOH control was used to identify DNA and RNA. This method contributed to determining the total volume of nucleic acids (DNA and RNA). In cases where the samples were treated solely with BaOH, only the amount of DNA was determined. Quantitative analysis of DNA and RNA in histological samples was carried out using the automated complex “Protva-PM11-DVK-3m” and the corresponding software. Scanning was performed with the following parameters: frame size 100×150 μm, probe 0.5 μm, step x=5 μm, and y=15 μm. Monochromatic light with a wavelength of 560 nm was also used. The determination of the amount of DNA and RNA was based on the extinction in the nucleus and cytoplasm.

For every batch of samples, fresh staining solutions were made for the gallocyanin-chromium gallate staining technique, and staining times were closely monitored. To keep an eye on the staining protocol’s consistency, positive and negative control slides were included with every staining run. Before the experimental samples were analyzed, the optical density measurements from the control

slides had to fall within specified acceptable ranges. Using standard solutions containing known concentrations of DNA and RNA, the automated complex “Protva-PM11-DVK-3m” was calibrated before each analysis to guarantee the accuracy and repeatability of the microspectrophotometric analysis. As a result, the nucleic acid levels in the tumor cell samples could be accurately quantified. Furthermore, each sample underwent double measurements in order to evaluate intra-sample variability. In order for these repeated measurements to be incorporated into the data analysis, the coefficient of variation had to be less than 10%.

To determine the amount of RNA in cells of primary tumors and their metastases, the difference in the mean values of the relative optical density of nuclei before and after BaOH treatment was applied. Thus, the difference in the amount of RNA between the cells of the primary tumor and its metastatic nodes was compared. The average amount of DNA in the nuclei of tumor cells of primary cancer and its metastases was estimated using the DNA accumulation index, representing the weighted average arithmetic amount of DNA in units of ploidy per nucleus, determined by comparing with the average DNA content in the nuclei of small lymphocytes in the analyzed sections.

Various scientific methods were employed in this study. Specifically, for the analysis of categorical and numerical data obtained during the study of DNA and RNA levels in tumor cells, the discrete statistics method was applied. This method helped establish relationships between data and determine their statistical significance. For a more detailed study of parameters such as levels of nucleic acids in primary and metastatic tumors, average nuclear diameter, coefficient of variation of the nuclear diameter of tumor cells, and DNA accumulation index, frequency analysis was applied. This methodological approach allowed for a quantitative assessment of the frequency of the occurrence of different indicators. The use of cluster analysis enabled a thorough investigation of the structure of the collected data, the identification of subgroups of samples based on the analysis of the similarity of different characteristics, and the determination of key factors influencing DNA and RNA indicators in tumor cells. To assess global trends in this field and support conclusions, a comprehensive review

of contemporary scientific publications was conducted. Information retrieval was performed using the bibliographic method, analyzing data from bibliometric databases.

Results

The analysis of data reflecting the level of DNA concentration, determined based on extinction, in the nuclei of primary and metastatic tumor cells indicates that in metastases, DNA concentration can be either higher (in 34.5% of cases) or lower (in 55.2% of cases), and in rare cases, even coincide with the indicators of the primary population of tumor cells (10.3%); the presented results are shown in Table 1. Similar findings suggest both polymorphism at the nuclear level and variability in DNA synthesis processes among different populations of tumor cells forming metastases. An effective indicator for assessing cell variability in the process of metastasis is the DNA accumulation index (DNAAI).[6] DNAAI determines the weighted arithmetic mean amount of DNA per nucleus in ploid units. The results shown in Table 2 demonstrate the fact that hetero- and polyploidy in metastases are not uniform. The uneven distribution of molecular-genetic material is also polymorphic in its structure, ranging from slight differences in metastatic cell populations to cases where DNAAI in a metastasis to a lymph node was 6.54, and in the lungs – 25.2.

