The Journal of Laryngology & Otology (2013), ***127*** (Suppl. S2), *S2–S7*.

doi:10.1017/S0022215113000753

REVIEW ARTICLE

Micro-ribonucleic acids in head and neck cancer: an introduction

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Abstract

Background and methods: Head and neck cancer is the sixth most common cancer worldwide. Advances in management have not greatly altered overall survival. Over the last decade, there have been significant scientific advances in our knowledge of cell cycle regulation and the complex oncogenic processes. MicroRNAs are small, non-coding RNAs which are integral to the regulation of gene expression and which play a part in carcinogenesis. The literature on the role of microRNA in head and neck cancer is reviewed.

Objective: To introduce the role and significance of microRNAs in head and neck cancer.

Results: The possibilities of incorporating microRNAs into clinical practice are discussed, including their potential role in diagnosis, prognosis, prediction of metastatic spread, therapy and tumour surveillance.

Conclusion: Discoveries in expression profiling of microRNA in head and neck oncology promise advancements in the diagnosis, prognosis and therapy of these cancers.

Key words: miRNA; Markers, Biochemical; Cancer of Head And Neck

Introduction

Head and neck cancer is now the sixth commonest malignancy worldwide.[1] Despite increasing molecular and genetic profiling of head and neck cancers over the last two decades, and trials of molecular markers and targeted therapies aimed at improving diagnostic and therapeutic options, the overall survival of head and neck cancer patients remains poor.

There is now an increasing body of literature allowing us a better understanding of the various molecular factors that impact on tumourigenesis and metastatic spread. The more well researched markers (e.g. tumour suppressor proteins p53 and p16, and the oncogene epidermal growth factor receptor) are now used in clinical practice. Oropharyngeal tumours positive for tumour suppressor protein p16 are now known to have a better prognosis, whilst epidermal growth factor receptor is used as the target receptor for cetuximab, enabling improved locoregional control.[2]

Recently, microRNAs (also known as 'miRNAs') have emerged as important regulators of gene function.[3] MicroRNAs are a group of endogenous ribonucleic acids which participate in the regulation of gene expression. The pathological involvement of microRNAs and their potential as prognostic biomarkers are being increasingly appreciated. This paper introduces microRNAs and their significance in head and neck cancer.

Micro-ribonucleic acids are small (18–22 nucleotide), non-coding RNAs which are vital for regulation of host genome expression at the post-transcriptional level.[4] Their role in normal cellular function occurs at various points on the pathway from gene to protein. Micro-ribonucleic acids do not directly code for proteins. Instead, they regulate protein expression by binding to complementary nucleotide sequences present in target messenger RNA (mRNA) molecules, thereby blocking the translation of mRNA into protein.[5] A single microRNA can regulate the expression of hundreds of different mRNAs. Furthermore, a single mRNA can contain binding sites for several microRNAs, typically within the 3′ untranslated region. It is now apparent that microRNAs are key modulators of biological processes including cellular proliferation, apoptosis, cell-to-cell communication and inflammation.[6,7] Micro-ribonucleic acids essentially 'fine-tune' the regulatory networks that control these processes, and as such are central to maintaining homeostasis.[8] Dysregulated microRNA patterns have now been demonstrated in many disease processes, including autoimmune disorders and other inflammatory conditions, as well as in carcinogenesis.[9,10]

Accepted for publication 12 February 2013 First published online 24 April 2013

Micro-ribonucleic acids have potential as biomarkers assisting early diagnosis, prognosis, decision-making and ongoing surveillance within the whole field of oncology. They may prove to have high specificity and sensitivity, and could possibly enable monitoring using easily accessible bodily fluids such as saliva and/or plasma.[11] Such innovations are yet to be introduced into clinical practice.

