Chapter 14

Noncoding RNAs in Lung Cancer Angiogenesis

Ioana Berindan-Neagoe, Cornelia Braicu, Diana Gulei, Ciprian Tomuleasa and George Adrian Calin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66529

Abstract

Lung cancer is the major death-related cancer in both men and women, due to late diagnostic and limited treatment efficacy. The angiogenic process that is responsible for the support of tumor progression and metastasis represents one of the main hallmarks of cancer. The role of VEGF signaling in angiogenesis is well-established, and we summarize the role of semaphorins and their related receptors or hypoxia-related factors role as prone of tumor microenvironment in angiogenic mechanisms. Newly, noncoding RNA transcripts (ncRNA) were identified to have vital functions in miscellaneous biological processes, including lung cancer angiogenesis. Therefore, due to their capacity to regulate almost all molecular pathways related with altered key genes, including those involved in angiogenesis and its microenvironment, ncRNAs can serve as diagnosis and prognosis markers or therapeutic targets. We intend to summarize the latest progress in the field of ncRNAs in lung cancer and their relation with hypoxia-related factors and angiogenic genes, with a particular focus on ncRNAs relation to semaphorins.

Keywords: noncoding RNAs, angiogenesis, lung cancer, semaphorins, therapy

1. Introduction

1.1. Noncoding RNAs (ncRNAs)—definition, biogenesis and classification

The noncoding RNAs evolved in the last few years as important regulators of numerous physiological and pathological processes with increased attention regarding cancer diagnosis, prognosis, and therapeutics [1]. The concept known as "dark matter" defined by the lack of function and lack of genetic information is now long gone, being replaced by the regulatory ncRNAs involved in cancer development and progression [1]. The transcription

of the noncoding regions produces RNA sequences that can vary in size, short, mid-size, and long noncoding RNAs, and are able to influence the expression of tumor suppressor or tumor promoting coding genes, activity that further classifies this class of RNAs into oncogenic or tumor suppressor sequences [2].

The noncoding niche is rapidly expanding as new sequences are discovered and characterized. The ncRNAs, as their name underline, are RNAs that do not codify for proteins but new molecular concepts are revealed regarding the interplay between these types of RNA sequences and protein coding genes [3]. ncRNAs are also known as regulatory RNAs.

One of the most studied ncRNAs class is represented by microRNAs (miRNAs) that are small single-stranded nucleotide sequences (18–22 nucleotide length) capable of gene regulation through sequence complementarity [2], being involved in all hallmarks of cancer [4]. The biogenesis mechanism is presented in **Figure 1**. The discovery of miRNAs has enabled new

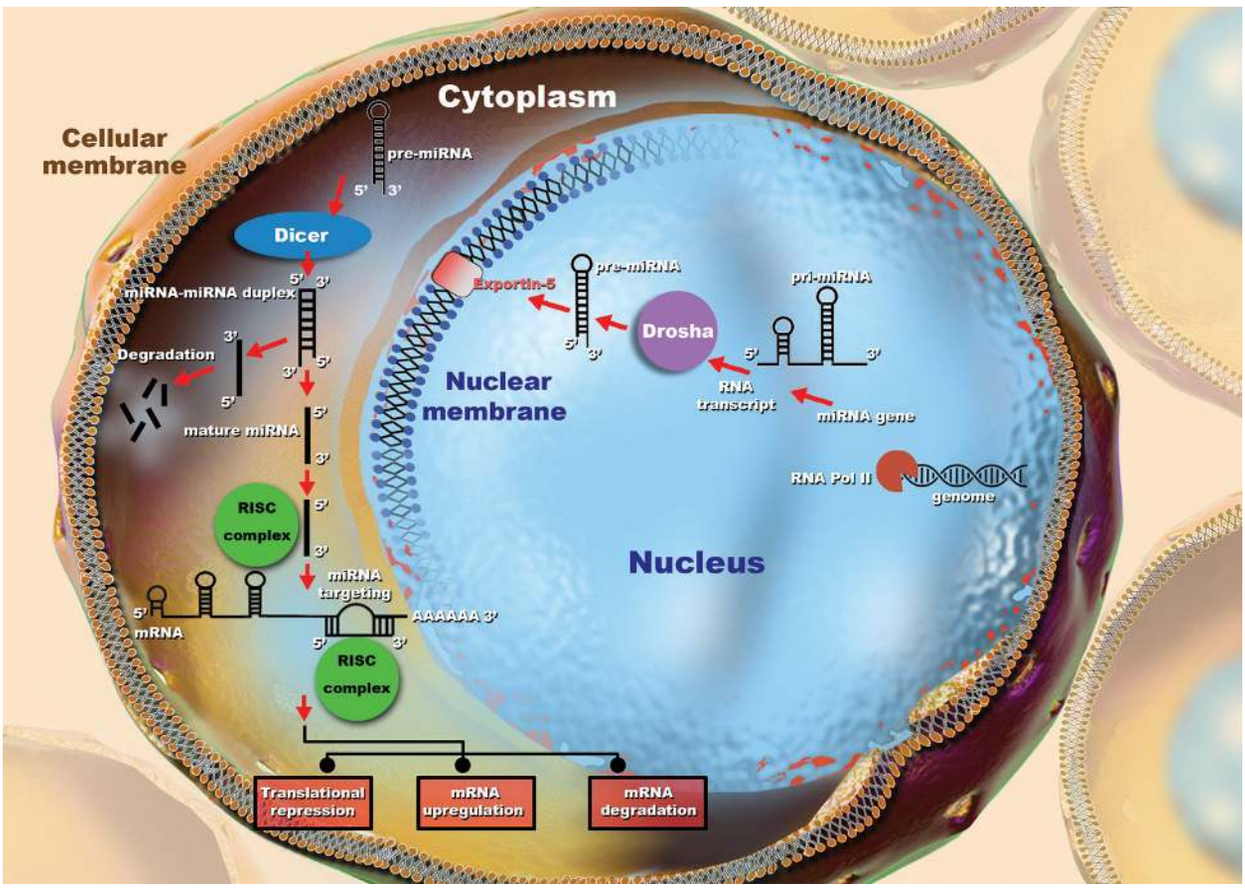

Figure 1. miRNA biogenesis mechanism. microRNAs are situated in the genome of the host as individual transcriptional units but also as clusters of a number of distinct microRNAs. For the first step, RNA polymerase II transcribes the target sequence resulting in a primary transcript named pri-miRNAs. This unprocessed sequence is then subjected to the activity of RNase III-type enzyme Drosha that transforms the pri-miRNA sequence into a transcript of approximatively 70 nt, pre-miRNA. This precursor is then transferred in the cytoplasm via Exportin-5, followed by another miRNA manipulation step governed by the RNase III protein Dicer, resulting in a double stranded RNA called miRNA-miRNA duplex. The less stable strand is further captured by the RISC complex, association that facilitates specific gene regulation through complementary interactions.

noninvasive diagnosis methods and also has conducted towards the development of more targeted therapeutics alternatives in a large number of cancers and other pathological states [4, 5]. Despite numerous discoveries in the ncRNA field, the two main noncoding fronts in cancer are still represented by microRNAs and the more recent characterized long noncoding RNAs (lncRNAs) [6]. As the technology advances, these last sequences are increasingly mentioned in pathological contexts, where differential expression levels are associated with malignant states and other diseases [6]. Despite the associations between lncRNAs expression patterns and different types of cancers, there are still many unknowns regarding the complex mechanism of action.

MiRNAs revolution has stimulated the investigation of other types of small ncRNAs such as small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs) [3, 7, 8]. These last two types of molecules are similar to miRNAs in length and function, where siRNAs mediate posttranscriptional inhibitory processes and piRNAs act particularly on transposable elements and are capable of forming complexes with Piwi proteins [7, 8]. piRNAs transcribed from kiwi clusters together with Piwi proteins are capable of transposon modulation through interruption of the specific transcript that will be no longer able to exercise their specific activity. Other types of ncRNAs, circularRNA (ciRNA) are formed through base pairing of intronic repeats that ends up with a complete circular fragment that is able to act as a miRNA sponge through complementary interactions [3, 9].

Supplementing the complex regulatory networks of miRNAs, ciRNAs have recently emerged as new cancer modeling tools through miRNA targeting, escaping from the initial characterization as transcriptional "noise" [9, 10]. These types of transcripts are ubiquitous present in eukaryotic cells and competitively bind microRNAs sequences, functioning like an inhibitory sponge; process that could attribute a significant therapeutic potential to these circular fragments [9, 10, 11, 12]. In this sense, specific microRNAs are eliminated from the regulatory networks, influencing the expression scheme of target genes. Competitive endogenous RNA (ceRNA) describes a new mechanism of gene regulation, being involved in physiological and pathological processes [13].

The traditional concept that RNA molecules are just intermediary sequences between DNA and proteins is now replaced with more advanced molecular data, where short- and long-noncoding sequences play a key role in normal development and disease progression [14]. SiRNAs and miRNAs are similar in length, approximatively 22 nucleotides, and are both processed by Dicer through cleavage. SiRNAs are derived from complementary dsRNA duplexes, where miRNAs originate from imperfect RNA hairpins from short introns or long transcripts [15–18]. Both small noncoding types of sequences associate with Argonaute proteins in order to manipulate gene expression (generally through 3′UTRs) [19], although siRNAs are also involved in viral defense and transposon regulation. piRNAs are the longest fragments from the small RNAs group, having approximatively 26–30 in length. This class associates with PIWI-clade Argonaute proteins in order to guide transposon activity and chromatin status [15, 17]. Long noncoding RNA group consist in all RNA sequences that are not responsible for protein generation and their length exceed

200 nucleotides, being further grouped in concordance with their genomic localization: intronic, intergenic, sense, and antisense ncRNAs to host gene locus [6, 20]. Biogenesis of lncRNAs is very similar with the processing activity of mRNAs molecule, being transcribed by RNA Pol II and also being subjected to the same epigenetic modifications and splicing signals. The functional roles of lncRNAs are more extended than in the case of small ncRNAs, a significant part being still incompletely understood. Briefly, this type of sequences is not so well conserved as miRNAs and also can control gene activity at different levels in a more complex scheme [2, 6, 16].

2. Lung cancer—molecular classification and survival rates

Lung cancer occupies the first place regarding the mortality rates from the oncological field, being characterized by an aggressive profile that ends with numerous deadly metastatic sites. One of the main reasons for the high mortality rates consists in the late diagnosis [21]. According to the characteristics of the cancer cells, this malignancy presents itself in two major forms, one being *small-cell lung cancer* (SCLC), and the other being named *non-small-cell lung cancer* (NSCLC) according to the histological classification and another rare subtype, *lung carcinoid tumor* (*LCT*) [22, 23]. NSCLC ranks as the number one diagnosed type of lung cancer in the oncological field, being further divided into three histologic types: *squamous cell carcinoma*, *large-cell carcinoma*, *and adenocarcinoma*. *Adenocarcinomas* represent the most common subtype of NSCLC, with an incidence of 35–40% from all lung cancer cases, being the most lethal type of cancer in male population, and the second in women. This type of pulmonary malignancy frequently presents distant metastases and pleural effusions. Between a quarter and 30% of all lung cancer cases belong to the squamous cell carcinoma category. These particular tumors are mostly located in the central areas of the lungs, and were shown to be connected to tobacco smoking [24]. Lung carcinoid tumors are very rare and represent about 5% of the lung cancers which grows very slowly and are rarely associated with metastasis [25]. Despite the frequency drop, pulmonary tumors remain the major cause of death and morbidity around the world, being very aggressive and refractory to standard oncologic therapy [26], due to the late diagnostic [27].

Environmental and occupational exposure to different agents and an individual's susceptibility for these agents were associated with a risk of lung cancer in approximately 9–15% of cases. The cigarette smoke is the primary risk factor for the development of lung cancer and is estimated to be responsible for approximately 90% of all lung cancers [24], followed by asbestos [28], and radon [27]. More than 300 harmful substances with 40 known potent carcinogens were discovered in tobacco smoke.

The classical therapeutic strategies like surgery and chemotherapy or radiation fail to accomplish their purpose in advanced pathological states. In the case of patients diagnosed early in the disease, the chances of survival are more promising, being observed a partial response to drugs based on platinum. However, even in this case, the final outcome is not necessary a positive one due to acquisition of treatment resistance. According to National Cancer Institute, survival rates for early stages of NSCLC are extremely low compared to other types

of cancer, where the rate for the late stages of the same malignancy can reach even 1%: the 5 years survival rate for stage IA is approximately 49%, 45% for stage IB, 30% for stage IIA and 31% for IIB. The next stages, IIIA and IIIB, are associated with even more dramatically numbers, 14% and 5% respectively (**Figure 2**). For the case of metastatic lung cancer, where the tumor has spread within different body sites, the survival rates are extremely low (1%) [29, 30]. Therefore, a critical part of lung cancer management is represented by the discovery of specific molecular carcinogenic pathways in order to precisely target key molecules that are responsible for tumor development and avoid treatment resistance. ncRNAs study represents an important research direction for achieving these goals.

