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CLINICAL ROLE OF miRNA BIOMARKERS IN LARYNGEAL SQUAMOUS CELL CARCINOMA (LSCC): LITERATURE REVIEW

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

Abstract

Introduction: Malignant transformations may result from gene dysregulation caused by genetic and epigenetic modifications. The use of miRNAs to treat cancer depends on the downregulation or upregulation of the miRNAs responsible for carcinogenesis (proliferation, angiogenesis, invasion, metastasis), providing the opportunity to suppress tumors. The purpose of this review is to list miRNAs identified so far as biomarkers in laryngeal squamous cell carcinoma (LSCC). This listing may help in predicting the evolution of miRNAs and developing anti-miRNAs and miRNA-mimics as anti-cancer therapies.

Material and methods: Relevant publications from 2008 to 2022 were retrieved from the PubMed and Google Scholar databases, using the key words miRNA (PubMed – 149,294 results; Google Scholar – 205,000 results), laryngeal squamous cell carcinoma (PubMed – 33,191 results; Google Scholar – 17,900 results), laryngeal squamous cell carcinoma biomarkers (PubMed – 6249 results; Google Scholar – 18,000 results), laryngeal squamous cell carcinoma miRNA (PubMed – 1338 results; Google Scholar – 17,500 results). Eventually 51 papers were selected for review.

Results: We identified 51 studies which have detected miRNA-based biomarkers for LSCC, and they are broadly reviewed here.

Conclusions: The identified laryngeal squamous cell-specific miRNA biomarkers appear to be promising for the detection, predicted course, and treatment of LSCC.

Key words: biomarkers • targeted therapy • miRNA • laryngeal squamous cell carcinoma • LSCC

KLINICZNA ROLA BIOMARKERÓW miRNA W RAKU PŁASKONABŁONKOWYM KRTANI (LSCC). PRZEGLĄD LITERATURY

Streszczenie

Wprowadzenie: Udowodniono, że transformacja złośliwa może wynikać z rozregulowania genów spowodowanego modyfikacjami genetycznymi i epigenetycznymi. Zastosowanie miRNA w leczeniu nowotworów może opierać się na regulacji w dół i w górę miRNA odpowiedzialnych za karcynogenezę (proliferację, angiogenezę, inwazję, przerzuty), co daje szansę na supresję nowotworu. Celem niniejszej pracy jest przedstawienie wybranych miRNA jako swoistych biomarkerów w raku płaskonabłonkowym krtani, które mogą pomóc w przewidywaniu jego ewolucji i w terapiach przeciwnowotworowych (anty-miRNA i miRNA-mimetyki).

Materiał i metody: Wszystkie istotne publikacje pobrano z baz PubMed i Google Scholar, korzystając ze słów kluczowych: miRNA (PubMed – 149 294 wyników; Google Scholar – 205 000 wyników), *laryngeal squamous cell carcinoma* (PubMed – 33 191 wyników; Google Scholar – 17 900 wyników), *laryngeal squamous cell carcinoma* biomarkers (PubMed – 6249 wyników; Google Scholar – 18 000 wyników), *laryngeal squamous cell carcinoma* miRNA (PubMed – 1338 wyników; Google Scholar – 17 500 wyników) z lat 2008–2022 i wybrano ostatecznie 51 prac.

Wyniki: W licznych badaniach wykryto specyficzne biomarkery oparte na miRNA dla raka płaskonabłonkowego krtani, które przedstawiono w tym przeglądzie.

Wnioski: Wykryte biomarkery miRNA specyficzne dla komórek płaskonabłonkowych krtani wydają się obiecujące w przewidywaniu i leczeniu raka płaskonabłonkowego krtani w przyszłości.

Słowa kluczowe: biomarkery • terapia celowana • miRNA • rak płaskonabłonkowy krtani • LSCC

Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck cancers in the world (6th of malignant tumors) and the second among respiratory tumors [1,2]. Among pharyngeal and laryngeal cancers, LSCC accounts for as much as 90% of cases [3]. It occurs in 3–4/100,000 people, more often in men, and is less common in children [4,5]. The tumor has a high tendency to recur [6]. Symptoms typically include hoarseness, impaired vocal function, breathing problems (dyspnoea), dysphagia, and odynophagia [5,7].