The uneven distribution of the molecular-genetic material can be attributed to several factors. Firstly, it may reflect the presence of different clones of tumor cells, each with its unique genetic profile and characteristics that influence their ability to metastasize and grow. This variation can be associated with the phenomenon of single nucleotide polymorphism, where point mutations occur on a nucleotide scale, leading to changes in the entire allele and morpho-functional properties of the cell. The risk of this phenomenon is directly proportional to the cell division rate, especially in the study of populations of malignant cells.[7] Secondly, it may be related to different microecological conditions in the metastatic development sites, which can either promote or inhibit the activity of tumor cells.[8] These conditions include factors of the microenvironment such as blood supply, oxygen saturation,

and the presence of inflammatory cells, which can affect the metabolic activity and replicative potential of tumor cells.[9,10]

Microspectrophotometric analysis of the nuclei of tumor cells in metastases and their comparison with each other and with primary tumors revealed that in most cases, metastases have distinct DNAAI, except for one case where the liver metastasis had DNAAI=1.61, and the pancreatic metastasis had DNAAI=1.65. This indicates the clonal nature of metastases, characterized by variable levels of DNA synthesis. Compared to primary tumors, metastases had a higher DNAAI in 55.2% of cases, lower in 41.4%, and equal in 3.4%, as demonstrated in Tables 1 and 2. Metastatic cell populations also showed a tendency to increase nuclear diameter and DNA quantity compared to primary tumors, indicating a prevalence of poorly differentiated, hetero- and polyploid cells. Information on the distribution of nuclear diameter among different cell groups is presented in Table 1. The above suggests a tendency to select less differentiated cells during the process of metastasis than in the primary population of cells. However, in some metastatic nodes, the opposite trend was observed, with signs of increased cell differentiation.

Since metastasis was detected in the liver in most cases, questions have arisen regarding the influence of the target organ on the DNA synthesis process in metastatic cell populations. The analysis showed that 44.4% of liver metastases had a higher DNAAI, 44.4% had a lower DNAAI, and 11.2% had a similar DNAAI to the primary tumor cell population. This indicates the relative autonomy of tumor cells from the target organ, as intracellular synthesis is genetically determined.[11]

Microspectrophotometric analysis to determine RNA levels in the cytoplasm and nucleus of tumor cells demonstrated significant variability in results, with no clear correlation with changes in DNA quantity in cells or among themselves. The results, as shown in Table 2, revealed a tendency to decrease the concentration of nuclear RNA and increase cytoplasmic RNA levels in metastatic cells, accounting for 53.3% and 56.6%, respectively. These results suggest that metastatic cells often exhibit an increase in DNA quantity, which is inversely proportional to RNA levels. In 36.6% of cases, the metastasis process was accompanied by a decrease in nuclear RNA concentration. Simultaneously,

an increase in cytoplasmic RNA was observed in 30% of cases. Thus, the correlation between DNA and RNA levels had some polymorphism depending on the type of RNA. It is also important to note that the correlation coefficient in these cases remained low ($r<0.3$). The concentration of RNA, both in the cytoplasm and in the nucleus (especially in the nucleolus), reflects the functional activity of cells, especially long non-coding RNAs (lncRNAs), which regulate a wide range of intracellular metabolic processes.[12,13]

Based on the analysis of the collected data, it can be stated that establishing a clear relationship between changes in DNA and RNA concentrations in tumor cells is not possible. The absence of a clear interaction may indicate the presence of noticeable polymorphism and significant functional diversity in both primary tumor cell populations and their metastases. When the concentration of one molecule changes, it does not necessarily coincide with a similar change in another, demonstrating the complexity of interactions between different molecular-genetic components of tumor cells. Additional investigation of the Nuclear-Cytoplasmic Index (NCI) in the metastatic cell population and its comparison with NCI in primary tumor cells, as well as with cytoplasmic RNA levels in metastases, revealed that in approximately half of the cases (46.7%), an increase in NCI was observed, indicating a decrease in the level of cell differentiation; these data are highlighted in Table 3. The described increase in NCI is often accompanied by a decrease in the concentration of cytoplasmic RNA in metastatic cell populations, which can be interpreted as a sign of suppression of their synthetic activity. Thus, this can also be considered a marker of a decrease in the level of cell differentiation in metastatic nodes. In general, the obtained data indicate the complexity and multiplicity of pathways through which tumor cells develop and adapt in both primary and metastatic foci. The large variability in the molecular profile and functional activity underscores the need for further detailed study of the mechanisms of oncogenesis and metastasis at the cellular level (Table 4).

