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MicroRNA Dysregulation in Squamous Cell Carcinoma of Head and Neck

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1. Introduction

Head and neck cancers refers to cancer arising in the head or neck regions including parnasal sinuses, nasal cavity, nasopharynx, oral cavity, salivary gland, oropharynx, pharynx, hypopharynx, larynx, and lymph node. Histologically, squamous cell carcinoma is the predominant form. The cancer progenitor cells are premalignant cells in the mucosa layer of head and neck. Cumulative genetic and epigenetic alterations lead to behavioural changes from hyperplasia to invasive carcinoma. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. It is the 4th most common cancer among men in the European Union (Black et al., 1997). In United State, over 12,000 patients died from HNSCC every year (Altekruse et al., 2008). Globally, there are approximately 650,000 new cases of HNSCC and 350,000 patients dying from HNSCC annually (Parkin et al., 2005). Most patients will develop local-regional disease with cervical lymph node involvements. HNSCC is heterogeneous in nature. Early disease might not have any symptoms. Further, the inconspicuous locations of some HNSCC make it difficult to be identified at the early stages. Thus, patients arrive at the clinic by large present late and have poor prognosis. The overall survival rate of HNSCC patients is about 50% (Stell, 1989; Argiris et al, 2004). Development of local recurrence, distant metastasis and secondary primary tumor is also common in HNSCC. Despite the advances of cancer treatment in last several decades, the overall survival rate of HNSCC did not have much improvement (Stell, 1989; Argiris and Eng, 2003).

HNSCC is a multifactorial disease. Major risk factors are alcohol consumption and tobacco use (Jaber et al., 1999). HNSCC is particularly common in countries with high alcohol and tobacco consumption e.g. southern Africa, Australia, Brazil, France, India, The Netherlands, Papua-New Guinea and Switzerland (Parkin et al., 2005). Smoking and drinking habit is associated with early onset of HNSCC (Farshadpour et al., 2007). Patients with alcohol and tobacco use generally have poor prognosis and poor survival rate (Farshadpour et al., 2011). Other risk factors of HNSCC include age, environmental exposures (including UV exposure and viral infection such as Epstein-Barr Virus and Human Papilloma Virus), sex, hygiene, industrial inhalants, and gender (Argiris et al., 2003). Regional lymph node involvement is also an indicator of poor prognosis. About 20–50% N0 patients will develop nodal

metastasis. The overall survival rate reduced to 50% in case if lymph node metastasis is observed (von Buchwald et al., 2002).

Management of HNSCC is based primarily on the tumor locations and stages (Akervall, 2005). For early HNSCC (stage I and II) surgical resection together with radiotherapy is the primary treatment regime. For advanced disease (stage III and IV) multidisciplinary treatment including surgery, radiation and chemotherapy is adopted (Posner, 2010). For loco-regionally advanced HNSCC, concurrent chemo-radiotherapy is used in case where the tumor is unresectable or adverse functional loss will be resulted from the operation (Wong et al., 2011). For oral squamous cell carcinoma, surgical excision of the primary tumor and/or selective neck dissection is the major treatment (Bilde et al., 2006). For pharyngeal and laryngeal SCC, radiotherapy and/or concomitant chemotherapy are commonly used (Lajer et al., 2011). It has been shown that the use of concomitant chemo- and radio-therapy is more effective in advanced HNSCC (Robbins, 2005).

2. MicroRNA

MicroRNA are small non-protein-coding RNA, which regulate mRNA at post-transcriptional level. MicroRNA are small epigenetic regulators usually about 19–22 or 19–25 nucleotides long (Ambrose, 2004). They are highly conserved molecules among different species including nematode, drosophila, vertebrate, and human indicating its significance in cellular functions. MicroRNA was first discovered in 1993 in nematode *Ceanorhabditis elegans* (*C. elegans*) (Lee et al., 1993). Later, the tumor suppressing microRNA let-7 was identified in *C. elegans* and mammalian models. By then, it was proposed that microRNA had a trans-regulatory role through direct binding to the target mRNA. Computational prediction suggested that microRNA are regulating about 30% of human genes (Lewis et al., 2005). Up till now (1 July 2011), 16,772 microRNA are reported in the miRBase (see miRBase at http://www.mirbase.org, Release 17) at which 8.9% (1,492) are human microRNA. MicroRNA could bind to the target mRNA in a partial or complete complementary manner. They regulate gene expression by promoting target mRNA degradation and/or hindering mRNA translation (Bushati and Cohen, 2007).

MicroRNA are transcribed in genomic DNA. The genes encoding microRNA are located throughout the human genome in intron, exon, coding / non-coding genes (Lee et al., 2002). MicoRNA are first transcribed by RNA polymerase II into long precursor microRNA. This long RNA will be cleaved by Dorsha (RNase III-type nuclease in the nucleus) generating primary microRNA (60–70 nucleotides hairpin molecules). The primary microRNA are later exported into the cytoplasma by Exportin-5 (a Ras-GTP-dependent dsRNA-binding protein). Primary microRNA will be further processed by Dicer (RNase complex) and TRBP [TAR (transactivation-responsive RNA of HIV-1) RNA-binding protein] forming an asymmetric microRNA:microRNA* intermediate duplex (microRNA* is usually functionless and are degraded subsequently). This duplex molecule is then incorporated with Argonaute-containing RNA-induced silencing (RISC) complex forming a functional post-transcriptional regulator (Bartel, 2004). This functional complex usually bind to the 3′ untranslated region of the target mRNA (Lim et al., 2005; Wightman et al., 1993). The complementary binding between microRNA and the target mRNA is not necessary perfect in order to carry out it function as negative regulator. The binding of seed sequence (2-7

nucleotides on the mature microRNA) of the microRNA with the target mRNA would suffice to induce mRNA destabilization and degradation (Filipowicz et al., 2008).

So far, microRNA were identified as negative regulator of specific mRNA (Lim et al., 2005). However, recent findings suggested that microRNA might also act as gene activator. Vasudevan *et al.* demonstrated that miR369-3 could activate translation (Vasudevan et al., 2007). Later, Place *et al.* observed that miR-373 could induce E-cadherin expression in prostate cancer cells (Place et al., 2008). MicroRNA Let-7 can induce upregulation of gene involved in cell cycle arrest (Vasudevan et al., 2007). Although such activating mechanisms are not yet clear, it revealed that many remain to be explored if we want to uncover the exact functions of microRNA in human cells.

3. MicroRNA and cancers

In comparison with the normal counterparts, cancer displays a differential microRNA expression patterns (Lu et al., 2005). Association between human cancers and microRNA dysregulation was first observed in leukemia. Downregulation of miR-15 and miR-16 was first discovered in peripheral blood of chronic lymphocytic leukemia (Calin et al, 2002). For HNSCC, study on individual microRNA was first performed by Jiang *et al*. in 2005 (Jiang et al., 2005). Later, *Tran et al*. performed microRNA expression profiling on head and neck cancer cell lines (Tran et al., 2007).

The microRNA profile of nasopharyngeal carcinoma, oral tongue carcinoma, and laryngeal carcinoma are emerging in the subsequent years (Li et al., 2010; Li et al., 2011; Liu et al., 2009; Rentoft et al., 2011; Scapoli et al., 2010). The underlying mechanism concerning microRNA dysregulation in head and neck cancers is not yet clear although it has been reported that the microRNA processing machinery is upregulated in head and neck cancers (Zhang et al., 2009). Zhang noticed that the microRNA processing enzymes Dicer and Drosha are overexpressed in salivary gland tumor (Zhang et al., 2009). Expression of Dorsha (micoRNA processor) will affect the phenotype of squamous epithelial cells (Muralidhar et al., 2011).

4. Mechanisms of MicroRNA dysregulation in head and neck cancers

4.1 Chromosomal rearrangement

Chromosomal abnormalities are associated with the development of head and neck caners (Akervall, 2005; Gollin et al., 2001). Common chromosomal gains in HNSCC include 3q, 5p, 7p, 8q, 9q, 11q13, and 20q. In comparison, losses of chromosomal region were frequently detected on 3p, 9p, 5q, 8p, 13q, 18q, and 21q. Cromer *et al*. reported that genes related to tumorigenesis and metastasis of hypopharyngeal carcinoma were located on 3q27.3, 17q21.2-q21.31, 7q11.22-q22.1and 11q13.1-q13.3. Chromosomal rearrangement (e.g. deletion or translocation) will result in dysregulation of the gene on the abbreviated loci (Akervall, 2005).

