Early detection of laryngeal cancer: prominence of miRNA signature as a new tool for clinicians

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Abstract

Early detection of laryngeal cancer, essential in the initial stages of the disease to achieve a high survival rate, is unfortunately hampered by the lack of specific symptoms. In this regard, there is a pressing need to dispose of reliable and non-invasive diagnostic and prognostic markers for a real time monitoring availing of accurate and reproducible methods, in order ensure applicability for early diagnosis and primary and secondary prevention. This review focuses on the recent reports that emphasize the crucial role of miRNAs in regulating laryngeal cancer tumorigenesis. In detail, we have reported the most characterized miRNAs with an established oncogenic or oncosuppressive role in cell biology of laryngeal cancer, also describing, for each of them, the main molecular mechanisms responsible for their specific function. We have also defined the potential of miRNAs as novel diagnostic and prognostic markers in virtue of their differential expression between laryngeal carcinoma tissues and the adjacent normal counterpart. Moreover, their proven stability in systemic circulation and other body fluids, as well as their easy detection and quantization, makes the analysis of miRNA signatures an excellent tool for clinicians as hallmark for cancer classification and diagnosis. Moreover, an eventual similarity between deregulated miRNAs in tumor tissues and in body fluids would allow to provide a considerable advantage for patients' compliance, replacing invasive tissue biopsies with simple assays on easily obtained blood products.

Introduction

Malignant tumors of the larinx are a worldwide leading cause of death. Current data about incidence and mortality, updated to 2012 and published by the European Network of Cancer Registries (EUCAN), indicate that the cases of laryngeal cancer in Europe are about 40,000 with more than 19,000 deaths each year linked to this disease and an high incidence (about 90%) in males [data available on European Cancer Observatory (ECO); http://eco.iarc.fr/eucan/Cancer.aspx?Cancer=17]. Early diagnosis is hampered by the lack of symptoms in the initial and pre-clinical stages. As regards etiology and pathogenesis, a strong influence is due to environmental and lifestyle-related factors, such as the use of tobacco, the assumption of ethanol and the exposure to toxic substances. Additionally, major oncogenic cofactors seem to be associated with diet, irradiation, papilloma virus infection and laryngopharyngeal reflux.[1] More than 95% of laryngeal tumors are squamous cell carcinomas (LSCC). Early detection is essential in the initial stages of the disease and is associated with a high survival rate of patients at five years from diagnosis. However, survival likelihood dramatically decreases when this cancer is detected at an advanced stage. Where possible, laryngeal cancers are treated surgically, but frequently this type of intervention become demolitive due to the wide invasiveness in 9 cases out of 10, when disease is diagnosed in advanced stages. Early intervention, which offers better chance of application to conservative surgery, preserves the functionality of organs and tissues affected by cancer.[2] However, the possibility of early intervention depends on the availability of reliable and easily identifiable markers that may result suitable for monitoring by clinical laboratories, using accurate and reproducible methods with sufficient sensitivity to ensure applicability to early diagnosis and to primary and secondary prevention. On these bases, the need to have non-invasive diagnostic and prognostic systems with wide applicability in these diseases, represents an objective of primary importance in oncology in view of improving clinical outcome. Therefore, it is essential to develop diagnostic procedures that allow early diagnosis of cancer.

Until a few years ago, the genes coding for proteins with oncogenic or tumor suppressor activity were considered the main factors responsible for the development and maintenance of tumor phenotypes. However, the recent discovery of thousands of genes transcribing non-coding RNA (including microRNA), makes it clear that biology underlying cancer is much more complex than previously thought. Several recent reports, whose main findings are discussed in the present review, have, in fact, emphasized the crucial role of miRNAs in the regulation of tumorigenesis, outlining their potential role not only as therapeutic targets, but also as useful prognostic and diagnostic biomarkers.[3-5]

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Clinics and treatment options of laryngeal squamous cell carcinomas

