SAINT-PETERSBURG UNIVERSITY

Manuscript

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EXPRESSION OF KISSPEPTINS IN LUNG TUMORS: DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE

Scientific specialty: 3.3.2. Pathological anatomy

Thesis for the degree of Candidate of Medical Sciences

Translation from Russian

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Saint Petersburg - 2023

CONTENTS

INTRODUCTION

A variety of tumors develop in the lung - both benign and malignant.

Benign lung tumors, which include adenoma and related tumors, account for about 10% of all benign and malignant tumors [17].

Approximately 1 in 500 chest x-rays show a solitary pulmonary nodule incidentally, and nearly half of these lung lesions are due to a tumor. Most are malignant neoplasms, most commonly primary lung cancer, followed by metastasis from extrapulmonary primary carcinomas [20]. Thorough diagnosis of single pulmonary nodes, including histological diagnosis, is mandatory for adequate management and treatment of patients with lung lesions.

Despite all recent improvements in treatments, lung carcinoma (cancer) is still the leading cause of morbidity and mortality among malignant diseases worldwide. Lung carcinoma is one of the most common oncological diseases, which is about 95% among lung tumors with poor prognostic outcome, and the highest probable cause of cancer mortality in Russia and around the world [80, 99].

The prognosis in patients with lung carcinoma is poor in most cases and a 5-year survival rate of only 14% makes lung cancer the primary cause of cancer death. Due to the poor prognosis, prevention and early diagnosis of lung cancer are critical to improving patient outcomes. A good knowledge of the epidemiology and etiology of lung tumors is key to preventive measures and to identify individuals at increased risk of developing lung cancer.

In 2018, about 2.1 million new patients with lung cancer were initially diagnosed [53, 99, 104]. Every year, more than 1.6 million people in the world die from lung cancer and its complications [40, 93]. In 2016, the mortality rate from lung cancer in Russia was 19.94‰.

In recent years, knowledge about the diverse biology of tumors has expanded significantly, thanks to the use of immunohistochemical (immunocytochemical) methods (IHC/ICC methods) and molecular biology [19]. These methods have contributed to improving the qualitative and quantitative characterization of heterogeneously differentiated lung tumors (e.g., neuroendocrine/blastomatoid sites, etc.). The heterogeneity of tumors is emphasized by the results obtained with the help of molecular genetic methods [19, 27].

The use of the IHC method to determine the immunophenotype of tumor cells significantly optimizes the differential diagnostic search, makes it possible to identify the pathogenetic mechanisms of tumor progression and molecular targets for the selection of modern and most effective therapy [7].

In recent years, there has been an increase in IHC studies, during which the expression of potential markers of malignancy is determined. Significant markers for the primary diagnosis of lung tumors are: CK7, CK20, thyroid transcription factor TTF-1, chromogranin A, synaptophysin, CD56; surfactant proteins; p63, CK5/6 and kisspeptins [103, 104].

Of interest are kisspeptins (CR), which belong to the group of peptides that occurred during the activation of the KISS-1 gene [5 , 15]. Kisspeptins and their receptor (KISS1R) play an important role in many physiological processes on the part of the reproductive system, vascular system, integral metabolism and others [18].

The KISS1/KISS1R signaling system can serve as a regulator of tumor metastasis and is a potential prognostic marker of tumor processes [14]. Suppression of KISS1 expression is also described in the progression of lung carcinomas and their metastasis. However, so far a small number of works have been devoted to kisspeptins and their role in the pathology of the respiratory system (in particular, lung tumors).

In this regard, the relevance of the chosen research topic is to conduct an in-depth study of the expression of kisspeptins and associated molecules in lung carcinomas to assess the diagnostic and prognostic value of kisspeptins in tumor growth.

The aim of the study.

The aim of the study is to determine the diagnostic and prognostic value of kisspeptins in lung carcinomas of varying degrees of differentiation.

To achieve this goal, the following tasks were set and consistently solved:

1. To establish correlations between the degree of differentiation of lung carcinomas, gender, age of patients and secondary changes in neoplasms;
2. To establish correlations between the level of expression of kisspeptin-1 and its associated signaling molecules – caldesmon and matrix metalloproteinase type 4 (MMP-4) in lung cancer;
3. To identify correlations between the expression of kisspeptin-1, caldesmon and MMP-4 in lung carcinomas and the degree of their differentiation;
4. To determine the relationship between metastasis and expression of kisspeptin-1, caldesmon and MMP-4 in lung tumors;
5. To evaluate the use of kisspeptin-1 as a biomarker for the prediction of lung carcinoma metastasis.

Scientific novelty of study.

For the first time, correlations were established between the degree of differentiation of tumors, gender, age of patients and secondary changes in neoplasms.

Correlations between the level of expression of kisspeptin-1 and its associated signaling molecules – caldesmon and matrix metalloproteinase type 4 (MMP-4) – were determined. Correlations between the expression of kisspeptin-1, caldesmon and MMP-4 in tumors and the degree of their

differentiation were revealed. The relationship between metastasis and expression of kispeptin, caldesmon and MMP-4 in lung tumors has been determined.

The possibility of using kisspeptin-1 as a biomarker for the prediction of metastasis of lung carcinomas has been developed.

The established correlations of the expression of kisspeptin-1 and associated signaling molecules with the degree of differentiation of lung carcinomas allow them to be used as biomarkers for assessing tumor progression.

Practical significance.

The results obtained made it possible to develop an algorithm for using the expression parameters of kisspeptin-1 and associated signaling molecules (caldesmon and MMP-4) in lung carcinomas as markers of the progression of malignant tumors.

Provisions for the defense

1. There are significant correlations between the degree of differentiation of lung carcinomas, gender, age of patients and secondary changes in neoplasms.

2. Correlations have been established between the level of expression of kisspeptin-1 and its associated signaling molecules – caldesmon and MMP-4 in lung carcinomas.

3. Correlations between the expression of kisspeptin-1, caldesmon and MMP-4 in lung carcinomas and the degree of differentiation of tumors were revealed. A monotonous increase in the expression of kispeptin-1 and caldesmon and a monotonous decrease in MMP-4 during the transition from a low to a high degree of differentiation of carcinomas were shown.

4. The relationship between metastasis and expression of kisspeptin, caldesmon and MMP-4 in lung tumors was determined. An inverse relationship was established between the presence of metastases and the expression of kisspeptin-1, caldesmon, as well as a direct relationship between the presence of metastases and the expression of MMP-4.

5. Kisspeptin-1 can be considered as an informative biomarker for the prediction of lung carcinoma metastasis.

6. An algorithm has been developed for using the expression of kisspeptin-1 and associated signaling molecules (caldesmon and MMP-4) in lung carcinomas as markers of the progression of malignant tumors.

The degree of reliability and approbation of the results.

The reliability of the results of the study is determined by the representative sample size, the use of modern methods and the use of adequate methods of statistical processing. The results of the dissertation research have been repeatedly reported at international and Russian scientific conferences.

Based on the materials of the dissertation research, 7 publications were published, including 2 articles in peer-reviewed scientific journals recommended by the Higher Attestation Commission of the Russian Federation and 1 article in a journal included in the SCOPUS and WEB OF SCIENCE databases.

The results of the dissertation research were reported at the VI Russian Congress with International Participation "Molecular Foundations of Clinical Medicine - Possible and Real", St. Petersburg, 2022.

Implementation of the results of the study.

The results of the dissertation were introduced into the scientific, clinical and pedagogical practice of the St. Petersburg Research Institute of Phthisiopulmonology of the Ministry of Health of the Russian Federation;

Anno NRC "St. Petersburg Institute of Bioregulation and Gerontology" and the Department of Pathology of the Medical University of St. Petersburg University.

Place of work and personal contribution of the applicant.

The dissertation work was carried out in 2020-2023 at the scientific and educational base of St. Petersburg University - St. Petersburg Research Institute of Phthisiopulmonology (Department of Translational Biomedicine) within the framework of the State Task No. 056-00013-18-00 "Signaling molecules as biomarkers of socially significant diseases of the respiratory system". The work carried out within the framework of the state assignment was approved by the decision of the Ethics Committee of the Federal State Budgetary Institution "SPb NIIF" No. 93.1 dated 11/23/2022.

The author carried out an analytical review of the literature, collection and systematization of tumor samples, and determined the design of the study. The analysis of microscopic images with automatic calculation of the area of expression of signal molecules was carried out, mathematical and statistical processing of the data obtained was carried out and the interpretation of the results was carried out.

Structure and volume of the dissertation.

The dissertation consists of an introduction, a literature review, two chapters of the results of one's own research, conclusions, conclusions, practical recommendations, and an index of the literature used. The volume of the thesis is 111 pages, includes 15 tables and 19 figures. The index of literature contains 122 sources, of which 23 are domestic sources and 99 are foreign sources.

Chapter 1. LITERATURE REVIEW. ROLE OF KISSPEPTINS AND THEIR ASSOCIATED MOLECULES IN THE DEVELOPMENT OF LUNG

1.1 Lung tumors: definition, etiology, pathogenesis, classification

A distinction is made between benign and malignant lung tumors. Benign lung tumors, which include adenomas and related neoplasms, account for 2% to 12% of all lung neoplasms [17].

Carcinoma or lung cancer (LR) is one of the most common oncological diseases and the highest probable cause of cancer mortality in Russia and around the world [80, 99].

In 2016, in the Russian Federation, in the structure of cancer incidence, RL ranked 3rd (10.1%) among both sexes, 1st among men (17.6%) and 10th among women (3.8%) [9].

There are different risk factors for developing lung cancer, including: genetic, occupational, gender, age, and others. However, tobacco smoking is the main cause of all major histological types of lung cancer [71]. The incidence of RL in smokers is 5-7 times higher than in non-smokers.

The five-year survival rate for lung cancer patients ranges from 4% to 17%. The main factor determining the prognosis of the survival rate of each individual patient is the stage of the disease [10]. Detection of RL at stages I - II contributes to an increase in the number of patients registered for 5 years or more, and a decrease in mortality [9].

Lung cancer, which is characterized by a fairly latent course and early appearance of metastases, is often diagnosed by chance or at a late stage, in most patients only after a medical examination.

Currently, the majority of patients (60-80%) with newly diagnosed RL in Russia are diagnosed with the disease at stage III-IV [13].

RL is a complex disease consisting of various histological and molecular types of clinical significance. The development of large-scale molecular profiling has helped to identify new molecular targets that can be applied to the treatment of specific patients with RL and has helped to change the pathological classification of lung cancer.

New directions include a revolution in immunotherapy, which has opened up prospects for new opportunities in cancer treatment, as well as for revising the classification of multiple tumors, including RL [71].

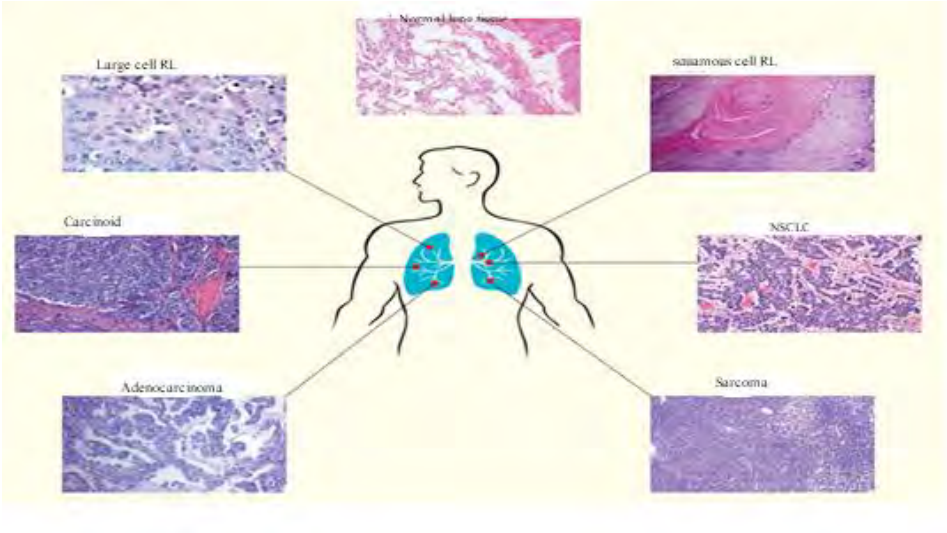


Fig. 1. International Histological Classification lung cancer [5].

However, despite progress in the field of molecular medicine, in 2016 the rate of active detection of RL in the Russian Federation was only 23.3%. These circumstances adversely affect the treatment process and determine the unfavorable prognosis of the course of the tumor process in a large number of patients.

RL is a heterogeneous disease with a wide range of clinical and pathological features [99, 100].

In 2021, the World Health Organization (WHO) Classification of Thoracic Tumors was published. Previous editions of the WHO were

published in 1967 and 1981 exclusively in relation to the lungs, then in 1999 the classification was divided into separately for the lungs and pleura, and in 2004 and 2015 for the lungs, pleura, thymus and heart. The classification has changed mainly due to significant advances in the understanding of genetics and molecular targeted therapy. Thanks to the introduction of immunohistochemistry and molecular testing throughout the classification, many of the more complex approaches to pathological diagnosis have led to more accurate pathological and genetic classification of lung tumors, which has allowed for the development of more effective therapeutic strategies.

The principles of RL classification are still based on the use of morphological criteria first, supplemented by immunohistochemical parameters, and then molecular methods.

In 2015, special attention was paid to the use of immunohistochemistry to improve the accuracy of classification.

In 2021, WHO paid more attention to advances in molecular pathology of all types of tumors. Individual molecular abnormalities are part of the diagnostic criteria for some rarer tumors, such as myxoid lung sarcoma with EWSR1-CREB1 expression, but although many molecular abnormalities do not yet affect the classification of specific tumor subtypes, they can affect patient management tactics.

According to the histological classification (Fig. 1), the following types of RL are distinguished:

1. Small cell carcinoma. A rare species that accounts for about 13% of the RL. It has an unfavorable prognosis with rapid progression and development of metastases.

2. Non-small cell carcinoma (NSCLC). The most common form of RL, it is more than 80 percent and does not develop as rapidly as the small cell form, but it is still believed to have an unfavorable prognosis [99].

NSCLC has a different location, and depending on the type of cells, it is divided into:

- Adenocarcinoma. Adenocarcinoma develops from the glandular epithelium of the lungs, is currently the main histological type, accounting for almost half of all cases. WHO in 2015 adopted a classification developed by the International Association for the Study of RL, the American Thoracic Society and the European Respiratory Society. This new classification of adenocarcinoma incorporates the latest advances in radiology, molecular biology and oncology, providing unambiguous diagnostic criteria and terminology. For resection specimens, new lesions such as in ***situ*** adenocarcinoma and minimally invasive adenocarcinoma were identified to designate adenocarcinomas, mostly non-nemcinous and ≤ 3 cm in size, either with pure scaly growth or with predominant scaly growth with invasion ≤ 5 mm, respectively. For invasive adenocarcinoma, the new classification introduced histological subtyping according to the predominant type of tumor cell growth: lipid (formerly non-nemucinous brochioloalveolar adenocarcinoma), acinar, papillary, micropapillary and solid. It should be noted that the micropapillary pattern is a completely new histological subtype. In addition, four variants of invasive adenocarcinoma are recognized, namely invasive mucinous (formerly mucinous brochioloalveolar adenocarcinoma), colloidal, fetal and intestinal. It has targets for targeted treatment, which is aimed at blocking the synthesis of epidermal growth factor (EGFR). Drugs - tyrosine kinase inhibitors.
- Squamous cell RL. Squamous cell RL is associated with high mortality and lack of treatments specific to the disease. Although repetitive molecular aberrations are present in squamous cell radar, efforts to develop targeted therapies against receptor tyrosine kinases,

signal transduction, and cell cycle checkpoints in squamous cell radar have encountered significant challenges. The current therapeutic landscape focuses on epigenetic therapies to modulate the expression of ancestry-dependent survival pathways and untreatable oncogenes. Another important therapeutic approach is the use of metabolic relationships unique to squamous cell radar. These new therapies can be combined with immune checkpoint inhibitors. For example, the recognition that changes in the KEAP1-NFE2L2 system in squamous cell radar affect antitumor immune responses has created unique opportunities for targeted, metabolic, and immune combinations.

- Large cell RL. Its name is due to the shape and size of the cells. Large cell RL is a rare subtype of malignant lung tumor. It has been reported that the incidence of large cell RL is 0.3-3%. Although large cell RL is classified as NSCLC, it is more aggressive and malignant than other types of NSCLC, and its biological behavior is similar to that of small cell RL. The clinical manifestations of this type of carcinoma are nonspecific. Visualized manifestations of tumors are often localized in the periphery and upper lobes, and enlargement of mediastinal or subcutaneous lymph nodes is common. The diagnosis is mainly based on histological signs and immunohistochemical criteria. Specific neuroendocrine markers such as chromogranin A (CgA), synaptophysin (Syn), and CD56 are usually diffusely positive in large cell RL, and insulinoma-associated protein (INSM1) and high levels of Ki-67 have also been found to be developmental predictors and diagnostic markers. A greater number of differential diagnoses also increases the difficulty of correctly diagnosing large cell radar. The spread of molecular typing of large cell RL in recent years may be useful for diagnosis and subsequent treatment.

- RL of unknown histological origin and unclassifiable cell differentiation accounts for less than 5% of NSCLC cases.

According to modern concepts, cells of epithelial malignant tumors (carcinomas) produce numerous signaling molecules [2,7], the expression of which is a key link in the mechanism of tumor progression and largely determines the prognosis of the development of a malignant neoplasm in each case.

In this regard, the study of molecular phenotypes of malignant tumors can be a promising direction in personalized medicine for choosing the optimal tactics for treating neoplasms and determining their prognosis in a particular patient.

Among the large number of signaling molecules, the production of which is described in normal and tumor lung tissue, of great interest is the family of proteins - kisspeptins and molecules associated with biological effects such as caldesmon and metalloproteinases.

The role of these molecules in the mechanisms of tumor progression has not yet been sufficiently studied.