About 50% of the microRNA are located in minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers (Calin et al., 2004). Recently, Persson *et al.,* has shown that t(6;9)(q22–23;p23–24) will lead to fusion of MYB oncogene to the transcription factor gene NFIB in head and neck cancers (Persson et al., 2009). As the 3′-

UTR of MYB is targeted by miR-15a/16, the chromosomal translocation allows the cancer cells to escape control by miR-15a/16. Lee *et al*., identified that miR-204 located in 9q21.1-22.3, a cancer genomic-associated region of head and neck cancers, is linked to progression of HNSCC (Lee et al., 2010). Loss of heterozygosity (LOH) in these loci are common in HNSCC (Bauer et al., 2008; Spafford et al., 2001).

4.2 DNA hypermethylation

DNA hypermethylation is usually found in the CpG island of tumor suppressor genes. Methylation of the clustered CpG dinucleotides in the CpG island would result in transcriptional silencing of the genes. It is now known that the methylated CpG dinucleotide could also link to the regulation of microRNA expression. Promoter methylation will affect binding of the transcriptional machinery (Zhang et al., 2011). Demethylation treatment of the nasopharyngeal carcinoma cells with demethylating agents would result in let-7 upregulation indicating the involvement of DNA methylation in regulating let-7 expression in nasopharyngeal carcinoma cells (Wong et al., 2011). Kozaki *et al*. identified that DNA methylation is linked to the transcriptional silencing of miR-137 and miR-193a, both of which are tumor suppressing microRNA associated with oral SCC (Kozaki et al, 2008). Apart from microRNA, the methylated microRNA promoter can also be used as a biomarker for HNSCC patients. Langevin *et al*. reported that methylated miR-137 promoter is associated with the clinical pathological characteristic of HNSCC patients (Langevin et al., 2010)

4.3 Genetic polymorphism of microRNA-encoding region

Similar to mRNA, microRNA are also encoded by genomic DNA. Theoretically, any variation in genomic materials will affect the biogenesis and final sequence of the mature microRNA (the seed sequence especially) and affect the specificity of microRNA to their target mRNA. Thus, any genetic variation in the microRNA biogenesis pathway gene, primary microRNA, precursor microRNA or mature microRNA sequence will eventually affect the microRNA regulatory pathways (Slaby et al., 2011)

4.3.1 Single nucleotide polymorphism (SNP) of microRNA-encoding genes

The association between microRNA and SNP has been demonstrated recently (Duan et al., 2007). It has already been demonstrated that SNP will affect the processing of pre-microRNA (Yu et al., 2007). In HNSCC, SNP of miRNA-146a (rs2910164; guanine to cytosine), miR-149 (rs2292832; guanine to thymine), miR-196a2 (rs11614913), and miR-499 (rs3746444; adenine to guanine) are associated with the risk of developing HNSCC (Liu et al 2010). Christensen *et al*. confirmed the association of SNP in miR-196a2 (rs11614913, C/T) with HNSCC. They demonstrated that miR-196a2 polymorphism is associated with the risk of HNSCC (Christensen et al., 2010).

4.3.2 Single nucleotide polymorphism (SNP) of microRNA-targeted genes

Apart from microRNA itself, SNP on microRNA target genes will also affect the binding efficacy of microRNA. Zhang *et al*. demonstrated that SNP associated with microRNA biogenesis pathway genes and microRNA-targeted genes are associated with the prognosis of HNSCC patients (Zhang et al., 2010). They proposed that microRNA-related genetic

polymorphisms might be used as predicative markers for secondary primary and/or recurrence in early HNSCC patients (Zhang et al., 2010).

4.4 MicroRNA dysregulation by candidate oncogene

MicroRNA expression could be controlled by oncogene such as myc. He *et al*. reported that the miR-17-92 cluster is transactivated by myc (He et al., 2007). In addition, p53 is also shown to be involved in microRNA dysregulation through inducing miR-34 expression (Chang et al., 2008; He et al., 2005; Melo and Estella, 2011; Suzuki et al., 2009).

5. MicroRNA as molecular markers in circulation and body fluids in HNSCC

With the advance of molecular techniques and understanding in cancers, molecular markers are now considered as an effective auxiliary test in conjunction to histological examination in assisting clinical decision making (Hui et al., 2010). The existence of differential microRNA patterns between cancer and the normal counterparts opens up the possibility of using the differential expressed microRNA in monitoring cancers (Krutovskikh et al., 2010). In view of the myriad of functions of microRNA in cancers, de Planell-Saguer and Rodicio suggested that microRNA is potentially useful as biomarkers for cancer onset, prognosis, risk of diseases and cancer classification (de Planell-Saguer and Rodicio, 2011).

MicroRNA is suitable cancer marker as it is highly stable and is resistant to degradation (Li et al., 2007). Significant amount of extracellular microRNA had been detected in peripheral blood, urine, saliva and semen (Mitchell et al., 2008; Hanke et al., 2009; Park et al., 2009; Zubakov et al., 2010) making it a candidate biomarker for detection and surveillance of HNSCC in a non-invasive manner. In addition, it could be extracted in formalin-fixed paraffin-embedded tissues (Li et al., 2007). Circulating microRNA have been found to be significantly elevated in the peripheral blood of head and neck cancer patients. However, there is no direct evidence showing that primary tumor is the only source of the circulating microRNA. Reduction of the circulating microRNA after removal of the primary tumor suggested that primary tumor is one of the major sources of circulating microRNA (Iguchi et al., 2010; Kosaka et al., 2010).

Peripheral blood has high RNase activity, however, circulating microRNA could still exist in cell-free form and remain stable in the blood (Mitchell et al., 2008). Circulating microRNAs are existed in membrane-bound vesicles (about 50 nm to 1 um) and are released from the cancer cells through exocytosis (Février & Raposo, 2004; Heijnen et al., 1999; Hunter et al., 2008). Recently, Turchinovich *et al*. performed physical analysis on the characteristics of the circulating microRNA. They noticed that circulating microRNA could exist independently without the vesicle provided that they are bound to the Ago2 protein (Turchinovich et al., 2011).

6. Examples of microRNA dysregulation in HNSCC

Depending on the functions, the dysregulated microRNA could be classified into oncogenic microRNA (onco-miR) and tumor suppressing microRNA (Kozaki et al., 2008; Iorio et al., 2005). Identifying the dysregulated microRNA patterns in HNSCC is useful in selecting suitable microRNA biomarkers for use in HNSCC monitoring (Chang et al., 2008; Ferdin et

al., 2010). We here performed a review on the potential oncogenic microRNA and tumor suppressing microRNA identified in HNSCC.

6.1 Let-7 Family

Human let-7 has multiple isoforms. They are let-7a-1[Mature sequence: 6 - ugagguaguagguuguauaguu – 27 (MI0000060)], let-7a-2 [Mature sequence: 5 - ugagguaguagguuguauaguu – 26 (MI0000061)], let-7a-3 [Mature sequence: 4 - ugagguaguagguuguauaguu – 25 (MI0000062)], let-7b [Mature sequence: 6 - ugagguaguagguuugugguu – 27 (MI0000063)], let-7c [Mature sequence: 11 - ugagguaguagguuguaugguu – 32 (MI0000064)], let-7d [Mature sequence: 8 - agagguaguagguugcauaguu – 29 (MI0000065), let-7e (Mature sequence: 8 - ugagguaggagguuguauaguu – 29 (MI0000066), let-7f-1 [Mature sequence: 7 - ugagguaguagauuguauaguu – 28 (MI0000067)], let-7f-2 [Mature sequence: 8 - ugagguaguagauuguauaguu – 29 (MI0000068)], let-7g (Mature sequence: 5 - ugagguaguaguuuguacaguu – 26 (MI0000433)], and let-7i [Mature sequence: 6 - ugagguaguaguuugugcuguu – 27 (MI0000434).