According to Marur et al. [8] and Woronkiewicz et al. [3], risk factors for LSCC include cigarette smoking, alcohol consumption, HPV (human papillomavirus), infections, exposure to asbestos, and family history. Analysis by Pagget-Bailly et al. [9] shows that polycyclic aromatic hydrocarbons, engine exhaust, textile dust, and work in the rubber industry also increase the risk of LSCC, although substances such as wood, cement dust, and formaldehyde appear not to have a significant impact. Zhou et al. [10] found a relationship between *Helicobacter pylori* infection and laryngeal cancer, which is probably caused by damage to the epithelium and mucosa, as well as inflammation, which leads to proliferation of epithelial cells. Their analysis showed that the risk of developing laryngeal cancer is 2.87 times higher in people with *H. pylori*. The clear cause of laryngeal cancer is gene dysregulation [11].

Over the years, surgical techniques of resection, imaging, chemotherapy, and radiotherapy have been increasingly refined, allowing for better treatment choices and improved chances of sparing healthy tissue and laryngeal function. Sometimes several treatments are necessary. However, the most important factors are the dimensions of the tumor, its location, and stage [1,2]. In the case of advanced lesions (T3 and T4 grade of the TNM scale), laryngectomy and laser microsurgery supported by radiotherapy or chemotherapy are most often used [12,13]. Advances in genetics has led to the development of predictive biomarkers and targeted therapies that help in diagnosis and treatment [11].

Power of the miRNA molecule

miRNAs (microRNAs; miRs) are small non-coding RNAs, a type of interference RNA capable of modulating gene expression by targeting mRNA (its translation inhibition or degradation of mRNA) which has an effect on cell homeostasis [14,15]. miRNA regulates 30% of the mRNA [16].

miRNA biogenesis involves the conversion of an inactive, primary miRNA transcript (pri-miRNA) into an active, mature miRNA. The primary miRNA transcript may contain several thousand nucleotides (most of them transcribed by RNA polymerase II). Almost half the miRNA has its own promoter located in chromatin domains, while the rest is located in introns (mirtrons) or exons. Within the cell nucleus, miRNA is cleaved and pre-miRNA is formed (precursor miRNA; approximately 70 nucleotides); this maturation process involves a microprocessor composed of Drosha nuclear RNAse III type protein and DGCR8 cofactor (DiGeorge Syndrome Critical Region 8; Pasha). The microprocessor-dependent process can bypass mirtrons and use splicing. pri-miRNA cleavage results in a stem-loop structure. pre-miRNA is transferred from the nucleus to the cytoplasm by Exportin-5. This causes the pre-miRNA to be cleaved into dsRNA with the help of Dicer and RNA polymerase III. The dsRNA contains the mature miRNA strand with its complementary strand and is bound by its dedicated domain by transactivating RNA binding protein (TRBP). With the participation of TRBP, Argonaute 2 (Ago2) is recruited, which is a component of RISC (RNA Induced Silencing Complex), a complex that selects the least stable RNA strands at the 5' end due to their lower susceptibility to degradation. Mature miRNA contains about 22 nucleotides (usually from 19 to 25) and is then loaded into the RISC. Inclusion in this complex allows for the inhibition of mRNA translation and degradation of the targeted mRNA. The miRNA binding sites are contained in the 3' untranslated regions (UTRs) of the mRNA. The imprecise complementarity of the miRNA to the target mRNA has its advantages – it allows the simultaneous inhibition of the expression of many different mRNAs [17–20].

Malignant transformation may be a result of gene dysregulation caused by genetic and epigenetic modifications (DNA methylations, histone modifications, RNA modifications and their stability and cleavage). Histone modifications (methylations and acetylations) can affect DNA accessibility, which is regulated by the two enzymes histone acetyltransferase (HAT) and histone deacetylase (HDAC). The process of histone acetylation increases gene transcription by loosening the nucleosomes and allowing the binding of transcription factors. Hence, histone deacetylase plays an important role in gene silencing as it causes condensation of chromatin and prevents transcription factors and proteins from binding. This enzyme also inhibits negative pathways for the development of cell differentiation and the maturation of antibody responses. miRNA and histone deacetylase can regulate each other, so it is important to maintain their cellular levels. Dysregulation of their levels can affect histone acetylation and miRNA expression, leading to pathology (and hence development and progression of the cancers). Gene demethylation typically leads to upregulation of genes that are involved in proliferation, migration, angiogenesis, and invasion of cancers. Thus, regulation of miRNA pathway proteins can inhibit dangerous cell proliferation. Although epigenetic modifications are inherited and reversible, miRNA is capable of targeting enzymes that modify epigenetic regulation via epi-miR-epi feedback. The correlation between miRNAs and epigenetics makes it possible to monitor gene expression profiles in cancers [20–23]. miRNA is divided into oncogenic miRNAs (OncomiR) and tumor suppressor miRNA (TS-miRNA, TS-miR). Depending on the specific tissue or cancer target, the same miRNA can function as a tumor suppressor or oncogene. Raised expression of OncomiR is responsible for the vast majority of tumors, so that miRNA therapy uses antisense miRNA (anti-miR) to repress OncomiR or restore TS-miR expression [23].