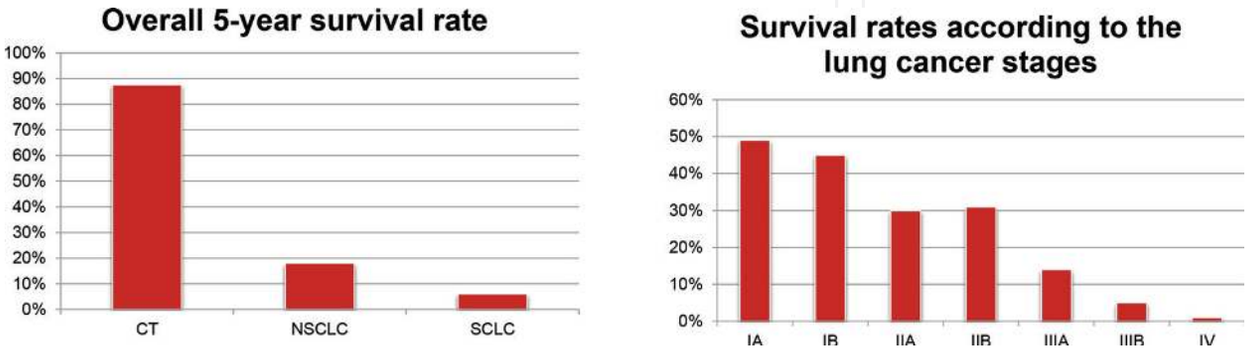

Figure 2. The overall survival rates associated with different lung cancer subtypes (NSCLC, SCLC) and the 5-year survival rate based on lung cancer stages.

3. Angiogenesis—beyond hallmarks of lung cancer

Nowadays, the cancer hallmarks are at the center of carcinogenesis: prolonged proliferation signals, escaping of growth inhibitors, apoptosis inhibition, indefinite replicative potential, vascular network development (angiogenesis) and activation of cell invasion, and thus metastasis (**Figure 3**) [31]. Although all of these hallmarks represent key elements without which tumorigenesis could not more or less advance, angiogenesis surpasses this listing of malignant processes: without the ability to receive oxygen and nutrients and evacuate waste products, the spreading of the tumor is naturally restricted. Moreover, the vessel network is one of the invasion routes used by transformed mesenchymal cell in order to evade from the original carcinogenic site and invade other tissues [31]. All these features stand at the base of the therapeutic concept, where angiogenesis is one of the main signaling pathway targeted in the treatment of cancer patients, including individuals with lung cancer. Inhibition of this malignant progression pathway through exogenous administration of targeted agents in the form of ncRNAs/anti-ncRNAs will enable the proper management of tumor spreading and will serve as a feasible therapeutic strategy for lung cancer [33].

The most promising proangiogenic target in lung cancer is VEGF (vascular endothelial growth factor), more precisely the interaction of VEGF with the transmembrane receptors or receptors downstream the signaling pathways. However, prolonged exposure to VEGF/VEGFR inhibitors may force tumor cells to find alternative pathways for vascular

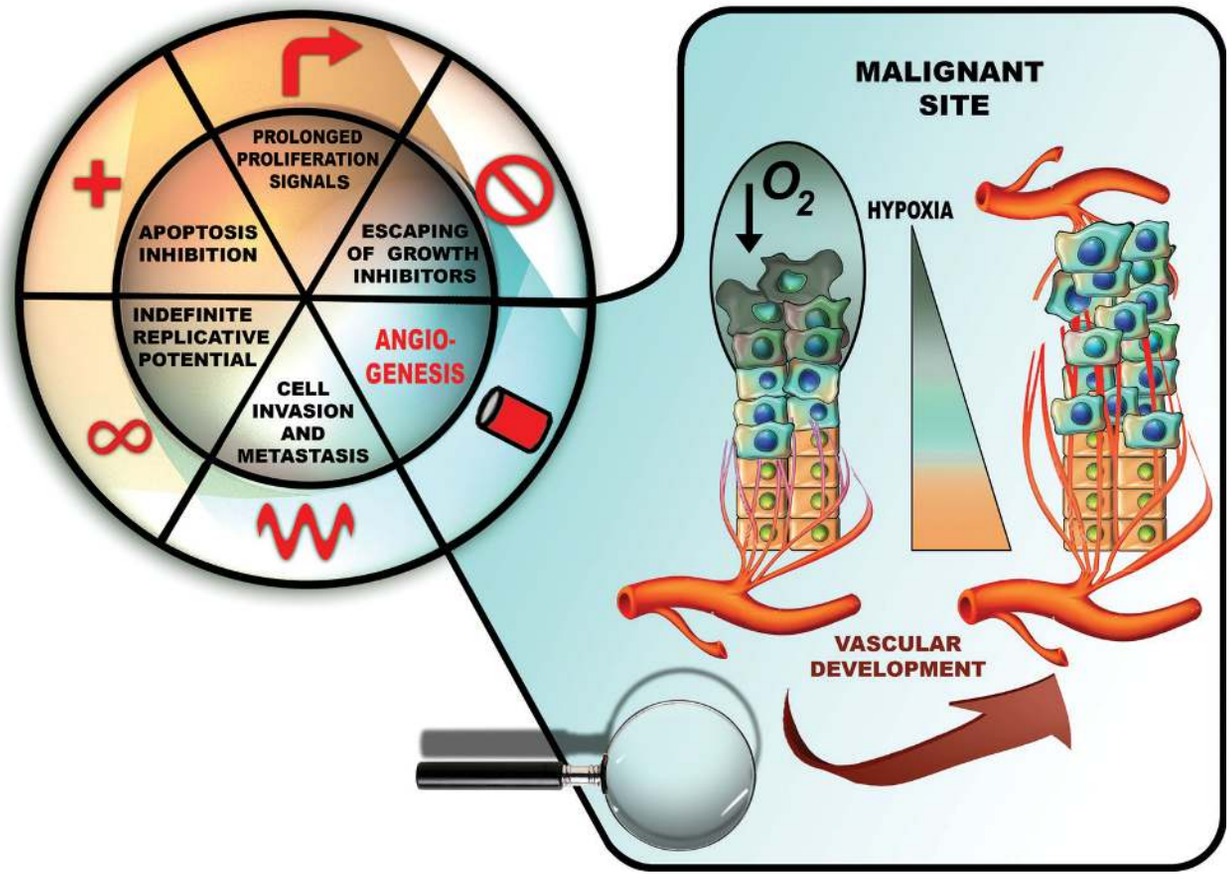

Figure 3. Lung cancer hallmarks with focus on angiogenesis.

development [34]. Additionally, some other angiogenic pathways have been explored with the same purpose, where FGFRs (fibroblast growth factor receptors), angiopoietin, PDGFRs (platelet-derived growth factor receptors), and, in the last few years, semaphorins and the related receptors captured the attention [32, 34]. The metastatic cascade, a multievent process that leads to the spreading of the tumor cells to numerous sites in the organism and causes death, represents the main challenge in cancer treatment and angiogenesis plays a major role in this progression [35].

3.1. Implication of ncRNAs in regulation of lung cancer angiogenesis

As a result of the limited success of the classical antiangiogenic therapies targeting VEGF and its related receptors [35, 36], researchers have deepened their knowledge by analyzing the expression of ncRNAs sequences in this pathology (**Figure 4**) [37, 38]. The mechanism of lung cancer angiogenesis is far from being completely deciphered and implicit the process of therapeutic inhibition via ncRNAs remains to be further investigated. Targeting ncRNAs will enable a more precise treatment and will avoid compensatory mechanisms retrieved in lung cancer [2, 37, 38].

http://dx.doi.org/10.5772/66529

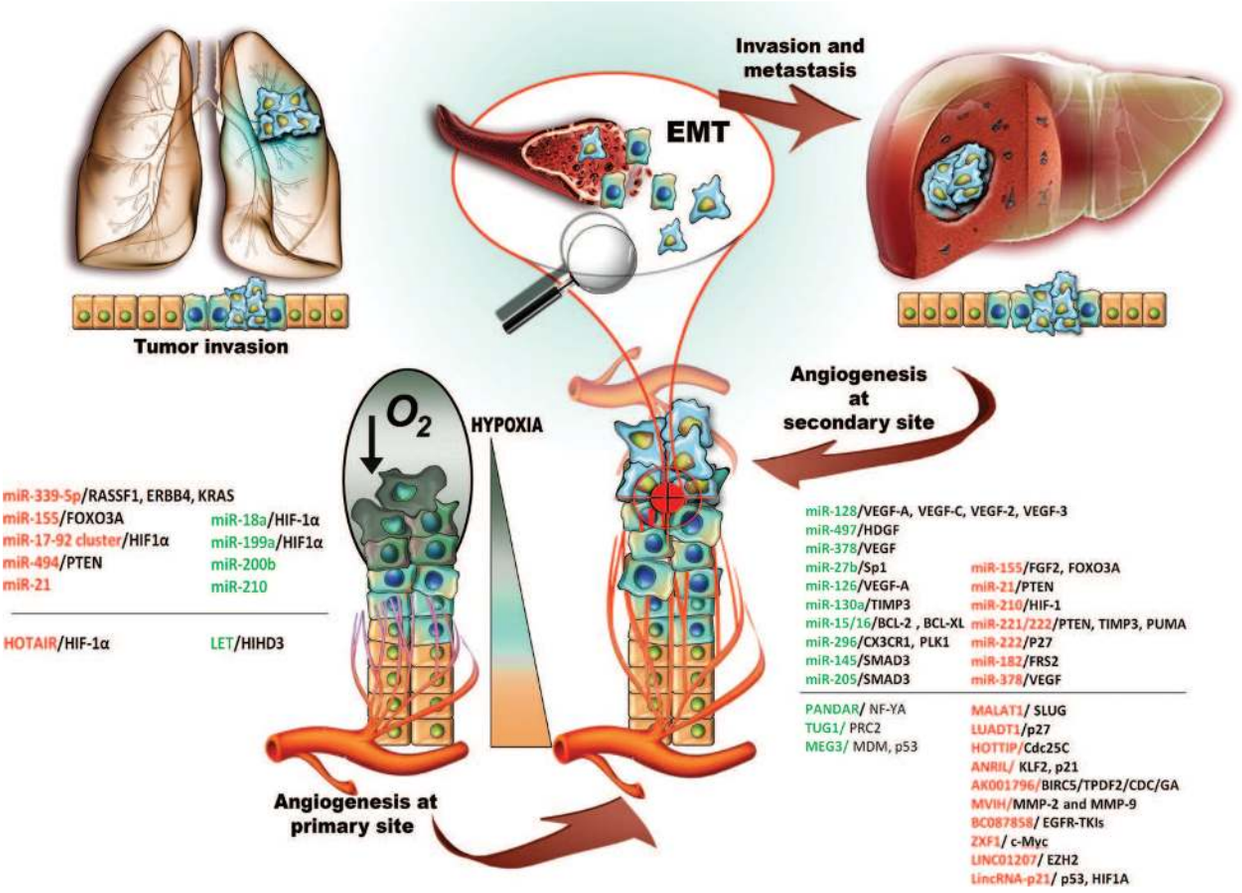

Figure 4. Evolution of vascular network within lung cancer. Malignant cells lacking nutrients and oxygen enter in hypoxic stress, state that promotes the signaling pathways related to angiogenesis in order to sustain cell proliferation. The same process is present at the metastatic sites, where mesenchymal cells that went through epithelial to mesenchymal transition are establishing new malignant formations. The complex malignant scheme is strictly regulated by noncoding RNAs (miRNAs and lncRNAs). Red - overexpressed ncRNAs; Green- downregulated ncRNAs.

3.2. miRNAs related to lung cancer angiogenesis

Among all types of ncRNAs, miRNAs molecules are the most intensive studied in what regards novel cancer therapies. Although the majority of the studies are concentrated on oncogenic miRNA inhibition via exogenous delivery of complementary (antisense) sequences through different vectors, it seems that another therapeutic alternative consists in miRNA replacement. This last type of targeted treatment may be even more effective due to the fact that the predominant pathological model consists more in dowregulated tumor suppressor sequences than overexpressed oncogenic genes [5, 21, 39].

Until this moment, several miRNA patterns involved in different lung cancer processes such as cell proliferation, resistance to therapy, invasion, metastasis, and angiogenesis have been identified. We will focus on some important miRNAs that presented the most aberrant expression related to lung cancer angiogenesis (**Tables 1** and **2**).