In human cancers, expression of let-7 family is usually reduced suggesting its tumor-suppressing role. In comparison with the normal nasopharyngeal cells, let-7 levels were significantly decreased in the nasopharyngeal carcinoma. Reduced expression levels of let-7 (-a, -b, -d, -e, -g, and -i) were detected in nasopharyngeal carcinoma cells compared to normal nasopharyngeal cells. Ectopic expression of let-7 in nasopharyngeal carcinoma cells reduced cell proliferation (Wong et al., 2011). Moreover, c-Myc expression was inhibited in NPC cells transfected with precursor let-7 (Wong et al., 2011). Association of let-7 with cell proliferation was also observed in oral cancer cells (Jakymiw et al. 2010). Let-7a microRNA expression was inhibited in both laryngeal squamous cancer tissues and in laryngeal cancer cell lines (Hep-2 and BEAS-2B). Let-7a could inhibit proliferation and induce apoptosis in laryngeal carcinoma cells (Long et al., 2009). In Hep-2 cells, overexpression of let-7a could also suppress RAS and c-MYC protein expression (Long et al., 2009). It was demonstrated that up-regulated RAS and c-MYC protein levels had inverse correlation with the down-regulated let-7a levels in cancer tissues (Long et al., 2009). Further, let-7a could enhance the chemosensitivity of head and neck cancer cells and might link to the stemness gene expression pathway (Yu et al., 2011).

6.2 MiR-15a [Mature sequence: 14 - uagcagcacauaaugguuugug - 35 (MI0000069)]

Regulation of miR-15a is altered in head and neck cancer cells (Persson et al. 2009). Expression of miR-15a was inversely correlated with protein kinase C which was usually overexpressed in primary HNSCC (Cohen et al., 2009). It has been shown that overexpression of miR-15a suppressed cyclin E protein expression and inhibition of miR-15a enhanced cyclin E protein expression in laryngeal cancer cell line. Precursor miR-15a could also affect DNA synthesis in laryngeal carcinoma cells but the related mechanisms are not yet identified (Cohen et al., 2009). These results indicated that miR-15a might function as a tumor suppressor through regulating the gene associated with the proliferation pathways of cancer cells (Cohen et al., 2009).

6.3 MiR-21 [Mature sequence: 8 - uagcuuaucagacugauguuga - 29 (MI0000077)]

Overexpression of miR-21 was first reported in human glioblastoma and miR-21 is now recognized as an potent anti-apoptotic factor (Fu et al., 2011). Upregulation of miR-21 is observed in multiple human cancers including breast, cervical, colon, leukemia, liver, lung, ovarian, pancreas, prostate, stomach and thyroid as well as head & neck (Krichevsky et al., 2009; Volinia et al., 2006). Elevated expression of miR-21 is observed in tongue squamous cell carcinomas. Suppressing miR-21 in tongue SCC cell lines (SCC-15 and CAL27) reduced cell survival and induced apoptosis (Li et al., 2009). It has been found that the expression level of miR-21 was reversely correlated with TPM1. The observation suggested that miR-21 may inhibit cell apoptosis partly via silencing the expression of TPM1 (Li et al., 2009). Furthermore, it has been shown that miR-21 expression was an independent prognostic factor associated with survival rate (Li et al., 2009). Moreover, repeated injection of miR-21 antisense oligonucleotide could inhibit tumor formation in nude mice (Li et al., 2009). Laryngeal cancer cell line (JHU-O11) transfected with miR-21 displayed enhanced cell growth (Chang et al., 2008).

6.4 MiR-29 [Mature sequence: 54 - uagcaccauuugaaaucgguua – 75 (MI0000735)]

MiR-29c expression was suppressed in nasopharyngeal carcinomas in comparison with normal healthy nasopharyngeal epithelia (Sengupta et al., 2008). The function of miR-29c is not yet clear. In HeLa cells, transfection of miR-29c precursor could suppress expression of collagen 3A1, 4A1, 15A1, laminin, and thymine-DNA glycosylase (TDG) linking to tumor cell invasiveness and metastatsis (Sengupta et al., 2008).

6.5 MiR-100 [Mature sequence: 13 - aacccguagauccgaacuugug - 34 (MI0000102)] and miR-125b [Mature sequence: 15 - ucccugagacccuaacuuguga - 36 (MI0000446)]

Suppression of both miR-100 and miR-125b were reported in HNSCC. Expression levels of miR-125b and miR-100 were decreased in oral squamous cell carcinoma cell lines and tumors of alveolar ridge, buccal mucosa, floor of mouth, retromolar trigone and tongue (Henson et al., 2009). Overexpression of miR-100 and miR-125b inhibited cell proliferation in buccal mucosa cell lines (Henson et al., 2009). Suppressed expression of miR-100 and miR-125b in oral cancer cells may lead to cancer progression and loss of sensitivity to ionizing radiation (Henson et al., 2009).

6.6 MiR-133 family

MiR-133 has 2 isoforms: miR-133a [Mature sequence: 53 - uuuggucccuucaaccagcug – 74 (MI0000450)] and miR-133b [Mature sequence: 66 - uuuggucccuucaaccagcua – 87 (MI0000822)]. Downregulation of miR-133 had been reported in HNSCC including tongue SCC (Child et al., 2009). Decreased expression of miR-133a and miR-133b was observed in tongue SCC cells. Tongue SCC cell lines (Cal27, HN21B and HN96) transfected with miR-133a and miR-133b precursors showed reduced proliferation rate and elevated apoptosis rate (Wong et al., 2008a). Overexpression of miR-133a and miR-133b reduced the expression of pyruvate kinase type M2 (PKM2) in tongue SCC cell lines (Wong et al., 2008a). The elevated expression of PKM2 in tongue SCC tissues was associated with the down-regulated expression of miR-133a and miR-133b (Wong et al., 2008a).

6.7 MiR-137 [Mature sequence: 59 - uuauugcuuaagaauacgcguag – 81 (MI0000454)]

Expression of mir-137 was downregulated in tongue carcinoma cells. Ectopic expression of miR-137 could inhibit cell growth cell growth in tongue SCC cell line HSC-6 and HSC-7 (Kozaki et al., 2008). MiR-137 is essential to cell cycle control of HNSCC. MiR-137 mimics enhanced the accumulation of G0-G1 phase cells, suggesting that it was associated with cell cycle arrest at the G1-S checkpoint (Kozaki et al., 2008). Expression of CDK6, E2F6, and NCOA2/TIF2 was suppressed by miR-137 in tongue SCC cell lines (Kozaki et al., 2008). Apart from the microRNA itself, the methylation status of miR-137 promoter has potential clinical value. Methylated miR-137 is a potential prognostic marker in HNSCC and is associated with survival (Langevin et al., 2011).

6.8 MiR-138 [Mature sequence: 23 - agcugguguugugaaucaggccg - 45 (MI0000476)]

MiR-138 is linked to cell invasion, cell cycle arrest and apoptosis of HNSCC (Liu et al. 2009). Reduced expression of miR-138 was reported in oral tongue cell lines UM1, UM2, Cal27, SCC1, SCC4, SCC9, SCC15, SCC25 (Liu et al., 2009). High level of miR-138 could reduce migration and invasion rate of tongue cancer cell UM1 and UM2 (Jiang et al., 2010). It has been demonstrated that overexpression of miR-138 could reduce expression of two key genes in the Rho GTPase signaling pathway, RhoC and ROCK2, leading to the reorganization of the stress fibers (Jiang et al., 2010). In contrast, inhibition the expression of miR-138 increased RhoC and ROCK2, contributing to an elongated cell morphology and enhanced cell migration and invasion (Jiang et al., 2010). The expression level of miR-138 was inhibited in hypopharyngeal carcinoma cell line (1386Tu) and oropharyngeal carcinoma cell line (686Tu) compared to non-tumorigenic cells (OKF4-E6/7 and NHOK) (Liu et al., 2009)

6.9 MiR-141 [Mature sequence: 59 - uaacacugucugguaaagaugg – 80 (MI0000457)]

Dysregulation of miR-141 was observed in head and neck cancer. However, its role in the pathogenesis remains unknown. Enhanced miR-141 expression was observed in NPC specimens in comparison with normal nasopharyngeal epithelium. Suppression of miR-141 affected cell cycle, apoptosis, cell growth, migration and invasion in NPC cells (Zhang et al., 2010). It has been shown that miR-141 directly targeted BRD3, UBAP1 and PTEN that are involved in NPC carcinogenesis (Zhang et al., 2010). Furthermore, inhibition of miR-141 affected the expression levels of some important molecules in the Rb/E2F, JNK2 and AKT pathways (Zhang et al., 2010). In contrast, Nurul-Syakima *et al*. demonstrated that miR-141 was downregulated in HNSCC and the results were different from those observed in NPC (Nurul-Syakima et al., 2011). Further studies are warranted to elucidate the role of miR-141 in head and neck cancers.