Generally, the clinical outcome of laryngeal cancer depends from its site of origin and its tendency to disseminate to local lymphatic vessels. Progressive and constant hoarseness is the most important symptom of laryngeal carcinoma, especially of the glottic one. Cough, swelling in the neck, dyspnoea and stridor, haemoptysis, pain, dysphagia, halitosis, tenderness of the larynx, reflective otalgia and weight loss can also occur during the course of disease.[6] Neck metastases are more common in supraglottic cancers due to copious lymphatic network that leads to early dissemi-

nation of primary tumor to the regional lymph nodes. On the contrary, glottic tumors only occasionally have regional neck metastases at the time of diagnosis, because of the poor lymphatic network. Staging of primary laryngeal tumors is strictly related to the surface extent of the lesion to different sites surrounding the same area of the larynx or from one area to the adjacent organs, such as the vocal cords.[6,7] About 70% of the patients with supraglottic tumors at the time of diagnosis display advanced (III-IV) disease stage, while 75% of the glottic tumors display localized (I-II) disease at initial stages. At the time of diagnosis a second primary tumor can be found within the respiratory tract, mainly in lung, that's why it is important to perform an appropriate radiological evaluation.[2] Of note, the extent of tumor cells on the perineural sheath correlates with the histopathology and clinical aggressiveness of LSCC. Our group has recently investigated the prognostic importance of perineural invasion, correlating the rate of infiltration with tumor histotype, site and tumor-node-metastasis stage and type of surgery (total or partial laryngectomy); we have also performed a retrospective analysis with the aim of evaluate the rate of disease-free survival according to the outcome of combined surgery and radiotherapy (RT) treatment, underlining the matter of performing an early clinical and instrumental follow-up in patients with laryngeal cancer whose histopathological examination is positive for perineural invasion.[8]

Surgery, radiotherapy and chemotherapy are the current strategies for LSCC management, respectively used in about 55, 70 and 10% of patients. These approaches could be adopted both as a single therapy, this is mainly the case of early-stage LSCC (I and II), or in a combination of them for more advanced disease (stages III and IV); in the last case, the most used treatment consists of a combination of surgery and irradiation.[2]

Only in recent times, innovative molecular approaches have been experienced and are currently under investigation. For instance, one of the most studied therapeutic interventions is based on the targeting of cellular tumor markers, with the aim of inhibit specific signaling pathways implicated in oncogenesis and cancer progression. Among these, there is the interfering of EGFR pathway, availing of monoclonal antibodies against the extracellular ligand binding domain, ligand-toxin conjugates, tyrosine kinase or antisense oligonucleotides against EGFR mRNA.[2,7] Another current molecular strategy for LSCC treatment is based on transient ectopic expression of wild-type p53 gene. This approach has been analyzed in head and neck tumors, mainly using a gene-delivery strategy based on recombinant adenovirus Ad-p53, demonstrating to be able to stimulate apoptosis and both radio- and chemo-sensitization in cancer cell lines. Therefore, the exploit of p53 gene therapy in association with radio- or chemotherapy could be accounted as a rational treatment option especially for the application in early laryngeal carcinogenesis where p53 mutations are frequently observed.[2,7] Nevertheless, the major issues correlated with these strategies, remain the absence of a really efficient, long-term gene-delivery system and the need of multitargeting agents capable of disrupting, simultaneously, multiple oncogenic signaling processes.

Biogenesis and role of miRNAs in laryngeal squamous cell carcinomas

Micro-RNAs are a class of non-coding RNAs with regulatory activity identified relatively recently; typically 19-25 nucleotides long, they work mainly by binding to specific messenger RNAs (mRNAs) and thus inhibiting translation through a partially complementary base pairing.[9]

miRNAs are synthesized in the nucleus where the primary RNA (pri-miRNA) is transcribed by RNA polymerase II (Pol II) and then processed by RNase III Drosha-DGCR8 complex, resulting in a miRNA precursor (pre-miRNA) that is exported to the cytoplasm by Exportin-5 and Ran-GTP61. pre-miRNA is subsequently spliced into mature double-stranded ~22-nucleotide miRNA by the RNase III Dicer, in complex with the transactivation-response RNA-binding protein (TRBP) enzyme. Among the two strands of mature miRNA, only the functional one is loaded together with Argonaute (Ago2) proteins into the RNA induced silencing complex (RISC), where miRNA guides RISC to bind the 3' UTR of a mRNA target, leading to either mRNA cleavage, translational repression, or deadenylation.[9]