1.2. The role of kisspeptins in the pathology of the respiratory system and the development of lung cancer

1.2.1 Functional characteristics of kisspeptins

Kisspeptin (KP) is a peptide that plays an important role in the functioning of the hypothalamic-pituitary-gonadal system (HGS). Kisspeptin is produced by two major populations of neurons located in the hypothalamus, the rostral periventricular region of the third ventricle, and the arcuate nucleus. These neurons project to gonadotropin-releasing

hormone (GnRH) neurons, activate them (acting through the kisspeptin receptor, KISS1R) in the hypothalamus and stimulate GnRH secretion.

Gonadal sex steroids stimulate kisspeptin neurons in the third ventricular region but inhibit kisspeptin neurons in the arcuate nucleus, a major positive and negative feedback mechanism, respectively, and it is now generally accepted that kisspeptin neurons in the arcuate nucleus act as a generator of GnRH impulses.

Because of the profound effect of kisspeptin on the HGS axis, the focus of recent research has been on afferent signals to kisspeptin neurons, and one particular area of interest has been energy balance, which is thought to contribute to effects such as fertility suppression in undernourished or, conversely, obese individuals.

On the other hand, evidence is accumulating for a direct role of kisspeptin in the regulation of energy balance and metabolism. Experimental data showed that mice with inhibited KISS1R, showed obesity and reduced energy expenditure. The mechanisms underlying these observations are currently unknown; however, KISS1R is expressed in adipose tissue. Separate studies have focused on the effects of kisspeptin signaling on behavior, as clinical evidence is emerging that kisspeptin affects sexual behavior [5, 15].

Expression of kisspeptins occurs upon activation of the KISS-1 gene. Kisspeptins were originally named metastatins because of their property of blocking the formation of tumor nodules in the lungs of mice that were inoculated with B16BL6 melanoma or Lewis lung carcinoma cells [15].

Kisspeptin, a product of the KiSS-1 gene, and its receptor bound to the G-protein GPR54 (Figure 2) play a major role in puberty and fertility. This 54 amino acid peptide, also known as antimetastatic because of its ability to suppress metastasis, also plays an active role in tumors. The various short peptides, which are grouped together under the name kisspeptins, share

a common C-terminal ligand that binds to KISS1R. The gene encoding KP is located on the long arm of chromosome 1 (1q32) and consists of four exons [26].

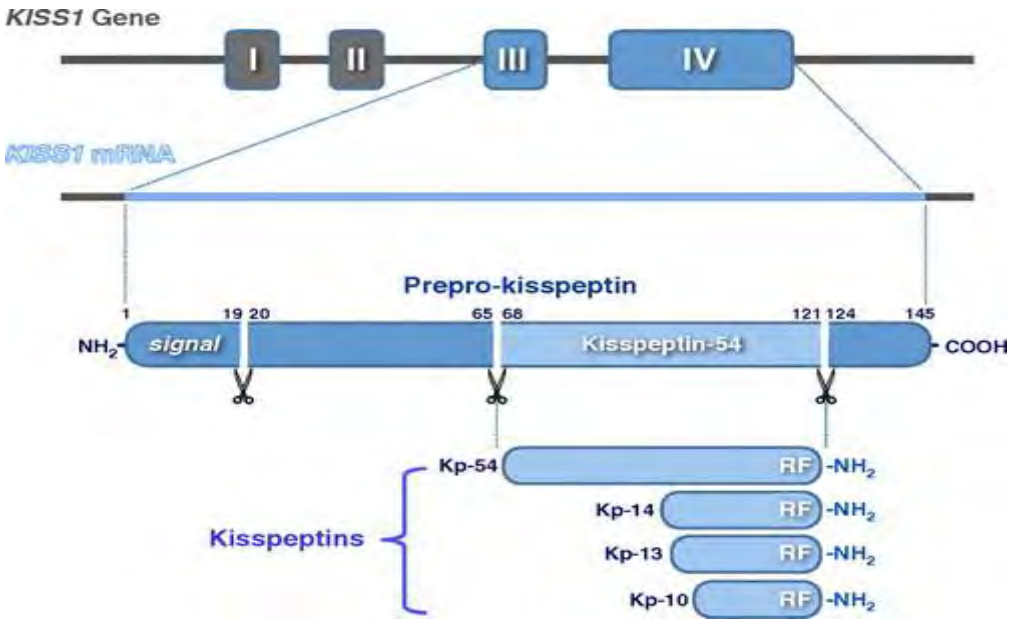


Fig 2. Expression and signaling pathway of kisspeptins [23].

KPs were discovered in the 1990s by Lee et al [68]. KPs are recognized as potent positive regulators of the reproductive neuroendocrine axis in mammals, and the role of KISS1 in suppressing metastasis in melanoma was first reported.

Since then, it has become apparent that KISS1, KP and KISS1R regulate the development and progression of several cancers, but interestingly, while these molecules act as suppressors of oncogenesis and metastasis in many cancers, they function as promoters in breast and liver cancer.

Given their roles, KISS1, KP and KISS1R represent important molecules in the development of new therapies and/or as prognostic markers in cancer treatment. However, a detailed understanding of the relationship between these molecules and different cancers is required to achieve this

goal. A new direction emerging from the reviewed studies is that the relationship between these molecules and a specific type of cancer is complex and depends on many factors such as the microenvironment and steroid receptor status of the cancer cell [68].

KPs include a common arginine-phenylamine at the C-terminus. The basic properties of CPs depend on the amino acid end residues [46].

KPs are neuropeptides localized in the preoptic area and arcuate nucleus of the hypothalamus. Kisspeptin produced in the brain does not enter the systemic bloodstream, but manifests its biological effect at the level of the hypothalamic-pituitary system [48].

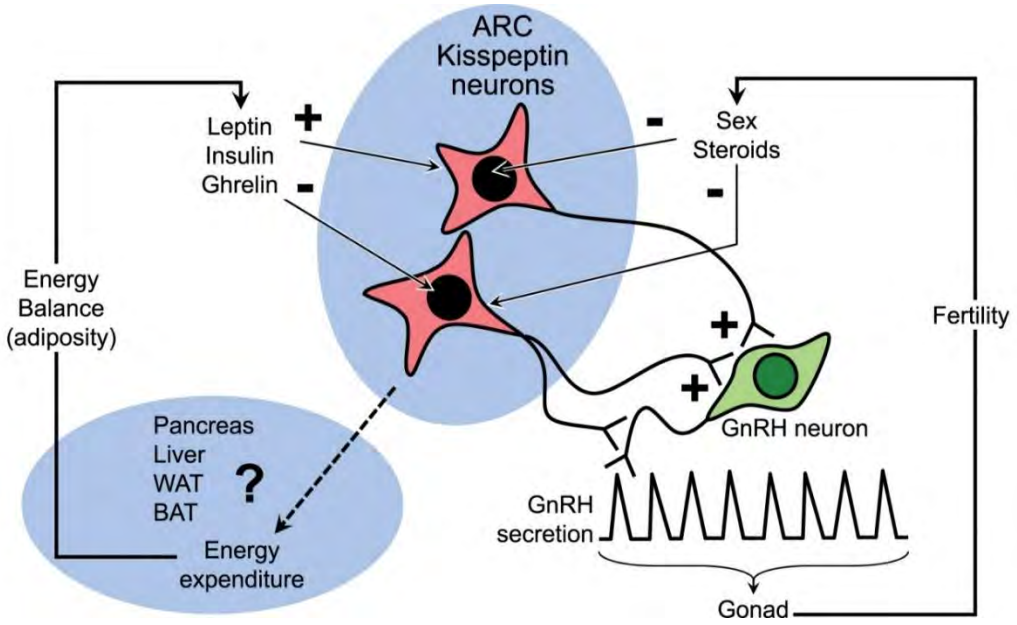


Figure 3. Synthesis and targets of kisspeptins in the human body [26].

The major and most common type of KP is kisspeptin-1 (KP-1).

Kisspeptin is a hypothalamic neuropeptide that acts through the hypothalamus to stimulate hypothalamic GnRH secretion and subsequent gonadotropin release (Figure 3) [16].

In a healthy state, KP-1 induces normal puberty and modulates ovulation in healthy women. Expression of KP-1 in the hypothalamus is reduced in several functional reproductive disorders. Thus, treatment of such conditions with kisspeptin is conceptually attractive.

Recent studies have demonstrated that KP-1 can induce a more physiologic degree of oocyte maturation during in vitro fertilization procedures, which may reduce the risk of potentially life-threatening complications such as ovarian hyperstimulation syndrome observed with human chorionic gonadotropin. In addition, chronic administration of KP-1 has the potential to restore reproductive health in women with hypothalamic amenorrhea, treat hyposexual attraction disorder in otherwise healthy men, and has potential indications in polycystic ovary syndrome, osteoporosis, and fatty liver disease associated with metabolic dysfunction [55]. It is also believed that during pregnancy, it is KP-1 that is the main regulator of gonadotropin-releasing hormone (GnRH) secretion [5, 55].

Thus, KISS1, KP, and KISS1R are actively involved in the functioning of many important organs and systems [59, 90]. Recently, there is evidence that KP may play an additional role in the regulation of glucose homeostasis. Contradictory reports have been published on the effect of KP on insulin secretion in experimental models, which cannot be fully explained by the different isoforms of kisspeptin and the range of kisspeptin doses used in these studies. Studies of glucose metabolism in humans have demonstrated an association between circulating kisspeptin levels and measures of insulin secretion and insulin resistance. Further studies are needed to elucidate the mechanisms underlying the effects of KP on pancreatic β-cells and to determine the therapeutic potential of KP receptor agonist in the treatment of glucose homeostasis disorders [90].

1.2.2. Kisspeptins in neoplastic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a prototypical chronic, progressive and fibrotic inflammatory lung disease. Healthy tissue is replaced by altered extracellular matrix and alveolar architecture is destroyed, leading to decreased lung elasticity, impaired gas exchange, and ultimately respiratory failure and death. In less than a decade, the

understanding of the pathogenesis and treatment of this disease has evolved and two therapeutic tactics have been approved for the treatment of this disease. Current research has contributed to a more detailed understanding of the mechanisms of pulmonary fibrosis, raising the hope that similar approaches will change the management of patients with other progressive fibrotic lung diseases.

The development of ILF can be caused by a history of lung inflammation or a complication from surgery for lung neoplasms.

ILF is a chronic, progressive disease of unknown cause characterized by ongoing scarring of the pulmonary parenchyma, leading to reduced quality of life and earlier mortality. ILF is an age-associated disease, and due to the aging population worldwide, the socioeconomic burden of ILF is expected to steadily increase in the future.

The mechanisms of fibrosis in ILF remain unclear, with prevailing concepts of disease pathogenesis involving repetitive microtrauma to the genetically predisposed alveolar epithelium followed by an aberrant reparative response characterized by excess collagen deposition. The first-line therapeutic tactic is the use of glucocorticoid hormones and cytostatics; however, there are many side effects and no proven efficacy base. There is a growing range of treatment options for ILF, with many profibrotic cytokines and growth factors involved in the pathogenesis of the disease, but these tactics may require a combination of therapeutic strategies with different mechanisms of action [67].

The development, presence and progression of ILF leads to the development of adenocarcinoma, squamous cell or small cell RL in about 15% of patients [60, 121]. Pathogenetically, there are common mechanisms for the development of these diseases, but the etiology is still unclear. The combination of ILF and RL has an extremely unfavorable prognosis [35].

The biological processes underlying ILF are thought to reflect an aberrant reparative response to repeated damage to the alveolar epithelium in a genetically susceptible aging individual, although many questions remain about how to determine susceptibility to this disease [50].

Current public health efforts are focused on early detection of ILF, potentially relying on combinations of biomarkers that include circulating factors, demographic data, and computed imaging data [67].

Existing therapeutic approaches ineffectively target metastatic cancer, often limited by their inability to eliminate non-proliferative, growth arrested, or therapy-resistant tumor cells that have already emerged. The development of effective approaches that target metastatic tumor cells has been the focus of oncologists for decades. However, progress has been limited due to limited understanding of the dormant tumor process. Studies on the status of the dormant tumor process have led to the identification of several regulators. One of them is KPs, namely KISS1, a metastasis suppressor gene that suppresses metastasis by keeping tumor cells dormant at ectopic sites. Its potential application as a therapeutic agent against metastatic cancer by killing resting cells or inducing prolonged remission in tumor cells has been discussed [31].

All native functional human CPs (kisspeptin-54, kisspeptin-14 and kisspeptin-13) contain 10 amino acids of kisspeptin-10 at their C-terminus. However, they are rapidly inactivated by matrix metalloproteinases (MMPs) by cleavage of the peptide bond between glycine and leucine, which limits their clinical use. MMP-resistant kisspeptin analogs are under development, which may provide better therapeutic outcomes with respect to fibrosis, inflammation, and cancer progression [92].

Considering the above, Lei et al. conducted a series of experiments to investigate whether kisspeptin-13 (KP-13) has antifibrotic properties.

It was experimentally shown that KP-13 can reduce inflammation and has antifibrotic properties in mice with pulmonary fibrosis caused by bleomycin.

Bleomycins are often used in cancer therapy, show good results and cause minor bone marrow suppression, but the main side effect is pulmonary toxicity. The mechanisms of cellular toxicity are well described based on ***in vitro*** experiments with DNA. The bleomycin molecule consists of two major structural components: a bithiazole component that is partially embedded in the DNA helix, separating the strands, and pyrimidine and imidazole structures.

Bleomycin is capable of causing cell damage independent of its effects on DNA by induction of lipid peroxidation. It is this property that may explain the negative inflammatory effects of bleomycin on the lungs. The lung damage observed after bleomycin administration involves interstitial edema with influx of inflammatory and immune cells. This can lead to the development of pulmonary fibrosis characterized by increased production and deposition of collagen and other matrix components. Several polypeptide mediators that can stimulate fibroblast replication or excess collagen deposition are involved, but the exact role of these mediators in the development of bleomycin-induced fibrosis remains to be investigated. Current therapy for bleomycin-induced lung injury is inadequate, with corticosteroids being used most commonly. Given the mechanism of action described above, antioxidants and iron chelators may be therapeutic endpoints to prevent the development of complications of bleomycin use. Although studies to date are equivocal and there is insufficient evidence for their clinical use. New drugs are being developed and it is hoped that they may prove more useful. One of these is KP-13, which has been experimentally proven to be an antifibrotic drug. Also in this study, it was shown that when KP-13 was inhibited, pulmonary fibrosis developed after

the use of bleomycin [69]. Therefore, the use of KP-13 as an antifibrotic agent has great prospects in the therapy of pulmonary fibrosis.

1.2.3. Kisspeptins in lung cancer

Lung cancer is the leading cause of cancer deaths worldwide. There are various methods of diagnosing the disease, but often RL is not detected until late stages. Treatment is determined by the subtype and stage of the cancer. There are several personalized treatments that did not exist just a few years ago. However, despite advances in radiation and chemotherapy, RL is still the leading cause of mortality. Therefore, the search for new treatment modalities is still relevant.

As mentioned above, kisspetins are involved in the regulation of collagenase activity, which degrades the extracellular matrix, which is important for metastasis. The expression and function of KISS1 are tissue specific [84, 117].

Ikeguchi et al. reported that the expression of KISS1 in esophageal cancer with lymph node metastasis was significantly lower than that without metastasis, suggesting that the absence of KISS1 was closely related to lymph node metastasis in esophageal cancer [56].

Ringel et al. reached a similar conclusion in a study of thyroid cancer [87]. On the contrary, KISS1 expression is elevated in breast cancer patients with aggressive tumors and high mortality [72].

Metalloproteinase-9 (MMP-9) has been found to have an angiogenic effect and can enhance the progression of tumorigenesis [120]. The role of kisspeptin in these processes has not been fully elucidated. Scientists led by Zheng studied the expression of MMP-9 and KISS1 in 85 cases of NSCLC and lymph node metastases to determine the relationship between the level of their expression and metastasis, as well as patient survival.

The study included patients from 41 to 80 years of age, with a mean age of 57 years at the time of surgery. The group was divided by gender as follows: 68 men and 17 women. Metastases to lymph nodes occurred in 66%. On pathomorphologic analysis, it was found that 60 cases were classified as squamous cell cancer, 22 as adenocarcinoma, and the remaining 3 cases as glandular squamous cell carcinoma. In terms of histologic grade, 7 cases were well differentiated, 47 cases were moderately differentiated and 31 cases were poorly differentiated. We studied 26 cases of stage I disease, 40 cases of stage II disease, 17 cases of stage III disease and 2 cases of stage IV disease.

When KISS1 mRNA and protein expression were analyzed, no statistically significant correlations were found with patient age or gender, tumor size, tumor subtype or histological grade ($p>0.05$).

Maximum expression of KISS1 was recorded at the beginning of the process, whereas it decreased at later stages of tumor progression. The synthesis of KISS1 was also influenced by the presence of metastasis process. Thus, in the absence of this process - KISS1 synthesis was higher than in the presence of tumor. The initial stage of tumor process was also characterized by high synthesis of KISS 1. On the contrary, the expression of MMP-9 was higher in NSCLC with metastasis, and increased expression was also observed in the progression of the disease compared to the initial stages (I-II).

The 5-year survival rate was highest (20.9%) in patients with initial stages (I-II) of NSCLC with high KISS1 expression, whereas the 5-year survival rate was only 2.4% in patients with later tumor stages (III-IV) and reduced KISS1 expression. The difference in 5-year survival between the two groups was statistically significant ($p <0.01$).

These data reflect the relevance of studying KISS1 and MMP-9 as possible molecular factors to determine the stage of tumorigenesis, as well

as the presence of metastatic process and progression, which is crucial to assess prognosis in NSCLC. The exact mechanism of suppression of tumor metastasis by kisspeptin has not yet been elucidated.

Loss of heterozygosity (LOH) in the region of chromosome 6q16.3-q23 was significantly correlated with suppression of KISS1 expression. Interestingly, loss of heterozygosity in chromosome 6q16.3 has been identified in some cases of non-small cell lung cancer, suggesting a similar role of KISS1 in this pathologic process [95].