6.10 MiR-184 [Mature sequence: 53 - uggacggagaacugauaagggu – 74 (MI0000481)]

MiR-184 was overexpressed in early oral SCC (Cervigne et al., 2009). Cervigne *et al.* demonstrated that miR-184 was upregulated during the progression of progressive dysplasia and oral SCC suggesting that miR-184 might potentially be used as a biomarker for malignant transformation. In tongue SCC, primary tumor has higher level of miR-184 in comparison with the paired normal epithelial cells. Inhibition of endogenous miR-184 in

tongue SCC cell lines (Cal27, HN21B, and HN96) resulted in reduced cell proliferation rate and enhanced apoptotic rate (Wong et al., 2008b). The observations that miR-184 levels were increased in the plasma before operation and decreased significantly after surgical treatment suggested that plasma miR-184 levels might serve as biomarker in oral tongue SCC patients (Wong et al., 2008b).

6.11 MiR-193a [Mature sequence 21 - ugggucuuugcgggcgagauga – 42 (MI0000487)]

The expression of miR-193a was inhibited in buccal mucosa cell line HO-1-N-1 cell line. Furthermore, HO-1-N-1 cell line transfected with miR-193a mimics displayed suppressed cell growth and induced apoptosis (Kozaki et al., 2008). In addition, miR-193a mimics reduced the protein levels of E2F6 and PTK2/FAK (Kozaki et al., 2008).

6.12 MiR-204 [Mature sequence 33 - uucccuuugucauccuaugccu – 54 (MI0000284)]

The expression of miR-204 was suppressed in tongue SCC cell lines (SCC58, SCC61, SCC151) and hard palate cell line SCC135 (Lee et al., 2010). Overexpression of miR-204 inhibited migration, adhesion and invasion of HNSCC cell (Lee et al., 2010). MiR-204 expression was reduced in NPC cell lines JSQ3 (Nasal cavity) and SQ38 (pyriform sinus) compared to samples of pooled normal buccal mucosa. NPC cell lines transfected with miR-204 mimics displayed suppressed cell-matrix interaction, motility and invasiveness (Lee et al., 2010).

6.13 MiR-205 [Mature sequence: 34 - uccuucauuccaccggagucug – 55 (MI0000285)]

MiR-205 is associated with the epithelial-mesenchymal transition of head and neck carcinoma (Zidar et al., 2011). It was proposed that high expression levels of miR- 205 can be used to detect HNSCC positive lymph nodes (Fletcher et al., 2008).

6.14 MiR-222 [Mature sequence: 69 - agcuacaucuggcuacugggu – 89 (MI0000299)]

MiR-222 is associated with the aggressiveness of tongue cancer cell lines (Liu et al., 2009b). Overexpression of miR-222 in UM1 resulted in reduced cell invasion (Liu et al., 2009b). It has been shown that miR-222 directly targeted metalloproteinase 1 (MMP1) and manganese superoxide dismutase 2 (SOD2) and suppressed their expression in oral tongue SCC cell lines (Liu et al., 2009b). These results indicated that miR-222 may serve as a novel therapeutic target for oral tongue SCC patients (Liu et al., 2009b).

6.15 Others microRNA dysregulation

It was recently shown that the expression levels of miR-221 to miR-375 could be used to distinguish tumor from normal tissue with high specificity and sensitivity (Avissar et al., 2009). The expression levels of miR-196b, miR-138, miR-155, miR-142-3p, and miR-18a were elevated and expression levels of miR-204, miR-449a, miR-34c-3p, miR-143, and miR-145 were reduced in NPC samples in comparison with normal nasopharyngeal tissues (Chen et al., 2009). Several biological pathways including TGF-Wnt pathways, G1-S cell cycle progression, VEGF signaling pathways, apoptosis and survival pathways, and IP3 signaling pathways are targeted by these down-regulated microRNA (Chen et al., 2009).

Tumor sites	Sub-sites	MicroRNA	Dysregulation	Related functions	References
oral cavity carcinoma	alveolar ridge	miR-100	down-regulated	proliferation	(Henson et al., 2009)
		miR-125b	down-regulated	proliferation	(Henson et al., 2009)
	buccal mucosa	miR-100	down-regulated	proliferation	(Henson et al., 2009)
		miR-125b	down-regulated	proliferation	(Henson et al., 2009)
		miR-193a	down-regulated	growth	(Kozaki et al., 2008)
	floor of mouth	miR-100	down-regulated	proliferation	(Henson et al., 2009)
		miR-125b	down-regulated	proliferation	(Henson et al., 2009)
		miR-138	down-regulated	migration, invasion	(Jiang et al., 2010; Liu et al., 2009a)
	hard palate	miR-204	down-regulated	migration, invasion	(Lee et al., 2010)
	retromolar trigone	miR-100	down-regulated	proliferation	(Henson et al., 2009)
		miR-125b	down-regulated	proliferation	(Henson et al., 2009)
	tongue	miR-100	down-regulated	proliferation	(Henson et al., 2009)
		miR-125b	down-regulated	proliferation	(Henson et al., 2009)
		miR-138	down-regulated	migration,	(Henson et al., 2009;
		miR-184	up-regulated	invasion	Liu et al., 2009a)
		miR-204	down-regulated	apoptosis,	(Wong et al., 2008b)
		miR-222	down-regulated	proliferation	(Lee et al., 2010)
		miR-21	up-regulated	migration,	(Liu et al., 2009b)
		miR-133a	down-regulated	invasion	(Li et al., 2009)
		miR-133b	down-regulated	invasion	(Wong et al., 2008a)
		miR-137	down-regulated	apoptosis,	(Wong et al., 2008a)
				survival	(Kozaki et al., 2008)
				proliferation, apoptosis	
				proliferation, apoptosis	
				growth	
naso pharyngeal carcinoma		miR-196b	up-regulated		(Chen et al., 2009)
		miR-138	up-regulated	proliferation	(Chen et al., 2009)
		miR-155	up-regulated	metastasis	(Chen et al., 2009)
		miR-142-3p	up-regulated	apoptosis,	(Chen et al., 2009)
		miR-18a	up-regulated	invasion	(Chen et al., 2009)
		miR-204	down-regulated	migration,	(Chen et al., 2009)
		miR-449a	down-regulated	invasion	(Chen et al., 2009)
		miR-34c-3p	down-regulated		(Chen et al., 2009)
		miR-143	down-regulated		(Chen et al., 2009)
		miR-145	down-regulated		(Chen et al., 2009)
		let-7 family	down-regulated		(Wong et al., 2011)
		miR-29c	down-regulated		(Sengupta et al.,
		miR-141	up-regulated		2008)
		miR-204	down-regulated		(Zhang et al., 2010)
					(Lee et al., 2010)

Tumor sites	Sub-sites	MicroRNA	Dysregulation	Related functions	References
pharyngeal carcinoma	oropharynx	miR-138	down-regulated		(Liu et al., 2009a)
	hypo pharynx	miR-138	down-regulated		(Liu et al., 2009a)
laryngeal carcinoma		miR-let-7a	down-regulated	Proliferation,	(Long et al., 2009)
		miR-204	down-regulated	apoptosis	(Lee et al., 2010)
		miR-21	up-regulated	migration,	(Chang et al., 2008)
		miR-15a	down-regulated	invasion	(Cohen et al., 2009)
				growth	
				proliferation	

Table 1. MicroRNA dysregulation in HNSCC

7. The role of viral-encoded microRNA in head and neck cancers

7.1 Epstein-Barr Virus (EBV)

EBV is a member of gamma-Herpes virus and is closely associated with the progression of undifferentiated nasopharyngeal carcinoma (Wei and Sham, 2005). EBV is the first identified oncogenic virus. Expression of EBV-encoded oncoproteins are linked to epithelial-mesenchymal transition of metastatic nasopharyngeal carcinoma (HoriKawa et al., 2011). EBV could alter somatic gene expression by controlling the microRNA biogenesis machinery of the host cells. Li *et al.* observed that LMP1 could induce expression of miR-10b and promote metastasis of nasopharyngeal carcinoma cells (Li et al., 2010). Du *et al*. reported that EBV oncoprotein LMP1 and LMP2A could activate miR-155 expression in nasopharyngeal carcinoma cells which is associated with the nodal status and metastasis of nasopharyngeal carcinoma patients (Du et al., 2011).