The main goal of developing miRNA therapy is to enable toxic-free delivery. Since miRNA regulates many cancer-related genes simultaneously, such a treatment seems promising. The aim is to make miRNA delivery organ-specific,

safe, and effective. Delivery to target cells is made easier due to the small size of the miRNA molecule. Limitations in delivery associated with miRNA mimics or antagonists are related to limited tissue bioavailability and permeability and instability of the load [24]. miRNA can be isolated from cells, tissues [22], and body fluids such as serum, plasma, saliva, and urine [23–26]. miRNA isolation kits provide a protocol for the isolation of total miRNA-containing RNA and a protocol for separating the small RNA enriched fraction (< 200 nucleotides) and the large RNA fraction (> 200 nucleotides). Isolation from body fluids is more difficult and requires a larger sample due to the lower content of miRNA in them [22]. Also, plasma has many inhibitors and proteins, which further reduces the effectiveness of this method. The binding of miRNA to protein complexes such as lipoproteins or RNA-binding proteins also interferes with the isolation and may hinder the quantitative measurements of miRNA [27].

Two types of strategies of delivery are differentiated – local and systemic. The advantage of local strategies is that the molecules go directly to the target tumor cells, thus eliminating the risk of toxicity, which is a common drawback of systemic delivery. However, the downside of local action is its low efficiency in late-stage metastatic cancer. The first strategy is the synthesis of chemically modified miRNAs or their antagonists such as anti-miRNA oligonucleotides (AMOs), which prevent destruction in the blood by miRNA inhibitors or mimics. Modified molecules also have a higher affinity for specific sequences. A second strategy is to synthesize a nanoparticle formulation that will pass into tumor tissues by passive diffusion. Due to leakage of tumor vessels, nanoparticles of the appropriate size are more likely to accumulate in the tumor compared to healthy tissues. A third option includes modification of the surface of the nanoparticles to allow specific binding to target cells and entry into tumor cells by endocytosis [24]. Following administration of miRNAs, unprotected miRNAs are rapidly degraded by serum nucleases in body fluids. While chemical modifications can protect miRNAs, these modifications may impair specificity and potentially introduce off-target effects. When the molecules reach their targets, the miRNA must separate to work [28].

The use of miRNAs is of great interest. Growing evidence from numerous studies suggests that miRNA-based therapies involving regulating its expression and activity are very promising.

Material and methods

Aim of study

The aim of the study is to identify LSCC-specific miRNAs as potential biomarkers of this cancer and their potential in diagnosis, prognosis, and treatment.

Eligibility criteria

We analysed studies published within the last 15 years, particularly the most recent. The focus was laryngeal squamous cell-specific miRNA biomarkers. We considered all types of observational studies.

Search strategy

The search was conducted using PubMed and Google Scholar. Keywords comprising "miRNA" (PubMed – 149,294 results; Google Scholar – 205,000 results), "laryngeal squamous cell carcinoma" (PubMed – 33,191 results; Google Scholar – 17,900 results), "laryngeal squamous cell carcinoma biomarkers" (PubMed – 6249 results; Google Scholar – 18,000 results) and "laryngeal squamous cell carcinoma miRNA" (PubMed – 1338 results; Google Scholar – 17,500 results) were used. The last time the source texts were reviewed was on 27/02/2023.

Data collection

Each author worked independently. One author focused on the potential of the miRNA molecule as a biomarker, the other searched for reliable and recent studies on specific miRNAs for LSCC. At the beginning, papers were selected and then abstracts and full articles for chosen studies were read.

The extracted data included the following information: identified LSCC biomarkers, their impact on carcinogenesis, and the possibility of their therapeutic use. There were 448,472 papers from the PubMed and Google Scholar databases which were retrieved from the last 15 years. Papers were searched by using combinations of the above key words. The publications were checked and included in the review beginning from the most recent. Only 1417 of the papers which we screened had titles relevant to the subject of this review and were focused on miRNA as a LSCC biomarker. After reading their abstracts, 252 of them appeared to be highly reliable – that is, studies on large groups of patients (diagnosed with LSCC but without other diseases) and determining cancer staging, comparative studies, and review papers based on the lastest studies. Full articles were then read. Only research works that received approval from a bioethics committee were included. Finally, 51 articles were selected due to their relevance to the topic of the work – the potential of miRNA as an LSCC biomarker. A flow chart of the search is shown in **Figure 1**.

The inclusion criteria used in the review were publication date (last 15 years, although we focused on the more recent studies), papers with full text available, compliance with the topic, approval of the bioethics committee, and high reliability. We excluded older studies, studies with low reliability, and those without the consent of a bioethics committee. Language of the papers was not a criterion.

Results

As shown in **Figure 1**, we included 51 studies in this review. This section summarises their findings.

It is assumed that in laryngeal cancer, the level of expression of genes such as miRNA-34a/c, miRNA-125b, miRNA-125a-5p, miRNA-138, and miRNA-153 is down-regulated, which increases the risk of development of laryngeal cancer and promotes tumor progression (Ding and Qi, 2019) [29]. Li and Liu [30] also mention down-regulation of miR-101, miR-129-5p, miR-139, miR-203, miR-205, miR-221, miR-24, miR-370, miR-375, miR-519b-3p, and

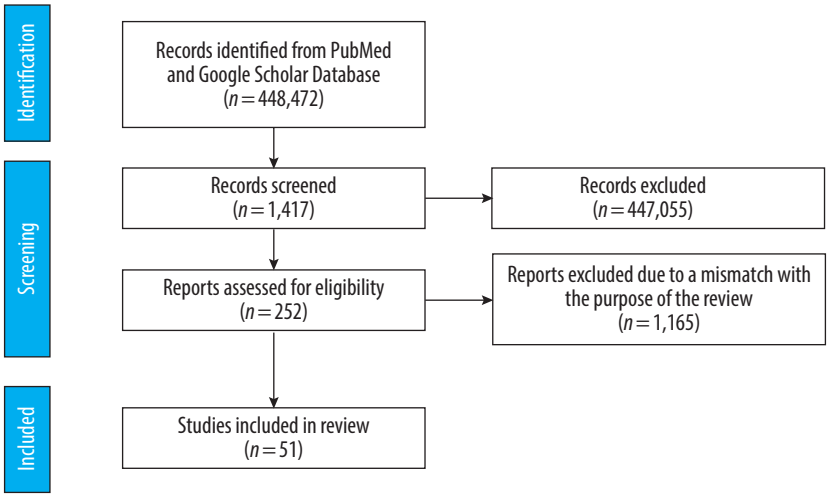

Figure 1. The search process

miR-874, while claiming that the expression of miR-106b, miR-1297, miR-155, miR-23a, miR-27a, miR-423-3p, miR-301a-3p is up-regulated.

A study by Grzelczyk et al. [31] showed that the expression of miR-31, miR-141, miR-149a, miR-182, LET-7a, miR-4853p, miR-122, and miR-33 were up-regulated, while the expression of miR-145, miR- 223, and miR-133a were down-regulated in blood serum of patients with LSCC, among which miR-31, LET-7a, miR-33 could be used as biomarkers (the study used RT-qPCR). The accuracy of these three biomarkers was 99–100%, while the sensitivity and specificity were in the range 98–100% [31].

Hu and Liu [32] hypothesize that miR-10a-5p and miR-34c-5p occur in laryngeal epithelial premalignant lesions (LEPLs) which helps classify low-risk lesions from high-risk ones. Because the tumor is initially asymptomatic, both miRs seem to be valuable markers in diagnosis and treatment [32].

Falco et al. [33] assume that miRNA abnormalities correlate with the occurrence of metastases in cancers (including LSCC); hence miRNAs could be used as biomarkers for the early detection of tumor progression and prediction of metastasis, enabling prompt intervention. In their analysis, the authors demonstrated, as a biomarkers in LSCC, the role of miR-138, miR-141, miR-145, miR-203, miR-204-5p, miR-143-3p, miR-101, miR-149, miR486-3p, miR-328, miR-376a, miR-493, miR-34a, miR-195, miR-449a, and miR-130a in downregulation, while miR-744-3p, miR-21, miR-618, miR-542-5p, let7b, miR-135a, miR-20b, miR-324-3p, miR-886-5p, miR-129-5p, miR-155, and miR-632 were involved in upregulation. These biomarkers were associated with tumor staging, progression, lymph node involvement, and metastasis, and might be useful in prognosis and estimated survival [33].

Downregulation of miR-141 has been associated with lymph node metastasis [34]. Chen et al. showed that this miRNA targets the HOXC6 gene and is downregulated in LSCC [34]. The increase in miR-141 expression may affect the reduction of HOXC6 and thus the inhibition of the TGF-β (transforming growth factor β) signaling pathway. In this mechanism, epithelial-mesenchymal transition (EMT) is also inhibited, which determines the inhibition of the spread of laryngeal cancer cells and metastases. Therefore, this mechanism might be used in anticancer therapies for laryngeal cancer [34].

Distant metastases might be associated with downregulation of miR-138 and upregulation of ZEB2. miR-138 inhibits the proliferation of LSCC cells by regulating the invasion factor ZEB2, so increasing miR-138 could decrease ZEB2 and inhibit LSCC invasion. miR-138 seems to be a promising therapeutic target [35].

Zhao et al. [36] hypothesise that miR-145 inhibits MYO5A in LSCC, which is associated with the inhibition of progression (invasion) and metastasis to neck lymph nodes and the promotion of apoptosis. Thus, miR-145 may be a predictive marker of metastasis and prognosis (where low levels of miR-145 may predict a worse prognosis). In the future, their findings could allow the determination of MYO5A in the serum of patients with LSCC and for planning therapy (including surgery). However, Zhao and colleagues point out that their study included only 132 samples and more research is needed to confirm the results [36]. This opinion is shared by Yu et al. [37], who further state that miR-145, which is downregulated in LSCCs, also regulates SOX2 in a negatively correlated manner, thereby causing cell proliferation and migration.

A study by Tian et al. [38] showed that miR-203 expression is lower in LSCC tissues (downregulation) and is associated with higher ASAP1 expression, which regulates EMT, E-cadherin, and CSC (cancer stem cell marker) levels; hence miR-203 is a tumor suppressor – the lower the expression of miR-203, the lower the miR-203 differentiation (and the higher the tumor stage, the more lymph node metastases and thus the worse the prognosis). In turn, overexpression of miR-203 inhibits cell proliferation and tumor spread and induces apoptosis, which makes

miR-203 a promising biomarker and a potential target for therapeutic intervention in LSCC [38].

According to [39] upregulation of miR-204-5p in the T3 and T4 stage of LSCC can reduce cell proliferation, invasion, and metastasis by downregulating the expression of FOXC1 (Forkhead C1) located in the nuclei and cytoplasm of tumor cells. The higher the stage, the higher the level of FOXC1 was observed. Increased expression of miR-204-5p or decreased expression of FOXC1 results in inhibition of tumor cell EMT and reduces tumor invasion. In an in vivo study of mice injected with HEp-2 or TU-177 cell xenografts, miR-204-5p inhibited tumor progression, making it a potential target for LSCC therapy [39].

Zhang and Cao [40] showed that among the miRNAs, the expression of miR-143-3p is most reduced in LSCC tissue compared to surrounding healthy tissues. It was found that the lower the miR-143-3p level, the higher the malignancy of the tumor, while upregulation of miR-143-3p is associated with reduced progression, reduced LSCC cell proliferation, and induction of apoptosis (via EMT inhibition). They found that k-Ras is a target of miR-143-3p and suppression of this miR can be lifted by upregulating k-Ras through targeting the signaling pathway miR-143-3p/k-Ras/Raf/MEK/ERK – mitogen-activated protein kinase k-Ras/Raf/protein kinase (MEK) and extracellular signal-regulated kinase (ERK) – which can reduce LSCC development and invasion. It also makes miR-143-3p a prognostic marker and a potential therapeutic target.

Xu et al. [41] conducted a study on miR-149 expression in patients after LSCC resection. The study was carried out on an in vitro culture of the Hep-2 cancer cell line, where the role of miR-149 was tested. Lower miR-149 expression was observed in LSCC tissues than in vocal cord polyp tissues. In addition, miR-149 expression was closely related to patient survival (lower expression = lower survival) and cancer development. In turn, inhibition of cell cycle progression was associated with ectopic expression of miR-149 in Hep-2 cells.

Shuang et al. [42] hypothesise that miR-195 may inhibit the growth and proliferation of laryngeal squamous cell carcinoma cells by targeting DCUN1D1 (which is upregulated in LSCC). The lower the expression of miR-195, the worse the prognosis of LSCC. Liu et al. [43] also assumed that miR-195 is strongly associated with the prognosis for a patient with LSCC – low expression of miR-195 is associated with growth, lymph node metastases, and a higher stage of cancer (LSCC cells have been shown to significantly reduce miR-195 compared to a normal human cell line keratinocytes). In addition, downregulation of the target gene for miR-195, ROCK1 (rho-associated kinase 1), had the same effect as miR-195 overexpression. The discovery of this molecular mechanism may open the way to new treatments for LSCC [43]. A study by Pang et al. showed also the downregulation of miR-195 in primary LSCC tumors [44]. The study was performed using miR-195 mimics to determine cell proliferation, survival time, migration, and invasion capacity as well as apoptosis in the AMC-HN-8 cell line; genetic analysis showed that upregulation inhibited pathological processes compared to the control group (miR-195 inhibitors) and negative control. This was done by inhibiting VEGFR2, the Raf/MEK/ERK pathway, SRC, and p-FAK in cell lines (which are responsible for proliferation, migration, invasion, and inhibition of apoptosis of LSCC cells). The study pointed to the role of miR-195 in the pathogenesis of cancer, as well as the potential of miR-195 as a biomarker and in targeted therapies [44].

A study by Li et al. [45] and Falco et al. [33] showed that among miR-7-1-3p, miR-196a/b, and miR-744-3p, high levels of miR-744-3p are associated with metastasis of LSCC to regional lymph nodes. Inhibition of miR-744-3p resulted, in mice, in a reduction of invasion, migration, and the number of metastatic nodules in the lung. According to the findings, the mechanism involved in the inhibition of PDCD4 (programmed cell death 4) by miR-744-3p (which is a p65 suppressor) – NF-κB – is responsible for this process. PDCD4 can suppress MMP-9 expression by inhibiting AKT activation. Reducing the expression of miR-744-3p may result in the return of expression of phosphatase and PTEN (a tensin homologue) by inhibiting AKT and MMP-9, which may effectively inhibit tumor metastasis through the AKT/mTOR and NF-κB pathways and reduce tumor aggression [33,45].

The important role of miR-31 as a LSCC biomarker is highlighted by Grzelczyk et al. [31] and also emphasized by Dioguardi et al. [46] and Yang et al. [47]. The overexpression of miR-31 is correlated with a poor prognosis (and is hence a survival biomarker). They are therefore hopeful that discoveries of miRNA biomarkers may well lead to new therapeutic approaches in the future [31,46,47].

Ma et al. [48] found that miR-140-3p is significantly associated with LSCC, in which it is downregulated. In turn, overexpression of miR-140-3p (using mimics) may inhibit cell proliferation and induce apoptosis of LSC-1 cells (an LSCC cell line). The potential anticancer effect of miR-140-3p could be used as a biomarker in LSCC (and possibly for therapeutic purposes) [48].

Re et al. [49] showed that miR-21-5p is upregulated and miR-let-7a is downregulated in LSCC tissue compared to healthy tissues around the neoplastic lesion. Downregulation of miR-34c-5p is associated with shorter survival and risk of recurrence. miR-21-5p plays an important role in tumor invasion, metastasis and growth, with its upregulation associated with tumor progression. These effects are likely related to the downregulation of PDCD4, TPM1, maspin, and PTEN, which are tumor suppressors. In addition, the authors found diagnostic value in the expression ratio of miR-21-5p to miR-let-7a – this ratio seems to be more important in distinguishing between healthy and cancerous tissue than each miRNA separately. miR-let-7a may be important in assessing the predisposition of a patient with LSCC to lymph node metastases. Re and colleagues assume that these three miRNAs are crucial in carcinogenesis [49].

The study by Janiszewska et al. [50] on LSCC cell lines and primary tumors showed overexpression of miR-21-3p, miR-21-5p, miR-1246, miR-1290, and miR-4317, and downregulation of miR-100-5p and miR-133a (compared to controls without LSCC). The researchers particularly

took into account miR-1290, which regulates the level of MAF and ITPR2 proteins at the mRNA level. Inhibition of miR-1290 expression resulted in an increase in MAF in cells, but a lack of ITPR2 protein was demonstrated (although this, according to the authors, may be due to the inhibition of ITPR2 by other miRNAs involved in the pathogenesis of LSCC) [50]. These ideas require further confirmatory research.

Discussion

The studies presented here have identified numerous potential miRNA biomarkers of LSCC. In summary, research has shown that the following are upregulated in LSCC: miR-10a-5p, miR-20b, miR-21, miR-21-3p, miR-21-5p, miR-23a, miR-27a, miR-31, miR-33, miR-106b, miR-122, miR-129-5p, miR-135a, miR-149a, miR-155, miR-182, miR-301a-3p, miR-324-3p, miR-423-3p, miR-542-5p, miR-618, miR-632, miR-744-3p, miR-886-5p, miR-1246, miR-1290, miR-1297, miR-4317, miR-4853p, and miR-let-7b. At the same time, the following are downregulated: miR-24, miR-34a, miR-34c-5p, miR-100-5p, miR-101, miRNA-125b, miRNA-125a-5p, miR-130a, miR-133a, miR-138, miR-139, miR-140-3p, miR-141, miR-143-3p, miR-145, miR-149, miRNA-153, miR-195, miR-203, miR-204-5p, miR-205, miR-221, miR-223, miR-328, miR-370, miR-375, miR-376a, miR-449a, miR486-3p, miR-493, miR-519b-3p, miR-874, and miR-let-7a. These biomarkers may be valuable prognostic indicators for LSCC. Studies have shown correlations of individual miRNAs with the progression and stages of cancer: lymph node metastases (miR-141, miR-145, miR-195, miR-203, miR-204-5p, miR-744-3p), distant metastasis (miR-138, miR-744-3p), and survival time (miR-31, miR-143-3p, miR-149, miR-195) [30–50]. miR-10a-5p and miR-34c-5p identified in laryngeal epithelial premalignant lesions may also have potential as LSCC biomarkers, especially in people from risk groups [32].

The differences in the results of studies on the direction of miR-129-5p, miR-141, and miR-let-7a regulation are controversial. Discrepancies were noted in the studies: according to Grzelczyk et al. [31], miR-141 is upregulated while according to Chen et al. [34] and Falco et al. [33] it is downregulated. Resolution will require further studies on miRNA expression in LSCC (although [34] did demonstrate an association of downregulation of miR-141 with lymph node metastasis [31,33]). Similarly, according to Re et al. [49], the marker miR-let-7a is downregulated, whereas according to [31] miR-let-7a expression in LSCC is increased [31,49]. Similarly, the issue of miR-129-5p expression in tumor tissues is contentious. Upregulation was documented by [33] and [30], while downregulation was observed by others [33,51]. Li et al. [51] demonstrated a significant increase in miR-129-5p expression in LSCC compared to non-cancerous tissues and a correlation of increased expression with cancer stages (T3 and T4 and the presence of lymph node metastases).

The general opinion is that specific miRNAs may allow rapid diagnosis of LSCC (even before the onset of disease symptoms). miRNA antagonists and mimics have the potential for early cancer prognosis, prompt therapeutic intervention, and development of targeted therapies.

Limitations

There are still only a few studies on miRNAs specific for LSCC, most of which are framed as hypotheses. Another limitation is the discrepancy over the direction of expression of individual miRNAs. It is therefore necessary to conduct more multicenter studies involving more patients with LSCC in order to identify and confirm specific biomarkers.

Conclusions

The discovery of a correlation between miRNA dysregulation and the onset, progression, and prognosis of cancers (including LSCC) opens up many therapeutic possibilities, mainly based on the antagonisation or mimicry of miRNA specific to a given cancer. In the future, the identified LSCC-specific miRNA biomarkers appear promising for the treatment of LSCC. There are still many challenges and the need to conduct related research to ensure the highest possible safety and best treatment options for patients with laryngeal cancer. The detection of biomarkers requires thorough clinical and pathological optimisation and unification of standards.

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