No.	Name	Location	Length (nucleotides)	Expression level	Target gene	Activity	Possible role in lung cancer angiogenesis	Clinical potential	References
1	miR-27b	9q22.32	22	▼	Sp1	Possible key miRNA regarding the development of lung cancer; ectopic expression reduced the cell growth and invasion	Sp1, a target gene of miR-27b, was associated with the angiogenic phenotype in gastric cancer, with key roles in the manipulation of this process; patients with high levels of Sp1 presented a more vascularized phenotype	Therapeutic target	[40, 41]
2	miR-126	9q34.3	22	▼	VEGF-A	Low expression of this miRNA is associated with high vascular density in NSCLC; this data were also observed in vitro	Due to direct targeting of miR-126 on VEGF-A, overexpression of this miRNA could be suitable for anti-angiogenic therapies	Therapeutic target and also prognosis tool	[42]
3	miR-130a	11q12.1	21	▼	MET	miR-130a downregulates the expression levels of two oncogenic miRNAs, miR-221 and miR-222; MET suppression	MET represents a key factor for vascular development and miR-221/222 cluster could also play an important role in angiogenesis due to the direct down regulation of TIMP3, an inhibitor of MET; miR-130 is able to reduce the levels of both this systems	Important therapeutic potential	[43, 44]
4	miR-15/16	13q14.3	–	▼	BCL-2 and BCL-XL	MiR-15/16 cluster was found as downregulated in NSCLCs; miR-15 directly targets BCL-2 and BCL-XL	BCL-2 has a suppressive action on VEGF and TP in lung cancer, both strongly implicated in angiogenesis development	Contradictory results; further studies needed	[45–47]
5	miR-378	5q32	21	▼	VEGF	Inhibition of lung cancer angiogenesis through VEGF targeting	Regulator of a central element in lung cancer angiogenesis	*In vivo* demonstrated therapeutic target	[48, 49]
6	miR-296	20q13.32	21	▼	CX3CR1, PLK1	Tumor suppressor role in lung cancer development targeting chemosensitivity and cell viability	MIR-296 has been associated with angiogenesis	Potential therapeutic target	[50–52]

No.	Name	Location	Length (nucleotides)	Expression level	Target gene	Activity	Possible role in lung cancer angiogenesis	Clinical potential	References
7	miR-128	2q21.3	23	▼	VEGF-A, VEGF-C, VEGF-2, VEGF-3	*In vitro* and *in vivo* overexpression of miR-128 led to significant suppression of angiogenesis due to down regulation of the target genes; furthermore miR-128 expression is correlated with the development stages of lung cancer	Current data shows that miR-128 could be used effectively as therapeutic target or prognostic tool	Therapeutic target for enhanced expression and prognosis tool	[53]
8	miR-497	17p13.1	20	▼	HDGF	Ectopic expression of this sequence in an animal model demonstrated positive effects through inhibition of cell proliferation and angiogenesis	Experimental data shows that miR-497 could be used with success as an antiangiogenic agent for lung cancer	Therapeutic target	[54]
9	let-7b	22q13.31	21	▼	RAS	Lung cancer tumors tissue revealed a downregulated pattern and was associated with increased vascular density	The collected data suggest a possible role for miR-7b as antiangiogenic tool in the moment of ectopic expression	Prognosis and therapeutic tool	[55]
10	miR-145	5q32	22	▼	SMAD3	Implicated in EMT and invasion	Enhancement of this miR expression could serve as a therapeutic strategy for lung cancer	Possible therapeutic target	[56]
11	miR-205	1q32.2	20	▼	VEGF	Implicated in EMT and invasion	Enhancement of this miR expression could serve as a therapeutic strategy for lung cancer	Possible therapeutic target	[57]

Table 1. The main tumor suppressor altered miRNAs implicated in lung cancer angiogenesis.

http://dx.doi.org/10.5772/66529

No	Name	Location	Length (nucleotides)	Expression level	Target gene	Activity	Possible role in lung cancer angiogenesis	Clinical potential	References
1	miR-221 miR-222	Xp11.3	23	▲	PTEN, TIMP3	Highly expressed in lung cancer cells; promotes invasion and migration	miR-221/222 cluster could have a role in angiogenesis promotion through down regulation of TIMP3, an inhibitor of MET, an angiogenesis promoter	Therapeutic target and patients stratification toll	[58]
					PUMA	Co-modulation of the two miRNAs on PUMA promotes cell proliferation and inhibits apoptosis			[59]
2	miR-222	Xp11.3	23	▲	P27	Increased miR-221/222 expression promotes H460 cells viability and proliferation	Considering the possible in vivo role of p27, where the overexpression of this gene impaired angiogenesis, miR-222 that inhibits the expression of p27 could become a potent therapeutic target regarding antiangiogenic strategies	Therapeutic target for inhibition	[60, 61]
3	miR-210	11p15.5	22	▲		Significantly up-regulated in lung cancer tissues and associated with angiogenic potential in other types of cancers	Due to the regulation by HIF-1 involved in hypoxia (event that triggers angiogenesis development), miR-210 could also become a therapeutic target	Still limited data	[62, 63]
4	miR-155	21q21.3	22	▲/▼	FGF2	MIR-155 expression is correlated with FGF2 levels, an important molecule for lung cancer angiogenesis. Also, mimR-155 was correlated with VEGF-A in the N+ subgroup of NSCLC	Several studies have investigated the role of this miR in angiogenesis	Prognosis and therapeutic tool	[64, 65]
					FOXO3A, SOCS1, SOCS6, and PTEN	Hypoxia promotes miR-155 increased expression concomitant with the downregulation of FOXO3A target			[65, 66]

No	Name	Location	Length (nucleotides)	Expression level	Target gene	Activity	Possible role in lung cancer angiogenesis	Clinical potential	References
5	miR-21	17q23.1	21	▲	PTEN, SOCS1, and, SOCS6	After antiangiogenic therapy this miR was observed as downregulated	Possible role in lung cancer angiogenesis observed after the post-antiangiogenic down regulation	Possible therapeutic target	[66]
6	miR-182	7q32.2	23	▲	FRS2	miR-182 directly targets FRS2 in lung cancer, gene that represents a key molecule for NSLC progression and antigenesis	N/A	Therapeutic and diagnosis tool	[67]
7	miR-106a	Xq26.2	22	▲	–	Augmented expression of miR-106 in NSCLC was reported in several studies	Previously associated with hypoxia progression in colon and breast cancer that could enable a possible role in lung cancer hypoxia/ angiogenesis	Possible therapeutic target	[67–69]

Table 2. The main oncogenic miRNAs involved in lung cancer angiogenesis.

http://dx.doi.org/10.5772/66529

3.3. LncRNAs related to lung cancer angiogenesis

The number of lncRNAs has significantly increased due to the progresses offered by sequencing methods in genomic research. Long noncoding transcripts act as gene regulators via a wide range of mechanism [70], those related to lung cancer being summarized in **Table 3**. The first long noncoding sequence associated with lung cancer was MALAT1 that through increased expression and gene targeting (caspase-8, caspase-3, BCL-XL, BCL-2, and BAX) promotes the proliferation and invasiveness of cancer cells. Recently it was emphasized to target SLUG gene via a competitively "sponging" miR-204 [71]. Following this initial lncRNA, a significant list of lncRNA was associated with lung cancer progression or inhibition through modulation of key mechanisms involved in the hallmarks of lung pathology. The regulatory process is complex, lncRNAs being able to escort chromatin modifying enzymes to target loci within the genome, to bind the promoter of genes and modify the transcription process, to be processed into miRNAs and further act as short noncoding transcripts, and finally to modify the stability of specific mRNAs through direct binding [70].

Recent evidences suggested the role of PANDAR in lung cancer cell proliferation through p53/PANDAR/NF-YA/Bcl-2 axis [72]. Another lncRNA positively regulated by p53 is TUG1, whose downregulation is associated with increased cell proliferation and poor survival rate in lung cancer patients [73]. Also, considering the antiangiogenetic role of the p53 gene and the positive correlation between the two sequences, there is a possible role for TUG1 in angiogenesis suppression, however further investigations are necessary. HOTTIP is a long noncoding transcript that is associated with tumor growth [74], process that involves the formation of new blood vessel network, fact that could transform HOTTIP into a new target for antiangiogenetic therapies. MVIH is associated with microvascular invasion in HCC, being upregulated in this type of cancer with an increased oncogenic potential. Further studies have investigated the possible role of the same lncRNA in lung cancer and the results were increasingly similar with the previous pathology, MVIH representing a biomarker for poor prognosis and associated tumor cell proliferation [75]. There are also other lncRNAs with tumor suppressor or tumor promoting roles in lung cancer malignancies, like MEG3 (tumor suppressor), ANRIL, and AK001796 (oncogenic role) that are involved in cell proliferation and cell viability, processes that go hand in hand with the angiogenetic transformation [76–78]. lnRNA BC087858 is overexpressed in NSCLC and was demonstrated to be connected with drug resistance via EGFR-TKIs axis [80]. MEG3 was proved to be downregulated in tumoral tissue, and directly related with high tumoral stage. Preclinical studies demonstrated a reduced proliferation rate in the case of MEG3 overexpression, by targeting MDM2 and p53 proteins. MEG3 is presented not only as prognostic marker but also as important therapeutic target [76]. ANRIL is overexpressed in lung cancer tissue, being correlated with tumor-node-metastasis stages and tumor size, but until now there are not presented data with a direct connection with angiogenesis [78].

3.4. Ultraconserved regions (UCRs)

Ultraconserved regions (UCRs) are genome sequences longer than 200 bp and, as the name suggests, are conserved within humans, rats, and mouse, preserving their nucleotide

lncRNA	Target gene	Biological role	Reference
TUG1	PRC2	Tumor suppressor lncRNA regulated by p53, gene that promotes the expression of TUG1	[73]
MEG3	MDM and p53	Tumor suppressor role through cell proliferation reduction and increased survival. P53 expression is frequently correlated with the expression of the MEG3, with possible cumulative role in angiogenesis suppression	[76]
MALAT1	SLUG	Promoter of EMT and metastasis, via miR-204	[71]
PANDAR	NF-YA	Inhibits tumor cell proliferation in the moment of overexpression through p53/PANDAR/NF-YA/ Bcl-2 axis	[72]
LUADT1	p27	Oncogenic role through promotion of cell proliferation; knockdown of the target gene significantly contribute to the reduced tumor size by inhibition of cell expansion	[79]
HOTTIP	Cdc25C	Promotes tumor growth and is overexpressed in lung cancers	[74]
ANRIL	KLF2 and p21	Oncogenic role; knockdown of this lncRNA reduced proliferation and increased apoptosis	[78]
MEG3	MDM and p53	Tumor suppressor role exercised through cell proliferation reduction and increased survival. P53 expression is frequently correlated with the expression of the MEG3, with possible cumulative role in angiogenesis suppression	[76]
AK001796	BIRC5/TPDF2/CDC/GA	Oncogenic role; involved in maintaining the tumor cell viability through complex mechanisms	[77]
MVIH	MMP-2 and MMP-9	Overexpressed in lung malignancies, being associated with increased cell proliferation and poor prognosis; previously recognized as angiogenesis promoter in HCCs	[75]
BC087858	EGFR-TKIs	Promotes cells invasion and induces drug resistance to EGFR-TKIs by activating PI3K/AKT and MEK/ ERK pathways and EMT via up-regulating ZEB1 and Snail	[80]
ZXF1	c-Myc	LncRNA ZXF1overexpression was connected with a relatively poor prognosis; Knockdown by siRNA has no effect on cell proliferation, but decreased the migration and invasion of lung cancer cells	[81, 82]
LINC01207	EZH2	Cancer initiation and progression	[83]
LincRNA-p21	p53 and HIF1A	Regulation of TP53-dependent apoptosis and Warburg effect and angiogenesis	[84]

Table 3. The main lncRNAs involved in lung cancer angiogenesis and possible therapeutic targets for inhibiting lung cancers.

succession during the evolution [85, 86]. Until this moment there are a number of 481 conserved sequences, a part of them being situated at sensitive sites regarding cancer susceptibility and are further transcribed (T-UCR) into pathological expression patterns. Considering this recent discovery, it has been postulated that the differential expression pattern could serve as stratification tool in the oncology domain, being able to differentiate between human cancers and possible between molecular subtypes of carcinomas [85, 86].

The exact mechanism that leads to aberrant expression of T-UCR is not fully deciphered, although it is thought that the primary regulation models are represented by miRNAs interactions and epigenetic modifications in CpG islands hypermethylation [85].

Calin et. al. were the first to discover the T-UCR spectrum in malignant cells compared with healthy ones and found significant differences between the two states [85]. So far, molecular analysis have revealed different T-UCR signatures in a number of carcinomas, including prostate, hepatocellular, and colorectal cancer, as well as in chronic lymphocytic leukemia and neuroblastoma. Presently was observed upregulation of several T-UCRs and demonstrated by multiple investigations to be related with increased risks for tumour occurrence and a high metastatic rate. Therefore, the main investigation area is focused on integration of synthetic antisense oligonucleotides (ASOs) to inhibit T-UCR functions [85]. In lung cancer, an important number of T-UCRs need to be characterized and then used for developing novel therapies. In spite of the interest on the T-UCR, there are only few investigations on T-UCR therapy.

4. ncRNAs related to hypoxia in lung cancer

Hypoxia is a preangiogenetic process driven by specific gene modifications, alterations that are able to induce the installation of the mesenchymal phenotype through epithelial to mesenchymal transition (EMT), acquisition of drug and radiation resistance, and propagation of lung cancer stem cells [87, 88]. Compared to other cancers, lung malignancy is severely sustained by the installation of hypoxia through complex interactions between specific molecules (HIF1α and miRNAs or other ncRNAs, as displayed in **Tables 4** and **5**) and establishment of noncoding regulatory networks related to connection with the cell cycle regulation, apoptosis or autophagy [88].

In terms of lung cancer hypoxia, miRNAs play a pivotal role through the ability to orchestrate extensive signaling networks involved in this carcinogenic step. MiR-200 family has been extensively characterized in numerous malignant scenarios and miR-200b member seems to have a role that could be exploited in the context of the clinical area regarding hypoxia induced EMT where cells acquire motility characteristic and are able to invade secondary sites within the organism promoting lung cancer metastasis [89]. Reinforced expression of the tumor suppressor miRNAs inhibited EMT through regulation of key genes involved in this pathway [88]. Another possible therapeutic target is represented by miR-21, that is elevated in NSCLC-derived cells grown under hypoxic conditions [90]. Hypoxic conditions also triggered miR-155 overexpression and downregulation of FOXO3A target gene, and protects lung cancer cells to irradiation, elucidating a possible course of treatment through inhibition of miR-155 combined with radiotherapy [65].

http://dx.doi.org/10.5772/66529

Type of miRNA	Expression level	Name	Target gene	miRNA role in lung cancer hypoxia	Possible clinical role of miRNAs in lung cancer	References
Tumor suppressor miRNAs	▼	miR-200b	–	Hypoxia-induced EMT in lung cancer, influencing the activity of key genes involved in mesenchymal transition miR-200 mimic blocks hypoxia-induced EMT	Novel therapeutic strategy via Nobiletin delivery	[89]
	▼	miR-210	–	Regulate cellular response under hypoxic conditions High levels connected with a positive outcome in NSCLC patients	Biomarker for prognosis and patient stratification	[63, 91]
	▼	miR-18a	HIF-1α	Connected with lung metastasis of breast cancer cells ability to decrease the hypoxic stress *in vitro* and reduce cell invasiveness	Possible prognosis factor of lung metastasis in breast cancer	[92]
	▼	miR-199a	HIF1α	Inhibition of cancer cell hypoxia induced proliferation in NSCLC cells	Possible prognosis factor	[93]
Oncogenic miRNAs	▲	miR-21	–	NSCLC derived cell lines grown under hypoxic condition showed an elevated miR-21 expression; modulates radiation resistance via hypoxic mechanism	Possible therapeutic and prognosis role	[87, 90]
	▲	miR-339	RASSF1, ERBB4, KRAS	Activity correlated with the process of response to hypoxia	Possible target for therapeutic strategies	[94]
	▲	miR-155	FOXO3A	Correlates with poor prognosis and protects hypoxic lung cancer cells to irradiation and conversely	Therapeutic potential for radio sensitization of hypoxic lung cancer cells	[65]
	▲	miR-17-92 cluster	HIF1α	Downregulation of HIF1α does not affect the cellular adaptation to hypoxia	Possible prognosis factor	[95]
	▲	miR-494	PTEN	Promotes angiogenesis through direct targeting of PTEN and activation of Akt/eNOS pathway; expression is induced during hypoxia	Possible prognosis and therapeutic tool	[96]

Table 4. Tumor suppressor and oncogenic miRNAs involved in lung cancer hypoxia with possible roles in diagnosis, prognosis, and therapy.

