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Review

Non Coding RNAs in Head and Neck Squamous Cell Carcinoma (HNSCC): A Clinical Perspective

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Abstract. *Background: Head and neck squamous cell carcinoma is a common cause of cancer death. Despite decades of clinical studies exploring new treatments and considerable advance in multimodality treatment, satisfactory curative rates have not yet been reached. In the last few decades the emerging data from both tumor biology and clinical trials led to growing interest for research of predictive biomarkers. However, no molecular markers were discovered to improve clinical outcomes and to be used as new anticancer agents. Non coding RNAs (ncRNAs) are promising biomarkers. They are important regulators both in normal biological process and in proliferation, metastasis, chemo-radioresistance. Materials and Methods: We revised the literature on this topic to summarize current findings on ncRNAs. Results: Several studies reported an altered regulation of ncRNAs and a specific cancer site signature has also been described among different primary head and neck squamous cell carcinomas (HNSCC). Moreover, expression of ncRNAs correlates with poor prognosis and resistance to treatment. Conclusion: ncRNAs are emerging potential molecular markers and anticancer agents.*

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cause of cancer mortality in the world and the 5th most commonly occurring cancer (1). Tobacco smoking, alcohol consumption and human papilloma virus (HPV) infections have been associated with the occurrence of HNSCC. Despite advances that have been made in HNSCC treatment, prognosis remains dismal. HNSCC often develops within pre-neoplastic fields of genetically-altered cells. Persistence of these fields after treatment forms a major clinical challenge since they may lead to local recurrences and second primary tumors that are responsible for a large proportion of relapses and deaths. In addition, there are currently no tools available to monitor HNSCC patients for early stages of local recurrences or distant metastases. There is a compelling need to identify novel biomarkers that can select for patients that would benefit from a given therapy and can predict the clinical outcome (2).

Non-coding RNAs (RNAs that do not code for proteins but regulate expression of coding genes) are promising candidates both for prognostic/diagnostic assessment and for therapy.

In the recent past, micro-RNAs (miRNAs), a novel class of non-coding small RNAs, have been assessed for their role in different areas, such as immune response, neural development, DNA repair, apoptosis, oxidative stress response and cancer. miRNAs are small, typically 22-nucleotide-long, endogenous, single-stranded RNAs. The majority of miRNAs are found intracellularly, although a significant number of miRNAs have been observed outside of cells, including various body fluids. Circulating miRNAs function as 'extracellular communication RNAs' that play an important role in cell proliferation and differentiation. The regulation of miRNAs is essential to many cellular processes and their escape from this regulatory network seems to be a common characteristic of several disease processes and malignant transformations including HNSCC. miRNAs play a key role in tumorigenesis by either enhancing the expression of oncogenes or subduing the expression of tumor suppressor genes (3). On the clinical side, because of their tumor-specific expression and stability in tissues and in the circulation, miRNAs might be used as novel diagnostic tools for classification and prognostic stratification of HNSCC (4). In recent years, the therapeutic potential of miRNAs has been demonstrated in various pre-clinical studies.

Abbreviations: EGFR, epidermal growth factor receptor; HDAC, hystone deacetylases; NR, not reported.

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In addition to miRNAs, long non-coding RNAs (lncRNAs) are emerging as critical regulators of gene expression and tumorigenesis (5). lncRNAs are involved in the regulation of many aspects of both cell physiology and pathology, through different mechanisms of action, such as imprinting, maintenance of pluripotency and cancer (6). Many nuclear lncRNAs are directly involved in gene expression control, some lncRNAs act as down-regulators recruiting gene silencing complexes (7, 8), while others as decoy precluding the access of regulatory proteins to DNA (9).

Some lncRNAs modify the activity of DNA binding proteins changing the expression of target genes (*e.g.* cyclin D1 gene -*CCND1*-). In the cytoplasm, lncRNAs have been described to modulate mRNA stability or to act as miRNA decoy (10). These areas are expected to be further explored in the upcoming years to assess the clinical value of ncRNA-based approaches in HNSCC and cancer in general.

Non coding RNAs are also a fascinating option for cancer treatment. Anti-miRNA oligonucleotides and miRNAs mimics have been found to have antitumor activity (11). Moreover, by exploiting tumor-specific expression of miRNA, efforts have been aimed at improving targeting of tumor cells by replicative oncolytic viruses while sparing normal cells.