Apart from the viral oncoprotein, the microRNA encoded by EBV virus itself is also playing a part in pathogenesis of nasopharyngeal carcinoma cells. EBV-encoded microRNA was first discovered in 2004 (Pfeffer et al., 2004). At present, 25 precursors and 44 mature microRNA were identified (Sanger database Release 16). The identified EBV microRNA are encoded in 2 major clusters: BHRF1 cluster and BART cluster (Lung et al., 2009). Barth *et al.* demonstrated that EBV-BART2 could target EBV DNA polymerase BALF5 hindering the lytic replication of EBV (Barth et al., 2008). EBV-encoded microRNA could regulate the activity of EBV and enhance the survival of the host cells (Lo et al., 2007). For example, BART5-5p could target pro-apoptotic gene PUMA contributing to the resistance to apoptotic agents (Choy et al., 2008).

As mentioned above, expression of the EBV oncoprotein LMP1 (Key viral oncoprotein linked to the pathogenesis of nasopharyngeal carcinoma) is critical in the pathogenesis of nasopharyngeal carcinoma. LMP1 act as tumor necrosis factor receptor (TNFR). It is the activator in multiple cancer-related pathways and could enhance proliferation, migration, and cell cycle progression in nasopharyngeal carcinoma cells (Kung et al., 2011). It is now known that LMP1 expression is partly controlled by the EBV-encoded microRNA. Lo *et al.* demonstrated that BART1-5p, BART16-5p and BART17-5p are involved in the regulation of LMP1 in nasopharyngeal carcinoma cells (Lo et al., 2007). In addition, LMP1 can suppress

somatic gene expression by inducing somatic microRNA expression (Anastasiadou et al., 2011; Motsch et al., 2007).

7.2 Human Papilloma Virus (HPV)

HPV is a DNA virus and could infect squamous epithelial cells (Muno et al., 2003; Tran et al., 2007). HPV infection was closely associated with cervical cancer and account for 70% of the cervical cancers (No et al., 2011). Recent data suggested that it could also play a role in HNSCC. In general, HPV could be found in about 30% of the HNSCC. According to Heller and Münger, HPV is associated with 24% oral cavity cancer and 36% in oropharynx cancer (Hellner and Münger, 2011). HPV infection has also been reported in nasopharyngeal carcinoma (Lo et al., 2010). Increasing evidence suggested that HPV is closely associated with tonsillar cancer with prevalence ranged from 50-100% (Hammarstedt et al., 2006; Nasman et al., 2009; Syrjanen, 2004). Alcohol and tobacco consumption is linked to the risk of HPV infection (Chaturvedi et al., 2008; Tran, 2007). HPV status greatly influences the clinical features and prognosis of head and neck cancer patients (Lajer and Buchwald, 2010). The viral-encoded oncoprotein is a sensitive and specific marker for identifying tonsillar carcinoma patients (Hellner and Münger, 2011).

HPV-infected HNSCC cells had a different microRNA expression pattern in comparison with the HPV-negative counterpart (Wald et al., 2011; Wang et al., 2008). It is now clear that HPV could affect the host microRNA expression patterns resulting in the distinct clinical features (Lajer and Buchwald, 2010). Similar to EBV, HPV-encoded microRNA could modulate the microRNA expression machinery of the host (Wang et al., 2009). Lajer *et al*. reported that HPV infection is closely associated with the alteration of miR-127-3p and miR363 in oral and pharyngeal SCC (Lajer et al., 2011). By interfering the E6-p53 and E7-pRb pathways, HPV E6 and E7 oncoproteins could control expression of miR-15/16 cluster, miR-17-92 family, miR-21, miR-23b, miR-34a, and miR-106b/93/25 cluster in the host cells (Zheng and Wang, 2011).

By the time of writing, there is still no HPV-encoded microRNA reported and its role is largely unknown. In addition, the oncogenic role of HPV is affected by geographic factors (Lajer et al., 2010). The prevalence of HPV-associated HNSCC varies between different geographic regions. Low prevalence is reported in Asia, Central Europe, and Latin America (Kreimer et al., 2005; Ribeiro et al., 2011). HPV is nearly undetectable in tonsillar carcinoma of the Chinese patients (Li et al., 2003). The data suggested that HPV infection is a risk factor for a subset of HNSCC and the molecular pathways associated with HPV-negative HNSCC remain to be elucidated.

8. Methods used in microRNA detection

Similar to gene expression patterns, head and neck cancers had specific microRNA expression patterns. With microRNA profiling, Lu *et al.* could distinguish poorly differentiated carcinoma from the rest (Lu et al., 2005). Thus, there is a need to develop molecular techniques to (1) detect and quantify known microRNA; and (2) identify novel microRNA; and (3) perform global and high throughput microRNA profiling. Since identification of the first microRNA in *C. elegan*, the technologies employed to examine microRNA are fast evolving. The following session will briefly describe the common

methods used in microRNA research. Among all the method, northern blotting is nearly the first use to detect and quantify specific microRNA expression (Lau et al., 2001). To date, this technique is largely replaced by others in detecting and quantifying microRNA. *In situ* hybridization detection is used to monitor the cellular and subcellular distribution of microRNA (Wienholds et al., 2005). *In situ* hybridization could be used on both frozen section and on archival formalin-fixed paraffin-embedded (FFPE) allowing localization of microRNA in clinical specimens. Real-time quantitative PCR is now the most commonly used technique in detecting and quantifying microRNA of interest. With the growing number of microRNA sequence published in the miRBase, real-time quantitative PCR primers and probe set could be designed to amplify specific microRNAs. For high throughout microRNA profiling, different form of microRNA array are commercially available. The oligo-nucleotide arrays allow detection of the whole miRBase library in a single run and are very suitable to use in examining the expression patterns of samples (Liu et al., 2008). Recently, next generation sequencing (deep sequencing) is employed to identify novel microRNA. The technique allows sequencing of the whole genome within weeks. In addition, deep sequencing can be used to identify posttranscriptional modifications in mature microRNAs. Initial studies have suggested that these post-transcriptionally modified, so-called isomiRs, might be evidence of tissue-specific or even tumor-specific distribution (Lee et al., 2010; Kunchenbauer et al., 2008). Commonly used system for microRNA identification includes Solexa (Illumina), SOLiD (ABI), and 454 (Roche) which allows detection of microRNA in low abundance (Fridlander et al., 2008).

9. MicroRNA and epigenetic therapies

MicroRNA could target multiple gene transcripts making it a good choice for systemic therapy of cancers. The rationale of microRNA-based therapy is similar to siRNA-based therapy. Based on the gene sequence, the microRNA/siRNA of a target gene could be synthesized chemically and delivered to the cancer patients. Synthetic microRNA can be used to restore the levels of basal tumor suppressing microRNA in cancer cells. In addition, microRNA antagonist (partially or completely complementary to specific microRNA sequences) can be designed based on the mature microRNA sequence to inhibit the overexpressed oncogenic microRNA in cancer cells (Krutzfeldt et al., 2005). The therapeutic microRNA could be packed into microvesicles and delivered to the cancer sites directly or through the circulation system (Skog et al., 2008). Cancer cell could take up the microvesicles at high efficiency as the constituent of microvesicle are similar to the plasma membrane (Thery et al., 2002). Elmén *et al.* tested this idea using mouse models and non-human primate models [African green monkeys (*Chlorocebus aethiops*)] (Elmén et al., 2008). They synthesized the miR-122 antagonist and delivered it into the animal model. MiR-122 is related to the cholesterol mechanisms in liver cells. The miR-122 antagonist could be taken by the liver cells resulting in decreased plasma cholesterol levels without any toxicity. Similar to drug treatment, the major challenge of microRNA-based therapy is the efficiency to deliver the therapeutic microRNA to cancer tissues as microvesicle in circulation is actively cleaned up by macrophage and kidney. Further, large microvesicles are difficult to pass through the capilliary endothelium and extracellular matrix of head and neck tissues (Bader et al., 2011). Advances in the microRNA delivery system are necessary in order to put microRNA-based therapy into clinical practice.