Type of lncRNA	Expression level	Name	Target gene	lncRNA role in lung cancer hypoxia	Possible clinical role of lncRNAs in lung cancer	References
Tumor supressor lncRNAs	▼	lncRNA-LET (Low expression in tumor)	HIHD3; hypoxia-induced histone deacetylase 3	Squamous-cell lung carcinomas downregulated by HIHD3 promotes hypoxia-induced cancer cell invasion	New methods for therapeutic intervention	[97]
	▼	GAS5-AS1	–	Downregulation of GAS5-AS1 contributes to hypoxia tumor metastasis in non-small cell lung cancer	Prognosis and therapeutic marker	[98]
	▼	LincRNA-p21	TP53 and HIF-1α	Target angiogenic mechanisms	Prognosis marker	[84]
Oncogenic lncRNAs	▲	HOTAIR (HOX transcript antisense intergenic RNA)	HIF-1α	HOTAIR is upregulated in hypoxic conditions and is a direct target of HIF-1α; Promotion of cancer cell proliferation and ability to migrate and invade other sites	Novel therapeutic target	[99, 100]
	▲	H19	HIF-1α	Possess oncogenic properties triggered by hypoxic stress Correlates with p53 tumor suppressor status	Prognosis/ Diagnosis marker	[101]

Table 5. LncRNAs involved in lung cancer hypoxia with possible roles in diagnosis, prognosis, and therapy.

Hypoxia management has led to reduced angiogenesis and thus obtuse the malignant cell proliferation and survival due to deprivation of nutrients and oxygen via various molecules including the noncoding transcripts represented by miRNAs and lncRNAs. Multiple targeting through ncRNAs that are able to influence the fate of the hypoxic microenvironment will bring new insights into the pathogenesis of lung cancer, permitting the development of new clinical tools for cancer management, improving the concerning survival rate of this pathology. The list of miRNAs and lncRNAs implicated in the vascular invasion of the pulmonary malignancy is presented in **Tables 4** and **5**.

LncRNAs have recently emerged as important prognosis and therapeutic tolls in different malignancies and even for specific carcinogenic processes as lung cancer hypoxia. One of the main studied lncRNAs is HOTAIR, pathological expressed in numerous malignant scenarios, being associated with tumor promoting roles and a negative outcome in oncological patients. It was demonstrated that this lncRNA is a direct target for HIF-1α that act as an enhancer of expression and contribute together to the securitization of hypoxia followed by cell proliferation, migration, and metastasis. This information could transform HOTAIR in a possible therapeutic target under hypoxic conditions for NSCLC, that is limited in what regards the therapeutic options [99, 100]. Another newly discovered lncRNAs in lung cancer hypoxia that is lncRNA-LET targeted by HIHDR. The interaction between these two molecules ends with reduction of histone acetylation at the promoter region of the noncoding transcripts and thus decreased expression. Moreover, the downregulation process secures the expression of nuclear factor 90 proteins, a key element for cell migration induced by hypoxia. This data suggest that lncRNA-LET can be used as a clinical tool against cancer promotion [97].

LincRNA-p21 impacts prognosis in resected nonsmall-cell lung cancer patients through angiogenesis regulation. LincRNA-p21 was proved to be activated by TP53 and HIF1A [84]. It was proved to target the apoptosis pathway via regulation by p53 and the Warburg effect. LincRNA-p21 is downregulated in tumor tissue, and has effect on the lung cancer patients via angiogenesis regulation [84].

Other important ncRNA structures with a significant role in the development of novel molecular therapies are represented by PIWI-interacting RNAs (piRNAs). piRNAs are recognized to be involved in transposon silencing and gene expression during development and the complete role on the somatic cells remains to be deciphered [8]. In a recent paper were emphases a different piRNASs expression profiles between normal bronchial epithelial cells and lung cancer cells. The most downregulated piRNAs in lung cancer cells was piRNA-like-163 (piR-L-163) having as direct target the phosphorylated ERM (p-ERM) [102]. S100A4-small interfering RNA (S100A4-siRNA) was proved to activate the apoptosis and increase the radiosensitivity of A549 lung cells. S100A4 may promote A549 cell proliferation but also invasion, and metastasis by regulating the expression of E-cadherin and p53 protein [103].

5. ncRNAs targeting semaphorines and its related receptors in lung cancer

Semaphorins are guidance molecules which were characterized initially as directing elements for axon outgrowth; however advances in genomic and translational medicine revealed a more complex role for these proteins, being involved in cell migration, vascular network, and tissue development [32, 104]. Considering their vital role in physiological processes is not surprising that these guidance proteins are also involved in similar pathological processes especially from the oncologic area, where they exercise the same functions, but in a negative manner [32]. Therefore, semaphorins are implicated in carcinogenic establishment, metastasis, and especially angiogenesis in numerous cancers, including lung

cancer. Regarding their role in angiogenesis, the family of semaphorins is divided into two main pathological classes: tumor suppressors inhibiting the angiogenic process and oncogenes through promotion of vascular invasion. Therefore, loss of expression in the case of antiangiogenic semaphorins and/or increased expression pattern for the procarcinogenic ones translates into sustaining of the malignant cells [106]. Immediately after the establishment of their newly discovered role, *in vitro* and *in vivo* studies confirmed the ability of semaphorins to serve as therapeutic targets in the form of suppression or enhancement [32]. Despite the fact that their role in pulmonary malignant processes is quite extensively studied, little is known about the ncRNAs regulatory action on the expression pattern of semaphorins. Deciphering the regulatory noncoding sequence panel for these proteins will enable a more advanced and specific molecular management of lung cancer, especially in angiogenesis that has a vital role regarding the maintenance of tumor cells integrity and proliferation.

The process of angiogenesis, can also occur through semaphorin receptors, neuropilins, and plexins (**Figure 5**). In the case of neuropilins, we encounter a multiple ligation system, this membrane proteins being able to bind both class-3 semaphorins, VEGF and growth factors. Also, this type of receptors that are essential to proper vascular development during organism development are generally mutated in lung cancer. On the other hand, *in vivo* suppression of neuropilins led to improper vascular network.

Among the first studies that elucidated the role of neuropilins in vascular development is the research where the authors observed that overexpressing of *Nrp1* was lethal for embryos due to extensive vascular defects like overdevelopment of blood vessel network and deformed hearts [107]. This discovery paved the way for further research in the area of cancer management with focus on targeted therapy. Therefore, it has been proven that a combined form of therapy represented by neuropilins inhibitors (semaphorin, anti-NRP, soluble NRP - B domain, and VEGF mimetics) administrated concomitant with anti-VEGF signaling molecules (kinase inhibitors, anti-VEGF, anti-VEGFR-2, and soluble VEGFR for VEGF) is more efficient than the classical antiangiogenic therapeutic strategy targeted towards VEGF alone [104]. Research studies demonstrated a role for NRP1 and NRP2 in lung cancer progression and angiogenesis where these two molecules were observed as normally expressed in bronchial basal cells, and as it progressed in the severity of the cell lesions, the level of neuropilins increased significantly, concomitant with VEGF expression [104]. NRP1 has been previously associated with cancer angiogenesis: overexpression of NRP1 in AT2.1 cells (*in vitro* model of prostate cancer) resulted in advanced vascular density, cell proliferation, and also inhibited apoptosis [108]; rat estrogen-induced pituitary tumors presented increased levels of NRP1, level that was also correlated in a positive manner with the aggressiveness of angiogenesis development [109].

The competitive binding of class-3 semaphorins and VEGF that in physiological conditions leads to the proper development of the vascular platform is changed during malignant scenarios where proangiogenic VEGF takes the lead due to mutations in the structure of the binding domain that decreases the complementarity with semaphorins or enhances the expression of receptors. Therefore, an alternative therapeutic pathway could be represented by the

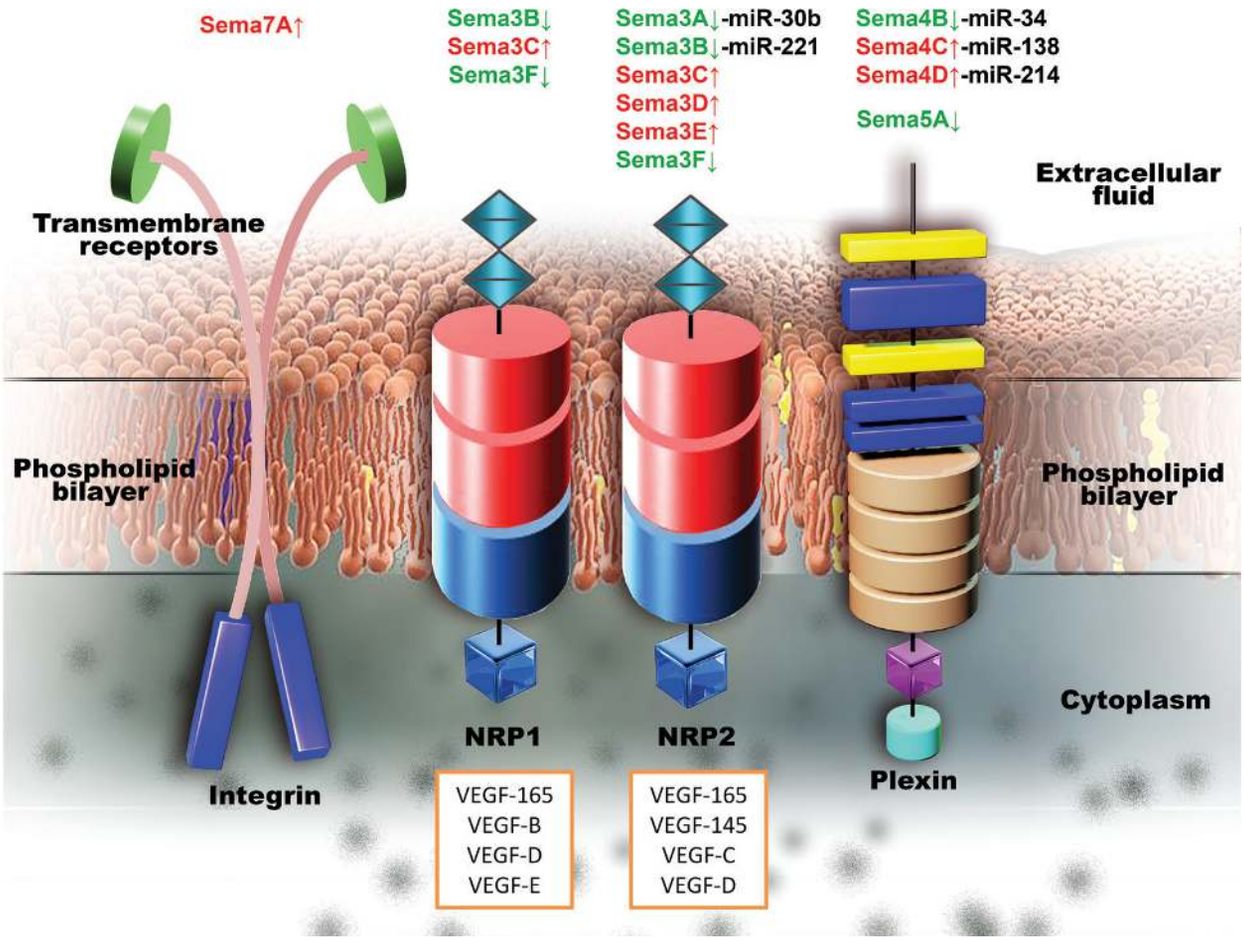

Figure 5. Semaphorin receptors and ncRNAs regulation. Green – downregulated genes; Red – overexpressed genes.

modulation of neuropilins (NRPs) expression. Furthermore, the specific malignant expression is most likely influenced by other molecules such as miRNAs and lncRNAs (**Table 6**).

Lung cancer therapies focused on semaphorins and their receptors are still an insufficiently explored domain that could hold great promises regarding the inhibition of cancer spreading. Considering the competitive binding between class-3 semaphorins and VEGF in vascular development, antiangiogenic strategies as antibodies for VEGF or NRP inhibition, soluble NRP or NRP blocking peptides have been tested with effective results [104, 106]. A more recent treatment compromising both VEGF and SEMA3A inhibitors have been applied *in vitro* and *in vivo* for colon cancer [105]. Another type of action could be represented by the induced internalization of the neuropilins through administration of dextran sulfate and fucoidan that significantly decreased the number of endothelial surface receptors, including VEGFR [131]. Although anti-VEGF molecules are well-known as efficient angiogenesis inhibitors, combining the modulation of VEGF/VEGFR with SEMA/NRP may hold significant clinical usage. Moreover, extension of the molecular insight regarding noncoding RNAs regulation of semaphorins and their receptor could improve even more the inhibition of angiogenesis if we take in consideration the ability of noncoding RNAs to regulate waste singling networks that involve more than one target gene.