According to the obtained data, in 36.7% of cases where the DNAAI increases in metastatic cell populations, there is also a significant decrease in nuclear RNA levels, which may indicate the

dominance of DNA synthesis processes in the nucleus. Concurrently, there is an increase in cytoplasmic RNA levels (30%), indicating the activation of synthetic processes within the cell. Therefore, it can be assumed that in the investigated metastatic nodes, the process of DNA synthesis in some tumor cells occurs through endomitosis, *i.e.*, DNA replication without morphological and functional changes in cells. The lack of a clear inverse correlation between DNA and RNA synthesis can be explained by the fact that in healthy cells, differentiation processes, and DNA synthesis activation are typically regulated by different biological pathways and regulatory mechanisms. This may involve the interaction of various genetic factors, signaling pathways, and molecular regulators that determine when and how a cell enters the process of division or replication of its genetic material.[14,15]

A comparison of the NCI and nuclear RNA concentration in the metastatic population of tumor cells with those in primary tumor cells is demonstrated in Table 4. It is also worth noting that an increase in NCI in metastases is usually accompanied by a decrease in nuclear RNA concentration. This may indicate the complex nature of metastatic processes, where changes in one aspect of cell function do not necessarily reflect or impact other processes to the same extent.

Thus, this section has highlighted the results of a significant number of microspectrophotometric studies on the levels of DNA and RNA in both primary and metastatic tumors. Several important aspects related to the content of nucleic acids in these cell populations have been discussed. It has been found that changes in DNA and RNA concentrations occur in metastases, and these changes do not always correlate with each other. This indicates the presence of distinct polymorphisms and functional heterogeneity in populations of tumor cells.[16] Changes in the concentration of nuclear and cytoplasmic RNA are particularly important, as they may serve as indicators of metabolic and synthetic processes. There is a tendency to decrease nuclear RNA concentration and increase cytoplasmic RNA concentration in metastases, indicating the activation of synthetic processes in cells and a decrease in their degree of differentiation. It has also been observed that alterations in DNA and RNA levels in metastases do not always reflect similar changes in the primary tumor. It is

established that DNA and RNA synthesis processes in tumor cells may occur independently of each other, indicating the presence of different regulatory mechanisms. These findings underscore the complexity of the molecular mechanisms underlying the development and progression of cancer, especially in the context of metastasis.

Discussion

The analysis and interpretation of the obtained data have demonstrated significant diversity in DNA and RNA concentrations in primary and metastatic populations of tumor cells, requiring further clarification regarding the mechanisms regulating the synthesis of these critically important molecular compounds. In this context, particular attention is deserved for understanding the role played by various types of nucleic acids, including Non-Coding RNAs (ncRNAs), in the development and progression of oncological diseases. Investigating the mechanisms through which nucleic acids influence tumor growth and metastasis may shed light on the complexity of oncogenesis and outline new perspectives for developing more effective approaches to oncodiagnosis and treatment.

In their study, Feunteun *et al.*[17] devoted considerable attention to the role of non-cell autonomous effectors in oncogenesis and metastasis. The authors examined various factors and mechanisms that impact the development and progression of cancer, but do not directly originate from tumor cells. These factors and mechanisms interact with malignant cells, originating from the surrounding cellular environment. Thus, the tumor microenvironment includes various types of cells: immune cells, fibroblasts, endothelial cells, extracellular matrix, and molecules secreted by these cells. The microenvironment significantly influences the growth, survival, and metastasis of tumor cell populations. Immune cells in the tumor microenvironment can either inhibit or promote the growth and spread of tumor cells. For example, some types of immune cells may secrete cytokines that support the tumor, while others may attack and destroy malignant cells. Signaling molecules released by micro-environmental cells can activate or suppress specific signaling pathways in tumor cell populations, affecting their division, survival, and migration.

The development of new blood vessels induced by the tumor (angiogenesis) is a key factor supporting tumor growth and metastasis. Endothelial cells and growth factors secreted by them in this process are examples of non-cell autonomous effectors. Tumor cells can alter the metabolism of surrounding healthy cells and tissues to support their growth and survival, representing an example of non-cell autonomous interaction. The overall state of the organism, including hormonal balance, nutrition, and general immune status, can also influence the patterns of tumor cell development. Researchers noted that non-cell autonomous effectors could serve as the basis for developing innovative methods to combat oncological diseases by directly impacting both tumor cells and their interaction with the microenvironment. Therefore, this research complements the current work on the mechanisms regulating tumor growth and metastasis. Nevertheless, it does not allocate sufficient attention to the molecular-genetic mechanisms of tumor cell functioning.