10. Concluding remarks

HNSCC is a complex disease caused by accumulating genetic, epigenetic and proteomic alterations. MicroRNA is a potent regulator controlling multiple biological processes including cell growth, differentiation, cell death, development and immune responses (Flynt et al., 2008; Stefani et al., 2008; Lodish et al., 2008). With emerging data supporting that microRNA plays a central role in gene dysregulation in human malignancies, unraveling the microRNA expression patterns in different HNSCC is essential and critical if we want to develop better diagnostic and prognostic system for our patients. On the other hand, gaining better insight into the regulatory mechanisms of microRNA would allow us to design therapeutic regime, which targets the disease with better outcome. We could anticipate that our knowledge to HNSCC will be changed with the increase in understanding of microRNA in the coming decades. Translating our knowledge into clinical management will be a beneficial to the treatment and prognosis of our patients.

11. References

Akervall, J. (2005). Gene profiling in squamous cell carcinoma of the head and neck. *Cancer Metastasis Reviews*, Vol.24, No.1, (January 2005), pp. 87–94, ISSN 0167-7659

Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK: *SEER Cancer Statistics Review 1975-2007*. National Cancer Institute. Bethesda, MD. Available from: http://www.seer.cancer.gov/csr/1975_2008/index.html

Ambros, V. (2004). The functions of animal microRNAs. *Nature*, Vol.431, No. 7006, (September 2004), pp. 350-355, ISSN 0028-0836

Anastasiadou, E., Boccellato, F., Vincenti, S., Rosato, P., Bozzoni, I., Frati, L., Faggioni, A., Presutti, C., and Trivedi, P. (2010). Epstein-Barr virus encoded LMP1 downregulates TCL1 oncogene through miR-29b. *Oncogene*, Vol.29, No.9, (March 2010), pp. 1316–1328, ISSN 0950-9232

Argiris, A., and Eng, C. (2003). Epidemiology, staging, and screening of head and neck cancer. *Cancer treatment and research*, Vol.114, (Jan 2003) pp. 15-60, ISSN 0927-3042

Argiris, A., Brockstein, B. E., Haraf, D. J., Stenson, K. M., Mittal, B. B., Kies, M. S., Rosen, F. R., Jovanovic, B., and Vokes, E. E. (2004). Competing causes of death and second primary tumors in patients with locoregionally advanced head and neck cancer treated with chemoradiotherapy. *Clinical Cancer Research*, Vol.10, No.6, (March 2004), pp. 1956-1962, ISSN 1078-0432

Avissar, M., Christensen, B. C., Kelsey, K. T., and Marsit, C. J. (2009). MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. *Clinical Cancer Research*, Vol. 15, No. 8, (April 2009), pp. 2850–2855, ISSN 1078-0432

Bader, A. G., Brown, D., Stoudemire, J., and Lammers, P. (2011). Developing therapeutic microRNAs for cancer. *Gene Therapy*, (June 2011), ISSN 0969-7128

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, Vol.116, No.2, (Jan 2004) pp. 281-97, ISSN 0092-8674

Barth, S., Pfuhl, T., Mamiani, A., Ehses, C., Roemer, K., Kremmer, E., Jäker, C., Höck, J., Meister, G., and Grässer, F. A. (2008). Epstein-Barr virus-encoded microRNA miR-

BART2 down-regulates the viral DNA polymerase BALF5. *Nucleic Acids Research*, Vol.36, No.2, (Feb 2008), pp. 666-675, ISSN 0305-1048

Bauer, V. L., Braselmann, H., Henke, M., Mattern, D., Walch, A., Unger, K., Baudis, M., Lassmann, S., Huber, R., Wienberg, J., et al. (2008). Chromosomal changes characterize head and neck cancer with poor prognosis. *Journal of Molecular Medicine*, Vol.86, No.21, (December 2008), pp. 1353–1365, ISSN 0946-2716

Bilde, A., Buchwald, von, C., Johansen, J., Bastholt, L., Sørensen, J. A. H. M., Marker, P., Krogdahl, A., Hansen, H. S., Specht, L., Kirkegaard, J., et al. (2006). The Danish national guidelines for treatment of oral squamous cell carcinoma. *Acta Oncologica* (Stockholm, Sweden), Vol. 45, No.3, (January 2006), pp. 294-299, ISSN 1651-226X

Black, R. J., Bray, F., Ferlay, J., and Parkin, D. M. (1997). Cancer incidence and mortality in the European Union: cancer registry data and estimates of national incidence for 1990. *European Journal of Cancer* (Oxford, England : 1990), Vol.33, No.7, (June 1997), pp. 1075-1107, ISSN 0959-8049

Buchwald, von, C., Bilde, A., Shoaib, T., and Ross, G. (2002). Sentinel node biopsy: the technique and the feasibility in head and neck cancer. *ORL J. Otorhinolaryngoly Related Specialities*, Vol.64, No.4, (June 2002), pp. 268–274. ISSN 0301-1569

Bushati, N., and Cohen, S. M. (2007). microRNA functions. *Annual Review of Cell and Developmental Biology*, Vol. 23, (January 2007), pp. 175-205, ISSN 1081-0706

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.99, No.24, (November 2002), pp. 15524-15529, ISSN 0027-8424

Calin, G. A., Sevignani, C., Dumitru, C. D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M., et al. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.101, No.9, (March 2004), pp.2999–3004, ISSN 0027-8424

Cervigne, N. K., Reis, P. P., Machado, J., Sadikovic, B., Bradley, G., Galloni, N. N., Pintilie, M., Jurisica, I., Perez-Ordonez, B., Gilbert, R., et al. (2009). Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. *Human Molecular Genetics*, Vol.18, No.24, (December 2009), pp. 4818-4829, ISSN 0964-6906

Chang, S. S., Jiang, W. W., Smith, I., Poeta, L. M., Begum, S., Glazer, C., Shan, S., Westra, W., Sidransky, D., and Califano, J. A. (2008). MicroRNA alterations in head and neck squamous cell carcinoma. *International Journal of Cancer*, Vol.123, No.12, (December 2008), pp. 2791-2797, ISSN 0020-7136

Chang, T.-C., Yu, D., Lee, Y.-S., Wentzel, E. A., Arking, D. E., West, K. M., Dang, C. V., Thomas-Tikhonenko, A., and Mendell, J. T. (2008). Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature Genetics*, Vol.40, No.1, (January 2008), pp. 43–50, ISSN 1061-4036

Chaturvedi, A. K., Engels, E. A., Anderson, W. F., and Gillison, M. L. (2008). Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *Journal of Clinical Oncology*, 2008 vol. 26 (4) pp. 612-619, ISSN 0732-183X

Chen, H. C., Chen, G. H., Chen, Y. H., Liao, W. L., Liu, C. Y., Chang, K. P., Chang, Y. S., and Chen, S. J. (2009). MicroRNA deregulation and pathway alterations in nasopharyngeal carcinoma. *British Journal of Cancer*, Vol.100, No.6, (March 2009), pp. 1002-1011, ISSN 0007-0920

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. (2009). Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma. *The American Journal of Pathology*, Vol.174, No.3, (March 2009), pp. 736-745, ISSN 0002-9440

Choy, E. Y.-W., Siu, K.-L., Kok, K.-H., Lung, R. W.-M., Tsang, C.-M., To, K.-F., Kwong, D. L.-W., Tsao, S.-W., and Jin, D.-Y. (2008). An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *The Journal of Experimental Medicine*, Vol.205, No.11, (Oct 2008), pp. 2551-2560, ISSN 2551–2560

Christensen, B. C., Avissar-Whiting, M., Ouellet, L. G., Butler, R. A., Nelson, H. H., McClean, M. D., Marsit, C. J., and Kelsey, K. T. (2010). Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. *Clinical Cancer Research*, Vol.16, No.14, (July 2010), pp. 3713-3720, ISSN 3713–3720.