MiRNAs are involved in a variety of biological processes, including development, differentiation, apoptosis, survival, senescence, and metabolism. To date, more than 720 miRNAs have been identified in humans and it is believed that they regulate from 30 to 60% of the whole genome. It has been shown that miRNAs can have specific expression profiles based on developmental stages, tissue type and pathological state. Studies carried out in various cancers, including laryngeal cancer, showed altered miRNA expression in tumor tissues as compared with the healthy ones, thus suggesting the involvement of these molecules also in carcinogenesis.[10] This evidence has also enabled the identification of two classes of miRNA: i) those targeting anti-apoptotic and tumor suppressor genes, that promote tumor growth, and ii) miRNAs able to bind and inhibit tumor oncogenes bearing onco-suppressor function. The latter category, in particular, could be helpful in the development of specific anti-cancer agents, able to mimic the function of tumor suppressor miRNAs in cancer cells. In addition, miRNAs offer the exciting prospective to be able to be used as diagnostic or predictive biomarkers in cancer diseases.[11] Several ongoing studies and recent reports are investigating on the correlation between deregulated miRNAs and the aggressiveness of LSCC. However, one of the major objectives is the attempt to identify a correlation between aberrantly expressed miRNAs and metastasization to lymph node in LSCC. The extent to which the cancer has spread to the lymph nodes is, in fact, an important prognostic factor and decision making for the subsequent surgical strategy of intervention. Moreover, LSCC that has spread to the lymph nodes at the time of diagnosis has less favorable outcomes than tumors that remain confined to the larynx.

Differentially expressed miRNAs with underlying molecular mechanisms in laryngeal squamous cell carcinomas

Differentially expressed miRNAs, as well as differentially expressed genes, act in concert in regulating development and metastasis of LSCC (Figure 1). miRNAs can modulate the expression of thousands of human target genes that may be easily predicted by the help of several data bases that include also the experimental validated miRNAs.[12,13] Intragenic miRNAs, both exonic and intronic, are generally transcribed in parallel with their host genes,[14] with whom they often share biological functions and the property to control specific signaling pathways.[15]

The analysis of an oligonucleotide microarray showed the differential pattern of microRNAs and mRNAs expressed in laryngeal carcinoma tissues compared to their adjacent normal ones.[10] In detail, it was established that miR-21 was overexpressed in laryngeal carcinoma tissues and that its inhibition by specific antisense oligonucleotides counteracted the prolifera-

tion of laryngeal carcinoma cell line HEp-2 through the downregulation of BTG2, a validated target of miR-21, thus inducing suppression of cell cycle progression without affecting cell apoptosis. Other miR-21 targets, PTEN, TPM1 and PDCD4, also showed a downregulation trend.[16] Also another suppressor of cell cycle progression, the cyclin-dependent kinase 2-associated protein 1 (CDK2AP1), an inhibitor of CDK2 and hence a G1/S transition inhibitor, was found negatively regulated in LSCC. Recently, CDK2AP1 was reported as an additional target gene of miR-21 in malignant human oral keratinocyte[17] and a recent study reported that the expression of this gene was downregulated by miR-205, thus outlining a CDK2AP1 targeting by miR-205 in LSCC. Therefore, oncogenic miR-205, through CDK2AP1 inhibition, mediated cell proliferation and invasion by increasing the activity of MMP2 and MMP9 and up-regulating c-Myc and CyclinD1 expression in LSCC cells.[18] Among the miRNAs overexpressed in laryngeal cancer, it was recently identified a role for miR-10b in the initiation of invasion and migration of HEp-2 cells. Zhang et al. revealed that miR-10b can promote the epithelial-mesenchymal transition (EMT) of HEp-2 cells by directly targeting the E-cadherin (E-cad) mRNA. The authors investigated, by Western blot analysis, the expression of both epithelial (E-cad and ZO-1) and mesenchymal (Vim, N-cad, and FN) markers in HEp-2 cells transfected with miR-10b, showing that miR-10b ectopic expression globally downregulated the first group and upregulated the second one. These results were further confirmed by qRT-PCR analysis.[19] An oncogenic role was also identified for miR-221 that is known for affecting several cancer pathways by modulating multiple gene targets such as estrogen receptor-α, p27, p57 and receptor tyrosine kinase kit 1 (c-kit).[20] In agreement with this individualized functions, it was reported that the silencing of endogenous miR-221 suppressed proliferation and promoted apoptosis and cell cycle arrest of laryngeal cancer cells. miR-221 was demonstrated to stimulate apoptosis resistance in LSCC by targeting Apaf-1, as demonstrated by bioinformatics prediction and luciferase assay. Of note, antitumor role of miR-221 was also confirmed in HEp-2 xenograft mouse models, since miR-221 inhibition decreased tumor volume and weights and improved survival rate.[21] The same authors also showed for the first time the correlation between miR-126 and LSCC. They assessed the levels of circulating miR-126 in LSCC patients' serum and focused their study on a miR-126 target protein, Camsap1, investigating both its function and mechanism, establishing that microtubules did not form aggregates in LSCC cells after miR-126 mimics transfection or Camsap1 knockdown. Moreover, they found a higher Camsap1 expression in cancer tissues compared with normal ones and related its expression with LSCC patients' prognosis and tumor metastasis.[22] miR-34c is down-regulated in human laryngeal carcinoma and also works as tumor suppressor. Its replacement, in laryngeal cancer cell models, inhibited growth and invasion by direct suppression of c-Met expression.[23] A member of the same family, with a well known ubiquitary oncosuppressive role, is miR-34a, whose expression in 71 LSCC patients was inversely correlated with cyclin D1 (CCND1) levels, as well as with lymph node metastases and clinical stage. CCND1 targeting by miR-34a was assessed by bioinformatic prediction and confirmed by Western blotting and luciferase reporter assay.[24] A molecular mechanism was also proposed for the oncosuppressor miR-340 that is able to promote p27 expression and to repress PI3K/Akt signaling in LSCC by targeting the histone methyltransferase EZH2, whose overexpression has been linked to aggressive phenotypes in several cancers. This mechanism was responsible for the inhibition of cell proliferation, invasion and migration, and for the induction of apoptosis in HEp-2 cells.[25] Other miRNAs involved in the suppression of migration and invasion in laryngeal cancer are miR-1, that acts through the targeting of fibronectin1,[26] and miR-206, that targets VEGF and whose expression has been inversely correlated with T stage and nodal metastasis of LSCC.[27] Also protein levels of cyclinD2 are under the direct control of miR-206 and the ectopic expression of this miRNA inhibits the proliferation by blocking the G1/S transition in HEp-2 cells and suppresses the growth in mice models.[28] In very recent times, it was also analyzed the molecular mechanism of Let-7a, a member of Let-7 miRNA family, and High-mobility group protein A2, HMGA2, in oncogenesis and progression of laryngeal cancer.[29] The negative correlation between low levels of Let-7a and high expression of HMGA2 mRNA in this neoplasm suggested their involvement in the promotion of invasion and metastasis. HMGA2 is, in fact, involved in changing the DNA structure through its binding, thus regulating DNA transcription, and can also directly bind transcription factors, still regulating DNA transcription. High HMGA2 expression was closely associated with clinical stage and lymph node metastasis in LSCC, as well as in numerous malignant

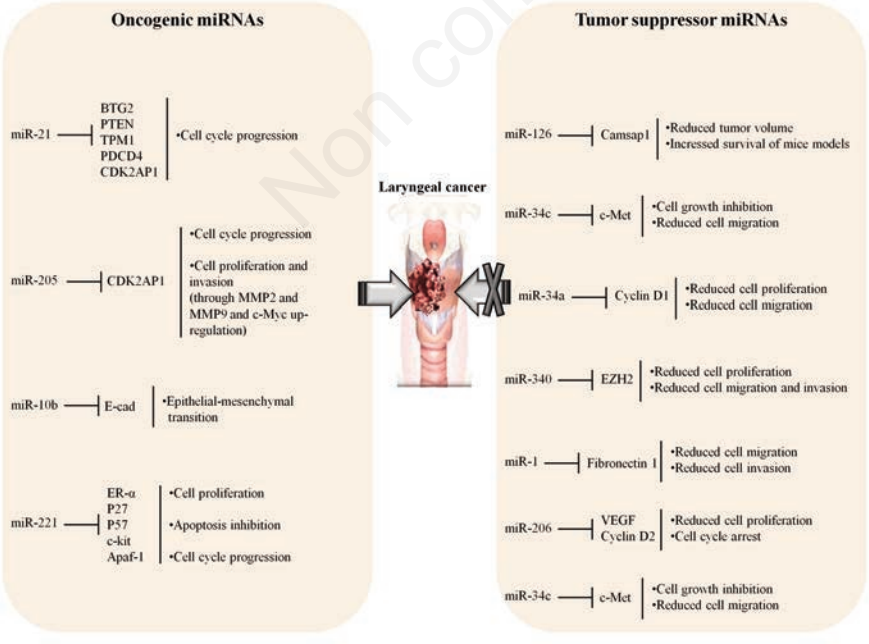

Figure 1. Main oncogenic and tumor suppressive miRNAs in laryngeal cancer representation of the main oncogenic and tumor suppressive miRNAs, along with the corresponding mRNAs target and the most important biologic effects found in tumor models.

tumors.[29] All these findings suggest the potential of miRNAs as a diagnostic markers and novel therapeutic target for patients with LSCC.

Circulating miRNAs: a new promise for cancer diagnosis

The diagnosis and staging of cancer, as well as the evaluation of response to therapy can be monitored through the dosing of specific molecular biomarkers, such as antigens, enzymes and lipids, easily retrievable from body fluids of cancer patients through minimally invasive methods. The recent detection of some circulating miRNAs in body fluids, including serum, plasma, urine, saliva and breast milk, has expanded the plethora of reliable biomarkers for early cancer detection and classification, opening a new scenario for the successfully use of nucleic acids. Circulating miRNAs are more resistant to RNase digestion as compared to tissue or cell miRNAs and their levels appear rather stable, reproducible and consistent among individuals of same species.[30] miRNAs' stability has been explained with the discovery of their package in microparticles, such as exosomes, microvesicles, and apoptotic bodies[31,32] or their association with RNA-binding proteins, such as Argonaute2 (Ago2)[33] or high-density lipoprotein complexes (HDL).[34,35] Cellular release and stability of extracellular miRNAs is an intense area of investigation that has also led to the fascinating idea that circulating miRNAs could have a key role in cell-to-cell communication, under an eventual selective targeting for secretion and an uptake by a distant target cell, possibly to regulate gene expression.[35] The released miRNAs can be detected and quantified developing a characteristic miRNome of a biological fluid that can be usefully paralleled to the diseased tissue's miRNA pattern. A possible similarity between deregulated miRNA expression detected in the tumor tissue and the corresponding modulation of circulating miRNAs, would allow to replace invasive tissue biopsies with straightforward assays on easily obtained blood products, with a clear advantage in terms of early warnings of tumorigenesis.

miRNAs as predictive biomarkers for early diagnosis of laryngeal squamous cell carcinomas