Discovery of microRNA

The nematode *Caenorhabditis elegans* has been widely used as a model organism for molecular and developmental biology research, and was first characterised at the genetic level in the early 1970s.[12,13] In 1993, research was conducted on two genes which controlled larval development events in a range of cell types.[14] This research demonstrated that one of these genes, *lin-4*, actually encoded two small RNAs (61 and 22 nucleotides) with antisense complementarity to sequences in an untranslated region of the second gene, *lin-14*. Further study demonstrated that RNA–RNA binding at this site actually blocked the protein expression of the second gene (*lin-14*).[15] These two small RNAs from *lin-4* were the founding members of the family of small regulatory RNAs that were eventually termed microRNAs.

Further research into microRNAs has continued exponentially, and there are now over a thousand (1587) different human microRNAs listed in the central miRBase database.[16]

MicroRNA biogenesis

Micro-ribonucleic acid biogenesis is complex and involves multiple processes. The key steps are as follows.

First, many microRNA genes are present in the intron region of each longer RNA transcript.[5,17] These RNA strands are transcribed from DNA by RNA polymerase II (Figures 1a to 2a).

Second, microRNA genes are transcribed by RNA polymerase II into primary microRNA transcripts (also termed 'pri-miRNAs') which are usually hundreds or thousands of nucleotides long and contain one or several hairpin loop structures (Figure 2b).[6,7]

Third, the primary microRNAs are cleaved in the nucleus by enzymes to produce precursor microRNAs (also termed 'pre-miRNAs') (Figure 2c and 2d).[18]

Fourth, a shuttle protein exports the precursor microRNA from the nucleus to the cytoplasm, at which point the precursor microRNA is processed by the Dicer RNAIII enzyme to yield imperfectly matched microRNA–microRNA* duplexes (Figure 2e, 2f and 2g).[7]

Last, the duplex is then separated into individual strands with the guide strand of the duplex being loaded into the Argonaute (Ago) protein to generate an RNA-induced silencing complex. This RNA-induced silencing complex interacts with the 3′ untranslated region of its target mRNA to regulate gene expression (Figure 3a and 3b).[19]

MicroRNA and cancer

Micro-ribonucleic acid expression has a significant biological impact, disruption of which can contribute to the development and progression of cancer. A microRNA that specifically binds to an mRNA which encodes for a protein with growth-suppressing roles can act as an 'onco-microRNA', because its expression can result in a bias towards growth-promoting processes associated with cancer development. Conversely, a microRNA that specifically binds to an mRNA with growth-promoting roles can act as a 'tumour suppressor microRNA' because its expression can result in a bias towards growth suppression and anti-neoplastic processes.[3,20]

The expression profiles of microRNA are certainly not the same in all cancers.[21,22] In 2002, studies on B-cell chronic lymphocytic lymphoma showed down-regulation of two key microRNAs (miR-15 and miR-16) in over 60 per cent of tumour samples.[23] In 2005, further work demonstrated that most microRNAs had lower expression in tumours compared with normal tissue, and that using expression profiles of microRNA could predict poorly differentiated tumour.[21]

MicroRNAs in head and neck cancer

The first work on microRNA in head and neck cancer was published in 2005.[24] A summary of microRNAs thought to be involved in head and neck cancer is

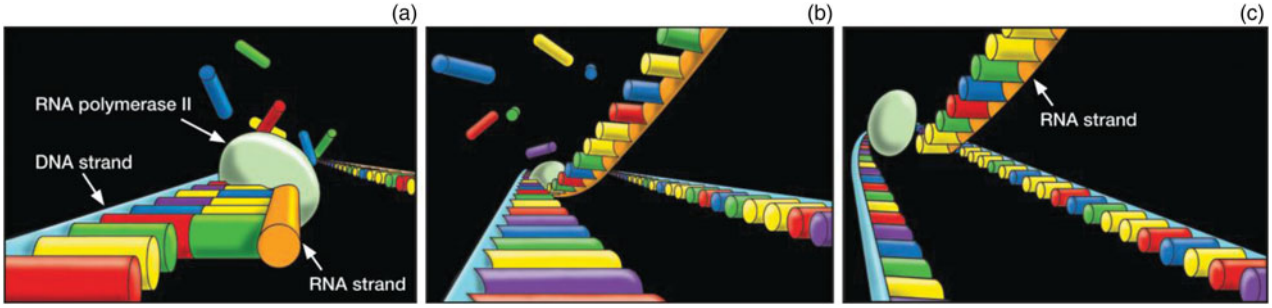

FIG. 1

Diagram showing DNA to RNA transcription by RNA polymerase II: (a) commencement of RNA strand transcription; (b) longer RNA transcript formation; and (c) release of RNA strand.

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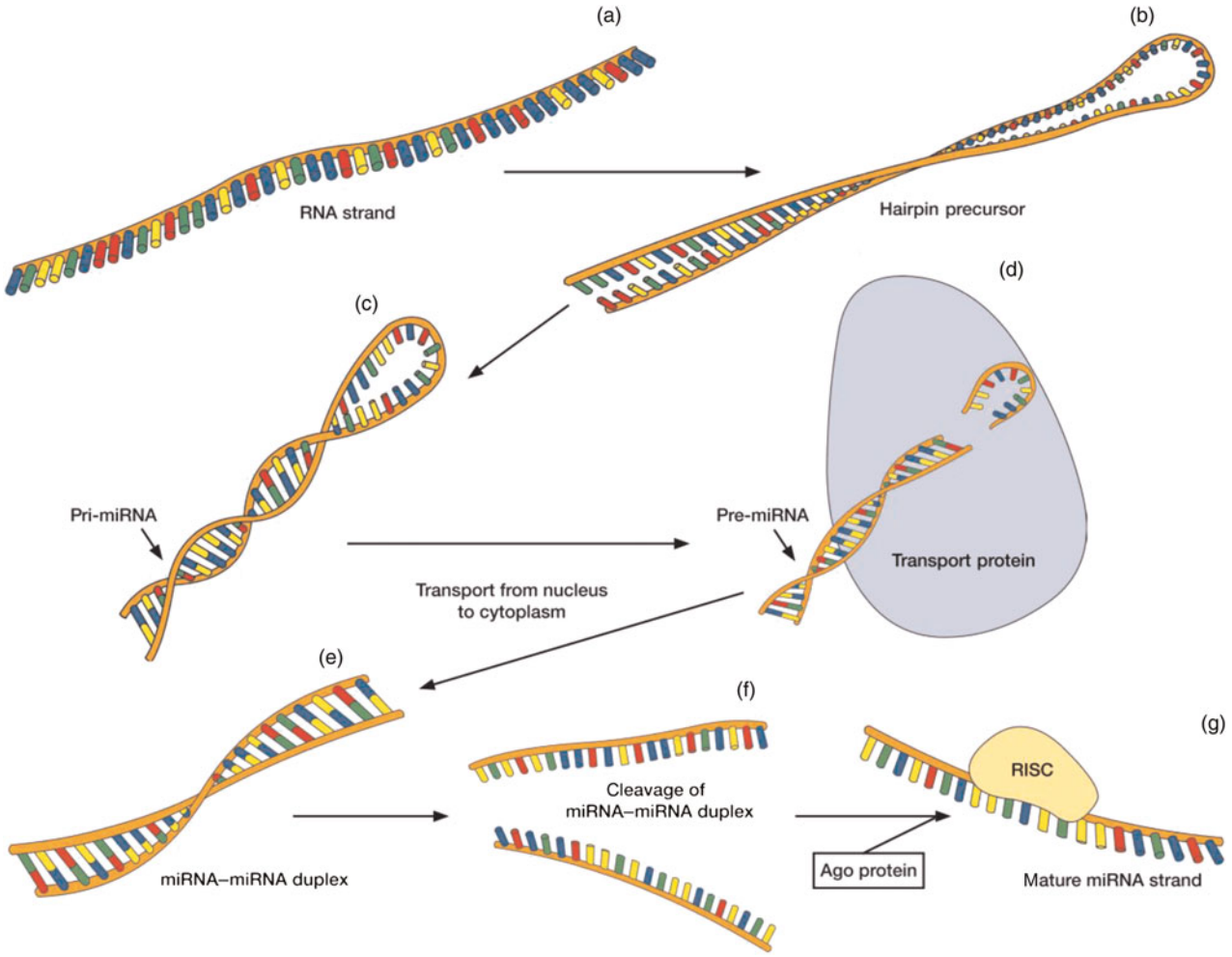

FIG. 2

Diagram showing biogenesis of microRNA (miRNA): (a) RNA strand; (b) hairpin precursor; (c) & (d) primary microRNA (pri-miRNA) cleaved to produce precursor microRNA (pre-miRNA) and transported from nucleus to cytoplasm by transport protein; (e) microRNA–microRNA duplex; (f) cleavage of microRNA–microRNA duplex; (g) mature microRNA strand together with Argonaute (Ago) protein generates RNA-induced silencing complex (RISC).

shown in Table I. There is no doubt that, in head and neck tumours, dysregulation of microRNA is associated with tumourigenesis.[22,25,29] However, the relevant studies do not always show the same trends in microRNA dysregulation; more detailed studies in this area are needed in order to determine the exact role of microRNA dysfunction at each particular site and subsite.

MicroRNA expression differs in normal and malignant head and neck tissue, a finding reported consistently by several authors.[11,21] At present, there is no published research assessing specific microRNA function in normal versus dysplastic tissue, and in successfully treated disease versus persistent disease.

The largest study of microRNA in head and neck cancer to date, published in 2009, studied samples from 104 head and neck cancer patients undergoing treatment with curative intent.[26] This study aimed to investigate the microRNA expression profile with respect to prognosis. It demonstrated that 43 microRNAs were expressed at lower levels than in normal tissues, while only 6 microRNAs were expressed at higher levels.

Further studies have demonstrated that microRNAs are involved in the specific molecular and biological processes that drive initial tumourigenesis, then subsequent invasion and eventual metastasis.[39]

Consistent down-regulation of the miR-21 microRNA has been found in several separate head and neck studies; miR-21 has also been found to be associated with tumourigenesis in a variety of non-head and neck cancers.[25–27] This microRNA is now known to alter tumour suppressor genes, resulting in reduced cell apoptosis.

Another microRNA of interest is miR-451, which has been shown to be down-regulated in head and neck patients with recurrent disease when compared with patients with an effective cure. Although further work is required, this molecular change may have a clinical role as a prognostic marker.[22]

Clinical relevance of microRNAs in otolaryngology and head and neck surgery

In spite of greater understanding of the molecular biology involved in head and neck cancer, 5-year

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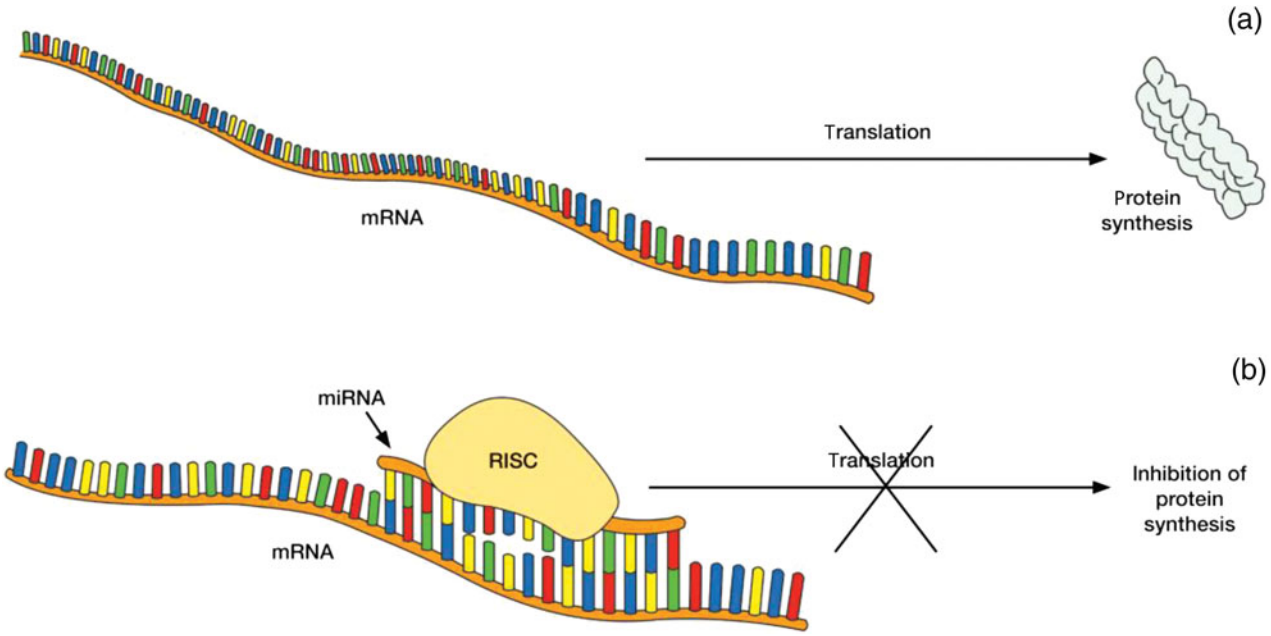

FIG. 3
Diagram showing (a) usual translational stage, with messenger RNA (mRNA) producing protein structures, and (b) interaction of mRNA with RNA-induced silencing complex (RISC) at translational stage, inhibiting protein synthesis.

TABLE I
MICRO-RNAS INVOLVED IN HEAD AND NECK CANCER

MicroRNA	Subsites	Expression profile in tumour or cell line	Cell cycle target genes; tumour phenotypes & outcomes	Refs
miR-21	Oral cavity, oropharynx, larynx, salivary gland	Up-regulated	PTEN, PDCD4, TPM1; reduces cell apoptosis	25–27
miR-205	Oropharynx, oral cavity	Up-regulated	Positive regional lymph nodes (linked to locoregional recurrence)	28
miR-155	Oral cavity, oropharynx	Up-regulated	APC	25
Let-7b	Oral cavity	Down-regulated	Over-expression of Dicer; facilitates cell proliferation	27, 29, 30
miR-133a	Oropharynx, hypopharynx, larynx	Down-regulated	PKM-2	26
miR-184	Oral cavity	Up-regulated	c-Myc	30
miR-142-3p	Oropharynx, oral cavity, larynx	Up-regulated		22, 25
miR-375	Oral cavity, oropharynx, larynx	Down-regulated	JAK2	31–33
miR-125-b	Oral cavity	Down-regulated	KLF13, CXCL11, FOXA1; reduces cell proliferation	12, 26, 34
miR-221	Oropharynx, larynx	Up-regulated	KIT, p27/KIP1, p57/KIP2	12, 33
miR-23b	Oropharynx, larynx, salivary gland	Up-regulated		12, 26
miR-16	Nasopharynx	Down-regulated	EGFR, MLLT1, NOTCH2	22
Let-7a & Let-7d	Larynx	Down-regulated	HMGA2, NF2, KRAS	35
miR-100	HNSCC	Down-regulated		19, 20, 22
miR-106b	HNSCC	Up-regulated		22
miR-137	Oral cavity	Down-regulated	CDK6, E2F6, NCOA2/TIF2; tumour suppression	36
miR-138	Oral cavity	Down-regulated	Increases cell migration & invasion	37, 38

PTEN = phosphatase and tensin homologue; PDCD4 = programmed cell death 4 (neoplastic transformation inhibitor); TPM1 = tropomyosin 1 (alpha); APC = adenomatous polyposis coli; PKM-2 = pyruvate kinase, muscle; c-Myc = v-myc myelocytomatosis viral oncogene homologue (avian); JAK2 = janus kinase 2; KLF13 = Kruppel-like factor 13; CXCL11 = chemokine (C-X-C motif) ligand 11; FOXA1 = Forkhead box protein A1; KIT = v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue; KIP = cyclin-dependent kinase inhibitor 1B (p27, Kip1); EGFR = epidermal growth factor receptor; MLLT1 = myeloid/lymphoid or mixed-lineage leukaemia (trithorax homologue, drosophila), translocated to 1; NOTCH2 = neurogenic locus notch homologue protein 2; HMGA2 = high mobility group AT-hook 2; NF2 = neurofibromin 2 (merlin); KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue; HNSCC = head and neck squamous cell carcinoma; CDK6 = cyclin-dependent kinase 6; E2F6 = E2F transcription factor 6; NCOA2 = nuclear receptor coactivator 2; TIF2 = translational intermediary factor 2

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survival has remained essentially unchanged, at approximately 50 per cent.[1] The changes occurring in the cell cycle that lead to tumourigenesis, invasion and metastasis are now better understood, and the success of translational research on markers such as p16 is now evident in clinical practice. The link between microRNA and the well-known tumour suppressors and proto-oncogenes that lead to cell cycle changes has been established in recent studies.[3] A classification system has been proposed for the microRNAs linked with head and neck cancers, which categorises them as causal, non-causal or 'not enough information'.[3,40] Under this classification system, five microRNAs are currently considered to be causal in head and neck cancer: miR-21, miR-100, miR-106b, miR-125b and miR-137. Understanding microRNAs and their significant contribution to cancer development may allow the development of clinical applications for determining diagnosis and prognosis, and for targeting therapeutics.

The potential role of microRNAs as diagnostic tools arises from the possibility of detecting differences in microRNA expression profiles between subsites of diseased and normal tissue, thus enabling early recognition of tumour. The presence of microRNAs linked to a subsite-specific cancer may determine the likelihood of occult nodal metastasis, or enable identification of primary sites in cases of unknown metastases. For example, miR-205, a specific marker for squamous epithelium, may be able to identify the presence of cervical lymph node metastasis with high specificity and sensitivity.[20] Some microRNA expression profiles are maintained between tissues: one study of squamous cell carcinoma from the tonsil, base of tongue, post-nasal space and nodal metastases found that all sites showed the same molecular changes.[36] It is possible that, once specific microRNA expression profiles are determined for primary head and neck cancer subsites, the location of an unknown primary tumour could be determined by molecular means.

The potential role of microRNAs as prognostic tools has also been studied. High levels of miR-21 expression in head and neck cancer have been found to be associated with decreased five-year survival.[31] Down-regulation of the microRNAs let-7d and miR-205 has also been found to correlate with shorter survival.[26]

In contrast to those molecular markers the clinical application of which requires tissue biopsies (e.g. for immunohistochemical analysis), microRNAs can be detected in circulating blood and saliva, facilitating easy sampling and inexpensive diagnosis.[41–43] Two studies of oral cavity squamous cell carcinoma detected reduced plasma microRNA levels following tumour resection.[42,44] This facility may enable specific microRNAs to play a role in the surveillance of head and neck tumours.

Another possible application of microRNAs is targeted therapy for tumours in which microRNAs promote oncogenes or tumour suppressor genes. This possibility has been explored for many different cancers. In head and neck cancer, epidermal growth factor receptor is blocked by cetuximab, a drug now widely utilised in the clinical setting. It is possible to up-regulate the expression of tumour suppressive microRNAs and to inhibit the over-expression of oncogenic microRNAs; thus, in future it may be possible to target therapy at the molecular level. For example, it may be possible to introduce synthetically designed microRNAs into cells to promote the expression of tumour-suppressive microRNAs.[44] The 'antago-miRs' or 'microRNA sponges' are synthetic microRNAs used as inhibitors, which act by binding to oncogenic microRNA and blocking its function.[45,46] Further research on microRNA in head and neck cancer may enable the development of microRNA-based therapy and/or microRNA-linked therapeutic agents.[47]

Conclusion

Micro-ribonucleic acids are important regulatory molecules in carcinogenesis. However, in head and neck cancer their expression profiling is still at a relatively early stage, compared with other areas of oncology. Discoveries thus far seem promising, and further molecular work in this field may enable the development of clinically applicable tools for head and neck cancer diagnosis, prognosis and therapy.

Acknowledgements

We thank David Heinrich (Graphic Artist, Medical Illustration and Media Unit, Flinders Medical Centre) for the illustrations. We also acknowledge Priya Ghosal (Research Assistant) for her contribution to the literature search on microRNAs in head and neck cancer.

References

1 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T *et al*. Cancer statistics. *CA Cancer J Clin* 2008;**58**:71–96
2 Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB *et al*. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;**354**:567–78
3 Babu JM, Prathibha R, Jijith VS, Hariharan R, Pillai MR. A miR-centric view of head and neck cancers. *Biochim Biophys Acta* 2011;**1816**:67–72
4 Calin GA. MicroRNAs and cancer: what we know and what we still have to learn. *Genome Med* 2009;**1**:78
5 Kim VN, Nam JW. Genomics of microRNA. *Trends Genet* 2006;**22**:165–73
6 Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 2005;**6**:376–85
7 Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009;**10**:126–39
8 Gantier MP, Sadler AJ, Williams BR. Fine-tuning of the innate immune response by microRNAs. *Immunol Cell Biol* 2007;**85**:458–62
9 Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. *Cell* 2009;**136**:26–36
10 O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010;**10**:111–22
11 Ramdas L, Giri U, Ashorn CL, Coombes KR, El-Naggar A, Ang KK *et al*. miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue. *Head Neck* 2009;**31**:642–54
12 Sulston JE, Brenner S. The DNA of *Caenorhabditis elegans*. *Genetics* 1974;**77**:95–104

https://doi.org/10.1017/S0022215113000753 Published online by Cambridge University Press

13 Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics* 1974;**77**:71–94
14 Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;**75**:843–54
15 Wightman B, Ha I, Ruvkun G, Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* 1993;**75**:855–62
16 miRBase: the microRNA data base. In: http://miRBASE.org [18 November 2011]
17 Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;**14**:1902–10
18 Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N *et al*. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004;**432**:235–40
19 Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell* 2009;**136**:642–55
20 Gomes CC, Gomez RS. MicroRNA and oral cancer: future perspectives. *Oral Oncol* 2008;**44**:910–14
21 Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D *et al*. MicroRNA expression profiles classify human cancers. *Nature* 2005;**435**:834–8
22 Chen HC, Chen GH, Chen YH, Liao WL, Liu CY, Chang KP *et al*. MicroRNA deregulation and pathway alterations in nasopharyngeal carcinoma. *Br J Cancer* 2009;**100**:1002–11
23 Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E *et al*. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002;**99**:15524–9
24 Jiang J, Lee EJ, Gusev Y, Schmittgen TD. Real-time expression profiling of microRNA precursors in human cancer cell lines. *Nucleic Acids Res* 2005;**33**:5394–403
25 Chang SS, Jiang WW, Smith I, Poeta LM, Begum S, Glazer C *et al*. MicroRNA alterations in head and neck squamous cell carcinoma. *Int J Cancer* 2008;**123**:2791–7
26 Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M *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**:736–45
27 Tran N, McLean T, Zhang X, Zhao CJ, Thomson JM, O'Brien C *et al*. MicroRNA expression profiles in head and neck cancer cell lines. *Biochem Biophys Res Commun* 2007;**358**:12–17
28 Fletcher AM, Heaford AC, Trask DK. Detection of metastatic head and neck squamous cell carcinoma using the relative expression of tissue-specific mir-205. *Transl Oncol* 2008;**1**:202–8
29 Jakymiw A, Patel RS, Deming N, Bhattacharyya I, Shah P, Lamont RJ *et al*. Overexpression of dicer as a result of reduced let-7 MicroRNA levels contributes to increased cell proliferation of oral cancer cells. *Genes Chromosomes Cancer* 2010;**49**:549–59
30 Wong TS, Ho WK, Chan JY, Ng RW, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. *Clin Cancer Res* 2008;**14**:2588–92
31 Avissar M, McClean MD, Kelsey KY, Marsit CJ. MicroRNA expression in head and neck cancer associates with alcohol consumption and survival. *Carcinogenesis* 2009;**30**:2059–63
32 Hui AB, Lenarduzzi M, Krushel T, Waldron L, Pintilie M, Shi W *et al*. Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas. *Clin Cancer Res* 2010;**16**:1129–39
33 Avissar M, Christensen BC, Kelsey KY, Marsit CJ. MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. *Clin Cancer Res* 2009;**15**:2850–5
34 Henson BJ, Bhattacharjee S, O'Dee DM, Feingold E, Gollin SM. Decreased expression of miR-125b and miR-100 in oral cancer cells contributes to malignancy. *Genes Chromosomes Cancer* 2009;**48**:569–82
35 Long XB, Sun GB, Hu S, Liang GT, Wang N, Zhang XH *et al*. Let-7a microRNA functions as a potential tumor suppressor in human laryngeal cancer. *Oncol Rep* 2009;**22**:1189–95
36 Barker EV, Cervigne NK, Reis PP, Goswami RS, Xu W, Weinreb I *et al*. MicroRNA evaluation of unknown primary lesions in the head and neck. *Mol Cancer* 2009;**23**:127
37 Liu X, Wang C, Chen Z, Jin Y, Wang Y, Kolokythas A *et al*. MicroRNA-138 suppresses epithelial-mesenchymal transition in squamous cell carcinoma cell lines. *Biochem J* 2011;**440**:23–31
38 Jiang L, Dai Y, Liu X, Wang C, Wang A, Chen Z *et al*. Identification and experimental validation of G protein alpha inhibiting activity polypeptide 2 (GNAI2) as a microRNA-138 target in tongue squamous cell carcinoma. *Hum Genet* 2011;**129**:189–97
39 Hurst DR, Edmonds MD, Welch DR. Metastamir: the field of metastasis-regulatory microRNA is spreading. *Cancer Res* 2009;**69**:7495–8
40 HeNeCan miRs – A database of integrative information on head and neck cancer associated microRNAs. http://tarmir.rgcb.res.in/henecan [19 April 2012]
41 Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E *et al*. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. *Clin Cancer Res* 2009;**15**:5473–7
42 Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. *Oral Dis* 2010;**16**:360–4
43 Wong TS, Ho WK, Chan JY, Ng RW, Wei WI. Mature miR-184 and squamous cell carcinoma of the tongue. *Scientific World Journal* 2009;**9**:130–2
44 Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. *Cancer Res* 2010;**70**:7027–30
45 Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschi T, Manoharan M *et al*. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005;**438**:685–9
46 Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 2007;**4**:721–6
47 MacDiarmid JA, Madrid-Weiss J, Amaro-Mugridge NB, Phillips L, Brahmbhatt H. Bacterially-derived nanocells for tumor-targeted delivery of chemotherapeutics and cell cycle inhibitors. *Cell Cycle* 2007;**6**:2099–105

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Competing interests: None declared