Cohen, E. E. W., Zhu, H., Lingen, M. W., Martin, L. E., Kuo, W.-L., Choi, E. A., Kocherginsky, M., Parker, J. S., Chung, C. H., and Rosner, M. R. (2009). A feed-forward loop involving protein kinase Calpha and microRNAs regulates tumor cell cycle. *Cancer Research*, Vol.69, No.1, (January 2009), pp. 65-74, ISSN 0008-5472

de Planell-Saguer, M., and Rodicio, M. C. (2011). Analytical aspects of microRNA in diagnostics: A review. *Analytica Chimica Acta*, Vol.699, No.2, (August 2011), pp. 134-152, ISSN 0003-2670

Du, Z.-M., Hu, L.-F., Wang, H.-Y., Yan, L.-X., Zeng, Y.-X., Shao, J.-Y., and Ernberg, I. (2011). Upregulation of MiR-155 in nasopharyngeal carcinoma is partly driven by LMP1 and LMP2A and downregulates a negative prognostic marker JMJD1A. *PLoS ONE*, Vol.6, No.4, (2011), ISSN 1932-6203

Duan, R., Pak, C., and Jin, P. (2007). Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-microRNA. *Human Molecular Genetics*, Vol.16, No.9, (May 2007), pp. 1124-1131, ISSN 0964-6906

Elmén, J., Lindow, M., Schütz, S., Lawrence, M., Petri, A., Obad, S., Lindholm, M., Hedtjärn, M., Hansen, H. F., Berger, U., et al. (2008). LNA-mediated microRNA silencing in non-human primates. *Nature*, Vol.452, No.7189, (April 2008), pp. 896–899, ISSN 0028-0836

Farshadpour, F., Hordijk, G. J., Koole, R., and Slootweg, P. J. (2007). Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. *Oral Disease*, Vol.13, No.2, (March 2007), pp. 239–243, ISSN 1354-523X

Farshadpour, F., Kranenborg, H., Calkoen, E. V. B., Hordijk, G. J., Koole, R., Slootweg, P. J., and Terhaard, C. H. (2011). Survival analysis of head and neck squamous cell carcinoma: influence of smoking and drinking. *Head & Neck*, Vol.33, No.6, (June 2011) pp. 817-823, ISSN 1043-3074

Ferdin, J., Kunej, T., and Calin, G. A. (2010). Non-coding RNAs: identification of cancer-associated microRNAs by gene profiling. *Technology in Cancer Research & Treatment*, Vol.9, No.2, (April 2010), pp. 123–138, ISSN:1533-0346

Février, B., and Raposo, G. (2004). Exosomes: endosomal-derived vesicles shipping extracellular messages. *Current Opinion in Cell Biology*, Vol.16, No.4, (August 2004), pp. 415–421, ISSN 0955-0674

Filipowicz, W., Bhattacharyya, S. N., and Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature Reviews Genetics*, Vol.9, No.2, (Feburary 2008), pp. 102–114, ISSN:1471-0056

Fletcher, A. M., Heaford, A. C., and Trask, D. K. (2008). Detection of metastatic head and neck squamous cell carcinoma using the relative expression of tissue-specific mir-205. *Translational Oncology*, 2008 vol. 1 (4) pp. 202-208, ISSN 1936-5233

Flynt, A. S., and Lai, E. C. (2008). Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nature Reviews Genetics*, Vol.9, No.11, (November 2008), pp. 831–842, ISSN 1471-0056

Friedländer, M. R., Chen, W., Adamidi, C., Maaskola, J., Einspanier, R., Knespel, S., and Rajewsky, N. (2008). Discovering microRNAs from deep sequencing data using miRDeep. *Nature Biotechnology*, Vol.26, No., 4, (April 2008), pp. 407–415, ISSN 1087-0156

Fu, X., Han, Y., Wu, Y., Zhu, X., Lu, X., Mao, F., Wang, X., He, X., Zhao, Y., and Zhao, Y. (2011). Prognostic role of microRNA-21 in various carcinomas: a systematic review and Meta-analysis. *European Journal of Clinical Investigation*, (April 2011), ISSN 0014-2972

Gollin, S. M. (2001). Chromosomal alterations in squamous cell carcinomas of the head and neck: window to the biology of disease. *Head & Neck*, Vol. 23, No.3, (March 2001), pp. 238–253, ISSN 1043-3074

Hammarstedt, L., Lindquist, D., Dahlstrand, H., Romanitan, M., Dahlgren, L. O., Joneberg, J., Creson, N., Lindholm, J., Ye, W., Dalianis, T., et al. (2006). Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *International Journal of Cancer*, Vol.119, No.11, (December 2006), pp. 2620–2623, ISSN 0020-7136

Hanke, M., Hoefig, K., Merz, H., Feller, A. C., Kausch, I., Jocham, D., Warnecke, J. M., and Sczakiel, G. (2010). A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. *Urologic Oncology*, Vol.28, No.6, (October 2010), pp. 655–661, ISSN 1078-1439

He, L., He, X., Lim, L. P., de Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., et al. (2007). A microRNA component of the p53 tumour suppressor network. *Nature*, Vol.447, No. 7148, (June 2007), pp. 1130–1134. ISSN 0028-0836

He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S. W., Hannon, G. J., et al. (2005). A microRNA polycistron as a potential human oncogene. *Nature*, Vol.435, No.7043, (June 2005), pp. 828–833, ISSN 0028-0836

Heijnen, H. F., Schiel, A. E., Fijnheer, R., Geuze, H. J., and Sixma, J. J. (1999). Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*, Vol.94, No.11, (December 1999), pp. 3791–3799, ISSN 0006-4971

Hellner, K., and Münger, K. (2011). Human papillomaviruses as therapeutic targets in human cancer. *Journal of Clinical Oncology*, Vol.29, No.13, (May 2011), pp. 1785–1794, ISSN 0732-183X

Henson, B. J., Bhattacharjee, S., O'Dee, D. M., Feingold, E., and Gollin, S. M. (2009). Decreased expression of miR-125b and miR-100 in oral cancer cells contributes to malignancy. *Genes Chromosomes & Cancer*, Vol.48, No.7, (July 2009), pp. 569–582, ISSN 1045-2257

Horikawa, T., Yoshizaki, T., Kondo, S., Furukawa, M., Kaizaki, Y., and Pagano, J. S. (2011). Epstein-Barr Virus latent membrane protein 1 induces Snail and epithelial-mesenchymal transition in metastatic nasopharyngeal carcinoma. *British Journal of Cancer*, Vol.104, No.7, (March 2011), pp. 1160–1167, ISSN 0007-0920

Hui, A. B. Y., Lenarduzzi, M., Krushel, T., Waldron, L., Pintilie, M., Shi, W., Perez-Ordonez, B., Jurisica, I., O'Sullivan, B., Waldron, J., et al. (2010). Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas. Clinical *Cancer Research*, Vol.16, No.4, (Feb 2010) pp. 1129-39

Hunter, M. P., Ismail, N., Zhang, X., Aguda, B. D., Lee, E. J., Yu, L., Xiao, T., Schafer, J., Lee, M.-L. T., Schmittgen, T. D., et al. (2008). Detection of microRNA expression in human peripheral blood microvesicles. *PLoS ONE*, Vol.3, No.11, (November 2008), pp. e3694, ISSN 1932-6203

Iguchi, H., Kosaka, N., and Ochiya, T. (2010). Secretory microRNAs as a versatile communication tool. *Communicative & Integrative Biology*, Vol.3, No.5, (September 2010), pp. 478–481, ISSN 1942-0889

Iorio, M. V., Ferracin, M., Liu, C.-G., Veronese, A., Spizzo, R., Sabbioni, S., Magri, E., Pedriali, M., Fabbri, M., Campiglio, M., et al. (2005). MicroRNA gene expression deregulation in human breast cancer. *Cancer Research*, Vol.65, No.16, (August 2005), pp. 7065–7070. ISSN 0008-5472

Jaber, M. A., Porter, S. R., Gilthorpe, M. S., Bedi, R., and Scully, C. (1999). Risk factors for oral epithelial dysplasia--the role of smoking and alcohol. *Oral Oncology*, Vol.35, No.2, (March 1999), pp. 151–156, ISSN 1368-8375

Jakymiw, A., Patel, R. S., Deming, N., Bhattacharyya, I., Shah, P., Lamont, R. J., Stewart, C. M., Cohen, D. M., and Chan, E. K. L. (2010). Overexpression of dicer as a result of reduced let-7 MicroRNA levels contributes to increased cell proliferation of oral cancer cells. *Genes Chromosomes & Cancer*, Vol.49, No.6, (June 2010), pp. 549–559, ISSN 1045-2257

Jiang, J., Lee, E. J., Gusev, Y., and Schmittgen, T. D. (2005). Real-time expression profiling of microRNA precursors in human cancer cell lines. *Nucleic Acids Re*search, Vol.33, No.17, (September 2005), pp. 5394–5403, ISSN 0305-1048