Growing evidence has suggested that miRNA signatures are useful as potential predictor for early diagnosis of laryngeal cancers. Recently, a number of research groups have shown attractive candidate miRNAs as powerful predictive biomarker (Table 1). Saito et al. described that miR-133b, miR-455-5p and miR-196a were differentially expressed between laryngeal tumors and their paired noncancerous tissues. Further studies indicated that miR-196a was over-expressed in cancer tissues if compared to precancerous dysplasias and benign laryngeal tissues.[36] A miRNA classifier, characterized using miRNA microarray and bioinformatic algorithms in early larynx carcinoma and normal esophageal mucosa tissue samples from 69 retrospectively selected patients, identified 47 miRNAs differentially expressed. Among them, hsa-miR-657 and hsa-miR-1287 were, respectively, highly up- and down-regulated, with high specificity and sensitivity for discriminating early laryngeal malignancies.[37] Moreover, another report showed that both miR-148a and miR-375 expression levels were strongly over-expressed in laryngeal carcinoma tissues.

Table 1. Summary of the most prominent predictive miRNAs in laryngeal cancer.

miRNAs	Regulation	Reference
miR-133b	Down	(36)
miR-455-5p	Up	(36)
miR-196a	Up	(36)
hsa-miR-657	Up	(37)
hsa-miR-1287	Down	(37)
miR-148a	Up	(38)
miR-375	Up	(38)
miR-423-3p	Up	(39)
miR-31-star	Up	(40)
miR-1264	Up	(40)
miR-3150b-5p	Down	(40)
miR-210	Down	(40)
miR-331-3p	Up*	(41)
miR-603	Up*	(41)
miR-1303	Up*	(41)
miR-660-5p	Up*	(41)
miR-212-3p	Up*	(41)
miR-27a	Up	(42)

*Upregulation due to its undetermined expression in normal samples.

Table 2. Summary of the most prominent prognostic miRNAs in laryngeal cancer.

miRNAs	Regulation	Prognosis	Reference
miR-152	Down	Poor pT and pN stage	(43)
miR-23a	Up	Lymph node metastasis, worse clinical stage, poor survival	(44)
miR-21	Up	Poor prognosis	(45)
miR-375	Down	Good prognosis	(45)
miR-296-5p	Up	Radio-resistance, recurrence	(46)
miR-101	Down	Poor prognosis	(47)
miR-9	Up	Poor prognosis	(48)

pT, primary tumor; pN, regional lymph nodes metastases.

Furthermore, association analysis revealed that miR-375 expression in advanced laryngeal cancer (stage III/IV) was much higher than in early cases (stage I/II). Conversely, miR-148a expression was also increased in early cancer tissues and its expression was not significantly altered compared to advanced cancers.[38] A study by Guan et al. exhibited that miR-423-3p was up-regulated in primary laryngeal carcinoma cell lines. The same group also revealed that miR-423-3p act as an oncological gene.[39] In HEp2 cells, a laryngeal cancer cell line, the expression of 49 miRNAs was significantly changed after treatment with paclitaxel. Remarkably, among these, miR-31-star, miR-1264, miR-3150b-5p, and miR-210 were the most deregulated.[40] To the best of our knowledge, Ayaz et al. have conducted the first study on miRNA profiling in plasma from patients with laryngeal cancer. In this investigation, several circulating miRNAs were determined and resulted abnormally expressed among plasma samples collected from laryngeal cancer patients compared to control plasma. Five circulating miRNAs, miR-331-3p, miR-603, miR-1303, miR-660-5p and miR-212-3p, were expressed in the pathological plasma, while they were undetectable in plasma derived from healthy subjects or patients suffering from any other diseases.[41] Finally, based on the previous evidence that miR-23a/24-2/27a cluster plays a crucial role in carcinogenesis acting as a novel oncogene, also confirmed by the finding that miR-27a can promote proliferation and suppresses apoptosis in HEp2 cells. A recent study investigated serum miR-27a levels in laryngeal cancer, finding that it was higher expressed than in healthy controls. Moreover, miR-27a over-expression appeared to be associated with clinical stage.[42]

Collectively, these miRNAs could be considered suitable as predictive molecular marker of laryngeal malignancies.

miRNAs as prognostic biomarkers in laryngeal squamous cell carcinomas patients management

Despite the improved therapeutic options of the past few decades, the treatment of laryngeal cancer and the patients' quality of life are still unsatisfying due to poor prognosis of laryngeal carcinoma. The deregulated miRNA expression patterns can also help clinicians to prognosticate cancer progression and outcome (Table 2).

Downregulation of miR-152 was observed in supragalottic laryngeal cancer tissues if compared with control mucosas. Additionally, miR-152 expression was associated with pT (primary tumor) stage (χ^2=26.88, P<0.001) and pN (regional lymph node metastases) stage (z=-3.56, P<0.001) in the patients suffering from supragalottic laryngeal carcinoma.[43] miR-23a expression was clearly increased in laryngeal carcinoma tissues if compared to their paired noncancerous ones, and overexpressed miR-23a was correlated with greater lymph node metastasis and shorter patient five-year survival. *In vitro* analysis revealed that up-regulation of miR-23a also accelerated cancer cell migration and invasion abilities.[44] Furthermore, Hu et al. (2015) showed that miR-21 was upregulated in freshly-frozen primary laryngeal tumors if compared to non-cancerous laryngeal squamous epithelial tissues, whereas miR-375 was down-regulated. Additionally, a miR-21/375 ratio was highly sensitive and specific for prediction.[45] A global miRNA expression profiling revealed that 4 miRNAs, miR-296-5p, miR-452, miR-183* and miR-200c, were aberrantly regulated in laryngeal cancer tissues from patients harboring radio-resistance. Interestingly, an additional validation test exhibited that miR-296-5p was associated with resistance to radiation and tumor recurrence in an early stage of laryngeal cancer.[46] Interestingly, down-regulated miR-101 was associated with nodal metastasis, T3-T4 tumor grade, and clinically advanced stage of laryngeal cancer in patients. In addition, relationship was observed between low miR-101expression level and poor prognosis. Exogenously up-regulated miR-101 suppressed cell proliferation and migration, and induced cell-cycle arrest and apoptosis. Moreover, miR-101 repressed tumor growth in a xenograft model of laryngeal cancer. Hence, miR-101 works as an oncosuppressor.[47] Wu et al. (2014) confirmed the previous reports about a significant higher expression of miR-9 in laryngeal carcinoma compared to the corresponding normal laryngeal tissues and also found a correlation between miR-9 up-regulation and progression and poorer prognosis of laryngeal cancer.[48]

Taken together, these intriguing results indicate that miRNAs are emerging as powerful prognostic biomolecules.

Conclusions

Functional studies of miRNA signatures reveal their significant role in the pathogenesis of laryngeal cancer and disclose their considerable contribute to clinical applications as hallmarks of cancer for the classification and diagnosis of several human tumors. Differential miRNA expression pattern between laryngeal carcinoma tissues and their adjacent normal counterpart has allowed to identify the main up- and down-modulated miRNAs, conferring them a precise tumor promoting or tumor suppressive role. Among the most characterized miRNAs with an established role in cell biology of laryngeal cancer there are the oncogenic miR-21, miR-205, miR-10b and miR-221 ([16-21]) and the oncosuppressive miR-126, miR-34c, miR-34a, miR-340, miR-1, miR-206 and Let-7a ([22-29]). For each of them we have described the main molecular mechanisms, to date identified, responsible for their function in LSCC. Knowledge of miRNA specific aberrations is crucial in view of undertake a miRNA-based therapy consisting in inhibition of overexpressed oncogenic miRNAs or ectopic re-expression of tumor suppressive miRNAs. Improvement in the stabilization and delivery methods is, therefore, a key point for the employment of miRNAs in the future clinical therapy. Moreover, we have also defined the potential of miRNAs as novel diagnostic and prognostic markers in LSCC, exploiting their stability in systemic circulation and other body fluids and their easy detection and quantization, developing a characteristic miRNome of a biological fluid that can be usefully paralleled to the diseased tissue's miRNA pattern. An assimilation among deregulated miRNAs derived from the tumor tissue and the body fluids will represent a significant innovation in view of replacing invasive tissue biopsies with easy assays that avail of readily available blood products, with a clear advantage in terms of early diagnosis and tumor stratification.

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