Semaphorin	Regulatory miRNAs	Predicted targeting miRNAs	Role in lung cancer	Potential clinical role in lung cancer	Ref.
Semaphorin 3A (SEMA 3A)	miR-30b	miR-95-3p miR-589-5p	NSCLC-anticarcinogenic activity; low expression of SEMA 3A correlates with lymph node metastasis	Biomarker for prognosis	[104, 110]
Semaphorin 3B (SEMA 3B)	miR-221	miR-155-5p miR-107 miR-187-5p miR-18a-3p miR-708 miR-3074-5p miR-106b-3p miR-340-3p miR-3074-5p	Cell proliferation and invasion Small-cell lung cancer-tumor suppressor role via induction of apoptosis and inhibition of angiogenesis	Marker for cancer progression	[111, 112]
Semaphorin 3C (SEMA 3C)	–	miR-4746-5p miR-500a-5p miR-187-5p miR-301a-5p miR-21-3p miR-106a-3p miR-4677-3p miR-3074-5p let-7g-3p miR-183-3p miR-29a-3p miR-519a-5p miR-200c-5p miR-4668-3p miR-16-2-3p miR-193a-3p miR-4326 miR-4417 miR-3664-3p miR-155-5p miR-590-5p miR-616-3p miR-3182 miR-103a-2-5p miR-501-5p miR-362-3p miR-330-5p miR-30e-5p	A549 lung cancer cells -p65-SEMA3C (cleaved SEMA3C) – protumorigenic activities	Novel antitumorigenic drug	[113]
Semaphorin 3D (SEMA 3D)	–	miR-484 miR-15b-3p miR-16-2-3pmiR-32-3p miR-32-5p miR-33a-5p miR-33b-3p miR-340-5p miR-4668-3p miR-345-5p miR-629-5p miR-18a-5p miR-1306-5p	Proangiogenic and metastatic role	Prediction of response and survival	[114, 115]

Semaphorin	Regulatory miRNAs	Predicted targeting miRNAs	Role in lung cancer	Potential clinical role in lung cancer	Ref.
		let-7a-3p miR-183-3p miR-21-3p miR-21-5p miR-4742-5p miR-425-5p miR-200a-5p miR-301a-5p miR-3619-3p miR-589-5p miR-3614-3p miR-141-5p miR-106a-3p miR-93-3p			
Semaphorin 3E (SEMA 3E)	–	miR-340-5p miR-1306-5p miR-15b-3p let-7d-5p miR-1307-3p miR-629-5p miR-629-3p miR-4677-3p let-7g-3p miR-301a-5p miR-15b-5p miR-19a-3p miR-16-2-3p miR-105-3p miR-18a-5p miR-1306-5p miR-19a-3p miR-1307-5p miR-505-5p	*In vivo* promotion of lung metastasis and tumor progression	Possible target for therapeutic strategies	[116]
Semaphoring 3F (SEMA 3F)	–	miR-29c-3p miR-191-3p miR-29b-1-5p miR-18a-5p miR-20a-5p miR-29b-2-5p miR-200c-5p let-7g-3p let-7a-3p let-7d-5p miR-7-5p miR-3619-3p miR-590-5p miR-29b-2-5p miR-30e-5p miR-676-3p miR-135b-3p miR-30c-1-3p miR-140-3p miR-135b-3p miR-140-3p	Role in TGF-beta1-induced EMT Antitumor role; Downregulated in lung cancer Targets HIF-1 and VEGF	Possible prognosis biomarker and therapeutic target	[117, 118]

Semaphorin	Regulatory miRNAs	Predicted targeting miRNAs	Role in lung cancer	Potential clinical role in lung cancer	Ref.
Semaphorin 4B (SEMA 4B)	miR-34	miR-34	NSCLC—inhibition of invasion and growth—prevention of metastasis-direct target of hypoxia-inducible factor 1 (HIF-1) miR-34/p53 axis	Novel therapeutic target for inhibition of metastasis and growth -Novel therapeutic target through inhibition of HIF-1	[119–121]
Semaphorin 4C (SEMA 4C)	miR-138	miR-138	NSCLC-cell proliferation and EMT	New target or prognosis marker for lung cancer treatment	[122]
Semaphorin 4D (SEMA 4D)	miR-214	miR-199b-3p miR-127-3p miR-185-5p miR-421 miR-500a-5p miR-22-3p miR-500a-5p miR-22-3p miR-505-5p let-7g-3p miR-1269a miR-18a-5p miR-3614-3p miR-331-3p miR-18a-5p miR-18a-3p	Highly expressed in lung cancer; promotion of angiogenesis; NSCLC-*in vitro* inhibition of cell proliferation, migration, and invasion	Possible early prognosis tool and therapeutic target	[124–126]
Semaphorin 5A (SEMA 5A)	-	miR-3677-3p miR-3200-3p miR-32-5p miR-29b-1-5p miR-183-3p miR-345-5p miR-454-5p miR-3614-3p miR-18a-3p miR-500a-5p miR-106b-3p miR-27b-5p let-7g-3p miR-660-5p miR-135b-3p miR-1306-5p miR-29a-3p miR-29b-2-5p miR-425-3p miR-365a-5p miR-3136-3p miR-93-3p miR-4787-3p miR-19a-3p	NSCLC-tumor suppressor role; low levels associated with poor survival rate	New biomarker for NSCLC	[127]

Semaphorin	Regulatory miRNAs	Predicted targeting miRNAs	Role in lung cancer	Potential clinical role in lung cancer	Ref.
Semaphorin 6A (SEMA 6A)	miR-27a/b	miR-1307-3p miR-940 miR-3187-3p miR-33a-5p miR-425-3p miR-99b-5p miR-99b-3p miR-183-3p miR-3176 miR-760 miR-345-5p miR-4461	Endothelial cell repulsion	New therapeutic target through manipulation of miR-27a/b expression	[128, 129]
Semaphorin 7A (SEMA 7A)	–	22_40957679_40957783 (novel miRNA)	Promotion of tumor microenvironment and metastasis through regulation of prototypic chitinase-like protein (Chi3l1)	Possible role as therapeutic target	[130]

Table 6. Semaphorin and the targeting miRNAs with implication in lung cancer.

6. ncRNAs therapies targeting lung cancer angiogenesis

Once considered the "trash" of the genome, the noncoding RNA sequences are now emerging as important therapeutic targets (**Table 7**). Due to the complex regulatory network involving ncRNAs and also because of the personalized pathological expression pattern among cancer types, subtypes and malignant stages, ncRNAs are subjected to numerous preclinical studies regarding their silencing or induced expression [2]. A lipid-based delivery vehicle for tumor suppressor miR-34 was developed in order to enhance the expression of the specific molecule in a mouse model of non-small-cell lung cancer [132]. This approach has demonstrated to be efficient in both locally and systemically administration, being observed a reinforced miR expression concomitant with downregulation of the specific targets. Moreover, the intravenous delivery of miR-34 mimic did not produce an immune reaction in mice, but unfortunately this was not the case in humans. Very recently, MRX34, the miR-34 mimic, was stopped to be administrated in a cancer clinical trial due to major immune reactions [133].

Another therapeutic alternative that is currently on the scientific spotlight consist in the manipulation of the ciRNAs that can function as microRNA sponges, modulating their oncogenic or tumor suppressor activity [136]. Despite the fact that there is a number of research studies focused on this type of noncoding RNAs, relatively little is known about the regulatory mechanism of circRNAs in cancer development. Future perspectives imply ciRNAs-based therapy that can stand as "super-sponges" and modulate the activity of extended regulatory miRNA networks, influencing at a superior level the carcinoma progression [136].

miRNAs	Lung cancer subtype	Experimental model	Therapeutic approach	Delivery system	Target gene	Obtained results	References
miR-128	NSCLC	NSCLC cells	Ectopic miR-128 overexpression		VEGF-C	Inhibition of VEGF-C expression concomitant with angiogenesis restriction	[53]
		HUVECs and NSCLC cells			VEGF-A, VEGFR-2 and VEGFR-3	Low expression of the target genes that are critical factors for angiogenesis	
		Nude mice (A549 cells)	*In vivo* replacement therapy			Inhibition of lymphangiogenesis	
miR-497	NSCLC	NSCLC cells	Over expression of the miRNA		VEGF-A	Decreases in the levels of VEGF-A protein with no significant changes for the VEGF-A mRNA; inhibition of cell invasion	[123]
			miRNA inhibition			Increased levels of VEGF-A protein with no significant changes for the VEGF-A mRNA; increased cell invasion	
		NSCLC cells SCID mouse xenograft model	Ectopic expression of the miRNA sequence		HDGF	Restoration of the miR-497 levels reduced tumor development and angiogenesis in both *in vitro* and *in vivo* experimental models	[54]
miR-378	NSCLC	Swiss nude immunodeficient murine model (NCI-H292-Luc cells overexpressing miR-378—subcutaneous xenografts)	Overexpression of miR-378	Lentiviral vectors particles (pEZX-MR03 backbone)	HMOX1	mir-378 over expression models presented tumors with decreased vascularisation compared to the models with HMOX1 induced over expression; increased oxygen partial pressure; increased MUC5AC, Ang-1,MMP12 levels and decreased TNF-α and IL-1β levels - all essential genes for angiogenesis	[48]
miR-126		A549, Y-90 and SPC-A1 cells	Overexpression of miR-126	mir-126 expression vector (LV-miR-126)	VEGF-A	Dowregulation of VEGF-A gene correlated with inhibited cell growth	[42]
		Tumor xenograft model (A549 infected with LV-miR-126)	mir-126 expression vector (LV-miR-126)				

miRNAs	Lung cancer subtype	Experimental model	Therapeutic approach	Delivery system	Target gene	Obtained results	References
miR-222	NSCLC	H460 cells	Inhibition of miR-222 expression	miR-222 inhibitor	p27 (*in vivo* over expression of p27 impaired angiogenesis)	Inhibition of oncogenic miR expression resulted in decreased cell viability and proliferation	[60, 61]
miR-27b	NSCLC	HEK293 cells	Cotransfection of miR-27b mimics and WT Sp1 in order to express both miRNA and target gene	psiCHECK-2 vector	Sp1	Expression of miR-27b resulted in suppression of cell growth and reduced invasion	[128]
miR-130a	NSCLC	A549,CALU-1, H1299 and A459 cells	Transfection with miR-130a in order to increase the endogenous levels of this sequence	pre-miR 130a	MET	Strong reduction of MET (angiogenesis promoter) mRNA and protein levels; down regulation of miR-221 and miR-222, that are able to inhibit TIMP3 expression, molecule that in turn inhibits MET	[44]
miR-210	Adenocarcinoma	A549 cells	Knocked down of miR-210 in hypoxic parameters	antimiR-210	SDHD (positive-regulatory loop between miR-210 and HIF-1α)	Decreased cell survival and alteration of mitochondrial phenotype	[63]
miR-155	Adenocarcinoma	A549 and H460 cells	Inhibition of miR-155 levels in cells preserved in hypoxic conditions	Synthetic antimiR-155	FOXO3A (associated with roles in angiogenesis)	miR-155 inhibition exercise a positive role through radiosensitization of the cells	[65, 134]
miR-21	NSCLC	A549	*In vitro* inhibition of miR-21	miR-21-sponge	PDCD4 (associated with angiogenesis development)	Inhibition of miR-21 ameliorates cell proliferation, migration, and invasion through PDCD4 modulation; knockdown of PDCD4 has been demonstrated to stimulate angiogenesis through positive regulation of Ang-2 (negative prognostic factor in lung cancer)	[66, 135]

Table 7. Some relevant miRNAs tested on cell culture and animal models as potential therapeutic agents in lung cancer.

http://dx.doi.org/10.5772/66529

The discovery of lncRNAs as regulators of cancer development has naturally conducted towards potential therapeutic alternatives using these long fragments as direct targets. The expression pattern of these sequences has been also investigated in lung cancer and the list of oncogenic and tumor suppressor pathological expressed lncRNAs is continuously growing. Administration of siRNA, shRNA, and miRNAs or antisense oligonucleotides in order to inhibit oncogenic lncRNAs is currently under investigation [137]. HOTAIR has been on the spotlight of artificial knockdown via siRNA delivery with great rates of success in lung cancer and also breast and pancreatic malignancies [100].

Moreover, the same approaches have been shown to be effective for the reverse of cisplatin resistance through reduced expression of p21 [138]. Downregulation of MALAT1 through shRNA delivery is also a potent therapeutic approach for lung cancer as it was shown reduced cell viability after this type of treatment [71]. MALAT1 has been also inhibited by exogenous antisense oligonucleotides, approach that induced reduced cancer progression through cell cycle arrest [151]. Considering that in lung cancer there are also downregulated tumor suppressor lncRNAs, the replacement therapy could also stand as an effective therapeutic approach for this type of carcinoma, but nonetheless the scientific information are quite limited regarding this area.

The discovery that miRNA sequences can act as key regulators in cancer pathways through aberrant expression has led to the idea that these fragments could serve as potent therapeutic targets [139]. In this sense, several strategies have been implemented until now: inhibition strategies—inhibitory antisense oligonucleotides and delivery vectors (miRNA sponges) and enhancement strategies—miRNA replacement therapy (**Figure 6**) [139, 140].