Both miRNAs and lncRNAs are excellent biomarker targets because they circulate stably in human body fluids and can be obtained with non-invasive methods. Moreover, a full elucidation of the complex ncRNA regulatory circuits could provide an additional treatment strategy in cancer treatment including HNSCC.

The aim of this review is to retrieve from the literature miRNAs and lncRNAs that have specifically been associated with HNSCC and group them according to their site of origin and association to smoking and HPV.

Materials and Methods

A literature search was conducted through the Medline database. The period considered was from January 1991 to June 2014. We chose 1991 as the starting point because, to the best of our knowledge, this was the first time that a correlation among miRNAs and HNSCC was described.

The following Medical Subject Heading (MeSH) terms and key words were used in the search: (("microRNA" or "miRNA") AND (head and neck cancer)) or ((“long non-coding RNA” or “lncRNA” or “ncRNA”) AND (head and neck cancer)).

We considered only English papers and excluded from the analysis studies devoted to other malignancies.

We analyzed these studies taking into account the previous reported reviews on this matter. The electronic search results were supplemented with hand searching of selected papers, expert consensus meeting notes, and reference lists from selected articles. Data extraction was performed by the first author.

Data analyses were performed by each author through the compilation and discussion of the manuscript and its tables. Drafting of the article was performed both during meetings and through mailing and web-conferences.

ncRNAs Network in HNSCC

The description of miRNAs’ and lncRNAs’ actions in HNSCC is rapidly emerging. Moreover, the regulation of lncRNAs’ expression and function by miRNAs and *vice versa* is only now coming into view (12). Several lncRNAs (such as *MALAT1, HOTAIR, H19, LOC285194*) are targets of miRNAs. For example in thyroid cancer cell lines, miR-574-5p over-expression reduces *PTCSC3*; the reduction of this lncRNA directly affect cell proliferation and tumorigenesis. In addition lncRNAs can compete with miRNAs for binding target mRNAs, as well as they can affect the levels and function of miRNAs (12).

Role of Specific miRNAs in HNSCC

microRNAs play an important role in all biological processes by post-transcriptional regulation of protein-coding genes. Recently miRNAs involvement in HNSCC has been demonstrated, as in other cancers; published papers are growing, although only few studies correlate profile expression with complementary techniques, such as RT-PCR (13).

Recent studies have identified aberrant miRNA expression profiles in HNSCC tissues and/or cell lines compared to matched normal controls, the mechanisms of which are becoming unveiled.

Moreover, a small number of dysregulated miRNAs have been implicated either as oncogenes or tumor suppressors, affecting the initiation progression and metastatization (4).

For example mi-R375 acts as a tumor suppressor in HNSCC and in other several cancers (haepatocellular, pancreatic, gastric), while it functions as oncogene in other cancers (lung, breast, neuroendocrine cancers). In addition, miR-375 has been reported to be lowered in non-malignant tissue compared with tumor tissue. Moreover, low miR-375 expression levels correlate significantly with cancer survival and distant metastasis. Harris *et al*. (2012) showed that a lower ratio among tumor (T) and normal tissue (N) is an independent negative prognostic factor. Also, adjusting the regression analyses for the strongest demographic and clinical prognostic factors for survival across all HNC tumor sites (age, tumor site, nodal metastases at diagnosis, sex and treatment regimen), patients with lower miR-375 T:N expression were more likely to die of disease (hazard ratio (HR): 12.8, 95%; confidence interval (CI): 3.4-48.6) than those with higher miR-375 T:N. In contrast, no significant association was observed with decreased expression of miR-375 in the tumor with head and neck cancer survival, locoregional recurrence or distant metastasis adjusting for tumor site, age, sex, nodal status, primary treatment and percent tumor (14).

In HNSCC, a correlation among some miRNAs and transcriptional factors has been described. For instance, activation of hypoxia-inducible factor-1 (HIF-1) and protein kinase Ca (PKCa) induce the transcription of miR-210 (15) and decrease miR-15a (16) expression, respectively. High expression level of miR-21 in tongue squamous cell carcinoma (TSCC), the most common subtype of oral SCC (OSCC), was found to be associated with low levels of tropomyosin 1 (*TPM1*) and phosphatase tensin homologue (*PTEN*) expression and diminished cell apoptosis (17). High miR-21 has been associated with significantly decreased 5-year survival in 169 patients (HR: 1.68; 95% CI: 1.04-2.77) in a model controlled for patient age, gender and tumor stage (18). miR-21 expression was independently associated with a poorer prognosis, even after adjusting for clinical parameters (perineural invasion and N-stage) in a multivariate analysis of 86 OSCC tumor samples (19).

Kozaki *et al*. (2008) documented that miR-137 and -193a, located around CpG islands, are silenced by DNA hypermethylation in OSCC (20).

miR-9 has been associated with the Wnt/β-catenin signaling pathway in OSCC. However, how the relationship works between remains largely unknown. Yu *et al*. (2013) demonstrated that lentivirus-mediated miR-9 over-expression inhibited proliferation of two tumor cell lines *in vitro* and *in vivo* (Tca8113 and SCC-9). Furthermore, miR-9 targets the CXC chemokine receptor 4 (CXCR4); in particular, miR-9 under-expression led to constitutive activation of β-catenin through activation of CXCR4 expression in OSCC cells (21).

Ganci *et al*. (2013) reported the association of 49 miRNAs with TP53 status. Among those, a group of 12 miRNAs (*e.g*. miR-17-3p, miR-18b-5p, miR-324-5p, miR-19a-3p, miR-200a-3p, miR-331-3p, miR-21-3p, miR-21-5p, miR-205-5p, miR-151a-3p, miR-96-5p and miR-429) and a group of 4 miRNAs (*e.g*. miR-17-3p, miR-21-3p, miR-21-5p and miR-139-3p) correlates with shorter recurrence-free survival and cancer-specific survival, respectively. The two groups share three miRNAs (miR-21-5p, miR-21-3p and miR-17-3p) (22).

Importantly, miRNAs that correlate with survival are independent prognostic factors either when considered individually or as signatures (22).

miR-205 and let-7d may be of outcome use to predict locoregional recurrence (18).

A prognostic role has been reported for miR-200, as it is implicated in epithelial-mesenchymal transition. Loss of miR-200s has been shown to enhance cancer aggressiveness and metastasis, whereas replacement of miR-200 has been shown to inhibit cell growth in several types of tumors, including HNSCC (23).

Lin28 is a developmentally regulated RNA binding protein that has recently emerged as key regulator in the biogenesis of the let-7 micro-RNA family. Alajez *et al*. (2012) demonstrated a direct regulation of selected genes (high mobility group AT-hook 2 -*HMGA2*-, Cycline 2 gene -*CCND2*-, insulin growth factor receptor -*IGF1R*- and insulin growth factor binding protein 2 -*IGF2BP2*-) *via* a let-7-Lin28b mechanism (24).

Notably, Lin28b promotes HNC progression *via* modulation of IGF survival pathway (up-regulation of several genes in the IGF pathway in Lin28b-expressing HNC cells was observed). Functional studies revealed significant increase in the survival of Lin28b-expressing cells when cultured under stress conditions, which was dependent on the presence of IGF1 (24).

In multivariate analyses on a larger independent case series of HNSCC tumors (169 pts), Avissar *et al*. (2009) demonstrated an increase of miR-375 expression with alcohol consumption ($p=0.002$), as well as a higher expression in tumors of pharyngeal and laryngeal origin compared with oral tumors ($p<0.05$ and $p<0.01$, respectively) (18).

miR-26a inhibits cell growth and tumorigenesis of nasopharyngeal carcinoma (NPC) through the repression of the enhancer of zeste homolog 2 (*EZH2*). Additionally, it works as anti-metastatic miRNA as its ectopic expression inhibited the migratory and invasive capacities of NPC cells *in vitro* (25).

miR-7 inhibits EGFR expression and downstream Akt and ERK1/2 activity in several cancer including HNSCC, leading to reduced cell proliferation and survival (16, 27). Furthermore, it acts synergistically with erlotinib to inhibit growth of erlotinib-resistant HNC cells (27).

Inhibition of Akt, Stat3 and Rho GTPases *via* Protein kinaseCε (PKCε) is performed by miR-107. An inverse relationship was revealed between miR-107 and PKCε in HNSCC cell lines. Delivery of miR-107 reduced PKCε levels in HNC cells. Treatment with miR-107 significantly blocked cell proliferation, DNA replication, colony formation and invasion in SCC25 and CAL27 cells (28).

miR-218 is a tumor suppressor miRNA that acts on the focal adhesion pathway (especially laminin-332). In cancer cells, the laminin-332-integrin alpha6beta4 (specific transmembrane receptor) interaction triggers a number of signaling cascades promoting both cell migration and cancer survival. Therefore, tumor suppressive miR-218 contributed to cancer cell migration and invasion (29).

Site-specific miRNAs have been identified, such as for laryngeal cancer (miR-133b, miR-455-5p and miR-196) (30), for nasopharyngeal cancer (miR-26a), for oropharynx (methylation of miR-9) and tongue (miR-21) (17).

In addition Lu *et al*. (2014) reported 8 miRNAs dysregulated in 42 laryngeal cancer samples (miR-21-3p and miR-106b-3p were up-regulated, while let-7f-5p, miR-10a-5p, miR-125a-5p, miR-144-3p, miR-195-5p and miR-203 were down-regulated) (31).

Similarly, supraglottic carcinoma microarray analyses highlighted nineteen differentially expressed miRNAs; among these three were significantly up-regulated (miR-21,

miR-19a, miR-33a) and two were down-regulated (miR-206, miR-375) (32).

Wong *et al*. detected the presence of miR-184 in the plasma of 80% of patients with tongue SCC compared to 13% of healthy patients, while Liu *et al*. (2012) reported high levels of miR-31 in oral cancer (33, 34).

Table I reports a classification of most common miRNAs based on dysregulation.

Table II reports a list of ncRNAs and clinical targets.

Table III reports a list of oncosuppressors.

Role of Specific lncRNAs in HNSCC

Long non-coding RNAs (lncRNAs) are a new class of non-coding RNA with size larger than 200 nt that contribute to cancer development and progression. The roles for several lncRNAs in cancers have been characterized and strategies targeting them have inhibitory effects to malignant cells *in vitro* and *in vivo*. Their ability to regulate essential pathways for tumor initiation and progression together with their tissue and stage specificity promotes them as valuable biomarkers and therapeutic targets (35). Additionally, various cancer-associated lncRNAs have been identified, such as metastasis-associated lung adenocarcinoma transcript 1 for lung cancer, providing the rationale for their use as biomarkers. Functional studies revealed a broad spectrum of mechanisms applied by lncRNAs, such as *HOTAIR* (HOX antisense intergenic RNA), *MALAT1* (metastasis associated lung adenocarcinoma transcript 1), *ANRIL* (antisense non-coding RNA in the *INK4* locus) or lincRNA-p21 to fulfill their functions (35).

Accumulating evidences show that lncRNAs also sustain proliferative signaling as the steroid receptor RNA activator (SRA) in breast tumors (36, 37), evade growth suppressor as growth arrest-specific transcript 5 (*GAS5*) in melanoma, prostate and breast cancers (38), enable replicative immortality as TERC and TERRA (39), activate invasion and metastasis as metastasis-associated lung adenocarcinoma trascript-1 (*MALAT-1*) in non-small cell lung cancer (40) and as homeobox (HOX) transcript antisense RNA (*HOTAIR*) in hepatocellular carcinoma (HCC) and esophageal SCC (ESCC) cell lines and patient samples (41, 42), induce angiogenesis as hypoxia inducible factor 1- α (HIF1-α) (43) and make cell resisting to death as prostate-specific transcript 1 (*PCGEM1*) in prostate cancer (44). PlncRNA-1 is one of lncRNAs that is associated with cell apoptosis and proliferation of prostate cancer and esophageal cancer (45). Alternative splicing (AS) was also described in buccal SCC (46). The lncRNA HOX transcript antisense RNA (*HOTAIR*) is up-regulated in esophageal SCC cell lines and patient samples and promotes ESCC cell proliferation and tumor metastasis (42). The lncRNA *MEG3* gene has been reported to be a tumor suppressor genes in various cancers, including tongue SCC (47). Additionally Fang *et al*. (2014) reported that the expression levels of lncRNA *UCA1* were significantly elevated in TSCC tissues ($p<.0001$) and were statistically correlated with lymph node metastasis ($p=.0371$). Over-expression of *UCA1* lncRNA could promote metastatic but not proliferation ability of TSCC cells (48).

Additionally, in prostate cancer, a role of lncRNA has been described in the impairment of double-stranded DNA break (DSB); this data appears an interest field of study. Li *et al*. reported that not only miR21 but also lncRNA *HOTAIR* induces laryngeal cancer progression (49).

Epigenetically-regulated ncRNA

Alterations in the epigenetic regulation of ncRNA are important in the pathogenesis of human disease. Both miRNAs and lncRNAs are modulated by hypermethylation or regulators of a biological process. Therefore, specific miRNAs are able to regulate the expression levels of hundreds of genes simultaneously (50). For instance, both the miRNA deregulation and several epigenetic mechanisms related to processing and expression of the mature miRNAs (including post-transcriptional methylation and single nucleotide polymorphisms (SNPs) and microsatellite alterations SNPs in pri- and pre-miRNAs) contribute to cancer initiation and progression (51, 52).

SNPs in mature miRNAs and miRNA binding sites function analogously to modulate the miRNA-mRNA interaction and create or destroy miRNA binding sites (53).

In HNSCC, only few reports demonstrated the association among methylation and miRNA activity, but this reflects both the huge amount of interest on this field in other big killer cancer and the little attention in the area of genome profiling in this field until few years ago.

miR-137 promoter methylation is associated with poorer overall survival in HNC patients although methylation did not correlate to site (oral cavity, pharynx, larynx) (54).

miR-124 diminishes the level of CDK6 and affects the phosphorylation status of CDK6-Rb (55).

The methylation of miR-124, let-7 family has been shown to influence the formation of an epigenetic field defect (18).

Therapy

Antagomir-Mimic combination with conventional therapies - Multidrug resistance and cetuximab resistance. Given the role of ncRNAs in epigenetic regulation of gene expression, ncRNAs have been proposed as possible candidates for drug targeting with the objective of interfering with biological processes regulated by the targeted ncRNAs.

The application of miRNA antagonists and/or their mimic agents in cancer treatments is an evolving field of interest.

In patients with HCV infections an antagomir that binds and inhibits miR-122, miravirsen, has been studied in a

Table I. *Classification of most common miRNAs based on dysregulation.*

miRNA	Dysregulation	Site-Target	References
miR-21	Up	It has been found to down-regulate the tumor suppressor programmed cell death protein 4 (PDCD4), thus aiding in the cancer's invasion, intravasation and metastasis. Increased expression of miR-21 causes reduction of PTEN and its transcriptional regulator Grhl3	Tran *et al*., 2007 (70) Chen *et al*., 2012 (64) Avissar *et al*., 2009 (18) Chang *et al*., 2008 (71) Li *et al*., 2009 (17)
miR-31	Up	Activates the HIF pathway through the suppression of its inhibitors (FIH), consequently promoting tumor angiogenesis and growth	Liu *et al*., 2010 (34)
Let-7 family	Up (down)	Let-7 has been demonstrated to be a direct regulator of *RAS* expression in human cells. Another oncogene, high mobility group A2 (*HMGA2*), has also been identified as a target of let-7. Removal of let-7 binding site by 3'UTR deletion causes over-expression of *HMGA2*. Let-7 regulates also cell cycle genes including cyclin A2 (*CCNA2*), *CDC25A, CDC34, CDK6*, Aurora A and B kinases (STK6 and STK12), *E2F5* and *CDK8* and apoptosis genes (*CASP3, BCL2, MAP3K1* and *CDK5*). Down-regulates c-Myc expression and might regulate also Kras	Chang *et al*., 2008 (71) Hebert *et al*., 2007 (60)
miR-16	Up	miR-16 has been shown to bind to a nine base pair to a complementary sequence in the 3' UTR region of *BCL2*	Hui *et al*., 2010 (72)
miR-18a	Up	miR-18a targets Kras not N and H-ras; moreover; it functions as a tumor suppressor by targeting Dicer	Chang *et al*., 2008 (71) Avissar *et al*., 2009 (18)
miR-106B-25 cluster	Up	miR-106b targets *CASP7* and influences focal adhesion-related pathways	Ramdas *et al*., 2009 (73) Hui *et al*., 2010 (72)
miR-130b	Up	Regulates HIF-1α. Levels of the miR-130 family are elevated under hypoxia and their target is *DDX6* mRNA, which is a component of the P-bodies	Avissar *et al*., 2009 (18) Hui *et al*., 2010 (72)
miR-142-3b	Up	Regulates D1 dopamine receptor; subtype miR-142-3p-mediated post-transcriptional regulation might regulate translation of D1 receptor protein in dendritic spines. It acts as miR-223 promoting T cell development through transcriptional regulators	Chang *et al*., 2008 (71) Hui *et al*., 2010 (72)
miR-146a	Up	It is a mediator of inflammation as miR-155. miR-146 is up-regulated by inflammatory factors, such as interleukin 1 and tumor necrosis factor-alpha. miR-146 down-regulates tumor necrosis factor receptor-associated factor 6 (TRAF6)	Chang *et al*., 2008 (71) Cervigne NK 2009 (74)
miR-155	Up	miR-155 is a commonly over-expressed oncomir in human cancers. In human breast cancer, it has been identified to target the *SOCS1* gene that encodes the suppressor of cytokine signaling 1 protein (SOCS1). Recent research suggests that miR-155 negatively regulates *SOCS1* but may be a feasible target in breast cancer therapy	Chang *et al*., 2008 (71) Janiszewska *et al*., 2013 (11) Liu *et al*., 2009 (75)
miR-184	Up	Regulates DNA methylation pathways. miR-184 negatively regulates miR-205. Promotes apoptosis, as well as hindering cell proliferation in cultured tongue SCC cells	Wong *et al*., 2008 (33)
miR-34b/c	Up	Hypermethylation and histone deacetilation. Targets cMyc CDK6	Pérez-Sayáns *et al*., 2012 (13)
miR-100	Down	Targets mTor	Janiszewska *et al*., 2013 (11)
miR-125a	Down	Targets Fyn, a member of the Src family kinases (SFKs)	Pérez-Sayáns *et al*., 2012 (13) Ramdas *et al*., 2009 (73)
miR-125b	Down	Down-regulates p53	Park *et al*., 2009 (76) Ramdas *et al*., 2009 (73)
miR-133a	Down	Several voltage gated K^+ channel	Childs *et al*., 2009 (77)
miR-200a	Down	miR-200a regulates epithelial to mesenchymal transition-related gene expression	Park *et al*., 2009 (76)
miR-221	Down	Targets CD117, which then prevents cell migration and proliferation in endothelial cells	Liu *et al*., 2009 (75)
miR-375	Down	miR-375 has been shown to target the *MTPN* gene, which encodes the myotrophin protein that regulates hormone release and exocytosis. miR-375 also lowers the level of the *PDK-1* (pyruvate deydrogenase) gene. Additionally it regulates AEG1/*MTDH*	Avissar *et al*., 2009 (18)

PTEN, Phosphatase and tensin homolog; HIF-1α, hypoxia inducible factor 1α; AEG1/MTDH, astrocyte elevated gene1/metadherin.

phase IIa trial. Miravirsen is a locked nucleic acid–modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, thereby inhibiting its function. It resulted in a dose-dependent reduction in HCV RNA levels that endured beyond the end of active therapy (56).

The resistance to chemotherapeutic agents is the major cause of failure in the treatment of cancer patients. Multidrug resistance (MDR) depend on over-expression of drug-expelling transporters in the plasma membrane, such as P-glycoprotein (Pgp), encoded by the *MDR1* gene and MDR-associated proteins (MRPs), glutathione S-transferase-p

Table II. *Classification of ncRNAs based on clinical implications.*

ncRNA	Site/stage	Etiology	Treatment response/prognosis	References
miR-375	Pharynx	Alcohol	Induces progression	Wong *et al*., 2008 (33) Avissar *et al*., 2009 (18)
miR-133b; miR-455-5p; miR-196	Larynx	NR	Induces progression	Saito *et al*., 2013 (30)
miR-26a	Oropharynx	NR	Induces progression	Li *et al*., 2009 (17)
miR-184	Tongue	NR	Induces progression	Wong *et al*., 2008 (33)
HOTAIR	Larynx	NR	Induces progression, inhibits apoptosis	Li *et al*., 2013 (49)
miR-155	OSCC	NR	Induces progression localized in the cancer nest, inflammatory area and vascular endothelium	Chang *et al*., 2008 (71) Shi *et al*., (78)
miR-203	Larynx	NR	Suppresses proliferation and induces apoptosis of tumors	Tian *et al*., 2014 (79)
miR-504	Hypopharynx	NR	Induces cell cycle arrest through targeting of TP53	Kikkawa *et al*., 2014 (80)

NR, not reported.

Table III. *Oncosuppressors.*

Oncosuppressor	Function and reference
miR-218	miR-218 contributes to cancer cell migration and invasion through regulating the focal adhesion pathway, especially laminin-332 (Kinoshita *et al*., 2013) (29)
miR-7	miR-7 regulates EGFR expression and Akt activity in a range of cancer cell types *via* its specific interaction with the *EGFR* mRNA 3'-untranslated region (3'-UTR). miR-7 acted synergistically with erlotinib to inhibit growth of erlotinib-resistant FaDu cells, an effect associated with increased inhibition of Akt activity (Kalinowski *et al*., 2012) (27)
miR-107	miR-107 significantly blocked cell proliferation, DNA replication, colony formation and invasion in SCC25 and CAL27 cells (Datta *et al*., 2012) (28)
Let-7	Inhibition of let-7-3 has additionally been shown to be associated with poor survival (Avissar *et al*., 2009) (18)
miR-34b	Hypermethylation and histone modification, HDAC (Kita *et al*., 2014) (23)
miR-200	Transcription repression, EMT (Epithelial Mesenchymal Transition), regulation of tumor angiogenesis (Cortez *et al*., 2014) (69)

(GST-p) and DNA topoisomerase II (topoII). In addition to all the molecules above, we now know that miRNAs also act by sensitizing or making tumor cells more resistant (57).

Ogawa *et al*. (2010) analyzed the head and neck squamous cell carcinoma cell line RPMI2650 and the cis-diamminedichloroplatinum (CDDP)-resistant cell line RPMI2650CR to identify resistant phenotype-related miRNAs and they identified miR-34a as down-regulated. Moreover, in 24 patients with intra-arterial infusion of CDDP, they identified a significant association between decreased expression of miR-34a and poor disease-specific survival, poor disease-free survival and poor local control rates. Furthermore, multivariate analyses demonstrated significant associations between miR-34a expression and the hazard ratios for disease-free survival (57).

Yu *et al*. (2011), using a microarray analysis, described high levels of let-7 family, miR-214 and miR-23a, in chemo-resistant cell lines of SCC of the tongue (58). They found that miR-23a is a potent inhibitor of topoII acting as a chemo-resistant agent. miR-214 is also implicated in chemoresistance; although the mechanism is not well known, it might induce cell survival and CDDP resistance through targeting the 3'untraslated region (UTR) of the *PTEN* gene, which leads to down-regulation of the protein and activation of AKT pathway (as showed in ovarian cancer) (59). By contrast, miR-21 and miR-342 over-expression showed generation of chemo-sensitive cells. Hebert *et al*. (2007) showed that high mobility group A2 (HMGA2) expression, a molecule associated with chemo-sensitivity to doxorubicin (a potent topoII inhibitor), is regulated in part by the expression of miR-98. HMGA2 expression is thwarted by hypoxia, coupled with over-expression of miR-98; in fact, transfection of pre-miR-98 during normoxia also decreases HMGA2 expression and promotes resistance to the topoII inhibitor (60). Furthermore, Dai *et al*. (2011) discussed the role of miRNAs in MDR, creating resistant HNSCC cell lines treated with doxorubicin, resulting in chemo-resistance that crossed over to other chemotherapeutic agents (61).

PCR analysis of miRNAs of these resistant cells, opposed to the original cells, showed over-expression of miR-181d and sub-expression of miR-100 and miR-130a. Another miRNA that has recently been linked to MDR is miR-212.

According to Hatakeyama *et al*. (2010), EGFR chronic inhibition by cetuximab induces up-regulation of its ligands, such as heparin-binding EGF-like growth factor (HB-EGF), controlled by miR-212. HB-EGF stimulation resulted in resistance to cetuximab in HNSCC cell lines and the addition of miR-212 sensitivity to resistant cells (62).

Phosphorylation of p63 is implicated in MDR in CDDP-treated HNSCC; high levels of phospho-p63 cause dysregulation of miRNAs (miR-630 up-regulation and down-regulation of miR-181a, miR-519a and miR-374a). Cisplatin exposure induced the ATM-dependent phosphorylation of p63a, which causes modulated expression of specific miRNAs in SCC cells exposed to CDDP. Moreover, along with transcription coactivators or corepressors, p63 induces gene promoters for miRNAs (miR-630 and miR-885-3p) or represses promoters for miRNAs (miR-181a-5p, miR-374a-5p and miR-519a-3p) (63). Chen *et al*. (2013) demonstrated that miR-27a modulates the MDR1/P-glycoprotein expression by inhibiting the FZD7/β-catenin pathway in haepatocellular carcinoma cells (64).

The possibility to predict chemotherapy response is a major challenge for oncologist. In vitro changes in circulating miRNA during radiochemotherapy has been reported (miR-590-5p, miR-574-3p, miR-425-5p, miR-885-3p, miR-21-5p, miR-28-3p, miR-195-5p, miR-191-5p, miR-150-5p and miR-142-3p) (65). These preclinical data requires further validation to confirm their predictive role in clinical practice.

Several miRNAs, such as miR-31-star, miR-1264, miR-3150b-5p and miR-210, influence endoplasmic reticulum and fatty acid biosynthesis. These two cell processes are associated with drug sensitivity. Xu *et al*. (2013) demonstrated their alteration, miRNA-induced, after paclitaxel treatment (66).

Radioresistance

The role of ncRNAs in the development of radioresistance has also been questioned. Niemoeller *et al*. (2008) showed that levels of miR-24-1, miR-144 and let-7i significantly increased following irradiation. In particular, miR-205 binds the 3'UTR of *PTEN* inhibiting cell cycle progression. Low levels of miR-125b also correlate with proliferation and radioresistance, mediated by the down-regulation of the intracellular adhesion molecule 2 (ICAM2) (67).

In radioresistant human cervical cancer cells, a specific miRNA signature consisting of miR-630, miR-1246, miR-1290 and miR-3138 has been reported by Zhang *et al*. (2013) (68). On the contrary, up-regulation of miR-200c correlates with increased cellular radiosensitivity by direct regulation of the oxidative stress response genes peroxiredoxin 2 gene-*PRDX2*-, GA-binding protein alpha chain nuclear factor erythroid 2 -*GAPB*/*Nrf2*- and *SESN1* in ways that inhibit DNA double-strand break repair, increases levels of reactive oxygen species and up-regulates p21 (69).

Discussion

miRNAs and lncRNAs represent relevant biomarkers that offer reliable assessment of risks for poor outcome or presence of metastatic disease. ncRNAs seem to be a very promising target both for early diagnosis and molecular therapies.

They target several pathways involved in tumor cell invasion and metastasis, such as EGF/EGFR/MAPK cascade and PI3K/AKT/mTOR pathway. Several studies showed a higher ncRNA expression in metastatic disease compared to localized cancer and in tumor tissue compared to normal one. However, there are no consolidated data to authorize their use in daily clinical practice. Further studies are needed to confirm their diagnostic and predictive role. From a therapeutic point of view, ncRNAs-based therapies warrant further investigation.

In addition, as for many other trial for biomarkers evaluation (www.clinicaltrials.com), in the near future ncRNAs will be used as diagnostic tools and/or therapeutic drugs.

Further interest will focus on epigenetically-regulated ncRNAs as epigenetic factors are ultimately responsible for the expression/ silencing of ncRNAs that affect their target gene levels, contributing to the normal or malignant phenotype. The clinical application of these ncRNA-regulated epigenetics will probably render them in the form of diagnostic and/or prognostic markers.

Although miRNAs and ncRNAs have been implicated in cell-fate determination and in various neoplastic diseases, little is known regardings the regulatory interaction networks among these classes of RNAs. Thus, a full elucidation of the complex ncRNAs regulatory circuits promises to be particularly exciting.

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