Jiang, L., Liu, X., Kolokythas, A., Yu, J., Wang, A., Heidbreder, C. E., Shi, F., and Zhou, X. (2010). Downregulation of the Rho GTPase signaling pathway is involved in the microRNA-138-mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma. *International Journal of Cancer,* Vol.127, No.3, (August 2010), pp. 505–512, ISSN 0020-7136

Kosaka, N., Iguchi, H., and Ochiya, T. (2010). Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Science*, Vol.101, No.10, pp. 2087–2092, ISSN 1347-9032

Kozaki, K.-I., Imoto, I., Mogi, S., Omura, K., and Inazawa, J. (2008). Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. *Cancer Research*, Vol.68, No.7, (April 2008), pp. 2094–2105, ISSN 0008-5472

Kreimer, A. R., Clifford, G. M., Boyle, P., and Franceschi, S. (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiology Biomarkers Prevention*, Vol.14, No.2, (February 2005), pp. 467–475, ISSN 1055-9965

Krichevsky, A. M., and Gabriely, G. (2009). miR-21: a small multi-faceted RNA. *Journal of Cellular Molecular Medicine*, Vol.13, No.1, (January 2009), pp. 39–53, ISSN 1582-1838

Krutovskikh, V. A., and Herceg, Z. (2010). Oncogenic microRNAs (OncomiRs) as a new class of cancer biomarkers. *BioEssays*, Vol.32, No.10, (October 2010), pp. 894–904, ISSN 0265-9247

Krützfeldt, J., Rajewsky, N., Braich, R., Rajeev, K. G., Tuschl, T., Manoharan, M., and Stoffel, M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. *Nature*, Vol. 438, No.7068, (December 2005), pp. 685–689, ISSN 0028-0836

Kuchenbauer, F., Morin, R. D., Argiropoulos, B., Petriv, O. I., Griffith, M., Heuser, M., Yung, E., Piper, J., Delaney, A., Prabhu, A.-L., et al. (2008). In-depth characterization of the microRNA transcriptome in a leukemia progression model. *Genome Research*, Vol.18, No.11, (November 2008), pp. 1787–1797, ISSN 1088-9051

Kung, C.-P., Meckes, D. G., and Raab-Traub, N. (2011). Epstein-Barr virus LMP1 activates EGFR, STAT3, and ERK through effects on PKCdelta. *Journal of Virology*, Vol.85, No.9, (May 2011), pp.4399–4408, ISSN 0022-538X

Lajer, C. B., and Buchwald, von, C. (2010). The role of human papillomavirus in head and neck cancer. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*, Vol.118, No.6-7, (June 2010), pp. 510-519, ISSN 0903-4641

Lajer, C. B., Nielsen, F. C., Friis-Hansen, L., Norrild, B., Borup, R., Garnæs, E., Rossing, M., Specht, L., Therkildsen, M. H., Nauntofte, B., et al. (2011). Different microRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. *British Journal of Cancer*, Vol.104, No.5, (March 2010), pp. 830–840, ISSN 0007-0920

Langevin, S. M., Stone, R. A., Bunker, C. H., Grandis, J. R., Sobol, R. W., and Taioli, E. (2010). MicroRNA-137 promoter methylation in oral rinses from patients with squamous cell carcinoma of the head and neck is associated with gender and body mass index. *Carcinogenesis*, Vol.31, No.5, (May 2010), pp. 864–870, ISSN 0143-3334

Langevin, S. M., Stone, R. A., Bunker, C. H., Lyons-Weiler, M. A., Laframboise, W. A., Kelly, L., Seethala, R. R., Grandis, J. R., Sobol, R. W., and Taioli, E. (2011). MicroRNA-137 promoter methylation is associated with poorer overall survival in patients with squamous cell carcinoma of the head and neck. *Cancer*, Vol.117, No.7, (April 2011), pp. 1454–1462, ISSN 0008-543X

Lau, N. C., Lim, L. P., Weinstein, E. G., and Bartel, D. P. (2001). An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science*, Vol.294, No.5543, (October 2001), pp. 858–862, ISSN 0036-8075

Lee, L. W., Zhang, S., Etheridge, A., Ma, L., Martin, D., Galas, D., and Wang, K. (2010). Complexity of the microRNA repertoire revealed by next-generation sequencing. *RNA*, Vol.16, No.11, (November 2010), pp. 2170–2180, ISSN 1355-8382

Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, Vol.75, No.5, (December 1993), pp. 843–854, ISSN 0092-8674

Lee, Y., Jeon, K., Lee, J.-T., Kim, S., and Kim, V. N. (2002). MicroRNA maturation: stepwise processing and subcellular localization. *The EMBO Journal*, Vol.21, No.17, (September 2002), pp. 4663–4670, ISSN 0261-4189

Lee, Y., Yang, X., Huang, Y., Fan, H., Zhang, Q., Wu, Y., Li, J., Hasina, R., Cheng, C., Lingen, M. W., et al. (2010). Network modeling identifies molecular functions targeted by miR-204 to suppress head and neck tumor metastasis. *PLoS Computional Biology*, Vol.6, No.4, pp. e1000730, ISSN 1553-734X

Lewis, B. P., Burge, C. B., and Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, Vol.120, No.1, (January 2005), pp. 15–20, ISSN 0092-8674

Li, G., Wu, Z., Peng, Y., Liu, X., Lu, J., Wang, L., Pan, Q., He, M.-L., and Li, X.-P. (2010). MicroRNA-10b induced by Epstein-Barr virus-encoded latent membrane protein-1 promotes the metastasis of human nasopharyngeal carcinoma cells. *Cancer Letter*, Vol.299, No.1, (December 2010), pp.29–36, ISSN 0304-3835

Li, J., Huang, H., Sun, L., Yang, M., Pan, C., Chen, W., Wu, D., Lin, Z., Zeng, C., Yao, Y., et al. (2009). MiR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. *Clinical Cancer Research*, Vol. 15, No.12, (June 2009), pp. 3998–4008, ISSN 1078-0432

Li, J., Smyth, P., Flavin, R., Cahill, S., Denning, K., Aherne, S., Guenther, S. M., O'Leary, J. J., and Sheils, O. (2007). Comparison of microRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. *BMC Biotechnolology*, Vol.7, (June 2007) pp. 36-41, ISSN 1472-6750

Li, L., Zhang, Z.-M., Liu, Y., Wei, M.-H., Xue, L.-Y., Zou, S.-M., Di, X.-B., Han, N.-J., Zhang, K.-T., Xu, Z.-G., et al. (2010). DNA microarrays-based microRNA expression profiles derived from formalin-fixed paraffin-embedded tissue blocks of squammous cell carcinoma of larynx. *Zhonghua Bing Li Xue Za Zhi*, Vol.39, No.6, (June 2010), pp. 391–395. ISSN 0529-5807

Li, T., Chen, J.-X., Fu, X.-P., Yang, S., Zhang, Z., Chen, K.-H., and Li, Y. (2011). microRNA expression profiling of nasopharyngeal carcinoma. *Oncology Report*, Vol.25, No.5, (May 2011), pp. 1353–1363, ISSN 1021-335X

Li, W., Thompson, C. H., Xin, D., Cossart, Y. E., O'Brien, C. J., McNeil, E. B., Gao, K., Scolyer, R. A., and Rose, B. R. (2003). Absence of human papillomavirus in tonsillar squamous cell carcinomas from Chinese patients. *The American Journal of Pathology*, Vol.163, No.6, (December 2003), pp. 2185–2189, ISSN 0002-9440

Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S., and Johnson, J. M. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, Vol.433, No.7027, (Feburary 2005), pp. 769–773, ISSN 0028-0836

Liu, C.-G., Spizzo, R., Calin, G. A., and Croce, C. M. (2008). Expression profiling of microRNA using oligo DNA arrays. *Methods*, Vol.44, No.1, (January 2008), pp. 22–30, ISSN 1046-2023

Liu, X., Chen, Z., Yu, J., Xia, J., and Zhou, X. (2009). MicroRNA profiling and head and neck cancer. *Comparative and Functional Genomics*, Vol.2009, No.837514, (June 2009), pp. 1-11, ISSN 1531-6912

Liu, X., Jiang, L., Wang, A., Yu, J., Shi, F., and Zhou, X. (2009). MicroRNA-138 suppresses invasion and promotes apoptosis in head and neck squamous cell carcinoma cell lines. *Cancer Letter*, Vol.286, No.2, (December 2009), pp. 217–222, ISSN 0304-3835

Liu, X., Yu, J., