Molecules **2014**, *19*, 5704-5716; doi:10.3390/molecules19055704

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Review

The Role of microRNA in Head and Neck Cancer: Current Knowledge and Perspectives

Giulia Courthod [1], Pierfrancesco Franco [2], Loredana Palermo [3,*], Salvatore Pisconti [4] and Gianmauro Numico [1]

[1] Medical Oncology Department, AUSL Valle d'Aosta, Aosta Postcode 11100, Italy; E-Mails: g.courthod@hotmail.it (G.C.); gnumico@ausl.vda.it (G.N.)

[2] Radiation Oncology Department, AUSL Valle d'Aosta, Aosta Postcode 11100, Italy; E-Mail: pfranco@ausl.vda.it

[3] Medical Oncology Unit—National Cancer Research Centre Istituto Tumori "Giovanni Paolo II", Bari Postcode 70124, Italy

[4] Medical Oncology Unit—S.G. Moscati Hospital ASL TA/1, Taranto Postcode 74100, Italy; E-Mail: salvatorepisconti@hotmail.it

* Author to whom correspondence should be addressed; E-Mail: palermo.loredana@libero.it; Tel.: +39-080-555-5498; Fax: +39-080-555-5419.

Received: 7 February 2014; in revised form: 28 March 2014 / Accepted: 24 April 2014 / Published: 5 May 2014

Abstract: Head and neck cancer is one of the most commonly diagnosed malignancies worldwide. Patients with advanced disease stages frequently develop recurrences or distant metastasis, which results a five-year survival rates of less than 60% despite considerable advances in multimodality therapy. A better understanding of molecular basis of tumorigenesis is required to improve clinical outcomes and to develop new anti-cancer drugs. microRNAs (miRNAs) are a class of small, non-coding, RNA molecules that modulate gene expression post-transcriptionally. They are important regulator in normal biological process; however miRNAs deregulation has been observed in many different tumors and is involved in tumorigenesis. miRNAs may act as tumor suppressors or as oncogenes. Several studies on head and neck cancer demonstrated how aberrant expression of miRNAs is involved in proliferation, metastasis, chemoresistence, and radioresistance. In addition, miRNAs are excellent biomarker targets because they circulate stable in human body fluids and can be obtained with non-invasive methods. Moreover, miRNAs up and down regulation has been correlated with specific cancer phenotype (poor prognosis, aggressiveness and resistance to treatment), playing a role as prognostic biomarkers. This

review summarizes current finding on miRNAs in head and neck cancer and their potential role as target for next drug therapy.

Keywords: microRNA; hand and neck cancer; biomarkers; chemoresistence; radioresistence

1. Introduction

Head and neck cancer (HNC) is a heterogeneous oncological setting arising from oral and nasal cavities, pharynx or larynx. It is the sixth most common cancer worldwide. The incidence of HNC in the European Union for 2012 was estimated in 73,014 new cases/year: Germany, France and Denmark showed the highest risk [1]. Squamous cell carcinoma is the most common histologic type (more than 90% of cases). Adenocarcinoma, basal cell and small cell, lymphomas, sarcomas and others are uncommon. Chronic tobacco, alcohol abuse and Human Papilloma-Virus (HPV) infection are the main risk factors for the development of squamous-cell HNC (SCHNC). In patients presenting with early, localized disease, surgery or radiotherapy achieve cure in the large majority of cases. Locally advanced disease is usually treated using integrated treatment modalities including chemotherapy with a worse long-term prognosis. Patients with loco-regional relapse or metastatic disease might benefit from palliative chemotherapy but usually cannot be cured.

Inactivation of tumor suppressor genes (e.g., p53, p16inK4a), activation of proto-oncogenes (e.g., cyclin D, Rb) and enhanced expression of the epidermal growth factor receptor (EGFR) have been observed in SCHNC [2]. Cetuximab, an anti-EGFR monoclonal antibody, has demonstrated activity when combined with radiotherapy in locally advanced disease [3] and in combination with chemotherapy in the relapsed/metastatic setting [4], and has been approved for the treatment of SCHNC [5]. On-going clinical trials are evaluating the efficacy of others EGFR monoclonal antibodies (panitumumab, zalutumumab) and EGFR tyrosine kinase inhibitors (gefitinib, lapatinib, afatinib, erlotinib) in these patients [6]. Despite the improvement of surgery, chemotherapy and radiotherapy, the 5-year survival rates are less than 60% [7]. In the last years, increasing interest has been focused on the role of microRNA (miRNA) in cancer. As in others malignancies, miRNA regulates several oncogenes and tumor suppressors, driving the growth, proliferation, metastatic attitude and drug resistance also.

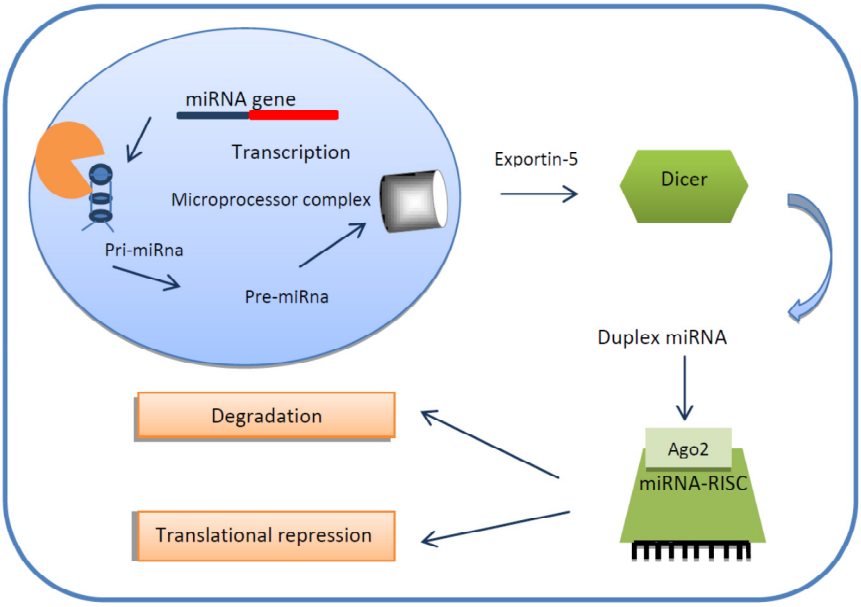
2. miRNA

miRNAs are a large family of high conserved non-coding, double-stranded RNA, consisting of about 18–25 nucleotides, that are able to regulate expression of multiple target genes in normal biological process (such as proliferation, differentiation and apoptosis) at post-transcriptional level. To date, 1,872 different miRNAs have been identified in human and the number is still rising [8].

miRNA genes are transcribed by RNA polymerase II (Poll) to form primary miRNA (pri-mRNA) characterized by a specially modified nucleotide at the 5′end and a polyadenilated at the 3′end (Figure 1). The Microprocessor Complex, formed by a nuclear protein known as DiGeorge Syndrome Critical region 8 (DGCR8) and an enzyme called Drosha ribonuclease (RNase III), convert pri-mRNAs

into a second precursor (pre-miRNA) [9,10]. This is then transported into cytoplasm through Ran-GTP-dependent transporter Exportin-5 where it is cleaved by the RNase III enzyme Dicer to create a miRNA-miRNA duplex. In order to form the final complex that performs gene silencing, the double-strained needs to be separated into the functional strand and loaded together with Argonaute proteins into the RNA-induced silencing complex (RISC). This complex mediates gene expression by binding target mRNAs and inducing mRNA degradation [11]. Several studies reported aberrantly expression of miRNAs in several cancers, where they play a role as tumor suppressors or as oncogenes. Up-regulation of oncogenic miRNAs results in down-regulation of tumor suppressor genes whereas down-regulation of tumor suppressive miRNAs results in up-regulation of oncogenes [12]. Endogenous circulating miRNAs are stable, and are protected from RNases, remaining steady even after being subjected to unfavourable conditions [13].

Figure 1. MiRNA biogenesis. MiRNAs genes are transcribed by RNA polymerase into primary miRNAs (pri-miRNA). They are converted into second precursors (pre-miRNA) by the Microprocessor Complex, composed by DiGeorge Syndrome Critical region 8 (DGCR8) and Drosha ribonuclease (RNase III). They are then exported to the cytoplasm by Exportin 5 and are processed into mat ure miRNA/miRNA duplex by DICER enzyme. One of the strands is combined with Argonaute proteins (Ago2) to RNA Induced Silencing Complex (RISC). This complex mediates gene expression by binding target mRNAs and inducing mRNA degradation.



miRNAs were discovered in 1993, within Ambros and Ruvhun's lab, on *Caenorhabditis elegans*. An intense research started in the following years aimed at understanding miRNAs functions and possible applications. miRNAs are implicated in several biological and pathologic processes. Great effort is focused on explanation of the potential role of miRNAs in inflammatory disease, atherosclerosis, metabolic disease and fibrosis [14]. They are also important regulator of immune system and angiogenesis. Moreover, miRNA involvement in tumorigenesis was demonstrated by the development

of lymphoproliferative tumors in transgenic mouse whose miR-155 gene was up-regulated [15]. As in hematologic neoplasms, solid tumors show aberrant expression of several miRNAs.

3. miRNA Deregulation in Head and Neck Cancers

A summary of recent studies regarding miRNA expression profiling in SCHNC is shown in Table 1 [16–37]. The vast majority of studies included a slender number of cases and informations are not conclusive yet. miR-21 has been shown to act as an oncogene by targeting PTEN in various cancer [38,39]. The miR-21 has also been frequently observed in HNC as reported in Table 1. Recent studies on SCHNC demonstrated that increased expression of miR-21 causes reduction of PTEN expression [27] and its transcriptional regulator Grhl3 [40]. PTEN is an inhibitor of the PI3K pathway. Its inactivation leads to accumulation of phosphatidylinositol 3,4,5-triphosfate (PIP_3) and consequently increased activity of serine/threonine protein kinase PDK-1 and AKT which promote cell cycle progression, proliferation and inhibit apoptosis. miR-31 activates the hypoxia-inducible factor (HIF) pathway through the suppression of its inhibitors factors (FIH), consequently promoting tumor angiogenesis and growth. As in other cancers, deregulation of miR-31 was observed in SCHNC [28,36]. miR-375 is frequently found as down-regulated in SCHNC and acts as tumor suppressor. Hui *et al.* demonstrated that the down-regulation of miR-375, observed in over 91% of HNSCC samples, would result in deregulated proliferation, chaotic growth and inhibited apoptosis [24]. Moreover, Nohata *et al.* suggested that miR-375 acts as tumor suppressor and regulates AEG1/MTDH (astrocyte elevated gene 1/metadherin), a mediator in different signaling pathways such PI3k/AK, NF-Kb and Wnt/b-catenin, promoting oncogenesis [30].

Table 1. miRNA expression profiling in SCHNC recent studies.

Reference	Materials	Methods	miRNA Deregulation
Avissar *et al.* [16]	16 tumors, 5 normal	microRNA array	↑ miR-21, miR-181d, miR-181b, miR-491, miR-455, miR-455, miR-18a, miR-130b, miR-221, miR-193b, miR-181a, miR-18b ↓ miR-375
Barker *et al.* [17]	12 primary tumors, 12 metastatic lymphnodes	TaqMan microRNA assay	↑ miR-103, miR-155, miR-191, miR-181b, miR-181d, miR-205
Cao *et al.* [18]	60 tumors, 54 normal	microRNA array	↑ miR-125, miR-145 ↓ miR-93, miR-205, miR-21, miR-708
Cervigne *et al.* [19]	12 tumors, 7 normal	TaqMan microRNA assay	↑ miR-21, miR-181b, miR-345, miR-146a, miR-184, miR-518b, miR-520g, miR-649
Chang *et al.* [20]	8 tumors, 4 normal	microRNA array	↑ miR-21, miR-155, miR-146, miR-29c, miR-18, miR-146b, let-7i, miR-142-3p ↓ miR-494
Childs *et al.* [21]	8 tumors, 8 normal	MicroRNA array	↑ miR-21, miR-24, miR-151, miR21, miR-199b ↓ let-7f, miR-142-3p, miR-324-5p, miR-368, miR-370, miR-373, miR-422b, miR-424, miR-95, let-7a, miR-16-2, miR-1, miR-133a
Christensen *et al.* [22]	513 tumors, 597 normal	TaqMan microRNA assay	↓ Let-7 family

Table 1. *Cont.*

Reference	Materials	Methods	miRNA Deregulation
Fletcher *et al.* [23]	19 tumors, 7 normal	qRT-PCR	↑ miR-205
Hui *et al.* [24]	51 tumors, 4 normal	TaqMan microRNA assay	↑ miR-423, miR-93, miR-106b, miR-16, miR-20a, miR-155, miR-193a, miR-25, miR-92, miR-17-5p, let-7i, miR-19b, miR-223, miR-27a, miR-142-3p, miR-210, miR-106a, miR-15a, miR-21, miR-29b, miR-130b, miR-205, miR-422b ↓ miR-125b, miR-375, miR-10a, let-7a, miR-140, miR-100, miR-143, miR-99a, miR-30c, miR-365, miR-127, let-7c, miR-199b, let-7e, miR-26a
Kozaki *et al.* [25]	18 tumors, 1 normal	qRT-PCR	↑ miR-374, miR-340, miR-224, miR-10a, miR140, miR-181a ↓ miR-27a, miR-34b, miR-34c, miR-203, miR-302c, miR-23a, miR-27b, miR-34a, miR-215, miR-299, miR-330, miR-337, miR-107, miR-133b, miR-138, miR-139, miR-223, miR-204, miR-370, let-7d, mir-95, miR-302a, miR-367*
Lajer *et al.* [26]	49 tumors, 39 normal	microRNA array	↑ miR-31, miR-21, miR-223, miR-503, miR-187, miR-1246, miR-146b-5p, miR-146a, miR-155, miR-424, miR-181a, miR-181b, miR-27a, miR-132, miR-106b, miR-345, miR-21 ↓ miR-375, miR-1224-5p, miR-617, miR-99a, miR-125b, miR-378, miR-27b, miR-125b-2
Li *et al.* [27]	10 tumors, 10 normal	microRNA array	↑ miR-21
Liu *et al.* [28]	10 tumors, 10 normal	TaqMan microRNA assay	↑ mir-31, miR-34c, miR-187, miR-135b, miR-372, miR-34b, miR-21, miR-371, miR-216, miR-301, miR-10a, miR-155, miR-130b, miR-223, miR-373, miR-96, miR-224, miR-147, miR-128b, miR-104, miR-183, miR-182 ↓ miR-100, miR-328, miR-99a, miR-124, miR-149, miR-139, miR-124a, miR-204, miR-211
Kikkawa *et al.* [29]	10 tumors, 10 normal	TaqMan microRNA assay	↑ miR-517c, miR-196a, miR-7, miR-196b, miR-650, miR-18a, miR-452, miR-183, miR-432, miR-301a, miR-21 ↓ miR-1, miR-375, miR-139-5p, miR-504, miR-125b, miR-199b, miR-100, miR-497, miR-30a, let-7c, miR-218, miR-10b, miR-126, miR-378, miR-328, miR-204, miR-143, miR-126, miR-99a, miR-195, miR-489, miR-203, miR-140-5p, miR-29a, miR-26a, miR-214, miR-30a, miR-26b, miR-30e, miR-30b, let-7b
Nohata *et al.* [30]	5 tumors, 5 normal	TaqMan microRNA assay	↓ miR-874, miR-133a, miR-375, miR-204, miR-1, miR-139-5p, miR-145, miR-143, miR-486-3p, miR-146a, miR-410, miR-126, miR-539, miR-134, miR-218, miR-146b-5p, miR-140-3p, miR-30a-3p, miR-191, miR-186, miR-148a, miR-30e-3p, miR-29c*
Ramdas *et al.* [31]	5 Tumors, 5 normal	microRNA array	↑ miR-7083, miR-7, miR-34b, miR-155, miR-182, miR-21, miR-181c, miR-181a, miR-25, miR-93, let-7i, miR-107, miR-103, miR-221 ↓ miR-23b, miR-7029, miR-125a, miR-125b
Rentoft *et al.* [32]	21 tumors, 8 normal	microRNA array	↑ miR-424, miR-21, miR-1301, miR-7, miR-142-5p, miR-105, miR-142-3p, miR-146b-3p, miR-659, miR-361-3p, miR-665, miR-146 b-5p* ↓ miR-617, miR-29b-2, miR-132, miR-548b-5p, miR-22, miR-629, mir-29b-1, miR-99a, let-7c*

Table 1. *Cont.*

Reference	Materials	Methods	miRNA Deregulation
Scapoli *et al.* [33]	15 tumors	microRNA array	↑ miR-489, miR-129, miR-23a, miR-214, miR-23b, miR-92, miR-25, miR-210, miR-212, miR-515, miR-146b, miR-21, miR-338 ↓ miR-520h, miR-197, miR-378, miR-135b, miR224, mir-34a
Tran *et al.* [34]	9 Tumors	microRNA array	↑ miR-21, miR-205, miR-16, let-7a ↓ miR-342, miR-346, miR-373
Wiklund *et al.* [35]	15 tumors, 7 normal	qRT-PCR	↑ miR-127, miR-200, miR-205 ↓ miR-375, miR-137
Wong *et al.* [36]	4 tumors, 4 normal	TaqMan microRNA assay	↑ miR-184, miR-34c, miR-137, miR-372, miR-124a, miR-21, miR-124b, miR-31, miR-128a, miR-34b, miR-154, miR-197, miR-132, miR-147, miR-325, miR-181c, miR-198, miR-155, miR-30a-3p, miR-338, miR-17-5p, miR-104, miR-134, miR-213 ↓ miR-133a, miR-99a, miR-194, miR-133b, miR-219, miR-100, miR-125b, miR-26b, miR-138, miR-149, miR-195, miR-107, miR-139
Xiao *et al.* [37]	20 oral leukoplakia, 7 malignant transformed oral leukoplakia	microRNA assay	↑ miR-31, miR-142-5p, miR-33a, miR-1259, miR-146b-5p, miR-886-3p, miR-886-5p, miR-519d, miR-301a ↓ miR-572, miR-611, miR-602, miR-675, miR-585, miR-623, miR-637, miR-1184

4. miRNAs as Prognostic Indicators

The prognosis of advanced SCNHC is dismal despite improvements in multimodal treatment. Recent studies showed a potential role of miRNAs as prognostic biomarkers.

Let-7 is a family of tumor suppressing miRNAs whose levels were found deregulated in SCHNC by different authors ([21–33] and others). Moreover Scapoli *et al.* [33] found a correlation between underexpression of Let-7, miR-155 and miR-146a and progression to metastatic tumors.

In a study on nasopharyngeal carcinoma cells, Wong *et al.* found low levels of Let-7 miRNA and suggested a role in regulating proliferation through the downregulation of c-MYC expression [41]. Low levels of Let-7 have a proliferative effect on nasopharyngeal cells trough the unsuccessful suppressing effect on c-MYC expression.

Moreover, recent studies on non-small cell lung cancer highlighted the role of Let-7 in the KRAS regulation [42]. A variant allele in the KRAS 3′ untranslated region, that binds the let-7 complementary site (KRAS-LCS6), is associated with an increased KRAS expression and low levels of Let-7. Christensen *et al.* demonstrated the presence of KRAS-LCS6 variant in SCHNC and its correlation with poor prognosis, especially in oral cancer [22].

TP53 is a tumor suppressor gene encoding for a protein that regulates the cell cycle. TP53 mutation is a common alteration in cancer with a frequency of 53% found in a large cohort of SCHNC analyzed by Poeta *et al.* TP53 mutations were more frequently in patients with tumors arising from hypopharynx (75%) and larynx (56.7%). Moreover, authors demonstrated a strong correlation between TP53 mutations in SCHNC and high risk of recurrence and poor survival [43]. Ganci *et al.* [44] found a strong association between 49 miRNAs and TP53 status with a particular correlation of a subset of 12 miRNAs with shorter recurrence free-survival whereas 4 of them were associated with lower

cancer-specific survival. A correlation between the expression of specific miRNAs, such as miR-375 and miR-210, and outcome of SCHNC patients was indeed reported: low levels of miR-375 correlated with poor survival and distant metastases as high levels of miR-210 correlated with locoregional recurrence [45,46].

5. miRNAs as Biomarkers

SCHNC is frequently diagnosed in advanced stage when metastases to regional lymph nodes are already present. Even with combination of surgery, chemotherapy and radiotherapy, these patients have a high risk of recurrence. Thus, an important goal would be the identification of biomarkers for early detection. Recent studies identified a potential role of some miRNAs as diagnostic biomarkers. Actually, miRNAs circulate stably in different human body fluids, such as blood, saliva, urine and breath. Hence they can be accessible with non-invasive methods.

Wong *et al.* detected the presence of miR-184 in the plasma of 80% of patients with tongue squamous cancer (all stages) compared to 13% of healthy patients [36]. In the saliva of patients with oral squamous cell cancer, miR-125a and miR-200 were significantly under expressed compared to controls [47]. Moreover, Liu *et al.* showed high levels of salivary miR-31 in oral cancer while levels of miR-31 in plasma were up-regulated [48,49]. Lastly, Clague *et al.* reported the correlation between one variant allele of miR-26a and an increased risk to develop premalignant oral lesions [50].

6. miRNAs and Resistance to Chemotherapy and Radiotherapy

The combination of chemotherapy and radiotherapy is the standard treatment for locally advanced SCHNC. However resistance to anticancer drugs oftenly leads to treatment failure, though alterations of different molecular pathways. Recent studies hypothesized a potential role of miRNAs in chemoresistance. Yu *et al.* found a different expression of miRNAs between cisplatin-sensitive tongue squamous cell carcinoma and cisplatin-resistant sublines: in particular increased levels of let-7 family, miR-23a, miR-214, miR-518c, miR-608 and decreased levels of miR-21 and miR-342 [51]. miR-21 and miR-214 have been already reported to alter the chemosensitivity in others tumors as cholangiocarcinoma and ovarian cancer respectively [52,53]. In this study, miR-214 might induce cell survival and cisplatin resistance through targeting the 3'-untranslated region (UTR) of the PTEN gene, which leads to down-regulation of the protein and activation of Akt pathway.

Several authors have also investigated the role of miRNAs in the development of radioresistance. miRNA expression profiles have been compared in 6 tumors cell lines (3 from gliomas and 3 from squamous cell carcinomas) after ionizing radiation. Levels of miR-24-1, miR-144, let-7i, and miR-1285 were significantly increased following irradiation, confirming their susceptibility to ionizing radiation [54]. miR-205 has been investigated in a radio-resistant nasopharyngeal carcinoma cell line (CNE-2P). High level of miR-205 has been detected compared to cell line control [55]. The 3'-UTR of PTEN contains a binding site for miR-205. PTEN is largely known as an inhibitor of cell cycle progression. Authors suggested that PTEN is responsible for radioresistance through miR-205. Low levels of miR-125b, observed in oral squamous cell carcinoma cells, are correlated with proliferation and radioresistance mechanism, mediated by the downregulation of the intracellular adhesion molecule 2 (ICAM2) [56]. Actually, miR-125b has complementary sequences for ICAM2 mRNA.

7. The Application of miRNA to Cancer Treatment

One key possibility for the future is the modulation of altered miRNAs concentration through molecules that replace downregulated miRNAs or using antagonists that binds overexpressed miRNAs. The first clinical trial, using a complementary molecule, was conducted in patients with chronic HCV infection [57]. Miravirsen is a short oligonucleotide that binds and inhibits miR-122. miR-122 is highly expressed in the liver and enhances HCV propagation. An ongoing phase II trial has the purpose to assess the antiviral activity and safety of Miravirsen.

To our knowledge there is only one clinical trial available in cancer patients. MRX34 is a molecule mimicking miR-34, which is found as downregulated in many tumors. miR-34 inhibits cancer proliferation and apoptosis by the deregulation of MYC, MET, BCLR and β-catenin. A phase I ongoing study is evaluating the maximum tolerate dose and the pharmacokinetic of MRX34 in patients with primary liver cancer or liver metastasis [58].

8. Conclusions

It is widely recognized that miRNAs are pivotal pawns in the cancer development of many tumors, including HNC. Several studies demonstrated considerable discrepancies in miRNAs levels between tumors and corresponding adjacent normal tissues. Moreover, a change in miRNAs expression has been observed in primary disease compared to metastatic sites, suggesting a role in the tumor progression. miRNAs are easily obtained with non-invasive methods because they circulate stable in blood, urine and saliva. Further studies will be needed to identify specific miRNAs that can be used as biomarkers for early detection or as biomarkers with predictive value. However, to date, there are no data mature enough to introduce miRNAs expression analysis in the current clinical management.

From a therapeutic point of view, miRNA-based therapies are currently in preclinical and clinical development with encouraging results. For miRNAs whose expression is reduced, re-introduction of mimics miRNAs could restore the correct gene targets modulation. Conversely, for miRNAs whose expression is increased, the strategy is aimed to the inhibition through use of anti-miRNAs.

Author Contributions

Giulia Courthod and Gianmauro Numico: study concept and design; Pierfrancesco Franco, Loredana Palermo and Salvatore Pisconti: acquisition of literature data; Giulia Courthod, Gianmauro Numico, Pierfrancesco Franco, Loredana Palermo and Salvatore Pisconti: data analysis and interpretation; Giulia Courthod and Gianmauro Numico: drafting of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ferlay, J.; Steliarova-Foucher, E.; Lortet-Tieulent, J.; Rosso, S.; Coebergh, J.W.; Comber, H.; Forman, D.; Bray, F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *Eur. J. Cancer* **2013**, *49*, 1374–1403.

2. Perez-Ordonez, B.; Beauchemin, M.; Jordan, R.C. Molecular biology of squamous cell carcinoma of the head and neck. *J. Clin. Pathol.* **2006**, *59*, 445–453.
3. Bonner, J.A.; Harari, P.M.; Giralt, J.; Cohen, R.B.; Jones, C.U.; Sur, R.K.; Raben, D.; Baselga, J.; Spencer, S.A.; Zhu, J.; *et al.* Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol.* **2010**, *11*, 21–28.
4. Vermorken, J.B.; Mesia, R.; Rivera, F.; Remenar, E.; Kawwecki, A.; Rottey, S.; Erfan, J.; Zabolotnyy, D.; Kienzer, H.; Cupissol, D.; *et al.* Platinum-Based Chemotherapy plus Cetuximab in Head and Neck Cancer. *N. Engl. J. Med.* **2008**, *359*, 1116–1127.
5. Numico, G.; Franco, P.; Cristofano, A.; Migliaccio, F.; Spinazzé, S.; Silvestris, N.; Cante, D.; Sciacero, P.; la Porta, M.R.; Girelli, F.; *et al.* Is the combination of Cetuximab with chemo-radiotherapy regimens worthwhile in the treatment of locally advanced head and neck cancer? A review of current evidence. *Crit. Rev. Oncol./Hematol.* **2013**, *85*, 112–120.
6. Numico, G.; Silvestris, N.; Grazioso Russi, N. Advances in EGFR-directed therapy in head and neck cancer. *Front. Biosci.* **2011**, *3*, 454–466.
7. Leemans, C.R.; Braakhuis, B.J.M.; Brakenhoff, R.H. The molecular biology of head and neck cancer. *Nat. Rev. Cancer* **2011**, *11*, 9–22.
8. Homo Sapiens miRNA (1872 Sequences). Available online: http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=hsa (accessed on 21 March 2014).
9. Lee, Y.; Kim, M.; Han, J.; Yeom, K.H.; Lee, S.; Baek, S.H.; Kim, V.N. microRNA genes are transcribed by RNA polymerase II. *EMBO J.* **2004**, *23*, 4051–4060.
10. Cai, X.; Hagedorn, C.H.; Cullen, B.R. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* **2004**, *10*, 1957–1966.
11. Kwak, P.B.; Iwasaki, S.; Tomari, Y. The microRNA pathway and cancer. *Cancer Sci.* **2010**, *101*, 2309–2315.
12. Garzon, R.; Calin, G.A.; Croce, C.M. microRNAs in cancer. *Annu. Rev. Med.* **2009**, *60*, 167–179.
13. Hu, Z.; Chen, X.; Zhao, Y.; Tian, T.; Jin, G.; Shu, Y.; Chen, Y.; Xu, L.; Zen, K.; Zhang, C.; *et al.* Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small cell lung cancer. *J. Clin. Oncol.* **2010**, *28*, 1721–1726.
14. Van Rooij, E.; Purcell, A.L.; Levin, A.A. Developing microRNA therapeutics. *Circ. Res.* **2012**, *110*, 496–507.
15. Costinean, S.; Zanesi, N.; Pekarsky, Y.; Till, E.; Volinia, S.; Heerema, N.; Croce, C.M. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in Eμ-miR155 transgenic mice. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7024–7029.
16. Avissar, M.; Christensen, B.C.; Kelsey, K.T.; Marsit, C.J. microRNA expression ratio is predictive of head and neck squamous cell carcinoma. *Clin. Cancer Res.* **2009**, *15*, 2850–2855.
17. Barker, E.V.; Cervigne, N.K.; Reis, P.P.; Goswami, R.S.; Xu, W.; Weinreb, I.; Irish, J.C.; Kamel-Reis, S. microRNA evaluation of unknown primary lesions in the head and neck. *Mol. Cancer* **2009**, *8*, 127.

18. Cao, P.; Zhou, L.; Zhang, J.; Zheng, F.; Wang, H.; Ma, D.; Tian, J. Comprehensive expression profiling of microRNAs in laryngeal squamous cell carcinoma. *Head Neck* **2013**, *35*, 720–728.
19. Cervigne, N.K.; Reis, P.P.; Machado, J.; Sadikovic, B.; Bradley, G.; Galloni, N.N.; Pintilie, M.; Jurisica, I.; Perez-Ordonez, B.; Gilbert, P.; *et al.* Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. *Hum. Mol. Genet.* **2009**, *18*, 4818–4829.
20. Chang, S.S.; Jiang, W.W.; Smith, I.; Poeta, L.M.; Begum, S.; Glazer, C.; Shan, S.; Westra, W.; Sidransky, D.; Califano, J.A. microRNA alterations in head and neck squamous cell carcinoma. *Int. J. Cancer* **2008**, *123*, 2791–2797.
21. Childs, G.; Fazzari, M.; Kung, G.; Kawachi, N.; Brandwein-Gensler, M.; McLemore, M.; Chen, Q.; Burk, R.D.; Smith, R.V.; Prystowsky, M.B.; *et al.* Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma. *Am. J. Pathol.* **2009**, *174*, 736–745.
22. Christensen, B.C.; Moyer, B.J.; Avissar, M.; Ouellet, L.G.; Plaza, S.L.; McClean, M.D.; Marsit, C.J.; Kelsey, K.T. A let-7 microRNA-binding site polymorphism in the KRAS 30 UTR is associated with reduced survival in oral cancers. *Carcinogenesis* **2009**, *30*, 1003–1007.
23. Fletcher, A.M.; Heaford, A.C.; Trask, D.K. Detection of metastatichead and neck squamous cell carcinoma using the relative expres-sion of tissue-specific miR-205. *Transl. Oncol.* **2008**, *1*, 202–208.
24. Hui, A.B.Y.; Lenarduzzi, M.; Krushel, T.; Waldron, L.; Pintilie, M.; Shi, W.; Perez-Ordonez, B.; Jurisica, I.; O'sullivan, B.; Waldrom, J.; *et al.* Comprehensive microRNA profiling for head and neck squamous cell carcinomas. *Clin. Cancer Res.* **2009**, *16*, 1129–1139.
25. Kozaki, K.; Imoto, I.; Mogi, S.; Omura, K.; Inazawa, J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylationin oral cancer. *Cancer Res.* **2008**, *68*, 2094–2105.
26. Lajer, C.B.; Nielsen, F.C.; Friis-Hansen, L.; Norrild, B.; Borup, R.; Garnaes, E.; Rossing, M.; Specht, L.; Therkildsen, M.H.; Nauntofte, B.; *et al.* Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: A prospective translational study. *Br. J. Cancer* **2011**, *104*, 830–840.
27. Li, J.; Huang, H.; Sun, L.; Yang, M.; Pan, C.; Chen, W.; Wu, D.; Lin, Z.; Zeng, C.; Yao, Y.; *et al.* miR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. *Clin. Cancer Res.* **2009**, *15*, 3998–4008.
28. Liu, C.J.; Tsai, M.M.; Hung, P.S.; Kao, S.Y.; Liu, T.Y.; Wu, K.J.; Chiou, S.H.; Lin, S.C.; Chang, K.W. miR-31 ablates expression of the HIF regulatory factor FIH to activate the HIF pathway in head and neck carcinoma. *Cancer Res.* **2010**, *70*, 1635–1644.
29. Kikkawa, N.; Hanazawa, T.; Fujimura, L.; Nohata, N.; Suzuki, H.; Chazono, H.; Sakurai, D.; Horiguchi S.; Okamoto, Y.; Seki, N. miR-489 is a tumour-suppressive miRNA target PTPN11 in hypopharyngeal squamous cell carcinoma (HSCC). *Br. J. Cancer* **2010**, *103*, 877–884.
30. Nohata, N.; Hanazawa, T.; Kikkawa, N.; Sakurai, D.; Fujimura, L.; Chiyomaru, T.; Kawakami, K.; Yoshino, H.; Enokida, H.; Nakagawa, M.; *et al.* Tumour suppressive microRNA-874 regulates novel cancer networks in maxillary sinus squamous cell carcinoma. *Br. J. Cancer* **2011**, *105*, 833–841.

31. Ramdas, L.; Giri, U.; Ashorn, C.L.; Coombes, K.R.; El-Naggar, A.; Ang, K.K.; Story, M.D. miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue. *Head Neck* **2009**, *31*, 642–654.
32. Rentoft, M.; Fahlen, J.; Coates, P.J.; Laurell, G.; Sjostrom, B.; Ryden, P.; Nylander, K. miRNA analysis of formalin-fixed squamous cell carcinoma of the tongue is affected by age of the sample. *Int. J. Oncol.* **2011**, *38*, 61–69.
33. Scapoli, L.; Palmieri, A.; Lo Muzio, L.; Pezzetti, F.; Rubini, C.; Girardi, A.; Farinella, F.; Mazzotta, M.; Carinci, F. microRNA expression profiling of oral carcinoma identifies new markers of tumor progressione. *Int. J. Immunopathol. Pharmacol.* **2010**, *23*, 1229–1234.
34. Tran, M.; McLean, T.; Zhang, X.; Zhao, C.J.; Thomson, J.M.; O'Brien, C.; Rose, B. microRNA expression profiles in head and neck cancer cell lines. *Biochem. Biophys. Res. Commun.* **2007**, *358*, 12–17.
35. Wiklund, E.D.; Gao, S.; Hulf, T.; Sibbritt, T.; Shalima, N.; Costea, D.E.; Villadsen, S.B.; Bakholdt, V.; Bramsen, J.B.; Sørensen, J.A.; *et al.* microRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma. *PLoS One* **2011**, *6*, e27840.
36. Wong, T.S.; Liu, X.B.; Wong, B.Y.; Ng, R.W.; Yuen, A.P.; Wei, W.I. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. *Clin. Cancer Res.* **2008**, *14*, 2588–2592.
37. Xiao, W.; Bao, Z.X.; Zhang, C.Y.; Zhang, X.Y.; Shi, L.J.; Zhou, Z.T.; Jiang, W.W. Upregulation of miR-31 is negatively associated with recurrent/newly formed oral leukoplakia. *PLoS One* **2012**, *7*, e38648.
38. Meng, F.; Henson, R.; Wehbe-Janek, H.; Ghoshal, K.; Jacob, S.T.; Patel, T. microRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* **2007**, *133*, 647–658.
39. Frankel, L.B.; Christoffersen, N.R.; Jacobsen, A.; Lindow, M.; Krogh, A.; Lund, A.H. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J. Biol. Chem.* **2008**, *283*, 1026–1033.
40. Darido, C.; Georgy, S.R.; Wilanowski, T.; Dworkin, S.; Auden, A.; Zhao, Q.; Rank, G.; Srivastava, S.; Finlay, M.J.; Papenfuss, A.T.; *et al.* Targeting of the tumor suppressor GRHL3 by a miR-21-dependent proto-oncogenic network results in PTEN loss and tumorigenesis. *Cancer Cell* **2011**, *20*, 635–648.
41. Wong, T.S.; Man, O.Y.; Tsang, C.M.; Tsao, S.W.; Tsang, R.K.; Chang, J.Y.; Ho, W.K.; Wei, W.I.; To, V.S. microRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through downregulating c-Myc expression. *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 415–422.
42. Chin, L.J.; Ratner, E.; Leng, S.G.; Zhai, R.H.; Nallur, S.; Babar, I.; Muller, R.U.; Starka, E.; Su, L.; Burki, E.A.; *et al.* A SNP in a let-7 microRNA complementary site in the KRAS 3' untraslated region increases non-small cell lung cancer risk. *Cancer Res.* **2008**, *68*, 8535–8540.
43. Poeta, M.L.; Manola, J.; Goldwasser, M.A.; Forastiere, A.; Benoit, N.; Califano, J.A.; Ridge, J.A.; Goodwi, J.; Kenady, D.; Saunders, J.; *et al.* TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* **2007**, *357*, 2552–2561.

44. Ganci, F.; Sacconi, A.; Bossel Ben-Moshe, N.; Manciocco, V.; Sperduti, I.; Strigari, L.; Covello, R.; Benevolo, M.; Pescarmona, E.; Domany, E.; *et al.* Expression of TP53 mutation-associated microRNAs predicts clinical outcome in head and neck squamous cell carcinoma patients. *Ann. Oncol.* **2013**, *24*, 3082–3088.
45. Harris, T.; Jimenez, L.; Kawachi, N.; Fan, J.; Chen, J.; Belbin, T.; Ramnauth, A.; Loudig, O.; Keller, C.E.; Smith, R.; *et al.* Low-level expression of miR-375 correlates with poor outcome and metastasis while altering the invasive properties of head and neck squamous cell carcinomas. *Am. J. Pathol.* **2012**, *180*, 917–928.
46. Gee, H.E.; Camps, C.; Buffa, F.M.; Patiar, S.; Winter, S.C.; Betts, G.; Homer, J.; Corbridge, R.; Cox, G.; West, C.M.; *et al.* Has-miR-210 is a marker of tumor hypoxia and a prognostic factor in head and neck cancer. *Cancer* **2010**, *116*, 2148–2158.
47. Park, N.J.; Zhou, H.; Elashoff, D.; Henson, B.S.; Kastratovic, D.A.; Abemayor, E.; Wong, D.T. Salivary microRNA: Discovery, characterization, and clinical utility for oral cancer detection. *Clin. Cancer Res.* **2009**, *15*, 5473–5477.
48. Liu, C.J.; Lin, S.C.; Yang, C.C.; Cheng, H.W.; Chang, K.W. Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma. *Head Neck* **2012**, *34*, 219–224.
49. Liu, C.J.; Kao, S.Y.; Tu, H.F.; Tsai, M.M.; Chang, K.W.; Lin, S.C. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. *Oral Dis.* **2010**, *16*, 360–364.
50. Clague, J.; Lippman, S.M.; Yang, H.; Hildebrandt, M.A.; Ye, Y.; Lee, J.J.; Wu, X. Genetic variation in microRNA genes and risk of oral premalignant lesions. *Mol. Carcinog.* **2010**, *49*, 183–189.
51. Yu, Z.; Zhong, L.; Zhang, P.; Chen, W.; Zhang, C. microRNAs contribute to the chemoresistance of cisplatin in tongue squamous cell carcinoma lines. *Oral Oncol.* **2010**, *46*, 317–322.
52. Meng, F.; Henson, R.; Lang, M.; Wehbe, H.; Maheshwari, S.; Mendell, J.T.; Jiang, J.; Schmittgen, T.D.; Patel, T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* **2006**, *130*, 2113–2129.
53. Yang, H.; Kong, W.; He, L.; Zhao, J.J.; O'Donnell, J.D.; Wang, J.; Weham, R.M.; Coppola, D.; Kruk, P.A.; Nicosia, S.V.; *et al.* microRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res.* **2008**, *68*, 425–433.
54. Niemoeller, O.M.; Niyazi, M.; Corradini, S.; Zehentmayr, F.; Li, M.; Lauber, K.; Belka, C. microRNA expression profiles in human cancer cells after ionizing radiation. *Radiat. Oncol.* **2011**, *6*, 29.
55. Qu, C.; Liang, Z.; Huang, J.; Zhao, R.; Su, C.; Wang, S.; Wang, X.; Zhang, R.; Lee, M.; Yang, H. miR-205 determines the radioresistance of human nasopharyngeal carcinoma by directly targeting PTEN. *Cell Cycle* **2012**, *11*, 785–796.
56. Shiiba, M.; Shinozuka, K.; Saito, K.; Fushimi, K.; Kasamatsu, A.; Ogawara, K.; Uzawa, K.; Ito, H.; Takiguchi, Y.; Tanzawa, H. microRNA-125b regulates proliferation and radioresistance of oral squamous cell carcinoma. *Br. J. Cancer* **2013**, *108*, 1817–1821.
57. Janssen, H.L.A.; Reesink, H.W.; Lawitz, E.J.; Zeuzem, S.; Rodriquez-Torres, M.; Patel, K.; van der Meer, A.; Patick, A.K.; Chena, A.; Zhou, Y. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* **2013**, *368*, 1685–1694.

58. Mirna Therapeutics, Inc. A Multicenter Phase I Study of MRX34, microRNA miR-RX34 Liposome Injectable Suspension. U.S. National Institutes of Health, October 2013. Available online: http://www.clinicaltrials.gov/ct2/show/NCT01829971 (accessed on 30 April 2014).