For the case of therapeutics that follow an antagonistic pattern, the activity of tumor promoting miRNAs that are hazardous expressed is inhibited via administration of single stranded oligonucleotides complementary with the specific molecule or with the target binding site on the mRNA molecule; in either situation, the interaction between miRNAs and mRNA molecules is blocked and the downstream pathological pathway is strongly affected [140].

Delivery of anti-miRNA oligonucleotides (AMOs) in the context of preclinical studies is still a problematic area considering the necessity of target administration, prolonged stability, and increased pharmacokinetic properties [140]. In this means, there is an urgent need for efficient delivery vectors/vehicles that are able to fulfill the reminded request in order to accomplish the treatment purpose. The majority of the freely administrated oligonucleotides is retrieved in the liver and kidneys and then eliminated through the urine. Also, the necessary dose of synthetic sequences is usually very high and the chance for off-target delivery is also increasing. Establishment of an effective delivery system will break the grounds of miRNAs therapeutics and also other noncoding treatment strategies [139]. The current strategy for *in vivo* administration implies conjugation-based methods, where miRNA sequences are conjugated with different molecules like cholesterol [141] and α-tocopherol [142]. Although these studies have demonstrated promising results, the efficiency of miRNA targeting is still limited. Another type of delivery method consists in liposome-mediated delivery of siRNAs, where the first attempt [143] was to inhibit the replication of hepatitis B virus (HBV) in an animal model through administration of siRNAs integrated in as polyethylene glycol (PEG)–lipid conjugate (SNALP). Since then, different liposome-based vehicles have been tested and the results are encouraging considering that the administration dose is significantly decreased

http://dx.doi.org/10.5772/66529

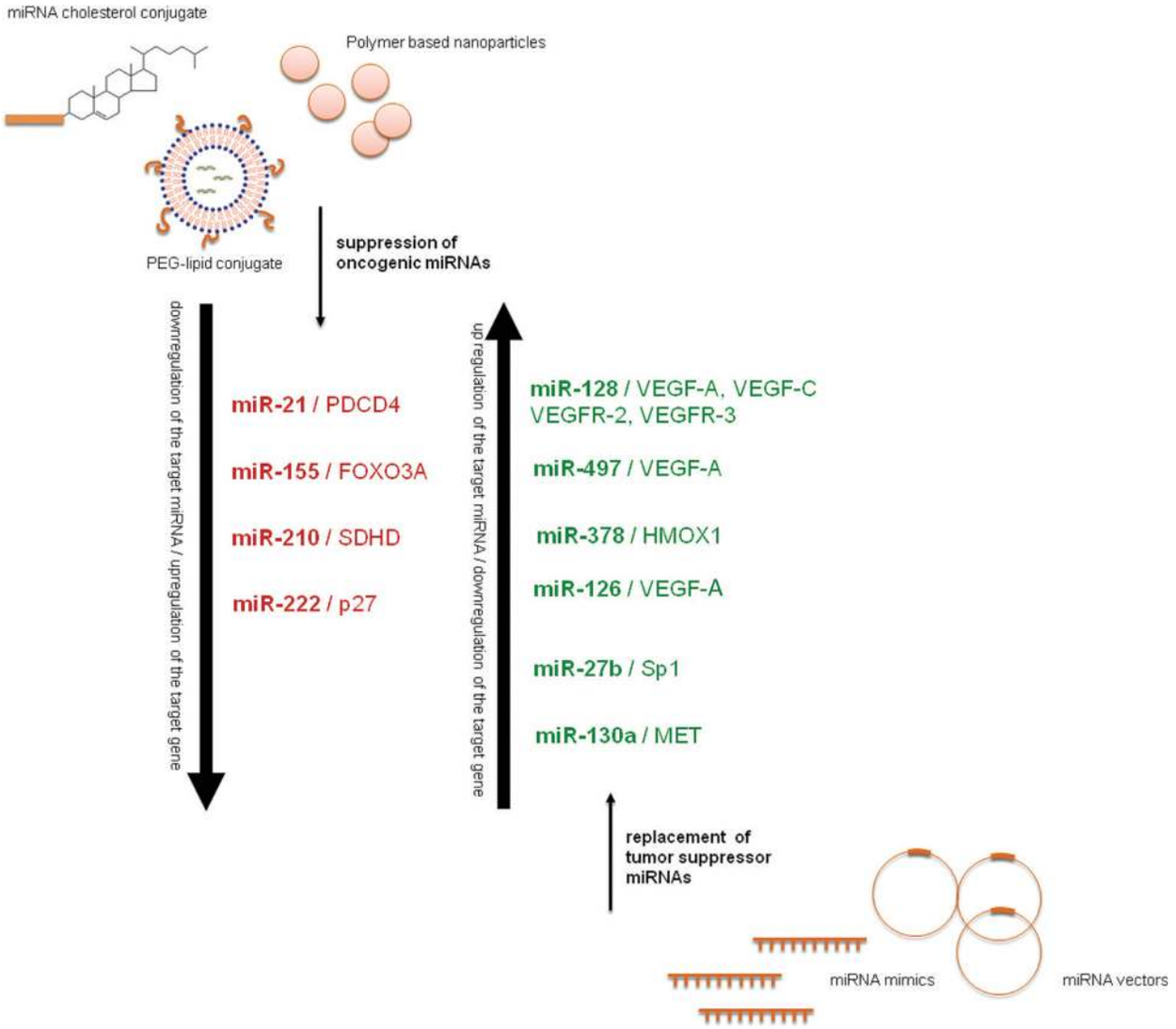

Figure 6. MicroRNAs have emerged as important regulators of lung cancer angiogenesis and also as key therapeutic targets regarding inhibition or enhancement strategies. MiRNAs sequences that are marked with green composed the tumor suppressor group that have been tested in the context of replacement therapies; the genes marked with the same color represent the target genes that have been downregulated after therapeutic modulation of miRNAs. Inversely, miRNAs sequences marked with red are oncogenic ones proposed for inhibition in what regards antiangiogenic programs; the genes marked with the same color also represent the target genes, but in this case their expression has been augmented.

comparing to the naked oligonucleotides [144–147]. Progresses in the area of material science produced a promising *in vivo* delivery system in the form of polymer-based nanoparticles that are more flexible than liposomes and also can be produced in a more homogenous manner regarding their size and form [139]. In respect of siRNAs and anti-miRs delivery, the size of the vehicle is very important in order to permit the passing through cellular compartments, where nanoparticles can fulfill this request having a size between 10 and 100 nm [139]. Moreover, in order to avoid the stimulation of the immune system, cyclodextrin–PEG conjugated nanoparticles have been developed and tested for the inhibition of EWS–FLI1 in an *in vivo* model of Ewing's sarcoma [148]. Attracting strategies for targeted therapies consist in the conjugation of siRNAs or anti-miR sequences with specific antibodies able to conduct the small fragments towards distinctive cells expressing the desired antigen [147, 149, 150].

MiRNA replacement therapy is more limited regarding the current attempts and results, although it seems to emerge as a more efficient form of treatment considering that the majority of pathological expressed miRNAs consist in downregulated or inhibited tumor suppressor sequences [140]. Even if the success of this therapeutic strategy could be greater than miRNA inhibition workflows, the requirements for the structure and composition of the replacement fragment are much more stringent considering the necessity of RISC uptake. Furthermore, the impediments regarding the delivery system for these oligonucleotides are the same as in the case of inhibitory antisense attempts.

7. Conclusions and future perspectives

Lung cancer remains the most deadly disease from the oncological field, being an aggressive form of cancer that is usually diagnosed in late stages with minimal therapeutic alternatives. Even in the case of early discovery, the classical treatments have failed numerous times due to compensatory mechanisms developed within the tumor environment leading to the same negative outcome. Therefore, we face a crisis situation where we need to develop new therapeutic tools for lung cancer management able to target key elements/pathways, but avoid in the same time the possibility of alternative carcinogenic pathways activation. One of the hallmarks of cancer is represented by angiogenesis, process that is in the sight of researchers for some time, but the classical inhibition of central molecules like VEGF has failed to deliver long-lasting results. Therefore, ncRNAs have emerged as potential lifesaving agents due to the capacity of extensive modulation, where the same ncRNA is able to target multiple genes and regulate their function. Also the same microRNA, or more recently discovered, the lncRNA can be encounter in different consecutive processes in pulmonary carcinogenesis, as in the case of hypoxia and angiogenesis. Development of novel therapeutic tools able to transform the pathological expression of ncRNAs, mainly through silencing of upregulated patterns, will enable a more extensive, and in the same time, specific approach that will probably excludes the installation of compensatory mechanism and significantly contribute to a better outcome in lung cancer patients. The concept of noncoding RNAs as therapeutic targets in the clinical context is now more feasible than ever, being supported by numerous preclinical studies. One of the main approaches should involve manipulation of miRNAs that are actively implicated in the regulation of VEGF genes expression, genes that hold a key role in the vascular development process. Even more, a heterogeneous approach that implies the administration of different miRNA sequences able to target multiple genes and naturally multiple pathological pathways within the angiogenic process will represent a more extended form of therapy that could modify extensive regulatory networks. This type of targeting will also minimize the compensatory mechanisms that are usually encountered after the implementation of classical therapeutic strategies due to concomitant regulation of multiple signaling pathways. Additionally, some other approaches may be used for the inhibition of angiogenesis. For example, semaphorins are now emerging as important regulators of vascular density in malignancies, with possible roles as prognostic tools or even therapeutic targets. Inhibition of procarcinogenic semaphorins would represent a novel course of action regarding cancer treatment considering their central role in vascular density. Moreover, the

http://dx.doi.org/10.5772/66529

receptors associated with semaphorins contain binding sites for both semaphorins and VEGF molecules, engaging the competitive binding between these types of molecules. Managing the expression of VEGF via miRNA therapy concomitant with the levels of neuropilins (semaphorins receptors) will enable a more dramatic approach that could have more drastic results for cancer development.

Current therapeutic programs are promoting the effectiveness of specific sequence inhibition or enhancement through administration of antisense oligonucleotides or supplementation of the same sequence through exogenous enhancement. Development of chemically modified oligonucleotides under the form of medication for individuals diagnosed with cancer is now at the close horizon. Administration of synthetic oligonucleotides for noncoding RNAs inhibition or upregulation will enhance the effect of the current therapeutic strategies by modulation of specific gene expression able to influence the carcinogenic process or even reverse the malignant installation. In this sense, it is now clearly understood that the major strategy towards cancer treatment is focused on taking advantage of the key roles of noncoding sequences regarding the modulation of entire aberrant regulatory networks through manipulation of central molecules.

Acknowledgments

This work was supported by the POC-P_37_796 grant, entitled "Clinical and economical impact of personalized targeted anti-microRNA therapies in reconverting lung cancer chemoresistance"-CANTEMIR.

Author details

Ioana Berindan-Neagoe[1,2,3]*, Cornelia Braicu[1], Diana Gulei[2], Ciprian Tomuleasa[1,4] and George Adrian Calin[5]

*Address all correspondence to: ioana.neagoe@umfcluj.ro

1 Research Center for Functional Genomics, Biomedicine and Translational Medicine, "Iuliu Hatieganu", University of Medicine and Pharmacy Iuliu-Hatieganu, Cluj-Napoca, Romania

2 Medfuture—Research Center for Advanced Medicine, University of Medicine and Pharmacy Iuliu-Hatieganu, Cluj-Napoca, Romania

3 Department of Functional Genomics and Experimental Pathology, The Oncological Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

4 Department of Hematology, The Oncological Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

5 Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

References

[1] Mattick, J.S. and I.V. Makunin, *Non-coding RNA*. Hum Mol Genet, 2006. **15 Spec No 1**: pp. R17–29.

[2] Braicu, C., et al., *NCRNA combined therapy as future treatment option for cancer*. Curr Pharm Des, 2014. **20**(42): pp. 6565–74.

[3] Choudhuri, S., *Small noncoding RNAs: biogenesis, function, and emerging significance in toxicology*. J Biochem Mol Toxicol, 2010. **24**(3): pp. 195–216.

[4] Berindan-Neagoe, I., et al., *MicroRNAome genome: a treasure for cancer diagnosis and therapy*. CA Cancer J Clin, 2014. **64**(5): pp. 311–36.

[5] Berindan-Neagoe, I. and G.A. Calin, *Molecular pathways: microRNAs, cancer cells, and microenvironment*. Clin Cancer Res, 2014. **20**(24): pp. 6247–53.

[6] Kung, J.T., D. Colognori, and J.T. Lee, *Long noncoding RNAs: past, present, and future*. Genetics, 2013. **193**(3): pp. 651–69.

[7] Liu, Y. *MicroRNAs and PIWI-interacting RNAs in oncology*. Oncol Lett, 2016. **12**(4): pp. 2289–2292.

[8] Ng, K.W., C. Anderson, E.A. Marshall, B.C. Minatel, K.S. Enfield, H.L. Saprunoff, W.L. Lam, and V.D. Martinez, *Piwi-interacting RNAs in cancer: emerging functions and clinical utility*. Mol Cancer, 2016. **15**: p. 5.

[9] Hansen, T.B., J. Kjems, and C.K. Damgaard, *Circular RNA and miR-7 in cancer*. Cancer Res, 2013. **73**(18): pp. 5609–12.

[10] Peng, L., X.Q. Yuan, and G.C. Li, *The emerging landscape of circular RNA ciRS-7 in cancer (Review)*. Oncol Rep, 2015. **33**(6): pp. 2669–74.

[11] Ebbesen, K.K., T.B. Hansen, and J. Kjems, *Insights into circular RNA biology*. RNA Biol, 2016 Dec 16:0. [Epub ahead of print]

[12] Hansen, T.B., et al., *Natural RNA circles function as efficient microRNA sponges*. Nature, 2013. **495**(7441): pp. 384–8.

[13] Shao, T., et al., *Identification of module biomarkers from the dysregulated ceRNA-ceRNA interaction network in lung adenocarcinoma*. Mol Biosyst, 2015. **11**(11): pp. 3048–58.

[14] Taft, R.J., et al., *Non-coding RNAs: regulators of disease*. J Pathol, 2010. **220**(2): pp. 126–39.

[15] Malone, C.D. and G.J. Hannon, *Small RNAs as guardians of the genome*. Cell, 2009. **136**(4): pp. 656–68.

[16] Carthew, R.W. and E.J. Sontheimer, *Origins and mechanisms of miRNAs and siRNAs*. Cell, 2009. **136**(4): pp. 642–55.

[17] Ghildiyal, M. and P.D. Zamore, *Small silencing RNAs: an expanding universe*. Nat Rev Genet, 2009. **10**(2): pp. 94–108.

[18] Sobecka, A., W. Barczak, and W.M. Suchorska. *RNA interference in head and neck oncology*. Oncol Lett., 2016. **12**(5): pp. 3035–3040.

[19] Meister, G. *Argonaute proteins: functional insights and emerging roles*. Nat Rev Genet, 2013. 14(7): pp. 447–59.

[20] Tordonato, C., P.P. Di Fiore, and F. Nicassio, *The role of non-coding RNAs in the regulation of stem cells and progenitors in the normal mammary gland and in breast tumors*. Front Genet, 2015. **6**: p. 72.

[21] Kilgoz, H.O., et al., *KRAS and the reality of personalized medicine in non-small cell lung cancer*. Mol Med, 2016. **22**.

[22] Nicholson, S.A., et al., *Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens*. Am J Surg Pathol, 2002. **26**(9): pp. 1184–97.

[23] Nicholson, A.G., et al., *The international association for the study of lung cancer lung cancer staging project: proposals for the revision of the clinical and pathologic staging of small cell lung cancer in the forthcoming eighth edition of the TNM classification for lung cancer*. J Thorac Oncol, 2016. **11**(3): pp. 300–11.

[24] Cheng, T.D., et al., *The international epidemiology of lung cancer: latest trends, disparities, and tumor characteristics*. J Thorac Oncol, 2016. 11(10):1653–71.

[25] Okereke, I.C., et al., *Outcomes after surgical resection of pulmonary carcinoid tumors*. J Cardiothorac Surg, 2016. **11**: p. 35.

[26] Govindan, R., et al., *Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database*. J Clin Oncol, 2006. **24**(28): pp. 4539–44.

[27] Urman, A. and H.D. Hosgood, *Curbing the burden of lung cancer*. Front Med, 2016. **10**(2): pp. 228–32.

[28] Landrigan, P.J., *Comments on the 2014 Helsinki Consensus Report on Asbestos*. Ann Glob Health, 2016. **82**(1): pp. 217–20.

[29] http://www.cancer.org/cancer/lungcancer-non-smallcell/detailedguide/non-small-cell-lung-cancer-survival-rates, accessed, august 2016

[30] http://globocan.iarc.fr/old/FactSheets/cancers/lung-new.asp, accessed, august 2016

[31] Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation*. Cell, 2011. **144**(5): pp. 646–74.

[32] Neufeld, G., N. Shraga-Heled, T. Lange, N. Guttmann-Raviv, Y. Herzog, and O. Kessler, *Semaphorins in cancer*. Front Biosci, 2005. **10**: pp. 751–60.

[33] Piperdi, B., A. Merla, and R. Perez-Soler, *Targeting angiogenesis in squamous non-small cell lung cancer*. Drugs, 2014. **74**(4): pp. 403–13.

[34] Cabebe, E. and H. Wakelee, *Role of anti-angiogenesis agents in treating NSCLC: focus on bevacizumab and VEGFR tyrosine kinase inhibitors*. Curr Treat Options Oncol, 2007. **8**(1): pp. 15–27.

[35] Lee, S.M., et al., *Anti-angiogenic therapy using thalidomide combined with chemotherapy in small cell lung cancer: a randomized, double-blind, placebo-controlled trial*. J Natl Cancer Inst, 2009. **101**(15): pp. 1049–57.

[36] Arnedos, M., et al., *Personalized treatments of cancer patients: a reality in daily practice, a costly dream or a shared vision of the future from the oncology community?* Cancer Treat Rev, 2014. **40**(10): pp. 1192–8.

[37] Li, H. and X. Lv, *Functional annotation of noncoding variants and prioritization of cancer-associated lncRNAs in lung cancer*. Oncol Lett, 2016. **12**(1): pp. 222–230.

[38] Stenvang, J., M. Lindow, and S. Kauppinen, *Targeting of microRNAs for therapeutics*. Biochem Soc Trans, 2008. **36**(Pt 6): pp. 1197–200.

[39] Zhang, B., X. Pan, G.P. Cobb and T.A. Anderson, *microRNAs as oncogenes and tumor suppressors*. Dev Biol, 2007 Feb 1. **302**(1): pp. 1–12.

[40] Wang, L., et al., *Altered expression of transcription factor Sp1 critically impacts the angiogenic phenotype of human gastric cancer*. Clin Exp Metastasis, 2005. **22**(3): pp. 205–13.

[41] Safe S. *MicroRNA-Specificity Protein* (*Sp*) *Transcription factor interactions and significance in carcinogenesis*. Curr Pharmacol Rep, 2015. **1**(2): pp. 73–78.

[42] Liu, B., et al., *MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo*. Lung Cancer, 2009. **66**(2): pp. 169–75.

[43] Zhou, Y.M., J. Liu, and W. Sun, *MiR-130a overcomes gefitinib resistance by targeting met in non-small cell lung cancer cell lines*. Asian Pac J Cancer Prev, 2014. **15**(3): pp. 1391–6.

[44] Acunzo, M., et al., *miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by down-regulating miR-221 and 222*. Oncogene, 2012. **31**(5): pp. 634–42.

[45] Sun, C.Y., X.M. She, Y. Qin, Z.B. Chu, L. Chen, L.S. Ai, L. Zhang, and Y. Hu. *miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF*. Carcinogenesis, 2013. **34**(2): pp. 426–35.

[46] Tafsiri, E., et al., *Expression of miRNAs in non-small-cell lung carcinomas and their association with clinicopathological features*. Tumour Biol, 2015. **36**(3): pp. 1603–12.

[47] Koukourakis, M.I., et al., *bcl-2 and c-erbB-2 proteins are involved in the regulation of VEGF and of thymidine phosphorylase angiogenic activity in non-small-cell lung cancer*. Clin Exp Metastasis, 1999. **17**(7): pp. 545–54.

[48] Skrzypek, K., et al., *Interplay between heme oxygenase-1 and miR-378 affects non-small cell lung carcinoma growth, vascularization, and metastasis*. Antioxid Redox Signal, 2013. **19**(7): pp. 644–60.

[49] Chen, L.T., et al., *MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis*. Med Oncol, 2012. **29**(3): pp. 1673–80.

[50] Luo, W., et al., *miRNA-296-3p modulates chemosensitivity of lung cancer cells by targeting CX3CR1*. Am J Transl Res, 2016. **8**(4): pp. 1848–56.

[51] Xu, C., et al., *miR-296-5p suppresses cell viability by directly targeting PLK1 in non-small cell lung cancer*. Oncol Rep, 2016. **35**(1): pp. 497–503.

[52] Suarez, Y. and W.C. Sessa, *MicroRNAs as novel regulators of angiogenesis*. Circ Res, 2009. **104**(4): pp. 442–54.

[53] Hu, J., et al., *microRNA-128 plays a critical role in human non-small cell lung cancer tumourigenesis, angiogenesis and lymphangiogenesis by directly targeting vascular endothelial growth factor-C*. Eur J Cancer, 2014. **50**(13): pp. 2336–50.

[54] Zhao, W.Y., et al., *Downregulation of miR-497 promotes tumor growth and angiogenesis by targeting HDGF in non-small cell lung cancer*. Biochem Biophys Res Commun, 2013. **435**(3): pp. 466–71.

[55] Xia, X.M., W.Y. Jin, R.Z. Shi, Y.F. Zhang, and J. Chen. *Clinical significance and the correlation of expression between Let-7 and K-ras in non-small cell lung cancer*. Oncol Lett, 2010. **1**(6): pp. 1045–1047.

[56] Hu, H., et al., *MiR-145 and miR-203 represses TGF-beta-induced epithelial-mesenchymal transition and invasion by inhibiting SMAD3 in non-small cell lung cancer cells*. Lung Cancer, 2016. **97**: pp. 87–94.

[57] Li, J., et al., *The role of miR-205 in the VEGF-mediated promotion of human ovarian cancer cell invasion*. Gynecol Oncol. 2015. **137**: pp. 125–133.

[58] Garofalo, M., et al., *miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation*. Cancer Cell, 2009. **16**(6): pp. 498–509.

[59] Zhang, C., et al., *PUMA is a novel target of miR-221/222 in human epithelial cancers*. Int J Oncol, 2010. **37**(6): pp. 1621–6.

[60] Zhong, C., et al., *MicroRNA-222 promotes human non-small cell lung cancer H460 growth by targeting p27*. Int J Clin Exp Med, 2015. **8**(4): pp. 5534–40.

[61] Goukassian, D., et al., *Overexpression of p27(Kip1) by doxycycline-regulated adenoviral vectors inhibits endothelial cell proliferation and migration and impairs angiogenesis*. FASEB J, 2001. **15**(11): pp. 1877–85.

[62] Devlin, C., et al., *miR-210: More than a silent player in hypoxia*. IUBMB Life, 2011. **63**(2): pp. 94–100.

[63] Puissegur, M.P., et al., *miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity*. Cell Death Differ, 2011. **18**(3): pp. 465–78.

[64] Donnem, T., et al., *Prognostic impact of MiR-155 in non-small cell lung cancer evaluated by in situ hybridization*. J Transl Med, 2011. **9**: p. 6.

[65] Babar, I.A., et al., *Inhibition of hypoxia-induced miR-155 radiosensitizes hypoxic lung cancer cells*. Cancer Biol Ther, 2011. **12**(10): pp. 908–14.

[66] Xue, X., Y. Liu, Y. Wang, M. Meng, K. Wang, X. Zang, et al. MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN. Oncotarget, 2016.

[67] Yanaihara, N., et al., *Unique microRNA molecular profiles in lung cancer diagnosis and prognosis*. Cancer Cell, 2006. **9**(3): pp. 189–98.

[68] Donnem, T., et al., *MicroRNA signatures in tumor tissue related to angiogenesis in non-small cell lung cancer*. PLoS One, 2012. **7**(1): pp. e29671.

[69] Patnaik, S.K., et al., *Evaluation of microRNA expression profiles that may predict recurrence of localized stage I non-small cell lung cancer after surgical resection*. Cancer Res, 2010. **70**(1): pp. 36–45.

[70] Sang, H., et al., *Long non-coding RNA functions in lung cancer*. Tumour Biol, 2015. **36**(6): pp. 4027–37.

[71] Li, J., et al., *LncRNA MALAT1 exerts oncogenic functions in lung adenocarcinoma by targeting miR-204*. Am J Cancer Res, 2016. **6**(5): pp. 1099–107.

[72] Han, L., et al., *Low expression of long noncoding RNA PANDAR predicts a poor prognosis of non-small cell lung cancer and affects cell apoptosis by regulating Bcl-2*. Cell Death Dis, 2015. **6**: p. e1665.

[73] Zhang, E.B., et al., *P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression*. Cell Death Dis, 2014. **5**: p. e1243

[74] Deng, H.P., et al., *Long non-coding RNA HOTTIP promotes tumor growth and inhibits cell apoptosis in lung cancer*. Cell Mol Biol (Noisy-le-grand), 2015. **61**(4): pp. 34–40.

[75] Nie, F.Q., et al., *Long non-coding RNA MVIH indicates a poor prognosis for non-small cell lung cancer and promotes cell proliferation and invasion*. Tumour Biol, 2014. **35**(8): pp. 7587–94.

[76] Lu, K.H., et al., *Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression*. BMC Cancer, 2013. **13**: pp. 461.

[77] Yang, Q., et al., *A novel long noncoding RNA AK001796 acts as an oncogene and is involved in cell growth inhibition by resveratrol in lung cancer*. Toxicol Appl Pharmacol, 2015. **285**(2): pp. 79–88.

[78] Nie, F.Q., et al., *Long noncoding RNA ANRIL promotes non-small cell lung cancer cell proliferation and inhibits apoptosis by silencing KLF2 and P21 expression*. Mol Cancer Ther, 2015. **14**(1): pp. 268–77.

[79] Qiu, M., et al., *A novel lncRNA, LUADT1, promotes lung adenocarcinoma proliferation via the epigenetic suppression of p27*. Cell Death Dis, 2015. **6**: pp. e1858.

[80] Pan, H., et al., *Long non-coding RNA BC087858 induces non-T790M mutation acquired resistance to EGFR-TKIs by activating PI3K/AKT and MEK/ERK pathways and EMT in non-small-cell lung cancer*. Oncotarget, 2016. **7**(31): pp. 49948–60.

[81] Zhang, L., et al., *Enhanced expression of long non-coding RNA ZXF1 promoted the invasion and metastasis in lung adenocarcinoma*. Biomed Pharmacother, 2014. **68**(4): pp. 401–7.

[82] Yang, Z.T., et al., *Overexpression of Long Non-Coding RNA ZXF2 Promotes Lung Adenocarcinoma Progression Through c-Myc Pathway*. Cell Physiol Biochem, 2015. **35**(6): pp. 2360–70.