In studies on tumors of other localizations, similar results were obtained, such that Dhar et al. found that gastric tumors with low KISS1 expression often had metastasis and tumor recurrence [39]. In addition, patients with tumors with low KISS1 expression were found to have significantly worse survival. In another study, high KISS1 expression was found to be an independent prognostic marker of recurrence-free and overall survival in patients with hepatocellular carcinoma [61]. Thus, kisspeptin can be considered as a possible prognostic marker for NSCLC.

A number of signaling molecules expressed in lung tissue are cascaded to the production of kisspeptins. These are primarily caldesmon and matrix metalloproteinase type 4 (MMP-4). Therefore, it is important to study their relationship with kisspeptin expression in the lung during malignant growth.

1.3. Caldesmon: general characteristics and role in oncogenesis

1.3.1. General characteristics

A thermostable multidomain 6eloq, which has a relative molecular mass of about 120-150 kDa, is found in smooth muscle, internal organs, and nonmuscle cells of many vertebrates [8, 24, 74]. Caldesmon, an actin-

binding protein, provides inhibition of myosin binding to actin and regulates contraction and relaxation of smooth muscle [107].

The caldesmon molecule is conventionally composed of three parts: the C-terminal part, which modulates almost all known functional properties of this molecule, such as actin and calmodulin binding, inhibition of actomyosin-ATPase activity and phosphorylation sites; the N-terminal part, which binds myosin and weakly interacts with actin and calmodulin [24, 65].

The middle part, known as the spacer, contains a highly charged repetitive sequence (Figure 4) and has no apparent binding properties. This middle part is only present in the smooth muscle form commonly referred to as high molecular weight caldesmon (H-caldesmon) [24, 113].

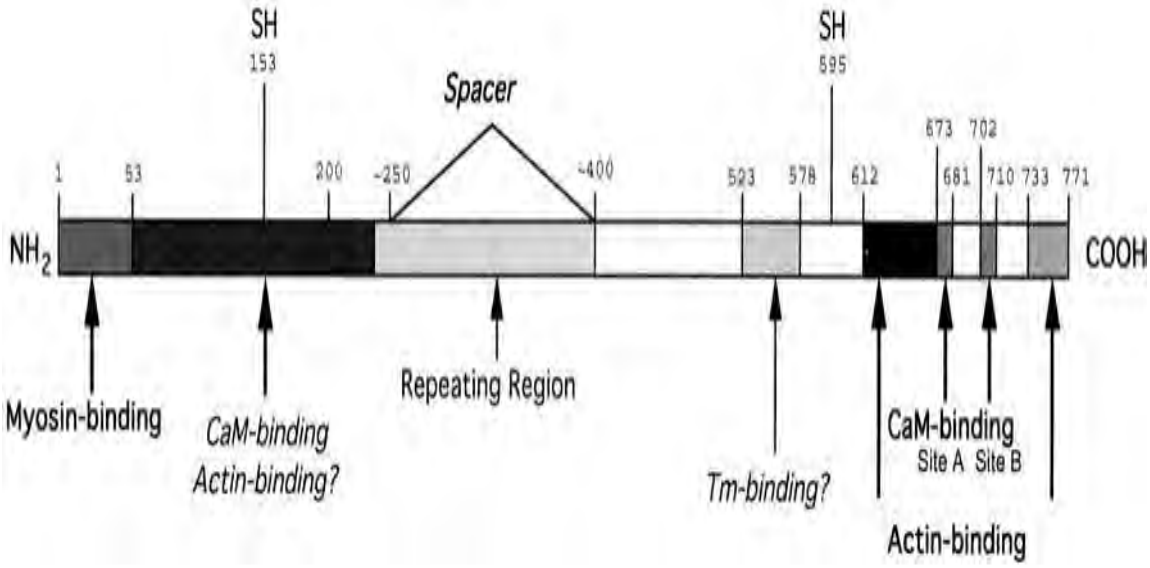


Figure 4. Molecular structure of caldesmon.

In visceral cells, the shorter version of caldesmon is expressed from a single gene by alternative splicing with deletion of the middle part, resulting in an isoform with a smaller molecular mass (L-caldesmon) [119]. The above two isoforms have virtually identical functional domains and are closely associated with their respective specific cell types. They are often used as markers of smooth muscle cell dedifferentiation during proliferation [24].

The caldesmon gene is located on human chromosome 7q33 and has 17 exons, and its isoforms (H-caldesmon and L-caldesmon) are mainly produced by alternative splicing of exons 7 and 8 [108] (Figure 3). Selective translation exon 7 encodes an average repetitive sequence that is specific for H-caldesmon (transcript 201,793 aa) [78].

The caldesmon gene has 24 transcripts (201-224). Transcript 201 can generate H-caldesmon, whereas transcripts 202-206 and 222 can generate L-caldesmon. L-caldesmon is further categorized into Fibro-type (WI-38) and HeLa-type depending on the promoters. Alternative splicing of the caldesmon gene determines the different structures and expression of isoforms [49, 107].

H-caldesmon is considered to be a specific biomarker that not only differentiates between leiomyoma and endometrial stromal sarcoma but also has diagnostic value in other tumors [91, 118]. In contrast, L-caldesmon is widely distributed in non-muscle tissues such as brain, spleen and lymph nodes [64].

Changes in the expression of the two isoforms correlate closely with phenotypic modulation of smooth muscle cells [110]. Caldesmon expression can switch from L-caldesmon to H-caldesmon or proceed in the reverse pathway from H-caldesmon to L-caldesmon during smooth muscle cell differentiation [101]. Consequently, different expression distributions determine different functions of H-caldesmon and L-caldesmon (Figure 5).

H-caldesmon and L-caldesmon also differ in their localization in cells and in tissues. H-caldesmon is expressed in vascular smooth muscle and internal organs and is not found in rhabdomyosarcoma, myofibroblasts, or neoplasms originating from myofibroblasts [42, 91].

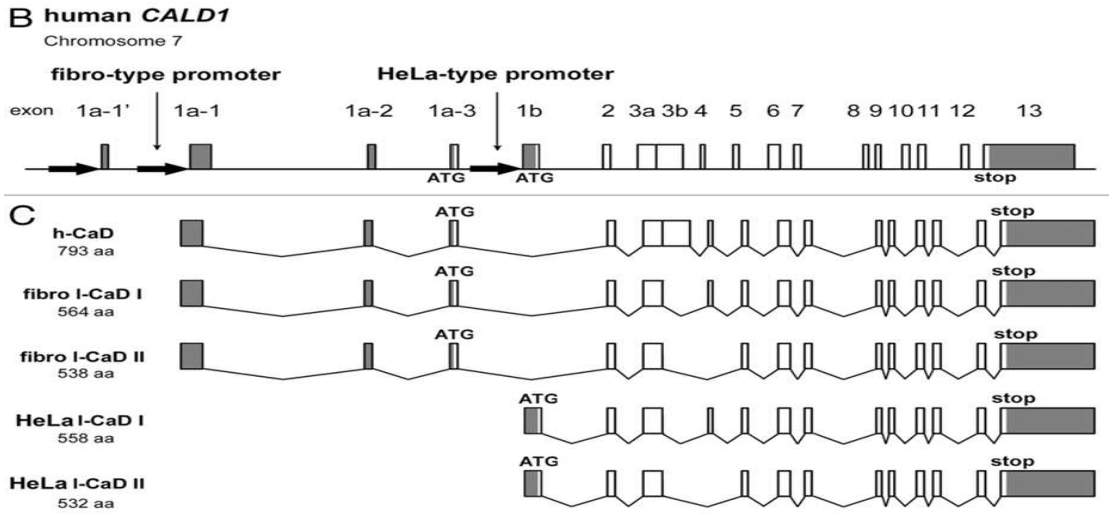


Figure 5. H-caldesmon and L-caldesmon

Caldesmon is a multifunctional 6e ndesmon that regulates smooth muscle contraction and various forms of nonmuscular motility, such as secretion, adhesion, migration, and cell division [2]. Two classes of isoforms obtained by alternative splicing of a single gene have been characterized.

Smooth muscle caldesmon isoforms with high molecular mass (89-93 kDa) are found exclusively in adult and fully differentiated smooth muscle cells.

Non-muscular caldesmon isoforms with low molecular mass (59-63 kDa) are found in non-muscle cells and in undifferentiated smooth muscle cells. The conserved regions of all isoforms contain caldesmon properties such as binding to actin, tropomyosin, Ca(2+)-calmodulin, myosin, and phospholipids. All isoforms are also very potent inhibitors of the actin-tropomyosin-activated myosin MgATPase.

Non-muscular and smooth muscle isoforms of caldesmon perform different functions in vivo. This can be reflected by the distinct cellular distribution of these classes of isoforms. Non-muscular caldesmon is a regulatory factor in the microfilament network and is thus involved in the assembly and stabilization of microfibers. Smooth muscle caldesmon

together with tropomyosin mediates the Ca(2+)-dependent inhibition of smooth muscle contraction [3, 24, 47].

Caldesmon is one of the few actin-binding proteins that is associated with podosomes but is excluded from focal adhesions. Caldesmon also inhibits the function of gelsolin and the Arp2/3 complex, which are essential for podosome formation [11].

The mechanism for reversing the putative inhibition of smooth muscle contraction by caldesmon depends on Ca2+/calmodulin and phosphorylation [97]. However, these processes are also dependent on Ca2+ concentration [96]. The action becomes possible when the concentration of free Ca2+ is less than 1 μM [96, 107]. The phosphorylation process also affects the activity of caldesmon [11].

Caldesmon is a substrate of cdc2 and Erk1/2 MAPK kinase, and phosphorylation of either of these kinases terminates the inhibitory effects of caldesmon. Cdc2-mediated phosphorylation of caldesmon and the resulting dissociation of caldesmon from actin filaments are required for M-phase progression during mitosis. Cells overexpressing the actin-binding carboxyterminal fragment of caldesmon cannot completely release this fragment from actin filaments during mitosis, resulting in a higher frequency of multinucleated cells. PKC-mediated phosphorylation of MEK/Erk/caldesmon is an important signaling cascade in the regulation of smooth muscle contraction. In addition, PKC activation in cultured vascular smooth muscle cells has been shown to remodel actin stress fibers into columns of podosomes enriched with F-actin [37, 115].

Podosomes are adhesive structures of the cytoskeleton associated with the release of metalloproteases and degradation of the extracellular matrix during cell invasion. Interestingly, caldesmon is one of the few actin-binding proteins that is associated with podosomes but is excluded from focal adhesions. Caldesmon also inhibits the function of gelsolin and the

Arp2/3 complex, which are essential for podosome formation [51]. Thus, caldesmon appears to be well positioned to play a modulatory role in podosome formation [75].

Determining the role of proteins that stabilize actin filaments, such as caldesmon and tropomyosin, in podosome formation should provide a more complete understanding of the molecular systems that regulate actin cytoskeleton remodeling during cell transformation and invasion [57, 73, 106].

1.3.2. The role of caldesmon in the formation of tumors and metastasis

Originally isolated from smooth muscle tissue, where it is the most abundant calmodulin-binding protein, caldesmon protein is widely distributed in actin- and myosin-containing cells, where it is localized in subcellular structures associated with motility, shape change, and exo- or endocytosis. Caldesmon is thought to be an actin-regulatory protein and binds with high affinity to actin or actin-tropomyosin. Caldesmon inhibits actin-tropomyosin activation of myosin-MgATPase activity, and the inhibition can be reversed by Ca2+calmodulin. The binding of caldesmon to smooth muscle proteins has been studied in detail, allowing the construction of a model that could explain the observed Ca2+ regulation of thin smooth muscle fibers. The abundance of caldesmon and Ca2+ regulation of its activity by calmodulin mean that it is potentially an important intracellular regulator of processes such as smooth muscle contraction, cell motility and secretion. However, many of its properties are still unexplored.

De Marchi et al. investigated the correlations between caldesmon and annexin-A1 in tamoxifen-resistant recurrent estrogen receptor (ER)-positive breast cancer [38]. Resistance to tamoxifen therapy is a major cause of death

in patients with recurrent estrogen receptor-positive recurrent breast cancer [22] [25].

Using high-resolution mass spectrometry, researchers created a prognostic signature with 4 proteins for the outcome of tamoxifen therapy in recurrent breast cancer. Annexin-A1 and caldesmon were the most differentially expressed proteins.

The clinical relevance of these markers was first assessed in a computer database, followed by immunohistochemical (IHC) staining of an independent set of tumors included in a tissue microarray (TMA) and regression analysis with respect to time to progression, clinical benefit, and objective response. The authors found that annexin-A1 and caldesmon proteins are independent markers of the outcome of tamoxifen therapy and are associated with rapid tumor progression [38].

Based on the fact that caldesmon inhibits myosin-ATPase activity and regulates cell motility, Schwappacher et al. investigated the effect of ZPKIβ and caldesmon on cell migration and invasion. The authors showed that inhibition of the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway (NO- cGMP - PKG) decreased the migration and invasion of human breast cancer cells, whereas activation of PKG increased their motility and invasion [94].

Small interfering RNAs mediated by knockdown of endogenous caldesmon exert pro-migratory and pro-invasive effects on human breast cancer cells. Restoration of wild-type caldesmon cells slowed migration and invasion; however, caldesmon containing the S12E phosphomimetic mutation failed to restore the pro-migratory and pro-invasive activity of caldesmon depletion.

Data from Schwappacher et al. indicated that via the caldesmon phosphorylation pathway, protein kinase Iβ (ZPKIβ) enhances the motility and invasive ability of breast cancer tumor cells [94].

According to the results of Kyung-Hee Kim et al, the expression level of L-caldesmon was significantly higher in tissues from patients with primary colorectal cancer and liver metastases compared to the level in the corresponding normal tissues [62]. L-caldesmon expression was increased in cancer tissues obtained from patients with poor response to chemoradiation therapy compared to that in the good response group [62].

Kokate et al. showed that caldesmon is an important component of actomyosin bundles in smooth muscle and non-muscle cells, which functions as a dynamic cross-linker between myosin-II and tropomyosin-actin filaments. Caldesmon depletion leads to aberrant lateral movement of myosin II filaments along actin bundles, resulting in an uneven distribution of myosin within stress fibers. This manifests as defects in stress fiber network organization and contractility and is accompanied by problems in cellular morphogenesis, migration, invasion, and mechanosensory perception. These results identify caldesmon as a critical factor that ensures the regular arrangement of myosin-II in actomyosin bundles of nonmuscle cells [65].

Caldesmon, as well as its two isoforms, are considered cancer biomarkers and a potent suppressor of cancer cell invasion by regulating podosome/invadopodia formation.

The invasion process is a hallmark of cancer progression, in which podosomes and invadopodia are partially involved [28]. The latter are highly dynamic cell adhesion structures that degrade the extracellular matrix by secreting matrix metalloproteases.

Consequently, podosomes and invadopodia allow cells to penetrate the basal membrane and migrate into tissues [28]. These adhesive structures are called "podosomes" in cells originating from monocytes, osteoclasts, smooth muscle cells and cells transformed by Rouse sarcoma virus, and "invadopodia" in cancer cells.

Because podosomes and invadopodia share common morphologic characteristics, functions, and molecular composition, they are considered related structures with different cellular contexts.

The above evidence suggests that actin and related proteins are important for the formation and dynamics of podosomes/invadopodia [111]. Recently, the actin-binding protein caldesmon has been shown to play a key role in the regulation of podosome formation and dynamics in smooth muscle cells and Rouse sarcoma virus-transformed fibroblasts [112].

T. Yoshio et al. demonstrated that caldesmon negatively regulates podosome formation in Rouse sarcoma virus-induced transformed fibroblasts by competing with the Arp2/3 complex, and that phosphorylation of caldesmon by p21-activated kinase 1/2 additionally enhances the effect of caldesmon. Moreover, researchers have also demonstrated that caldesmon acts as a potent repressor of cancer cell invasion [111].

Thus, the authors suggest that decreased caldesmon expression promotes:

- Podosome/invadopodia formation and cancer cell invasion;

- Caldesmon is a potent repressor of podosome/invadopodia formation;

- Caldesmon overexpression suppresses podosome formation, MMP degradation and cancer cell invasion.

Increased expression of caldesmon is commonly observed in various cancers. However, the significance of reduced caldesmon expression in cancer progression is unclear. Reduced expression of caldesmon is found in blood vessels of malignant melanomas compared to both benign melanocytic tumors and normal tissues [107].

1.3.3. Caldesmon and vascular invasion of tumors

The presence of vascular invasion is considered an indicator of poor prognosis in many malignant tumors [41]. Vascular smooth muscle contains both H-caldesmon (>75%) and L-caldesmon (<25%) [45]. H-caldesmon is the most specific and sensitive marker for endotheliocyte verification [45, 82].

The structural integrity and functional maturity of blood vessels are determined by the presence of normally functioning endothelial cells, as well as the involvement of interendothelial junctions and wall cells (smooth muscle cells or pericytes) [119]. Caldesmon knockdown caused severe defects in vasculogenesis and angiogenesis in morphant Danio fishes, while vascular integrity and blood flow were simultaneously impaired [119].

The expression level of H-caldesmon in melanoma blood vessels showed a negative correlation with the incidence of metastasis [63]. Endothelial cells of blood vessels in melanoma foci were found to be fragile compared to normal tissues by electron microscopy [63]. It should be noted that the fragility of blood vessels may enhance metastasis.

In a study by Hou Q et al. [54] identified biomarkers of gastric cancer metastasis using a quantitative proteomic approach. Proteins isolated from a panel of 4 gastric cancer cell lines, 2 derived from primary cancer (AGS, FU97) and 2 from metastasis to lymph nodes (AZ521, MKN7), were labeled with iTRAQ reagents (8-plex) and analyzed by 2D LC-MALDI-TOF/TOF MS analysis.

A total of 641 proteins were identified with at least 95% confidence. Using threshold values of >1.5 and <0.67, 19 proteins were found to be activated and 34 were suppressed in metastatic and primary gastric cancer cell lines, respectively. Some of these dysregulated proteins, including caldesmon, were identified by western blotting.

Caldesmon expression was found to be downregulated in 2 cell lines derived from metastases, and this was confirmed by further analysis of 7 gastric cancer cell lines. In addition, immunohistochemical staining of 9 pairs of primary gastric cancer and corresponding lymph node metastasis tissue also confirmed this observation [16].

Finally, siRNA knockdown of caldesmon in AGS and FU97 gastric cancer cells resulted in increased cell migration and invasion, while overexpression of caldesmon in AZ521 cells resulted in decreased cell migration and invasion.

Thus, this study established a potential role for caldesmon in gastric cancer metastasis. Further functional studies are currently underway to determine the underlying mechanism of action of this protein [54]. Thus, caldesmon can be considered as a promising therapeutic oncomic target.

1.3.4. Caldesmon as a possible tumor biomarker

Recently, caldesmon has attracted the attention of oncologists as a potential oncobiomarker. Caldesmon has been described to be of high value in the diagnosis of myoma [88, 107], as H-caldesmon is a highly sensitive and specific marker of smooth muscle differentiation and mesenchymal cell nature in uterine sarcoma [32, 79, 107].

H-caldesmon expression may be useful in differentiating endometrial stromal tumors from smooth muscle neoplasms. However, it should be considered that in some cases H-caldesmon is expressed in some nonmyogenic tumors, such as gastrointestinal stromal tumors, malignant pleural mesothelioma, and adult ovarian granulosa cell tumors [113].

It can be concluded that the presence of H-caldesmon is not a proven marker and the diagnosis should be supplemented with other markers to clarify the diagnosis [114]. In addition, L-caldesmon is also considered a potential serum marker of glioma [119].

Despite the controversial results, different caldesmon isoforms may be promising biomarkers for the diagnosis and prognosis of various mesenchymal tumors [107].

1.3.5. Caldesmon and lung tumor

The discovery of molecular biomarkers for early diagnosis and treatment of lung cancer remains a challenge.

Using antibodies to H-caldesmon, Comin, C. E. et al. examined 70 cases of epithelial mesotheliomas and 70 cases of lung adenocarcinomas. In addition, immunohistochemistry for muscle markers such as desmin, alpha smooth muscle actin, muscle-specific actin, myoglobin, myogenin, myosin, and MyoD-1 was performed in all mesothelioma cases. Reactivity to H-caldesmon was obtained in 68 (97%) of 70 epithelial mesotheliomas but in none of the adenocarcinoma cases.

All mesothelioma cases were negative for the other muscle markers tested. The authors concluded that H-caldesmon is a highly sensitive and specific marker and suggested its inclusion in the immunohistochemical panel for differential diagnosis of epithelioid mesothelioma and lung adenocarcinoma [34].

In their work, Zhang et al. tried to find out the relationships between the expression of inducible nitric oxide synthase (iNOS), caldesmon, matrix remodeling protein-osteopontin (OPN) and clinical parameters, especially lung cancer metastasis. They found that the expression of iNOS, caldesmon and OPN were closely related to lung cancer metastasis.

Intracranial metastatic lung cancer tissue samples showed significantly higher expression of iNOS, caldesmon and OPN. Flow cytometry analysis of peripheral blood of lung cancer patients showed an increased number of EPCAM+/OPN+ cells in the bloodstream of patients

with bone metastases compared to patients without metastases, indicating circulating cancer cells.

Serum OPN concentration was also positively associated with lung cancer metastasis to bone. These results suggested that iNOS, OPN, and caldesmon may be considered as biomarkers of lung cancer metastasis [16] [116].

1.4. Matrix metalloproteinases

The family of human matrix metalloproteinases (MMP) includes several tightly regulated classes of proteases. These enzymes and their specific inhibitors play an important role in tumor progression and metastatic process by promoting degradation of the extracellular matrix. As scientific understanding of MMP has increased, therapeutic strategies aimed at blocking these enzymes with MMP inhibitors (MMPI) have rapidly evolved [76].

1.4.1. Structure and functions of matrix metalloproteinases

Matrix metalloproteinases (MMP) are a family of zinc-dependent endopeptidases that are involved in the degradation of various proteins in the extracellular matrix. As a rule, MMP have a propeptide sequence, a zinc-catalyzed metalloproteinase domain, a hinge region or linker peptide, and a hemopexin domain [10].

MMP are generally classified based on their substrates and the organization of their structural domains into collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMP and other MMP [6, 23, 66, 89].

The functions of MMP are diverse with respect to both endothelium, collagen, and the muscular vascular system [4, 6, 21]. Their involvement in morpho- and angiogenesis as well as in the regeneration process has been noted, as well as in fibrotic disorders, osteoarthritis and cancer. An increase in specific MMP may play a role in arterial remodeling, aneurysm formation, venous dilation, and lower extremity venous dysfunction. MMP also play an important role in leukocyte infiltration and tissue inflammation [122].

MMP have been detected in cancer processes, and elevated MMP levels have been associated with tumor progression and invasiveness [86].

MMP have been proposed as biomarkers for numerous pathologic conditions and are being studied as potential therapeutic targets in various cardiovascular and musculoskeletal diseases as well as in cancer [23, 58].

Compared to invertebrate MMP, vertebrate MMP have diverse subtypes and complex functions [9].

Remodeling of the extracellular matrix is crucial for many physiological (cell migration, proliferation, growth and development) and pathological (cardiac remodeling, carcinogenesis, metastasis, etc.) events. Thus, the interaction between cells and extracellular matrix plays a key role in the normal development and differentiation of the organism, as well as in the mechanisms of many pathological conditions [10,12].

Changes in the extracellular matrix are regulated by a system of proteolytic enzymes that are responsible for proteolysis of a huge number of components of the extracellular matrix. MMPs represent the major group of regulatory proteases in the extracellular matrix. The ability of matrix metalloproteinases to alter the structural integrity of tissues is essential for certain aspects of normal physiology and pathology [44, 102]. The ability to process molecules such as growth factors, receptors, adhesion molecules, other proteinases and proteinase inhibitors makes MMPs powerful regulators of physiological and pathological processes in the cellular

microenvironment. MMP hyperactivation is associated with numerous pathological conditions [21, 81, 85].

MT4-MMP deserve special attention [83], as an increase in MT4-MMP synthesis has been observed in various types of tumor process; however, in the context of our study, the most interesting fact is the increase of this marker in breast cancer metastasis to the lungs. It is assumed that MT4-MMP stimulate the progression of tumor process and metastasis [109].

1.4.2. MT4-MMP in tumors of various organs

MT4-MMP belongs to the membrane-type matrix metalloproteinases (MT-MMPS), a distinct subset of the MMP family that is attached to the cell surface, in this case by a glycosylphosphatidylinositol terminus. MT4-MMP contain a furin sequence (R-X-K/R-R-R) and can be activated by furin. The precursor form (69 kDa) is found in the Golgi complex, while the processed form (58 kDa) is present on the membrane. MT4-MMP biosynthesis follows a unique pathway that terminates in the Golgi complex [29, 52].

MT4-MMP expression in various cancers is well understood. However, the molecular mechanisms by which MT4-MMP contributes to tumor development need further investigation. Researchers aim to summarize the contribution of MT4-MMP to oncogenesis by focusing on the molecular mechanisms triggered by the enzyme during tumor cell migration, invasiveness and proliferation, in the tumor vascular network and microenvironment, and during metastasis [77].

In particular, they highlight putative processed substrates and signaling cascades activated by MT4-MMP that may underlie these malignant processes and compare this to what is known about its role during embryonic development.

Finally, MT4-MMP is an important biomarker of tumor malignancy that can be used to monitor cancer progression in patients and also serve as a potential target for future therapeutic drug development. [105].

MT4-MMP has been described to possess aggrecanase activity, indicating its role in cartilage homeostasis. Both MT4-MMP mRNA and MT4-MMP protein itself have been found to be upregulated in various types of osteoatritis. MT4-MMP transcripts were found in human cartilage in osteoarthritis but not in intact control cartilage without pathology. Increased synthesis of proinflammatory molecules significantly increases MT4-MMP expression in articular cartilage discs derived from the femoral patellar sulcus of calves (experimental data). MT4-MMP is able to cleave the ADAMTS4 p68 isoform, which has relatively low aggrecanase activity, to form the ADAMTS4 p53 isoform, which is the highly active form. The amount of total ADAMTS4 protein is not modulated in inflammatory conditions. However, the ADAMTS4 p53 isoform is found in higher amounts than the p68 isoform, reflecting increased aggrecanase activity. The formation of the p53 isoform is directly related to MT4-MMP expression. All these data indicate the role of MT4-MMP in the regulation of cartilage remodeling in osteoarthritis under inflammatory conditions and open new therapeutic perspectives in the treatment of osteoarthritis and cartilage cancer [43].

MT4-MMP is expressed in lipid rafts of the highly metastatic colorectal cancer cell line HM-7, suggesting a role for MT4-MMP in the metastatic spread of colorectal cancer [83].

The role of MT4-MMP in the regulation of vascular stability in oncology and vascular disease is well known. Recently, its role in the development of thoracic aortic aneurysms and dissections was reported in a screening study of 58 patients with an inherited predisposition to aortic aneurysm. The R373H mutation in the mt4-mmp gene, which prevents

protease expression, was identified. Reduced MT4-MMP is associated with the development of hypotension, aortic dissection and fibrosis formation, and cardiovascular oncology. In addition, MT4-MMP expression is detected in periaortic precursors during embryogenesis, suggesting a role for MT4-MMP in early aortic wall construction and cardiovascular maturation. In another model of vascular injury induced by carotid artery ligation, MT4-MMP-null mice exhibit altered vascular remodeling characterized by more pronounced neointima in carotid arteries and increased vascular wall proliferation. Atherosclerosis may be associated with aortic dissection. Interestingly, the absence of MT4-MMP expression correlates with lipid deposition in atherosclerotic plaques [29, 83]. A direct correlation between the presence of oncological process in the cardiovascular system and the level of MT4-MMP is also noted, which indicates a promising study of this molecule as a marker of onco diagnostics and a possible target for optimization of antitumor therapy.

1.4.3. MT4-MMPs in the process of lung tumor formation

The specific role of MMP and their inhibitors in cancer progression is widely recognized. Although the human MMP family consists of 25 enzymes, only five MMPs, including the four soluble forms (MMP-1, -2, -9, and -13) and the membrane form (MT1-MMP), have been intensively studied. This interest in these MMP is due to their overexpression, which was revealed on the basis of genomic and transcriptomic data collected from various types of human cancers [109].

MT4-MMP has unique functions and different substrates that contribute to various pathologies, especially during tumor progression. All these data support the development of blocking molecules to counteract the effects of MT4-MMP. The pathologic functions of this protease depend

mainly on its proteolytic activity. Interestingly, the catalytic domain of MT4-MMP has a sequence different from that of other MMPs, which allows the development of specific inhibitors as antibodies to block the function of MT4-MMP [33].

MT4-MMP expression has been shown to induce the development of lung metastases by destabilizing the blood network, characterized by increased blood vessel lumen and pericyte detachment. Although no differences were found in the production of key angiogenic modulators (VEGF, PDGFR, FGF and their receptors), the expression of human thrombospondin-2 (TSP-2) was reduced in MT4-MMP xenografts. This result is consistent with a decrease in this antiangiogenic factor, which has been associated with impaired vascular integrity and permeability [30].

Other data show that in breast cancer cells, MT4-MMP promotes primary tumor growth and metastasis to the lung. Although the transport and internalization of type 1 transmembrane MMP have been extensively investigated, little is known about the regulatory mechanisms of MT4-MMP. MT4-MMP forms homophilic complexes at the cell surface and is internalized in early endosomes, with a portion of the enzyme either self-degraded or recycled to the cell surface.

These data indicate that MT4-MMP is internalized by clathrin-independent transporters of early endosomal complexes, a mechanism that differs from that responsible for the internalization of other membrane-type MMP members. Although MT4-MMP localizes with caveolin-1, MT4-MMP internalization was not affected by inhibitors of the caveolin-1 or clathrin endocytosis pathways but was reduced by silencing of CDC42 or RhoA with a small interfering RNA. This provides a novel mechanistic insight into the regulatory mechanisms of MT4-MMP, which may have implications for the development of new therapeutic strategies in metastatic breast cancer. [70, 98].

Thus, the analysis of literature data indicates the relevance and timeliness of studying kisspeptins and associated signaling molecules as modulators of tumor growth in general (and of lung cancer formation and development).

In this context, they should also be considered as potential biomarkers for optimizing the molecular diagnosis of different types of lung cancer and personalized assessment of their prognosis.

Chapter 2. MATERIALS AND METHODS

2.1 Characterization of the material under study

Thirty-four samples of lung tumors (adenocarcinomas only) served as material for the study. Of these, 15 (44.1%) belonged to men and 19 (53.9%) to women. The mean age of men in the study was 65.1±4.6 and women 68.3±5.8 years-old, respectively. Differences by sex and age in the formed groups did not exceed the limits of statistical error ($p<0.05$).

Samples of lung tumors were obtained in the pathology department, and molecular and morphological examination of the samples was performed in the department of translational biomedicine of the research and teaching base of St. Petersburg State University - FGBU "St. Petersburg Research Institute of Phthisiopulmonology" of the Ministry of Health of the Russian Federation.

The comparative analysis of expression levels of the studied signaling molecules in tumor samples took into account the size of tumors (according to TNM nomenclature), the degree of their differentiation (according to Grading system) and the nature of secondary changes in neoplasms.

2.1.1. T - tumor size according to TNM classification

The TNM classification is approved by the International Agency for Research on Cancer (Lyon, France). It defines the characterization of tumors by their size, regional and distant metastases.

T – tumor. The letter T is the basis of the classification and means that the tumor is primary. As the tumor grows from the initial stage to subsequent stages, refining characteristics are added to this designation:

- **Tx** – insufficient or no data to describe the tumor.
- **T0** – stage zero, or pre-cancer.
- **Tis** – stages of cancer ***in situ***. This is a non-invasive type of cancer; the tumor localizes only within the epithelial tissue without growing into the organ.
- **T1** – this group includes tumors ranging in size from 0.1 mm to 2 cm and are also non-invasive. The **T1** group includes several subcategories: these are **T1a** (0.1 to 0.5 cm), **T1b** (0.5 to 1 cm), and **T1c** (1 to 2 cm).
- **T2** – the stages of cancer when malignant cells already tend to be invasive and the tumor spreads to the tissues of the affected organ, but not beyond it. Lymph nodes may be affected, but to a certain, moderate extent and only nearby. The tumor size does not exceed 2-5 cm, such neoplasms are fully operable.
- **T3** – refers to potentially dangerous stages, in this case the tumor grows to 5–7 cm or more, and spreads outside the organ, to the lymph nodes. This stage is already characterized by metastases, including the detection of foci in distant organs.
- **T4** – are referred to the terminal stages of cancer, when the fight against the disease is often really ineffective, and the meaning of any therapy is reduced to the relief of the patient's condition. The size of the tumor itself, the degree of its invasion, sprouting into tissues, vessels and organs is of secondary importance since malignant cells are present in the lymph.

2.1.2. Grading system for determining the level of tumor differentiation

The Grading system is approved by the International Agency for Research on Cancer (Lyon, France). Using light microscopy, this system is used to determine the degree of malignancy, both in terms of the shape of the cancer cells and, in general, in the modified tissue itself. The higher the value of the index, the more aggressive and active the tumor behaves.

In this system, the grade ranges from 1 to 4 degrees.

When the modified tissue and cellular conglomerates appear close to normal, the tumor tissue is called "well-differentiated" and is grade 1. This grade has a good prognosis and a low rate of progression of the cancer process.

"Poorly/poorly differentiated" (Grade 2 and/or Grade 3) or "undifferentiated" (Grade 3 or 4) tumor cells and tissues have the worst prognosis and a rapid degree of metastasis, a highly aggressive process.

Hence, the Grading system denotes the "malignancy" of the cancer process:

- GX — undetermined (little data);
- G1 — highly differentiated (non-aggressive);
- G2 — moderately differentiated (moderately aggressive);
- G3 — low-differentiated (highly aggressive);
- G4 — undifferentiated tumor (highly aggressive).

2.2. Methods

Sections (5 μm) were prepared from tumor samples (paraffin blocks). Sections were processed in a standardized manner, and an IHC reaction was

performed. Primary monoclonal AT to Ki-67 protein (an integral marker of cell proliferation), kisspeptin-1, caldesmon and matrix metalloproteinase type 4 (all Abcam, 1:100) were used.

To verify immunohistochemical staining, a universal kit containing biotinylated Ig (EnVision Detection System, Peroxidase/DAB) was used. Diaminobenzidine was used to detect horseradish peroxidase.

The immunohistochemical study protocol is outlined below.

2.2.1. Immunohistochemical method

- Prepare three 200ml containers of distilled water, hydrogen peroxide (20ml mother liquor + 180ml distilled water) and buffer for washing samples (WB).
- Quickly transfer the slices to WB and leave at room temperature for 10 minutes.
- Rinse in distilled water.
- Place in H_2O_2 peroxide (15%) for 10 minutes.
- Transfer to WB for 10 minutes.
- Remove excess moisture from the windows.
- Circle the slices with a hydrophobic marker before applying primary antibodies.
- - Apply primary antibodies at the desired concentration to the slices (20-40 µl per glass depending on the number of slices) and incubate in a humid chamber for 12 hours at 3ºC.
- Rinse in distilled water.
- Immerse the tripod with slices in fresh WB for 10 min.
- Remove excess moisture from the windows.
- Apply secondary antibodies to the slices (20-40 µl).
- Incubate for 30 min at room temperature in a humid chamber.
- Rinse in distilled water and repeat step 10.
- Remove excess moisture from the windows.