Studies in this field were also conducted by Li *et al.,*[18] focusing on factors stimulating the development and further metastasis of gastrointestinal tumors. In this context, researchers emphasized the leading roles of immune cells, endothelial cells, and fibroblasts. Regarding immune cells, Penetrating Lymphocytes (PLs) play a significant role, participating in both the immune masking of the tumor and its recognition, destruction, and elimination. Macrophages are classified into M1 and M2, with M1 macrophages typically suppressing the development of tumor nodes, while M2 macrophages promote tumor progression by secreting growth factors, including those stimulating angiogenesis and tumor invasion.[19-21] Neutrophils can both promote and inhibit the formation and further development of tumors by limiting the tumor microbiota and thus suppressing the progression and metastasis of tumor cell populations. As for Tumor-Associated Fibroblasts (TAFs), these cells are among the main cellular components of tumor growth, producing chemokines and cytokines that interact with tumor cells, promoting angiogenesis, growth, and tumor metastasis. Angiogenesis is critically important for the progression of oncological pathology. This process is significantly influenced by Tumor-Associated Endothelial Cells (TAECs) that produce growth factors, enhancing angiogenesis and thereby promoting the progression and metastasis of tumors. Scientists have

emphasized that the pathogenesis of cancer involves a complex interaction between tumor cells and their microenvironment. Various components of the microenvironment can both promote the growth and spread of tumor cells and inhibit them, affecting the overall balance between oncogenesis and tumor suppression. While this work complements existing data on the role of intercellular interaction in the development of gastrointestinal cancers, it lacks information on the molecular-genetic mechanisms of these processes.

The impact of macrophages on the growth and metastasis of tumor cells was also explored by Fu *et al*.[22] The researchers emphasized the existence of a distinct fraction of macrophages playing a leading role in tumor development, known as Tumor-Associated Macrophages (TAMs). TAMs create an immunosuppressive environment around tumor cell populations by producing signaling molecules that inhibit anti-tumor cellular and humoral responses. Importantly, the concentration of TAMs increases proportionally with tumor development and is associated with unfavorable clinical outcomes. TAMs also participate in angiogenesis through factors such as Vascular Endothelial Growth Factor A (VEGF-A), Placental Growth Factor (PlGF), Interleukin 1 Beta (IL-1β), Epidermal Growth Factor (EGF), Tumor Necrosis Factor Alpha (TNF-α), Transforming Growth Factor Beta (TGF-β), Interleukin 8 (IL-8), CXCL8, Chemokine Ligand 2 (CCL2), and CXCL12. TAMs play a crucial role in the metastatic process by forming premetastatic niches and stimulating epithelial-mesenchymal transitions, enhancing the invasion of tumor cells. Thus, the provided material complements research on the influence of TAMs on oncogenesis and metastasis.

Regarding the molecular-genetic aspect of metastasis and oncogenesis, significant contributions were made by Abdi and Latifi-Navid,[23] who examined the role of Long Non-Coding RNAs (lncRNAs) and Single Nucleotide Polymorphisms (SNPs) in this context. LncRNAs are sequences of over 200 nucleotides that do not encode proteins but serve various regulatory functions. These RNAs can act as oncogenic factors or tumor suppressors in different types of cancer. They are categorized into pseudogenes, signaling, guiding, and scaffold lncRNAs based on their molecular mechanisms of action. Signaling lncRNAs, crucial in this context, can activate or suppress other transcripts and

exhibit expression specificity depending on the cell type. They can compete with transcription factors and RNA-binding proteins, influencing processes such as cell proliferation, differentiation, angiogenesis, metastasis, and chemotherapy resistance. In terms of the interaction between SNPs and lncRNAs, the latter can affect the function and structure of SNPs, altering their interaction with Messenger RNAs (mRNAs). Researchers emphasized that lncRNAs can serve as targets for chemotherapy and potential targets for oncological preventive interventions.[24] Therefore, this study complements existing knowledge about the molecular-genetic characteristics of tumor processes, although the focus on lncRNAs has led to insufficient coverage of DNA changes during the tumor process.

Expanding on the topic of molecular-genetic determinants of oncological processes, it is worth paying attention to mRNAs. In their study, Mazziotta *et al*.[25] investigated these sequences in the context of signaling pathways related to the growth and differentiation of cell populations. mRNAs are a class of non-coding RNAs that play a crucial role in regulating gene expression. They constitute 1 to 5% of the human genome and influence the regulation of 30 to 60% of protein-coding genes. The impact of mRNAs on signaling pathways is crucial for understanding the mechanisms of differentiation and tumor growth, particularly through fundamental signaling pathways such as Transforming Growth Factor Beta Bone Morphogenetic Protein (TGF-β/BMP) and *Wnt*/β-catenin. In the TGF-β/BMP signaling pathway, mRNAs regulate receptors and ligands of BMP and TGF-β, influencing the development patterns of tumor cells. For example, mRNAs such as mRNA-153 and mRNA-100 selectively affect BMP Type II Receptor (BMPR2), while mRNA-195-5p affects BMP Type IA Receptor (BMPR1A), modulating the growth and development of undifferentiated cell populations. In the TGF-β signaling pathway, mRNAs such as mRNA-10b, which promote cell growth and differentiation, are targeted at SMAD2, while mRNA-221-5p and mRNA-708 inhibit cell growth and differentiation, affecting SMAD3. In the *Wnt*/β-catenin signaling pathway, mRNAs such as mRNA-1297, mRNA-9-5p, mRNA-16-2-3p regulate *Wnt* receptors and ligands, including *Wnt3a* and *Wnt5a*, influencing the development patterns of undifferentiated cells. They can either activate or suppress

the growth of cell populations. mRNAs also affect transcription factors involved in the *Wnt* pathway, such as β-catenin and LEF/TCF. For instance, mRNA-129-5p, mRNA-24, and mRNA-132 influence these factors, modulating cell growth. mRNAs targeting *Wnt* signaling inhibitors, such as GSK-3β and APC, regulate β-catenin levels and influence the development of cell populations. Researchers emphasized that mRNAs are potential targets for therapeutic and preventive interventions in oncology. This work expands the understanding of molecular-genetic mechanisms in oncopathology through the analysis of the role of mRNAs, although other aspects of cell growth regulation were not addressed by the authors.

In summary, significant advancements have been made in expanding existing knowledge of the molecular-genetic mechanisms underlying the development of malignant tumors and their metastasis. The role of non-cell autonomous effectors in oncogenic cell transformation processes, their interactions with each other and the surrounding environment, and their impact on intracellular signaling pathways, gene expression profiles, proliferation, differentiation, and migration of tumor cells have been thoroughly analyzed. Additionally, the study focused on the roles of lncRNAs and mRNAs in oncogenesis, metastasis, differentiation, and cell growth. The obtained results significantly deepen the understanding of tumor biology and open new therapeutic strategies for combating cancer.

Conclusions

Morphometric and microspectrophotometric analysis of primary cancer cells and their metastases from different locations showed wide variability in the content of DNA, nuclear, and cytoplasmic RNA. Pronounced heterogeneity of DNA content in different metastases, as well as inconsistency in changes in the concentration of DNA and RNA in the cells of individual metastases, were established. Since metastases are dominated by cells with a lower level of differentiation and a higher degree of hetero- and polyploidy, and individual metastases often have unique characteristics based on these indicators, it is worth concluding about the selection of highly metastatic clones of cells that are less

differentiated than the bulk of cells of the primary tumor focus. This emphasizes the complexity and dynamics of the process of cancer occurrence, development, and spread, as well as the importance of understanding the molecular and cellular changes that occur during these processes and their potential for developing effective strategies for the diagnosis, treatment, and prevention of oncological pathology.

Thus, an in-depth analysis of metastatic cell populations in comparison with cells of the primary tumor center revealed that in metastases, an increase in the size of cell nuclei, a decrease in DNA concentration, and an increase in DNAAI are more often observed; there is also a decrease in the concentration of RNA in both the cytoplasm and the nucleus. These observations may indicate a tendency towards an increase in the number of less differentiated cell clones in the process of metastasis compared to the primary tumor focus.

Adding to this awareness of the importance of non-cell-autonomous effectors in the process of oncogenesis and metastasis, it is possible to gain a more profound understanding of the interaction between tumor cells and their microenvironment. Thus, immune cells, fibroblasts, endothelial cells, as well as the extracellular matrix and molecules secreted by these cells have, a significant impact on the development and progression of cancer. Understanding these interactions may open up new opportunities for oncology therapy targeting not only tumor cells but also their interactions with the microenvironment. Therefore, the results of this study, which encompass the analysis of DNA and RNA levels in primary and metastatic tumor cells, indicate a complex interplay between different types of nucleic acids, revealing substantial heterogeneity in the molecular mechanisms regulating cancer growth and progression, while highlighting the importance of additional studies in this area, especially considering the role of non-cell autonomous effectors and mRNA in these processes.

It is important to acknowledge several potential limitations. First off, even with a reasonable sample size, the results could not be as broadly applicable as they could be, especially for less common tumor types or subtypes. Furthermore, because the analysis is retrospective in nature, a longer time span was used to gather the samples, which may increase variability over time due to adjustments made to

sample processing or measurement methods. Additionally, the study only used microspectrophotometric analysis of nucleic acid content; it did not use other molecular profiling techniques, such as sequencing or gene expression analysis, which could have given researchers a more thorough understanding of the underlying transcriptomic and genomic changes.

As for the direction of future research, it is important to focus further efforts on expanding the understanding of the role of mRNA in the processes of tumor growth and development. Special attention should be paid to researching the mechanisms of interaction between tumor cells and their microenvironment, in particular, the role of the immune system and metabolic changes in these processes. An important direction is also the search for new biomarkers for early diagnosis of cancer and monitoring the effectiveness of treatment, in particular the use of dncRNA and SNP as potential targets for therapy.

References

1. Sulaieva ON, Artamonova O, Dudin O, et al. Ethical navigation of biobanking establishment in Ukraine: Learning from the experience of developing countries. 2023. Available from: https://jme.bmj.com/content/early/2023/11/09/jme-2023-109129
2. Bondarenko LB, Karatsuba TA, Khavich OO, et al. The effect of ethyl 2-(quinazolin-4-yl-amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate hydrochloride on DNA, RNA, chromatin proteins and contents of non-protein and protein-bounded SH-groups in rats' sarcoma 45 and unchanged skin tissues. Pharmacol Drug Toxicol 2020;14:97-105.
3. Kirkilevskyi SI, Sulaeva OM, Lukyanchuk OV, et al. Immunoscore and personification of chemotherapeutic treatment in patients with gastric cancer. Ukr J Radiol Oncol 2020;28:199-207.

4. Dumanskyi YuV, Kirkilevskyi SI, Sulaeva OM, et al. Impact of tumor molecular phenotype on survival of patients with gastric cancer after radical surgery. Exp Oncol 2022;44:324-6.
5. Kulak MO. Prospects for the development of pharmaceuticals based on the mechanism of RNA interference. Kyiv: National Aviation University; 2020. Available from: https://er.nau.edu.ua/handle/NAU/46746
6. Zhang C, Zhao Y, Xu X, et al. Cancer diagnosis with DNA molecular computation. Nat Nanotechnol 2020;15:709-15.
7. Aragon IM, Cendón Y, Lorente D, et al. Implications of single nucleotide polymorphisms (SNPs) in androgen related-genes in outcome of metastatic castration-resistant prostate cancer (mCRPC) patients treated with abiraterone (Abi) and enzalutamide (Enza). Ann Oncol 2019;30:v346.
8. Schulz M, Salamero-Boix A, Niesel K, et al. Microenvironmental regulation of tumor progression and therapeutic response in brain metastasis. Front Immunol 2019;10:1713.
9. Chulenbayeva L, Ilderbayev O, Taldykbayev Z, et al. Phytocorrection of immunological and biochemical changes in the combined impact of coal dust and high dose of radiation. Georgian Med News 2018;1:141-50.
10. Shen Z, Ma Q, Zhou X, et al. Strategies to improve photodynamic therapy efficacy by relieving the tumor hypoxia environment. NPG Asia Mater 2021;13:39.
11. Sevastre AS, Manea EV, Popescu OS, et al. Intracellular pathways and mechanisms of colored secondary metabolites in cancer therapy. Int J Mol Sci 2022;23:9943.
12. Hussain S, Afzal O, Gupta G, et al. Long non-coding RNAs in lung cancer: unraveling the molecular modulators of MAPK signaling. Pathol Res Pract 2023;249:154738.
13. Dykstra PB, Kaplan M, Smolke CD. Engineering synthetic RNA devices for cell control. Nat Rev Genet 2022;23:215-28.
14. Epum EA, Haber JE. DNA replication: the recombination connection. Trends Cell Biol 2022;32:45-57.

15. Khavinson VK, Popovich IG, Linkova NS, et al. Peptide regulation of gene expression: a systematic review. Molecules 2021;26:7053.
16. Svyatova G, Berezina G, Urazbayeva G, Murtazaliyeva A. Frequencies of diagnostically significant polymorphisms of hereditary breast cancer forms in BRCA1 and BRCA2 genes in the Kazakh population. Asian Pacif J Cancer Prev 2023;24:3899-907.
17. Feunteun J, Ostyn P, Delaloge S. Tumor cell malignancy: a complex trait built through reciprocal interactions between tumors and tissue-body system. iScience 2022;25:104217.
18. Li J, Chen D, Shen M. Tumor microenvironment shapes colorectal cancer progression, metastasis, and treatment responses. Front Med 2022;9:869010.
19. Altayeva AA, Issenova SS, Machtejeviene E, et al. Results of using the Robson classification in Kazakhstan. Open Access Macedonian Journal of Medical Sciences 2021;9:663-9.
20. Pappuswamy M, Chaudhary A, Meyyazhagan A, et al. DNA damage on buccal epithelial cells, personal working in the rubber industry occupationally exposed to carbon disulfide (CS2). Asian Pacif J Cancer Prev 2023;24:357-61.
21. Salimi M, Rastegarpouyani S. E74-like factor 5 promoter methylation in circulating tumor DNA as a potential prognostic marker in breast cancer patients. Asian Pacif J Cancer Prev 2023;24:4035-41.
22. Fu LQ, Du WL, Cai MH, et al. The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. Cell Immunol 2020;353:104119.
23. Abdi E, Latifi-Navid S. Emerging long noncoding RNA polymorphisms as novel predictors of survival in cancer. Pathol Res Pract 2022;239:154165.
24. Hirna HA, Maltsev DV, Natrus LV, et al. Study of the immunomodulating influence of preparation alpha/beta-defensins on chemo/radiotherapy of patients with oral and oropharyngeal cancer. Fiziologich Zhurn 2021;67:86-96.

25. Mazziotta C, Lanzillotti C, Iaquinta MR, et al. MicroRNAs modulate signalling pathways in osteogenic differentiation of mesenchymal stem cells. Int J Mol Sci 2021;22:2362.

Table 1. The amount of DNA in metastases cells compared to that in primary tumor cells.

Indexes	**The amount of DNA in metastases compared to the primary tumor**			**r**
	Bigger	**Smaller**	**Equal**	
The average diameter of the nuclei	48.3	41.4	10.3	0.1
The coefficient of variation of the diameter of tumor cell nuclei	53.3	26.7	20	0.3
DNA concentration	34.5	55.2	10.3	0.3
DNA accumulation index	55.2	41.4	3.4	0.3

Source: created by the authors

Table 2. The amount of RNA in the cells of metastases compared to the cells of the primary tumor, depending on their DNAAI parameters (number of observations/%).

DNAAI	**Bigger**			**Smaller**			**Equal**		
The amount of RNA in metastases compared to the primary tumor	Bigger	Smaller	Equal	Bigger	Smaller	Equal	Bigger	Smaller	Equal
Concentration of RNA in the nucleus of tumor cells	5/6.7	11/6.7	2/16.6	5/16.6	5/3.4	1/3.4	1/3.4	0	0
Concentration of RNA in the cytoplasm of tumor cells	9/30	4/13.3	4/13.3	8/26.6	2/6.7	2/6.7	0	0	1/3.4

Source: created by the authors

Table 3. The amount of RNA in the cytoplasm of tumor cells of metastases compared to the primary tumor, depending on the Nuclear-Cytoplasmic Index (NCI) (abs/%).

Relative quantity	**The value of NCI in metastasis in comparison with the primary tumor**			**In total**
	Bigger	**Smaller**	**Equal**	
Bigger	0/0	7/23.4	1/3.3	8/26.7
Smaller	14/46.7	6/20	1/3.3	21/70
Equal	0/0	0/0	1/3.3	1/3.3
In total	14/46.7	13/43.4	3/9.9	30/100

Source: created by the authors

Table 4. The amount of RNA in the nuclei of tumor cells of metastases in comparison with the primary tumor, depending on the Nuclear-Cytoplasmic Index (NCI) (abs/%).

Relative quantity	**The value of NCI in metastasis in comparison with the primary tumor**			**In total**
	Bigger	**Smaller**	**Equal**	
Bigger	4/13.3	6/20	0/0	10/33.3
Smaller	11/36.7	5/16.7	1/3.3	17/56.7
Equal	2/6.7	1/3.3	0/0	3/10
In total	17/56.7	12/40	1/3.3	30/100

Source: created by the authors