[83] Wang, G., H. Chen, and J. Liu, *The long noncoding RNA LINC01207 promotes proliferation of lung adenocarcinoma*. Am J Cancer Res, 2015. **5**(10): pp. 3162–73.

[84] Castellano, J.J., et al., *LincRNA-p21 impacts prognosis in resected non-small-cell lung cancer patients through angiogenesis regulation*. J Thorac Oncol, 2016. **11**(12):2173–82

[85] Calin, G.A., et al., *Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas*. Cancer Cell. **12**(3): pp. 215–229.

[86] Peng, J.C., J. Shen, and Z.H. Ran, *Transcribed ultraconserved region in human cancers*. RNA Biol, 2013. **10**(12): pp. 1771–7

[87] Jiang, S., et al., *MicroRNA-21 modulates radiation resistance through upregulation of hypoxia-inducible factor-1alpha-promoted glycolysis in non-small cell lung cancer cells*. Mol Med Rep, 2016. **13**(5): pp. 4101–7.

[88] Kenneth, Niall S. and S. Rocha, *Regulation of gene expression by hypoxia*. Biochem J, 2008. **414**(1): pp. 19–29.

[89] Gao, X.J., et al., *Nobiletin inhibited hypoxia-induced epithelial-mesenchymal transition of lung cancer cells by inactivating of Notch-1 signaling and switching on miR-200b*. Pharmazie, 2015. **70**(4): pp. 256–62.

[90] Haigl, B., et al., *Expression of microRNA-21 in non-small cell lung cancer tissue increases with disease progression and is likely caused by growth conditional changes during malignant transformation*. Int J Oncol, 2014. **44**(4): pp. 1325–34.

[91] Eilertsen, M., et al., *Positive prognostic impact of miR-210 in non-small cell lung cancer*. Lung Cancer, 2014. **83**(2): pp. 272–8.

[92] Krutilina, R., et al., *MicroRNA-18a inhibits hypoxia-inducible factor 1alpha activity and lung metastasis in basal breast cancers*. Breast Cancer Res, 2014. **16**(4): p. R78.

[93] Ding, G., et al., *MiR-199a suppresses the hypoxia-induced proliferation of non-small cell lung cancer cells through targeting HIF1alpha*. Mol Cell Biochem, 2013. **384**(1–2): pp. 173–80.

[94] Guo, W.G., et al., *Bioinformatics analyses combined microarray identify the desregulated microRNAs in lung cancer*. Eur Rev Med Pharmacol Sci, 2013. **17**(11): pp. 1509–16.

[95] Taguchi, A., et al., *Identification of hypoxia-inducible factor-1 alpha as a novel target for miR-17-92 microRNA cluster*. Cancer Res, 2008. **68**(14): pp. 5540–5.

[96] Mao, G., et al., *Tumor-derived microRNA-494 promotes angiogenesis in non-small cell lung cancer*. Angiogenesis, 2015. **18**(3): pp. 373–82.

[97] Yang, F., et al., *Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis*. Mol Cell, 2013. **49**(6): pp. 1083–96.

[98] Wu, Y., et al., *Downregulation of the long noncoding RNA GAS5-AS1 contributes to tumor metastasis in non-small cell lung cancer*. Sci Rep, 2016, **6**: p. 31093.

[99] Zhou, C., et al., *Long noncoding RNA HOTAIR, a hypoxia-inducible factor-1alpha activated driver of malignancy, enhances hypoxic cancer cell proliferation, migration, and invasion in non-small cell lung cancer*. Tumour Biol, 2015. **36**(12): pp. 9179–88.

[100] Yao, Y., J. Li, and L. Wang, *Large intervening non-coding RNA HOTAIR is an indicator of poor prognosis and a therapeutic target in human cancers*. Int J Mol Sci, 2014. **15**(10): pp. 18985–99.

[101] Matouk, I.J., et al., *The oncofetal H19 RNA connection: hypoxia, p53 and cancer*. Biochim Biophys Acta, 2010. **1803**(4): pp. 443–51.

[102] Mei, Y., et al., *A piRNA-like small RNA interacts with and modulates p-ERM proteins in human somatic cells*. Nat Commun, 2015. **6**: p. 7316.

[103] Qi, R., T. Qiao, and X. Zhuang, *Small interfering RNA targeting S100A4 sensitizes non-small-cell lung cancer cells (A549) to radiation treatment*. Onco Targets Ther, 2016. **9**: pp. 3753–62.

[104] Geretti, E. and M. Klagsbrun, *Neuropilins: novel targets for anti-angiogenesis therapies*. Cell Adh Migr, 2007. **1**(2): pp. 56–61.

[105] Nguyen, Q.D., et al., *Inhibition of vascular endothelial growth factor (VEGF)-165 and semaphorin 3A-mediated cellular invasion and tumor growth by the VEGF signaling inhibitor ZD4190 in human colon cancer cells and xenografts*. Mol Cancer Ther, 2006. **5**(8): pp. 2070–7.

[106] Geretti, E., A. Shimizu, and M. Klagsbrun, *Neuropilin structure governs VEGF and semaphorin binding and regulates angiogenesis*. Angiogenesis, 2008. **11**(1): pp. 31–9.

[107] Kitsukawa, T., et al., *Overexpression of a membrane protein, neuropilin, in chimeric mice causes anomalies in the cardiovascular system, nervous system and limbs*. Development, 1995. **121**(12): pp. 4309–18.

[108] Miao, H.Q., et al., *Neuropilin-1 expression by tumor cells promotes tumor angiogenesis and progression*. FASEB J, 2000. **14**(15): pp. 2532–9.

[109] Banerjee, S.K., et al., *Overexpression of vascular endothelial growth factor164 and its co-receptor neuropilin-1 in estrogen-induced rat pituitary tumors and GH3 rat pituitary tumor cells*. Int J Oncol, 2000. **16**(2): pp. 253–60.

[110] Han, F., et al., *MicroRNA-30b promotes axon outgrowth of retinal ganglion cells by inhibiting Semaphorin3A expression*. Brain Res, 2015. **1611**: pp. 65–73.

[111] Loginov, V.I., et al., *Tumor suppressor function of the SEMA3B gene in human lung and renal cancers*. PLoS One, 2015. **10**(5): pp. e0123369.

[112] Cai, G., S. Qiao, and K. Chen, *Suppression of miR-221 inhibits glioma cells proliferation and invasion via targeting SEMA3B*. Biol Res, 2015. **48**: p. 37.

[113] Mumblat, Y., et al., *Full-length semaphorin-3C is an inhibitor of tumor lymphangiogenesis and metastasis*. Cancer Res, 2015. **75**(11): pp. 2177–86.

[114] Meert, A.P., et al., *[Difficulties and limitations in conducting translational research in thoracic oncology. A practical example]*. Rev Mal Respir, 2016. **33**(7): pp. 594–9.

[115] Foley, K., et al., *Semaphorin 3D autocrine signaling mediates the metastatic role of annexin A2 in pancreatic cancer*. Sci Signal, 2015. **8**(388): p. ra77.

[116] Christensen, C., et al., *Proteolytic processing converts the repelling signal Sema3E into an inducer of invasive growth and lung metastasis*. Cancer Res, 2005. **65**(14): pp. 6167–77.

[117] Nasarre, P., et al., *Neuropilin-2 Is upregulated in lung cancer cells during TGF-beta1-induced epithelial-mesenchymal transition*. Cancer Res, 2013. **73**(23): pp. 7111–21.

[118] Clarhaut, J., et al., *ZEB-1, a repressor of the semaphorin 3F tumor suppressor gene in lung cancer cells*. Neoplasia, 2009. **11**(2): pp. 157–66.

[119] Jian, H., et al., *SEMA4B inhibits growth of non-small cell lung cancer in vitro and in vivo*. Cell Signal, 2015. **27**(6): pp. 1208–13.

[120] Jian, H., B. Liu, and J. Zhang, *Hypoxia and hypoxia-inducible factor 1 repress SEMA4B expression to promote non-small cell lung cancer invasion*. Tumour Biol, 2014. **35**(5): pp. 4949–55.

[121] Jian, H., et al., *SEMA4b inhibits MMP9 to prevent metastasis of non-small cell lung cancer*. Tumour Biol, 2014. **35**(11): pp. 11051–6.

[122] Li, J., Q. Wang, R. Wen, J. Liang, X. Zhong, W. Yang, et al. *MiR-138 inhibits cell proliferation and reverses epithelial-mesenchymal transition in non-small cell lung cancer cells by targeting GIT1 and SEMA4C*. J Cell Mol Med, 2015. **19**(12): pp. 2793–805.

[123] Gu, A., J. Lu, W. Wang, C. Shi, B. Han, M. Yao. *Role of miR-497 in VEGF-A-mediated cancer cell growth and invasion in non-small cell lung cancer*. Int J Biochem Cell Biol, 2016. **70**: pp. 118–25.

[124] Ruan, S.S., et al., *Expression and clinical significance of Semaphorin4D in non-small cell lung cancer and its impact on malignant behaviors of A549 lung cancer cells*. J Huazhong Univ Sci Technolog Med Sci, 2014. **34**(4): pp. 491–6.

[125] Liu, Y., et al., *MiR-214 suppressed ovarian cancer and negatively regulated semaphorin 4D*. Tumour Biol, 2016. **37**(6): pp. 8239–48.

[126] Fazzari, P., et al., *Plexin-B1 plays a redundant role during mouse development and in tumour angiogenesis*. BMC Dev Biol, 2007. **7**: p. 55.

[127] Lu, T.P., et al., *Identification of a novel biomarker, SEMA5A, for non-small cell lung carcinoma in nonsmoking women*. Cancer Epidemiol Biomarkers Prev, 2010. **19**(10): pp. 2590–7.

[128] Veliceasa, D., et al., *Therapeutic manipulation of angiogenesis with miR-27b*. Vasc Cell, 2015. **7**: p. 6.

[129] Urbich, C., et al., *MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A*. Blood, 2012. **119**(6): pp. 1607–16.

[130] Ma, B., et al., *Role of chitinase 3-like-1 and semaphorin 7a in pulmonary melanoma metastasis*. Cancer Res, 2015. **75**(3): pp. 487–96.

[131] Narazaki, M., M. Segarra, and G. Tosato, *Sulfated polysaccharides identified as inducers of neuropilin-1 internalization and functional inhibition of VEGF165 and semaphorin3A*. Blood, 2008. **111**(8): pp. 4126-36.

[132] Wiggins, J.F., et al., *Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34*. Cancer Res, 2010. **70**(14): pp. 5923–30.

[133] AUSTIN, Texas, BUSINESS WIRE - *Mirna Therapeutics*, Inc. (Nasdaq:MIRN) September 20, 2016

[134] Potente, M., et al., *Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization*. J Clin Invest, 2005. **115**(9): pp. 2382–92.

[135] Krug, S., et al., *Knock-down of Pdcd4 stimulates angiogenesis via up-regulation of angiopoietin-2*. Biochim Biophys Acta, 2012. **1823**(4): pp. 789–99.

[136] Wang, F., A.J. Nazarali, and S. Ji, *Circular RNAs as potential biomarkers for cancer diagnosis and therapy*. Am J Cancer Res, 2016. **6**(6): pp. 1167–76.

[137] Ricciuti, B., et al., *Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy*. Med Oncol, 2016. **33**(2): p. 18.

[138] Liu, Z., M. Sun, K. Lu, J. Liu, M. Zhang, W. Wu, et al. *The long noncoding RNA HOTAIR contributes to cisplatin resistance of human lung adenocarcinoma cells via downregualtion of p21(WAF1/CIP1) expression*. PLoS One, 2013. **8**(10): p. e77293.

[139] Li, Z. and T.M. Rana. *Therapeutic targeting of microRNAs: current status and future challenges*. Nat Rev Drug Discov, 2014. **13**(8): pp. 622–38.

[140] Roberts, T.C. and M.J. Wood. *Therapeutic targeting of non-coding RNAs*. Essays Biochem, 2013. **54**: pp. 127–45.

[141] Krutzfeldt, J., N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, et al. *Silencing of microRNAs in vivo with 'antagomirs'*. Nature, 2005. **438**(7068): pp. 685–9.

[142] Bagga, S., J. Bracht, S. Hunter, K. Massirer, J. Holtz, R. Eachus, et al. *Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation*. Cell, 2005. **122**(4): pp. 553–63.

[143] Morrissey, D.V., J.A. Lockridge, L. Shaw, K. Blanchard, K. Jensen, W. Breen, et al. *Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs*. Nat Biotechnol, 2005. **23**(8): pp. 1002–7.

[144] Akinc, A., et al., *A combinatorial library of lipid-like materials for delivery of RNAi therapeutics*. Nat Biotechnol, 2008. **26**(5): pp. 561–9.

[145] Peer, D., et al., *Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target*. Science, 2008. **319**(5863): pp. 627–30.

[146] Palliser, D., et al., *An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection*. Nature, 2006. **439**(7072): pp. 89–94.

[147] Kumar, P., et al., *A single siRNA suppresses fatal encephalitis induced by two different flaviviruses*. PLoS Med, 2006. **3**(4): pp. e96.

[148] Hu-Lieskovan, S., et al., *Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma*. Cancer Res, 2005. **65**(19): pp. 8984–92.

[149] Song, E., et al., *Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors*. Nat Biotechnol, 2005. **23**(6): pp. 709–17.

[150] Yao, Y.D., et al., *Targeted delivery of PLK1-siRNA by ScFv suppresses Her2+ breast cancer growth and metastasis*. Sci Transl Med, 2012. **4**(130): p. 130ra48.

[151] Gutschner, T., et al., *The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells*. Cancer Res, 2013. **73**(3): pp. 1180–9.

IntechOpen