- Dilute chromogen at a rate of 1:50, apply to preparations.
- Incubate for about 1 min (check the progress of the reaction under the microscope).
- Place the tripod with glasses in a container of distilled water.
- Immerse the preparations in a container with hematoxylin for 1-2 min.
- Show hematoxylin staining for 10 min in running water. This dye stains basophilic cellular structures that contain nucleic acids (DNA and RNA) blue: cell nucleus, ribosomes and RNA-rich areas of the cytoplasm.
- Immerse the preparations in a container of fresh distilled water for 3 min.
- Dry the slices in a thermostat at 60°C.
- Soak for 30 min in xylene for clarification.
- Blot the slices with clean filtered paper before encapsulation (to avoid bubbles on the finished preparation).
- Enclose in a mounting environment (UltraKitt, J.T. Baker).

2.2.2. Morphometry of microscopic images

For quantitative morphometric study of the results of immunohistochemical reactions, a hardware-software complex based on an Olympus IX73 inverted microscope with an Olympus DP80 digital camera and Olympus CellSens image archiving and analysis software (Olympus) was used.

The Olympus DP80 camera is dual-sensor (monochrome and color at the same time), and thanks to its two CCD sensors, it can capture both color and monochrome images, providing high resolution brightfield imaging with enzymatic cell receptor imaging systems and sensitive detection of biomarker molecules with fluorescent dyes.

The 6.45 μm square pixel size and active cooling provide the high sensitivity and high signal-to-noise ratio needed to capture the volume and

localization of molecular markers. The ability to combine color and monochrome images allows the creation of superimposed images to confirm the position of molecules in correlation with morphological structure.

Olympus CellSens Standard software allows you to capture images, make adjustments and work with images in real time, archive files to a gallery in JPEG, TIFF and PNG formats, work with multi-layer images, analyze images by color or pattern, make interactive measurements of angles, distances, areas, record these data on the image with the possibility of saving the original image..

CellSens Standard software also displays data beyond a single image using advanced image capture processes (e.g., interval capture) and control of motorized and coded microscope components.

The relative area of expression of signaling molecules (determined by immunohistochemistry) to the total area of cells in the field of view was measured. Relative expression area was expressed as a percentage.

2.2.3. Methods of statistical processing

The obtained results were checked for compliance with the normal distribution law using the Shapiro-Wilk criterion. If the data conformed to the normal distribution law, the typical value was presented as M±σ, and the groups were compared using Student's criterion. If the data did not conform to the normal distribution law, the typical value was presented as median and interquartile range, and the groups were compared using the Mann-Whitney criterion.

Additionally, the chi-square (χ^2) criterion was applied.

Fisher's angular transformation was also performed. The null hypothesis and CI were evaluated in a standardized manner.

Discriminant analysis, A. Wald sequential analysis method modified by E.V. Gubler and neural network model were used to solve the problem of adenocarcinoma metastasis prediction. The method of global optimization, random search, creation of algorithms was used.

A combined global optimization method was used to create the neural network, which consisted of applying the methods of conjugate gradients, random search, inertial and genetic algorithms at different iterations.

The method of sequential analysis of A. Wald modified by E.V. Gubler is based on T. Bayes formula for independent signs, the method of sequential analysis of A. Wald and the statistics of S. Kulbak.

The essence of the statistical method is to determine information about the presence or absence of metastasis by the value of a particular sign. Thus, if point A is taken as the presence of metastases, and point B as the absence, then if in point A the sign is maximal, and in point B is insignificant, it is a marker of metastasis. If the ratio of signs in point A and B is equal, or almost equal, the sign cannot be considered a marker of diagnosis.

This method can also be used to determine the likelihood of disease from a collection of signs:

$$ДК_i = 5 * \lg \frac{P_j(A)}{P_j(B)} \qquad (2.1)$$

ДКi is the diagnostic coefficient calculated for each of the gradations of the question, P j (A) is the probability of the respondent getting into group A with the gradation of the answer Sj; Pj (B) - - the probability of the respondent getting into group B with the gradation of the answer Sj

This method relies on the differences in the occurrence of features in the two subgroups, and if the values in groups A and B are similar, the informativeness of the feature in dividing the population is reduced.

S. Kulbak's criterion was used to assess the informativeness of the trait. The informativeness of the feature gradations was calculated using the so-called prognostic coefficient according to:

$$r(Xij) = 0,5[P2 - P1] \cdot 5lg(P2/P1), \quad (2.2)$$

P2 - the likelihood of a "no metastasis" state; P1 - probability of the condition "occurrence of a case of IUT disease".

And further, the information content of the entire feature was calculated:

$$R(x_i) = \sum_{k=1}^{n} r(X_{ij}) \quad (2.3)$$

The decision to categorize patients into groups ("no risk of metastasis" or "increased risk of metastasis") was made as follows: a card was filled out for each patient, diagnostic coefficients for each individual respondent were summarized, and total diagnostic coefficients (TDC) were determined.

Next, a comparative analysis was performed with the put value, which was chosen as 0. Anything less than 0 indicated no risk of metastasis. Anything greater than 0 indicated a high risk of metastasis.

Chapter 3. STUDY RESULTS AND DISCUSSION

3.1. Analysis of general biologic and gender parameters of tumors

Analysis of the results of morphologic examination showed that metastases were observed in 5 (14.7%) cases. The presence of metastasis depending on gender is presented in Table 1.

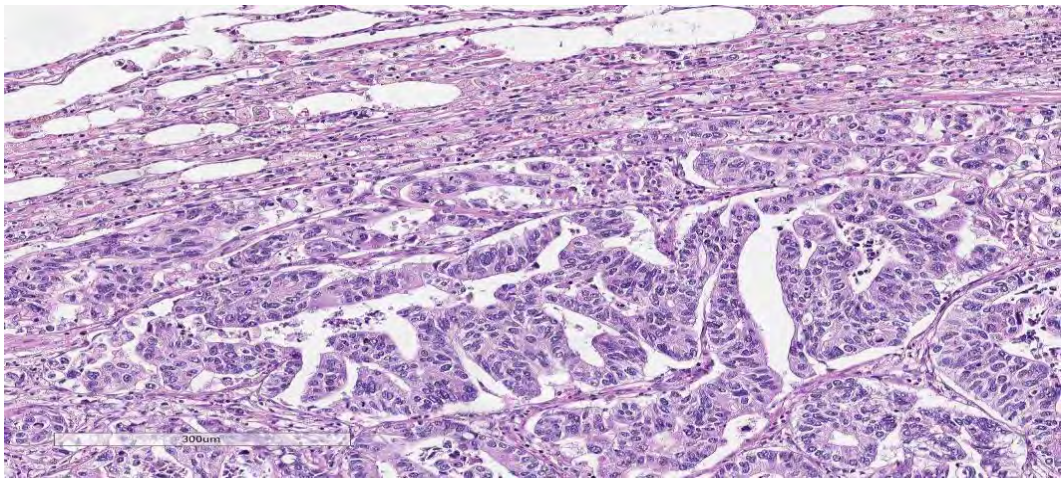
Table 1. Presence of metastasis according to patient gender.

The presence of metastasis	Gender			
	Men		Women	
	Absolute	%	Absolute	%
Yes	3	20,0	2	10,5
No	12	80,0	17	89,5
Note: *differences in indicators at p<0.05				

As shown in Table, the study found no statistically significant difference in the incidence of metastasis in males and females.

Tumors of moderate degree of differentiation were the most common, 19 (55.9%), followed by low grade, 12 (35.3%) and high grade (8.8%).

The distribution of detected tumors by Grade depending on sex is presented in Table 2, and their histological picture is shown in Fig. 6.



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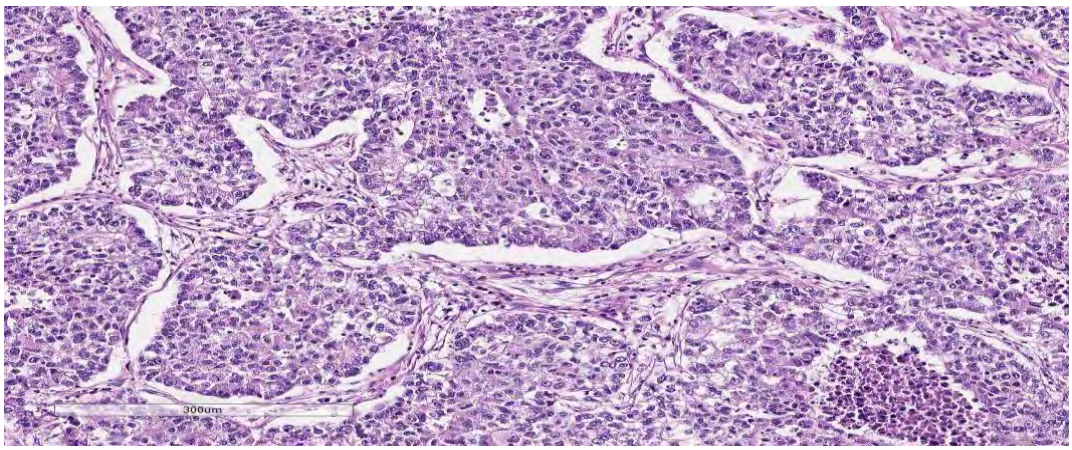

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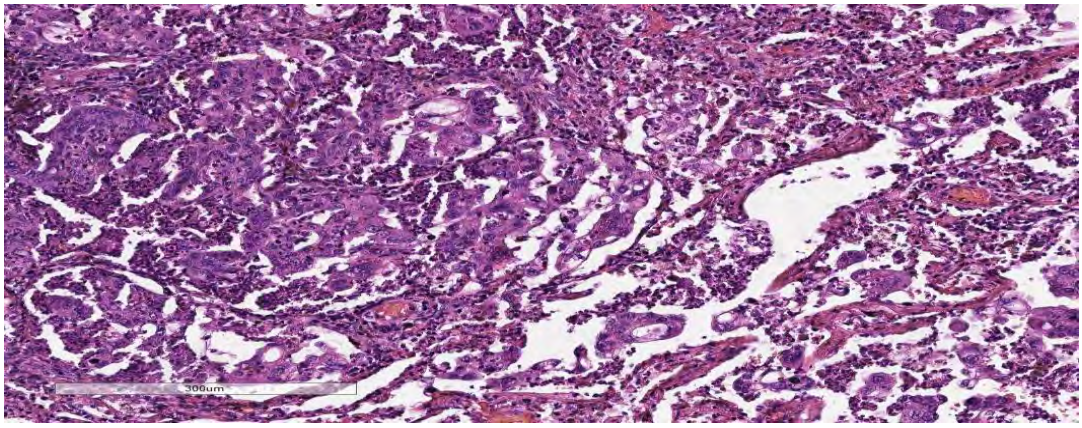


C

Figure 6. Lung carcinoma with different degrees
of differentiation. A – G1; B – G2; C – G3.
Hematoxylin-eosin staining. X200.

As shown in Table 2, the study found no statistically significant differences in the degree of differentiation between men and women.

Analysis of tumor size showed monotonically decreasing frequencies - 18 (52.9 %) for T1, 11 (32.4 %) for T2, 3 (8.8 %) for T3 and 2 (5.9 %) for T4.

Table 2. Degree of tumor differentiation according to gender.

Degree of differentiation	Gender			
	Men		Women	
	Absolute	%	Absolute	%
Low	4	26,7	8	42,1
Moderate	10	66,7	9	47,4
High	1	6,7	2	10,5
Note: *differences in indicators at p<0.05				

The distribution of tumor size according to gender is shown in Table 3.

Table 3: Size of tumors according to the patient's gender.

Tumor size	Gender			
	Men		Women	
	Absolute	%	Absolute	%
T1	9	60,0	9	47,4
T2	6	40,0	5	26,3
T3	-	-	3	15,8
T4	-	-	2	10,5
Note: *differences in indicators at p<0.05				

As shown in Table 3, the study found no statistically significant difference in tumor size between males and females.

Signs of lymphovascular invasion and necrosis were detected in most cases (23) and amounted to 67.6%. In the study, the differences in these parameters among men and women did not exceed the statistical error (p>0.05).

Inflammatory process was characterized in majority 24 (70.4%) and hemorrhage in half 20 (58.8%) cases.

The distribution of secondary tumor changes according to gender is shown in Table 4.

Table 4. Secondary tumor changes according to gender.

Secondary changes	Gender			
	Men		Women	
	Absolute	%	Absolute	%
Lymphovascular invasion	10	66,7	13	68,4
Necrosis	11	73,3	12	63,2
Hemorrhages	10	66,7	10	52,6
Inflammation	12	80,0	12	63,2
Note: *differences in indicators at $p<0.05$				

As shown in Table 4, the study found no statistically significant differences in secondary tumor changes between men and women.

3.2. Analysis of gender peculiarities of proliferative activity and immunophenotypes of tumor cells

The proliferative activity of tumors by Ki-67 expression averaged 24.0±9.0 %. Relative areas of expression of kisspeptin, caldesmon and matrix metalloproteinase of membrane type 4 were 13.6±2.6%, 7.1±1.2% and 6.6±0.9% respectively. The distribution of immunohistochemical parameters depending on sex is presented in Table 5.

Table 5. Relative area of expression (%) of signaling molecules in lung tumors according to patient gender.

Immunohistochemical parameters	Gender	
	Men	Women
Ki-67	26,3±14,7	22,3±10,8
Kisspeptin	12,9±4,0	14,2±3,6
Caldesmont	6,7±2,0	7,4±1,6
Membrane-type matrix metalloproteinase 4	7,1±1,5	6,2±1,2
Note: *differences in indicators at p<0.05		

As shown in Table 5, the study found no statistically significant differences in immunohistochemical parameters in males and females.

Thus, the formed groups of men and women with adenocarcinomas were comparable in all studied parameters.

The most frequent (55.9% of cases) were moderately differentiated tumors; metastases were observed in 14.7% of cases. More than half (52.9%) of the tumors presented were classified as T1.

Secondary manifestations of lymphovascular invasion and necrosis were detected in the majority of cases (67.6 %). The inflammatory process was characteristic in the majority (70.4 %), and hemorrhages in more than half (58.8 %) of the studied preparations.

The proliferative activity of tumors by the level of Ki-67 expression averaged 24.0±9.0 %. Relative expression areas of kisspeptin, caldesmon and matrix metalloproteinase of membrane type 4 were 13.6±2.6 %, 7.1±1.5 % and 6.6±0.9, respectively.

3.3. Analysis of the prognostic significance of expression of kisspeptin-1 and associated molecules in lung tumors

During the study, a correlation between metastasis and the degree of tumor differentiation was established (R=0.5, p<0.05). Thus, the presence of metastases was characteristic only for low degree of tumor differentiation.

Table 6: Relative area of expression of signaling molecules (%) and degree of tumor differentiation.

Immunohistochemical parameters	Degree of differentiation		
	Low	Moderate	High
Kisspeptin*	0,3±0,2	9,9±2,8	22,6±2,2
Caldesmon*	0,4±0,2	5,9±2,8	10,5±1,1
Membrane-type matrix metalloproteinase 4*	18,4±2,3	8,6±1,5	0,5±0,3
Note: *differences in indices at p<0.05 at transition from low to moderate and high tumor differentiation degree to moderate and high degree of tumor differentiation			

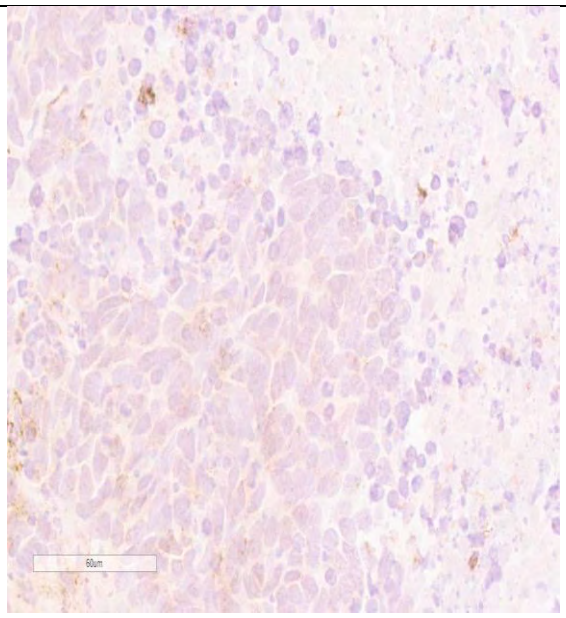
At the same time, the age structure analysis showed that the second degree of tumor differentiation occurred at older ages (70.7±3.8 years old) than the first (60.5±6.2 years old) or third (68.3±11.8 years old) (R=0.4, p<0.05). The indicated feature was characteristic for both men and women.

Analysis of changes in the relative area of expression of kisspeptin and caldesmon depending on tumor differentiation suggests a high degree of correlation (R=-0.8, p<0.05). The evaluation of immunohistochemical markers and tumor differentiation degree is presented in Table 6.

As can be seen from Table. 6, there is a monotonous increase in the relative area of expression of both kisspeptin and caldesmon and a decrease in the relative area of expression of membrane type 4 matrix metalloproteinase during the transition of tumors from a low to a high degree of differentiation (Fig. 7-9).

In addition, an inverse relationship (R=-0.5, p<0.05) was established between the presence of metastases and the relative area of expression of kisspeptin and caldesmon, as well as a direct relationship (R=0.4, p<0.05) between the presence of metastases and the relative area of expression of matrix metalloproteinase type 4 membranes (Table 7.)

According to Table. 7 in the presence of metastases, higher expression levels of both kisspeptin and caldesmon were found, as well as lower expression levels of membrane-type 4 matrix metalloproteinase (p<0.05).



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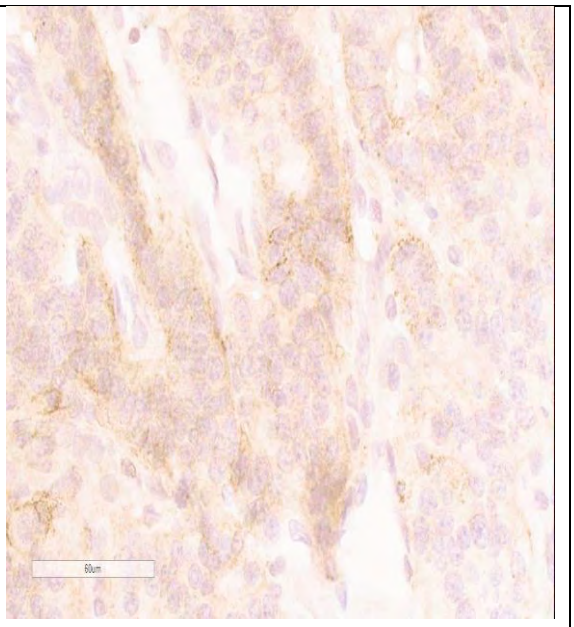

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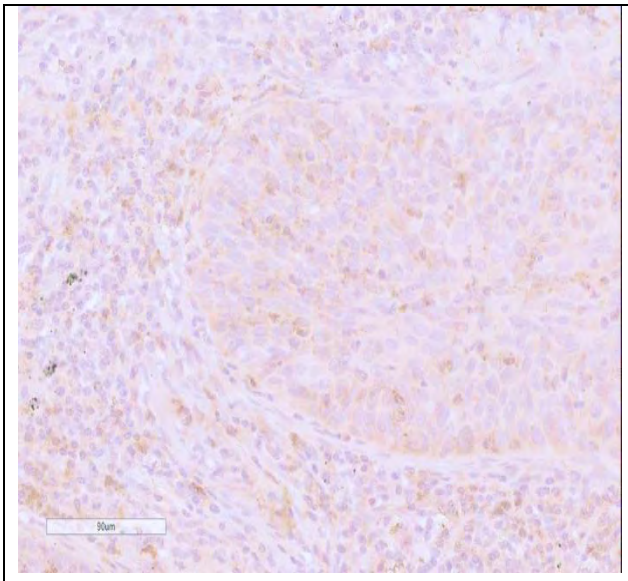
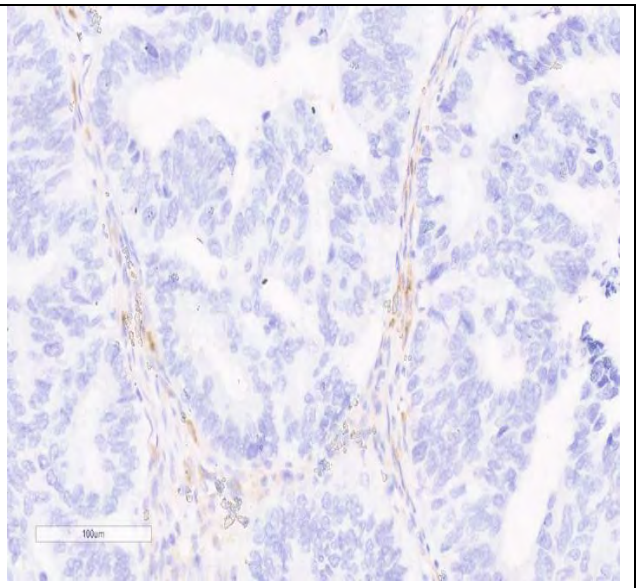
Figure 8. Expression of kisspeptin in low (A) and high (B) differentiated lung adenocarcinoma. X200.





А	Б

Figure 9. Expression of membrane-type 4 matrix metalloproteinases at low (A) and high (B) differentiated lung adenocarcinoma. X200.

Table 7: The relative area of expression of signaling molecules (%) and the presence of tumor metastases.

Immunohistochemical parameters	Metastasis	
	Presence	Absence
Kisspeptin*	25.0±3.4	11.6±2.4
Caldesmon*	11.8±1.2	6.3±1.2
Membrane-type 4 matrix metalloproteinase	4 4.1±2.6	7.3±0.9
Note: *Differences in scores at p<0.05 when comparing the presence and absence of metastasis		

Assessment of secondary changes accompanying carcinogenesis showed that inflammation was in the first place - 24 (70.6%), necrosis - in the second - 23 (67.6%) and hemorrhage - in the third - 20 (58.8%)

Analysis of lymphovascular invasion, as a precursor of metastasis, helped to reveal the relationship with secondary changes accompanying carcinogenesis.

Secondary changes accompanying lymphovascular invasion are presented in Table. 8.

Table 8. Secondary changes in lymphovascular invasion.

Secondary changes	Lymphovascular invasion			
	Presence		Absence	
	Абс	%	Абс	%
Necrosis*	20	87,0	3	27,3
Hemorrhage*	18	78,3	2	18,2
Inflammation*	19	82,6	5	45,5
Note: *Differences in scores at $p<0.05$ when comparing the presence and absence of metastasis				

The differences in the level of tumor differentiation, the relative area of expression of kisspeptin, caldesmon, and membrane-type 4 matrix metalloproteinase in the groups that differ in the presence of metastasis, revealed during the study, suggested the existence of factors contributing to the prognosis of metastasis.

As can be seen from Table. 8, the frequency of secondary changes (necrosis, inflammation and hemorrhage) is statistically significantly higher in lymphovascular invasion.

Table 9. Integral indicators of the relative area of expression (%) of immunohistochemical markers and the degree of tumor differentiation.

Иммуногистохимические показатели	Степень дифференцировки		
	Низкая	Умеренная	Высокая
Кисспептин*	0,3±0,2	9,9±2,8	22,6±2,2
Кальдесмон*	0,4±0,2	5,9±2,8	10,5±1,1
Матриксная металлопротеиназа мембранного типа 4*	18,4±2,3	8,6±1,5	0,5±0,3
Примечание: *различия в показателях при $p<0,05$ при переходе от низкой к умеренной и высокой степени			

In the course of the discriminant analysis, a factor (kisspeptin) was established that allows for prognosis by dividing groups with and without metastasis based on discriminant functions (1,2).

$$P1 = 0.6\ K - 10 \qquad (3.1)$$

$$P2 = 0.3\ K - 1.9 \qquad (3.2)$$

where – P1 and P2 are discriminant functions that allow predicting the presence (P1) and absence (P2) of metastasis, K is the area of kisspeptin expression (in %).

The study showed a correct assessment of the prognosis in 91.1% of cases, however, in the group with metastasis, the correct prognosis was less - in 75% of cases, which requires further research in this direction.

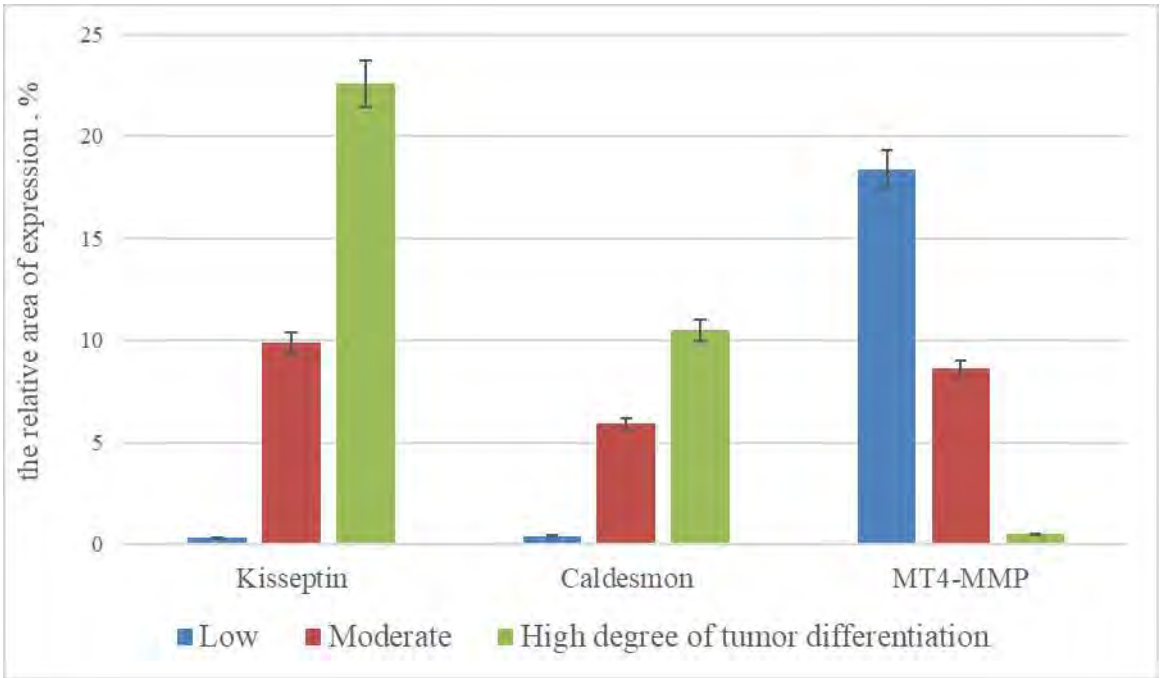


Figure 10. Indicators of the expression of signaling molecules in the tumor tissues with different degrees of neoplasm differentiation.

Table 10. Relative area of expression of immunohistochemical markers (%) and presence of tumor metastasis.

Immunohistochemical markers	Metastasis	
	Presence	Absence
Kisspeptin*	25,0±3,4	11,6±2,4
Caldesmon*	11,8±1,2	6,3±1,2
Membrane-type 4 matrix metalloproteinase	4,1±2,6	7,3±0,9
Note: *Differences in scores at $p<0.05$ when comparing the presence and absence of metastasis		

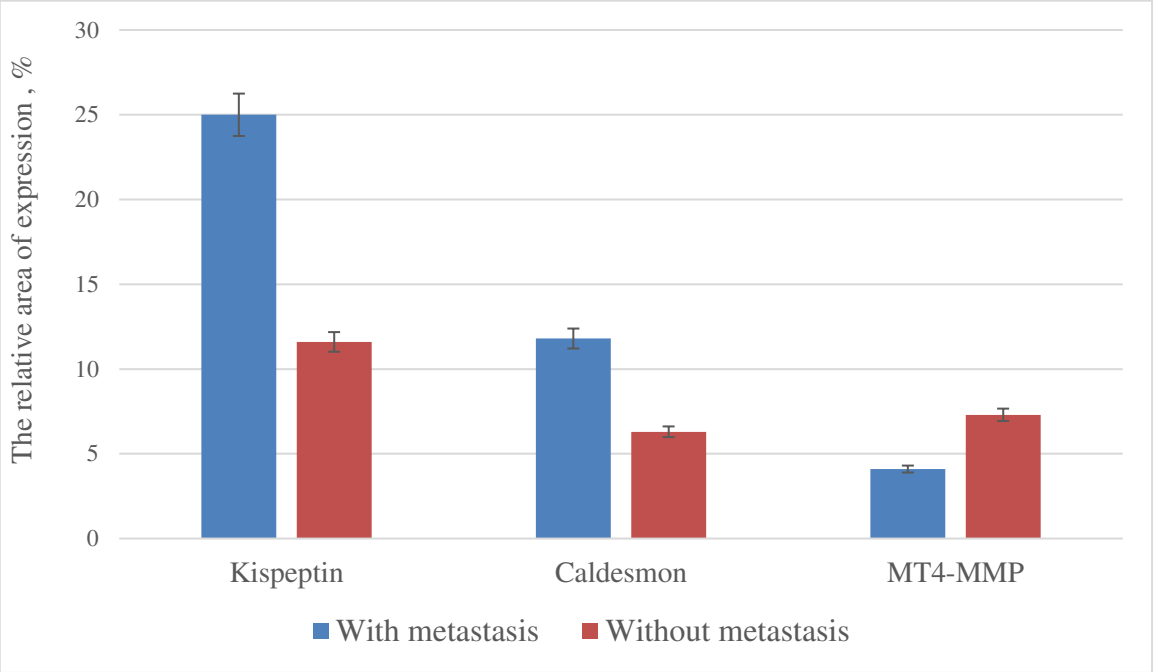


Figure. 11. Indicators of the expression of signaling molecules in the tumor tissues with/without metastasis

Table 11. Secondary changes in lymphovascular invasion.

Secondary changes	Lymphovascular invasion			
	Presence		Absence	
	Abs	%	Abs	%
Necrosis*	20	87,0	3	27,3
Hemorrhage*	18	78,3	2	18,2
Inflammation*	19	82,6	5	45,5
Note: *Differences in the manifestation of secondary manifestations in the presence and absence of lymphovascular invasion at $p<0.05$				

Thus, the study found that the second degree of tumor differentiation occurred at older ages. A monotonous increase in the relative area of expression of both kisspeptin and caldesmon and a monotonous decrease in the relative area of expression of matrix membrane-type metalloproteinase 4

during the transition from a low to a high degree of differentiation were revealed.

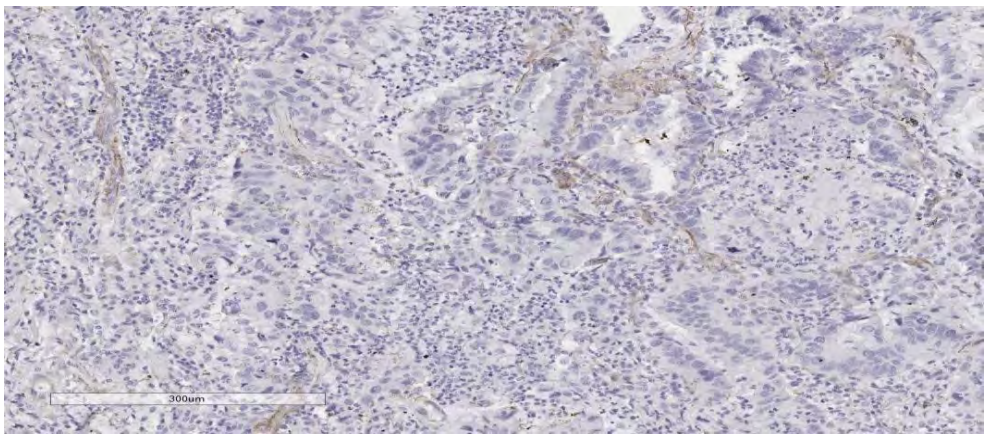


Figure.12. Pronounced expression of kisspeptin at high differentiated lung adenocarcinoma. X200.

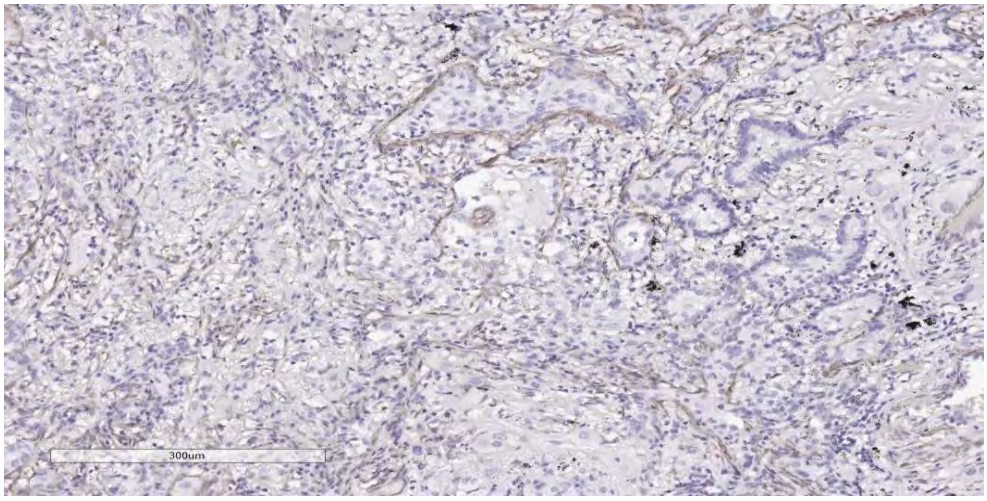


Figure13. Pronounced expression of caldesmon at high differentiated lung adenocarcinoma. X200.

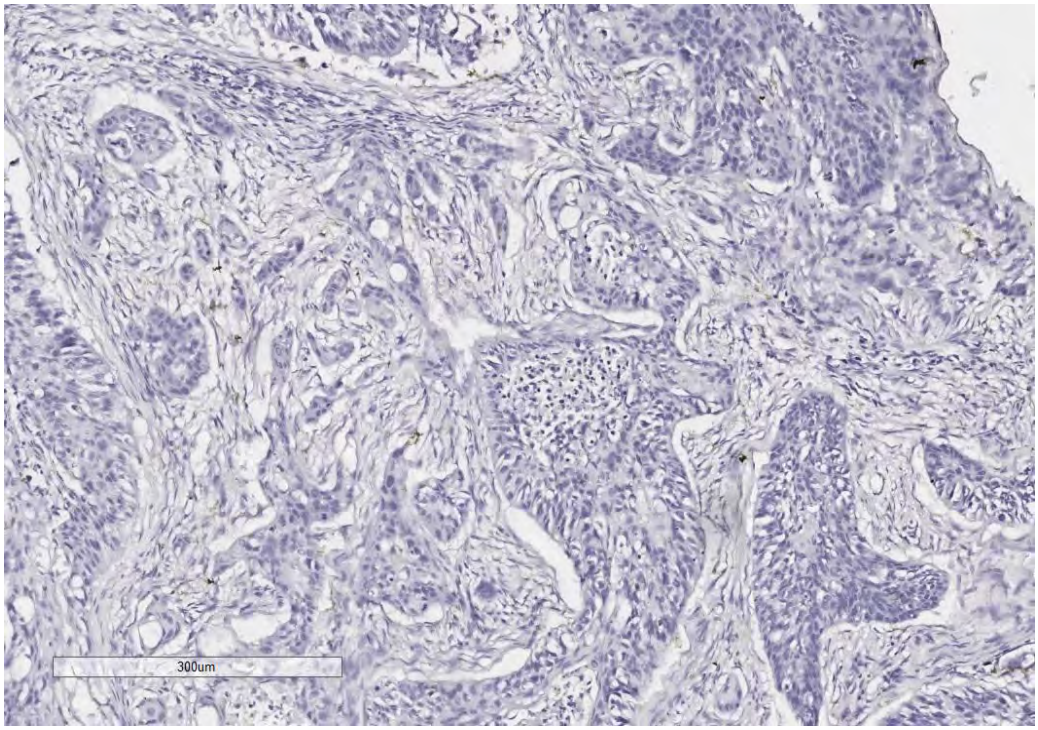


Figure 14. Low level of expression MT4-MMP at high differentiated lung adenocarcinoma. X200.

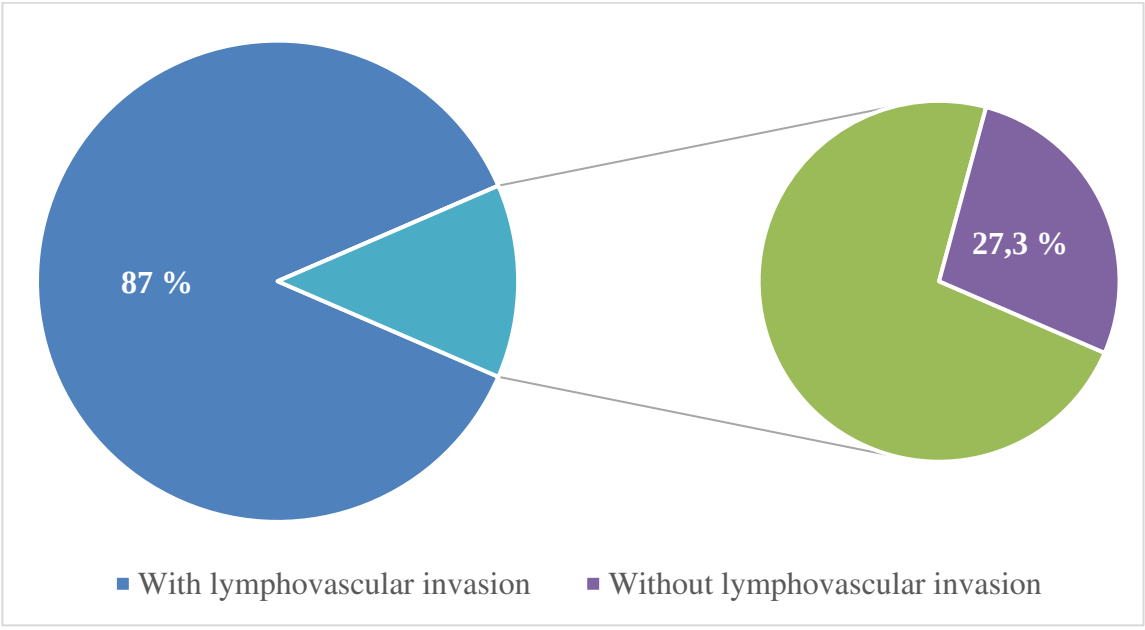


Figure 15. Total expression of signaling molecules in tumor tissue during tumor necrosis.

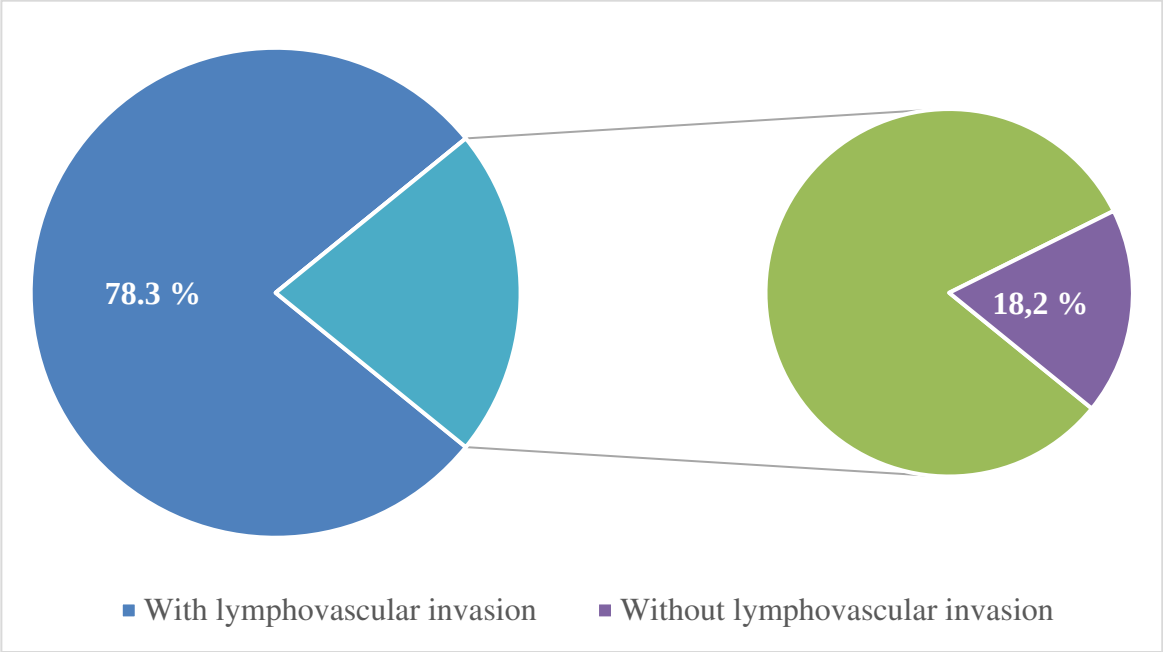


Figure 16. Total indicators of expression of signaling molecules in tumor tissue during intratumoral hemorrhages.

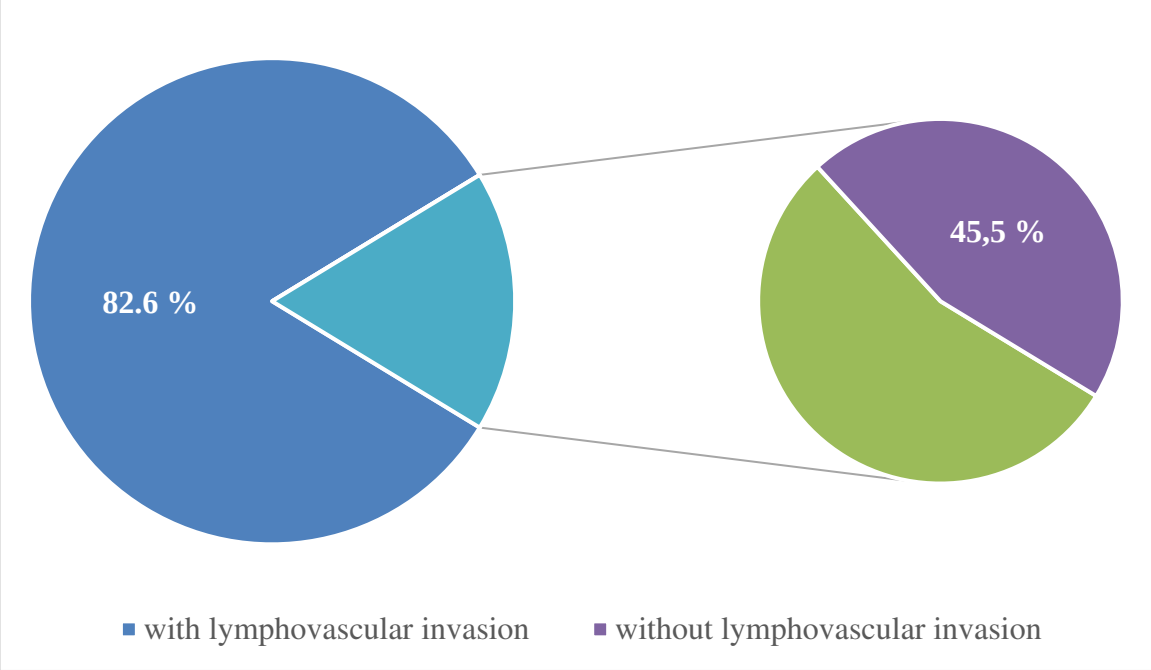


Figure 17. Total indicators of expression of signaling molecules in tumor tissue during intratumoral inflammation.

The relationship between the degree of tumor differentiation and the relative area of expression of kisspeptin, caldesmon, and matrix membrane-type ô metalloproteinase was shown. The relationship between metastasis

and the relative area of expression of kisspeptin, caldesmon, and matrix membrane-type 4 metalloproteinase was determined (Fig. 10-14). It was confirmed that secondary changes (inflammation, hemorrhage and necrosis) are statistically significantly more common in lymphovascular invasion (Tables 9-11, Figures 15-17).

3.4. Assessing the prognosis of lung carcinoma metastasis

The differences in the level of tumor differentiation, the relative area of expression of kisspeptin, caldesmon, and matrix membrane type 4 metalloproteinase in the groups that differ in the presence of metastasis, revealed during the study, suggested the existence of factors contributing to the prognosis of metastasis.

When predicting metastasis, the entire study population (34 people) was divided into two subgroups: training - 38.2% (13 people), and testing - and 61.8% (21 people). Informativeness of features was assessed using the Kullback coefficient.

Diagnostic criteria (at R not less than 1) are presented in Table. 12.

The most informative signs that allow predicting metastasis were: the relative area of expression of kisspeptin and caldesmon (for both, R=2.0), the degree of tumor differentiation by grade (R=1.3).

Table 12. Diagnostic coefficients for predicting the occurrence of metastasis (a fragment of the diagnostic table).

Kullback coefficientR	Questions of diagnostic table	Diagnostic coefficients
1,3	Tumor differentiation by grade: 1) low 2) moderate and high	1) 2,6; 2) -2,6
2,0	Relative area of kisspeptin expression (%): 1) Less than 18; 2) 18 and over	1) -3,0; 2) 3,3

2,0	Relative area of caldesmon expression (%): 1) Less than 9; 2) 9 and over	1) -3,2; 2) 3,8

By calculating the diagnostic coefficients, the resulting diagnostic coefficient was obtained. A positive coefficient indicated a high risk of metastasis, a negative coefficient - a low one.

A comparative analysis with the verification group was carried out. The correct result was determined in 81% of cases (Table 13).

In the course of the discriminant analysis, a factor (kisspeptin) was established that allows for prognosis by dividing groups with and without metastasis based on discriminant functions (1,2).

$$P1 = 0.6\ K - 10 \quad (3.3)$$

$$P2 = 0.3\ K - 1.9 \quad (3.4)$$

where – P1 and P2 are discriminant functions that allow predicting the presence (P1) and absence (P2) of metastasis, K is the area of kisspeptin expression (in %).

The study showed a correct assessment of the prognosis in 91.1% of cases, mainly due to high (96.5%) specificity, but the correct prognosis in the group with metastasis was 60%, which required further research in this direction.

Table 13**.** The results of checking the diagnostic table for predicting metastasis on the test group.

Indicator	Testing group	
	Relative values, %	Number of persons
Sensitivity	100	2
Specifity	84	16

False negative response	0	0
False positive response	16	3

A feature of the constructed models of A. Wald in the modification of E.V. Gubler and discriminant analysis is the assumption of the linearity of the interaction of factors within the model. This feature can significantly reduce the accuracy of the forecast, therefore, to eliminate these shortcomings, a neural network model was chosen, and the input parameters previously used in the A. Wald model were used to build and train it.

The structure of the neural network included an input layer of 3 neurons, hidden layers of 4 and 6 neurons, and an output layer of one neuron (Fig. 18). From the total population, three groups were formed, of which two were directly involved in the training: the training group (18 people) and control (8 people), as well as the testing (checking) group - 8 people. The correct result in the training group was 100%, control 100% and testing - 100%.

The results of testing the neural network on the testing group are presented in Table. 14.).

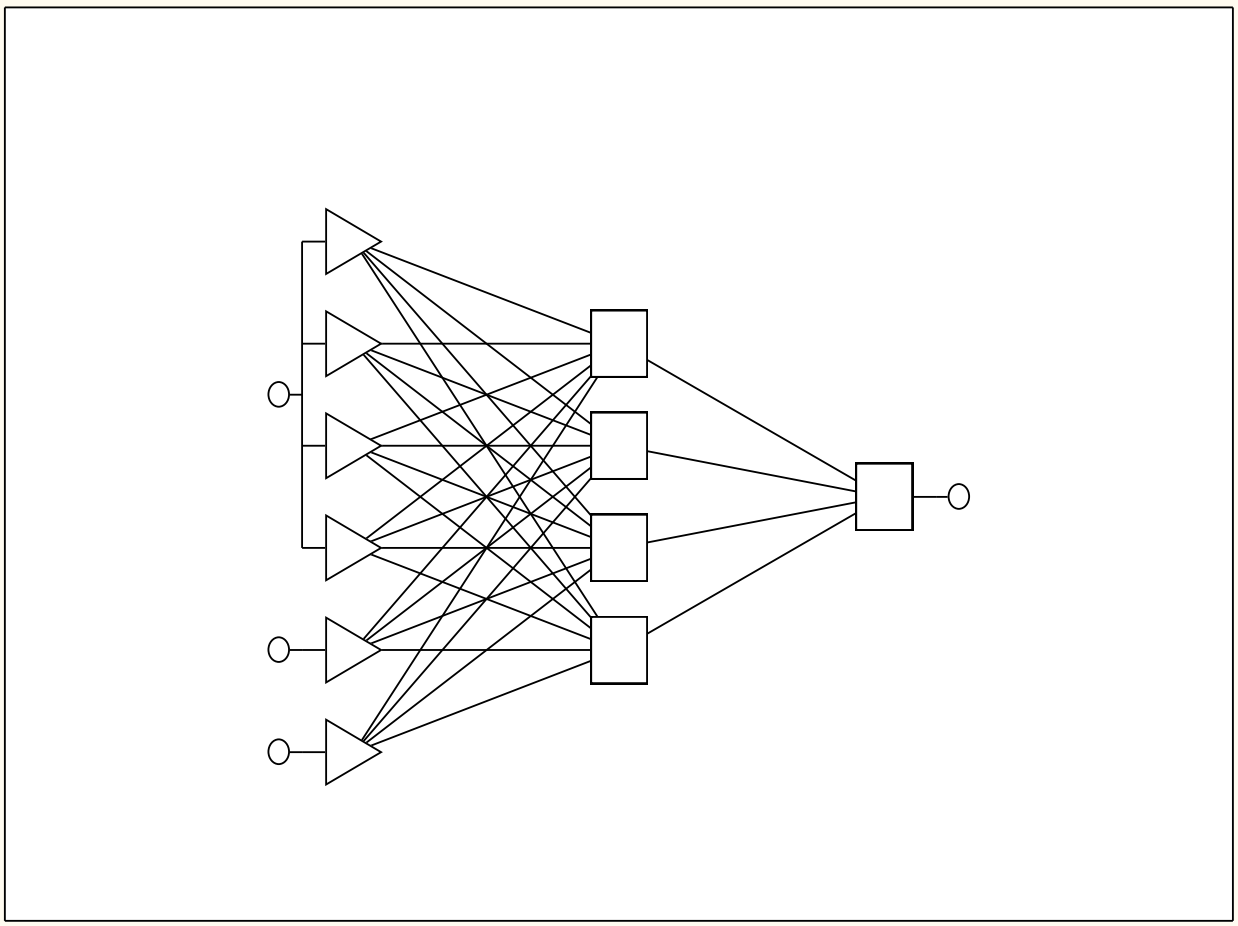


Figure 18. The structure of the neural network predicting metastasis.

The sensitivity of the neural network was 100%, the specificity was 100%. The sensitivity of individual variables in the neural network model was determined as the ratio of neural network errors in the testing group without the factorial feature of interest to the network errors with the factorial feature of interest. The greater the sensitivity, the greater the value of the studied predictor, the smaller the prediction error of the neural network.

The informative value of the factors included in the neural network is presented in Table 15.

Table 14**.** The results of testing the neural network for predicting metastasis on the testing group.

Indicator	Testing group	
	Relative values, %	Number of persons
Sensitivity	100	1
Specifity	100	7
False negative response	0	0
False positive response	0	0

Table 15. Results of sensitivity analysis predictor factors of neural network.

Factor	Sensitivity	Rank
Relative area of expression of kisspeptin	54	1
Secondary manifestations: necrosis	18	2
Presence of lymphovascular invasion	16	3

When predicting metastasis using a neural network, the most informative factor was the relative area of kisspeptin expression, in second place was the presence of necrosis, as a secondary manifestation of the tumor process.

It was found that the chosen processing model allows to correctly predict cases of metastasis in 81%, and discriminant analysis - in 91% of cases. At the same time, taking into account the non-linearity of the relationships between the relative area of kisspeptin expression and secondary manifestations of the tumor process made it possible to increase the accuracy of metastasis prediction up to 100%.

Most of the works consider kisspeptins (and especially KiSS-1) as a powerful intracellular inhibitor of tumor progression and metastasis, acting mainly due to the degradation of matrix metalloproteinases, especially MMP-4 and 9, caused by them (Fig. 19).

However, it should be noted that kisspeptins play an ambiguous role in the development of various neoplasms. For example, KiSS-1 stimulates metastasis in hepatocellular carcinoma and breast cancer, which may be associated with impaired estrogen synthesis in these tumors.

Moreover, it is generally accepted that tumor cell heterogeneity can cause differences in tumor growth rate, invasiveness, drug sensitivity, and neoplasm prognosis. KiSS-1R was found to be upregulated in mesenchymal stem cells and osteoblasts when co-cultured with multiple myeloma cells. Cross-talk between tumor cells and the bone tissue microenvironment has shown that KiSS-1 can be considered as a plausible biomarker for assessing the progression of multiple myeloma. (by its expression it is possible to evaluate the activity of fibroblasts, endotheliocytes and tumor-infiltrating lymphocytes).

Thus, it can be assumed that the CR expression pattern in each specific case contributes to the functioning of the tumor microenvironment and, thus, is a target for optimizing targeted therapy for malignant neoplasms.

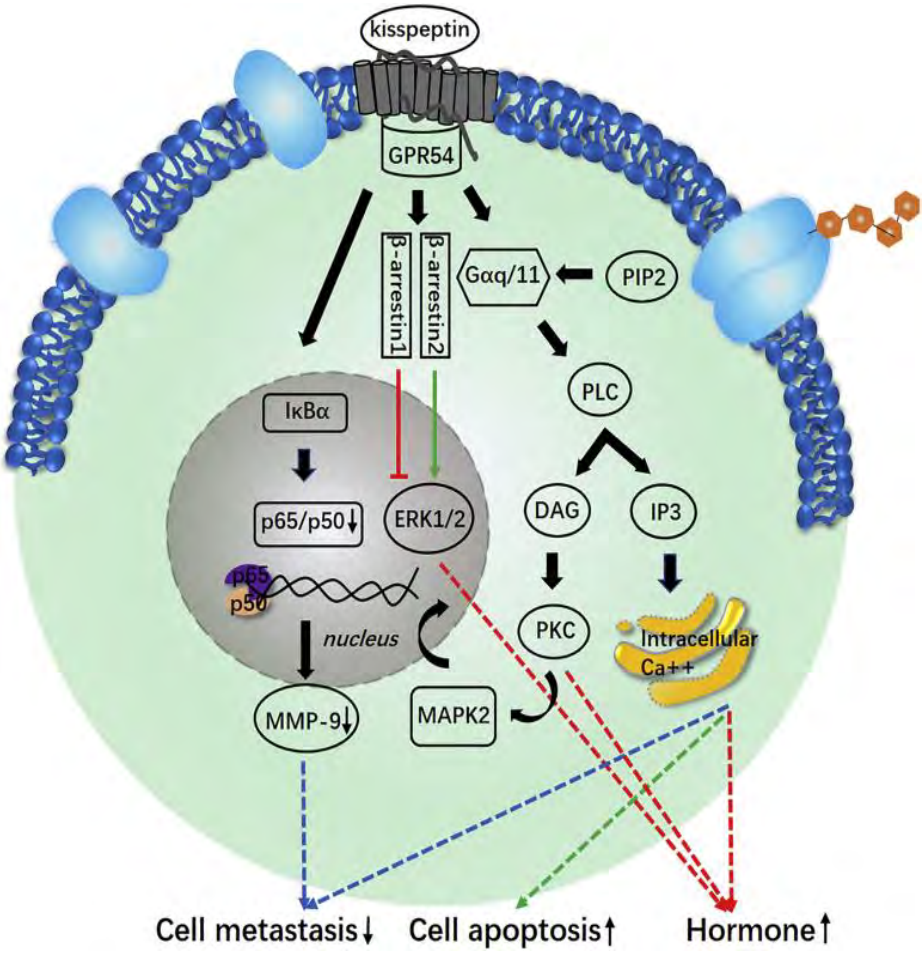


Figure 19. Scheme of participation of KiSS-1 in intra- and intercellular metabolic processes [26].

Tumor metastasis is a complex process involving loss of intercellular adhesion, invasion of the extracellular matrix, spread through lymphatic or vascular channels, and colonization at the site of metastasis, where secondary tumors continue to grow and exacerbate the primary neoplasm. It has been shown that changes in oncogenes and tumor suppressor genes are involved in the process of metastasis. It was found that the process of metastasis depends on a certain class of proteins that play an important role in the suppression of the metastatic phenotype. These proteins are encoded by metastasis suppressor genes, defined as genes that suppress metastasis in vivo without inhibiting the growth of the primary tumor.

The KISS1 gene, originally identified in cultured melanoma cells by Lee et al. in 1996. The authors indicated that it acts as a metastasis suppressor gene in a number of malignancies, including tumors of the thyroid, liver, stomach, esophagus, kidneys and bladder. It is believed that the function of a metastasis suppressor is realized by inhibiting chemotaxis and local invasion of malignant cells. Interestingly, KISS1 appears to be involved in the regulation of collagenase activity, which degrades the extracellular matrix, which is important in metastasis.

Expression and function of KISS1 are cell, tissue, and organ specific. It is reported that KISS1 expression in esophageal cancer with lymph node metastases was significantly lower than in tumors without metastases. Similar data are presented in studies of the dynamics of the progression of thyroid cancer. On the contrary, an increase in KISS1 expression was registered in breast cancer patients with aggressive tumors and high mortality.

MMP-4 and 9 have been shown to be involved in the degradation of the extracellular matrix and promote tumor growth and metastasis due to their angiogenic properties. The expression of these markers was found to correlate with the stage of the tumor process, metastasis to the lymph nodes, and survival. An inverse relationship was observed between the expression indices of KISS1 and MMP-4 and 9. KISS1 expression was significantly higher at stages I-II of tumor growth compared with stages III-IV, indicating an inverse relationship between KISS1 expression and the progression of non-small cell LC.

In addition, KISS1 expression was significantly higher in primary tumors than in metastases, supporting the notion that KISS1 acts as a metastatic suppressor in LC.

Our results show that KISS1 and MMP-4 can serve as informative biomarkers for assessing the metastatic potential and prognosis in patients with LC.

The mechanisms of suppression of KISS1 production during tumor metastasis have not been fully elucidated. Loss of heterozygosity in the region of chromosome 6q16.3-q23 correlated with a decrease in KISS1 expression. Interestingly, loss of heterozygosity on chromosome 6q16.3 has been identified in some cases of LC, indicating a similar mechanism for this phenomenon in the regulation of KISS1 expression in lung cancer. Decreased KISS1 expression has been associated with cancer progression and metastasis to other sites, as it was found that gastric tumors with low KISS1 expression often had distant metastases and recurred. In addition, the survival of patients with tumors expressing low levels of KISS1 was significantly lower than those with high expression. It has also been shown that high expression of KISS1 is an independent predictor of relapse-free and overall survival in patients with hepatocellular carcinoma.

The role of MMP-4 and 9 in the degradation of the extracellular matrix and stimulation of angiogenesis and growth of metastatic tumor cells is well known. Expression of MMP-4 and 9 increased with tumor size and its expression was significantly higher in LC cases with metastases compared to patients without metastases. In addition, MMP-4 and 9 expression was significantly higher in lymph node metastases than in primary lesions. Taken together, these results support the notion that MMP-4 and 9 play an important role in PD progression.

An inverse relationship was also found between the expression of KISS1 and MMP-4 proteins. These data indicate that KISS1 has an inhibitory effect on the process of RL metastasis, while MMP-4 stimulates metastasis. An imbalance between the expression of these two proteins seems to be an important reason for the spread of RL. Survival in patients

with low KISS1 expression was lower than in patients with high KISS1 expression, while low MMP-4 expression indicated a favorable prognosis. Thus, the assessment of KISS1 and MMP-4 expression provides important prognostic information for patients with LC.

In recent years, information has appeared on the role of a new member of the metalloproteinase family, MMP-26, the expression of which is also closely associated with the expression of CR. MMP-26 was first cloned from a human endometrial tumor cDNA library in 2000. The 3-genomic MMP-26 gene is located at the 11p15.3 locus and encodes a 30 kDa protein consisting of 261 amino acid residues. MMP-26 has been shown to cleave several components of the extracellular matrix, including fibronectin, type IV collagen, vitronectin, gelatins, and fibrinogen, as well as non-matrix proteins such as insulin-like protein-1, transforming growth factor, α-1 inhibitor proteases.

These properties reflect the possible key role of MMP-26 for tumor progression and formation of its angiogenesis. It has been described that MMP-26 expression is significantly higher in carcinoma tissues of various localizations than in normal tissues, although expression patterns differ among themselves in different tissues. MMP-26 expression in esophageal squamous cell carcinomas and ovarian carcinomas is significantly higher in infiltrative carcinomas than in non-invasive tumors; at the same time, expression of MMP-26 in the breast and prostate is significantly higher in preinvasive carcinomas than in infiltrating tumors.

It was shown that the level of MMP-26 expression is significantly higher in lung carcinoma tissue than in normal lung tissue, which reflects the possible role of MMP-26 overexpression in the development of LC.

It was also found that a high level of MMP-26 expression was 87.5% in patients with metastases to the lymph nodes, while in patients without metastases, a similar level was recorded only in 26.4%, which indicates a

significant role of MMP-26 in RL metastasis. At the same time, the mechanisms of MMP-26 overexpression that promote invasion and metastasis of cancer cells remain unclear.

It is noted that functionally blocking specific antibodies (anti-MMP-26 and anti-MMP-9) reduce the invasiveness of ARCaP tumor cells through the stabilization of fibronectin and type IV collagen.

MMP-26 expression in LC was found to be significantly higher at stages III–IV of tumor development than in patients with LC at stages I–II. In addition, it has been shown that higher expression of MMP-26 correlates with tumor recurrence, and lower expression correlates with relapse-free survival. MMP-26 expression is highly correlated with the depth of tumor invasion, lymph node metastasis, and distant metastasis.

MMP-9 has been shown to be secreted by various cells in an inactivated form as a 10 kDa propeptide that can be activated by other MMPs or the tissue plasminogen activator (tPA)-plasmin system due to its biological function of gelatin cleavage, which promotes cancer motility. cells.

In many studies, MMP-9 has been considered as an important prognostic marker and therapeutic target for cancer. Basic research has shown that high expression of MMP-9 is positively associated with the aggressiveness of malignant cells in solid tumors. With the exception of its well-known extracellular matrix degradation function, in vivo studies in MMP-9 deficient mice have shown that tumor metastasis is enhanced by implantation of MMP-9 expression in the bone marrow, which may promote angiogenesis to facilitate cancer cell migration.

In addition, MMP-9 can be obtained from stromal cells such as inflammatory cells and fibroblasts [31, 32]. Furthermore, although MMP-9 is a soluble enzyme, it can also bind to CD44 to activate the TGF-β pathway to enhance angiogenesis and epithelial-to-mesenchymal transition [33, 34]

and can also downregulate the expression of E-cadherin as a transcription factor [35].

Although all of the above data strongly support the fact that MMP-9 can increase the aggressiveness of cancer, there is still controversy among clinical observational studies. Retrospective analysis did not find any significant correlation between total serum MMP-9 expression and clinicopathological features or survival. Since MMP-9 activity could not be detected by ELISA, gelatin zymographic analysis was performed, and high MMP-9 activity was a risk factor for late tumor stage and distant metastases.

A meta-analysis was performed to determine the predictive value of MMP-9 in NSCLC; however, none of the included studies determined its tissue or serum activity. Our systematic analysis showed that MMP-9 expression in tumor tissue was positively associated with T category, tumor stage, and 3- and 5-year OS, while high serum MMP-9 levels were not associated with any of the these factors. options.

The increase in circulating MMP-9 is released by tumor or stromal cells, and its activity can be suppressed by many extracellular factors, such as MMP-1, 3, 7, 10, 26, trypsin-2, and neutrophil elastase [37]. Among them, tissue inhibitors of metalloproteinases 1 (TIMP1) are the most important endogenous inhibitors of MMP-9. TIMP1 belongs to a family of naturally occurring MMP inhibitory proteins that consists of four members (TIMP1, TIMP2, TIMP3, and TIMP4).

In fact, several studies have shown that TIMP1 not only blocks the extracellular effect of MMP-9 cleavage, but also inhibits the process of membrane protein cleavage and the regulatory effect of the MMP-9 cellular signal [38-40]. Although anti-MMP-9 behavior initially led to TIMP1 being considered as a tumor suppressor gene, researchers later found that TIMP1 also functions independently of MMP-9, promoting tumor growth and inhibiting apoptosis [41].

TIMP1 expression has been increased in solid tumors such as lung cancer, breast cancer, and colorectal cancer and has been recognized as a risk factor for poor outcome [42-44]. Its high expression not only prevents MMP-9 activity, but also potentiates the aggressiveness of malignant cells. Based on our own data and the meta-analysis performed here, serum MMP-9 activity was associated with tumor stage and metastatic status, but its total serum expression was not associated with clinicopathological parameters or survival. Therefore, circulating MMP-9 activity may be disproportionate to the amount detected by ELISA.

All these data allow us to consider the MMP family as potentially significant biomarkers for monitoring the progression of assessing the prognosis of the development of LC and other tumors.

In general, our studies of the KiSS-1 expression profile showed that the overexpression of KiSS-1 and its receptor correlates with the progression of LC.

The data obtained by us and other researchers suggest that KiSS-1, in addition to its diagnostic significance as a biomarker, may be a potential molecular target for targeted therapy to reduce the risk of lung carcinoma metastasis.

CONCLUSIONS

In recent years, there has been an increase in IHC studies performed, during which the expression of potential markers of malignancy is determined. Significant markers for the primary diagnosis of lung tumors are: CK7, CK20, thyroid transcription factor TTF-1, chromogranin A, synaptophysin, CD56; surfactant proteins; p63, CK5/6 and kisspeptins.

Kisspeptins are of great interest. Suppression of KISS1 expression has also been described in the progression of lung carcinomas and their metastasis. The KISS1/KISS1R signaling system can serve as a regulator of tumor metastasis and is a potential prognostic marker of tumor processes. However, so far, few works have been devoted to kisspeptins and their role in the pathology of the respiratory system (in particular, lung tumors).

An analysis of literature data indicates the relevance and timeliness of studying kisspeptins and their associated signaling molecules as modulators of tumor growth in general (and, in particular, the formation and development of lung cancer). In this context, they should also be considered as potential biomarkers for optimizing the molecular diagnosis of various types of lung cancer and personalized assessment of their development prognosis.

In this regard, the relevance of the chosen research topic is to conduct an in-depth study of the expression of kisspeptins and their associated molecules in lung carcinomas to assess the diagnostic and prognostic value of kisspeptins in tumor growth.

In the course of the study, correlations were established between the level of expression of kisspeptin-1 and its associated signaling molecules, caldesmon and MMP-4, in lung carcinomas. Correlations were found between the expression of kisspeptin-1, caldesmon, and MMP-4 in lung carcinomas and the degree of their differentiation. A monotonous increase in the expression of kisspeptin-1 and caldesmon and a monotonous decrease

in MMP-4 during the transition from a low to a high degree of differentiation were shown. The relationship between metastasis and expression of kisspeptin, caldesmon and MMP-4 in lung tumors was determined. An inverse relationship has been established between the presence of metastases and the expression of kisspeptin-1, caldesmon, as well as a direct relationship between the presence of metastases and the expression of MMP-4.

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The data obtained by us and other researchers suggest that KiSS-1, in addition to its diagnostic significance as a biomarker, may be a potential molecular target for targeted therapy to reduce the risk of lung carcinoma metastasis.

Thus, the results obtained allow us to propose an algorithm for using the expression indices of kisspeptin-1 and its associated signaling molecules (caldesmon and MMP-4) in lung carcinomas as markers of malignant tumor progression.

The algorithm is as follows. In the morphological study of all types of malignant epithelial lung tumors, in addition to the standard staining of preparations with hematoxylin-eosin, an immunohistochemical study of the expression of kisspeptin-1 and its associated signaling molecules (caldesmon and MMP-4) in the tumor tissue is performed. With positive immunostaining for the above biomarkers, the pathologist, in addition to making a histological diagnosis, indicates a variant of the possible development of the process for assessing the expression of kisspeptin-1, caldesmon and MMP-4.

FINDINGS

1. Correlations have been established between the degree of differentiation of lung carcinomas, gender, age of patients and secondary changes in neoplasms.
2. Correlations were established between the level of expression of kisspeptin-1 and its associated signaling molecules – caldesmon and MMP-4 in lung carcinomas.
3. Correlations between the expression of kisspeptin-1, caldesmon and MMP-4 in lung carcinomas and the degree of their differentiation were revealed. A monotonous increase in the expression of kispeptin-1 and caldesmon and a monotonous decrease in MMP-4 during the transition from a low to a high degree of differentiation were shown.
4. The relationship between metastasis and expression of kisspeptin, caldesmon and MMP-4 in lung tumors was determined. An inverse relationship was established between the presence of metastases and the expression of kisspeptin-1, caldesmon, as well as a direct relationship between the presence of metastases and the expression of MMP-4.
5. The possibility of using kisspeptin-1 as a biomarker for the prediction of metastasis of lung carcinomas has been established. Taking into account the nonlinearity of the relationship between the relative area of kisspeptin expression and the secondary manifestations of the tumor process makes it possible to increase the correctness of the prognosis of metastasis.
6. The results obtained made it possible to develop an algorithm for using the expression parameters of kisspeptin-1 and associated signaling molecules (caldesmon and MMP-4) in lung carcinomas as markers of the progression of malignant tumors.

RECOMMENDATIONS

1. The results obtained allow us to propose an algorithm for using the expression of kisspeptin-1 and associated signaling molecules (caldesmon and MMP-4) in lung carcinomas as markers of the progression of malignant tumors.

2. The algorithm is as follows: in the morphological study of all types of malignant epithelial lung tumors, except for the standard staining of preparations with hematoxylin-eosin, an immunohistochemical study of the expression of kisspeptin-1 and associated signaling molecules (caldesmon and MMP-4) in tumor tissue is carried out. With positive immunostaining for the above biomarkers, the pathologist, in addition to making a histological diagnosis, indicates the possible development of the process for assessing the expression of kisspeptin-1, caldesmon and MMP-4.

LIST OF ABBREVIATIONS AND SYMBOLS

DNA – deoxyribonucleic acid

IPF – idiopathic pulmonary fibrosis

ICC - – immunocytochemical method method

IHC - – immunohistochemical method

KP – kisspeptin

KP-10 – kisspeptin-10

KP-13 – kisspeptin-13

MMP/MMP - metalloproteinases

MMP-4 – matrix metalloproteinase type 4

NSCLC – is a non-small cell lung cancer

PCG - protein kinase G

RNA – ribonucleic acid

RL – lung cancer

RTK – receptor tyrosine kinases

cGMP cyclic – guanosine monophosphate

DDR – receptors of the discoidin domain

EGFR – epidermal growth factor receptors

H-caldesmon - high molecular weight caldesmon

iNOS – inducible nitric oxide synthase

KISS1R - kisspeptin receptor

LOH - loss of heterozygosity

L-caldesmon - low molecular weight caldesmon

NO - nitric oxide

OPN - osteopontin

TGF – tumor growth factor

TMA – tissue microchip

α-SMA - α-smooth muscle actin

WB – buffer for washing samples

α-SMA - α-smooth muscle actin

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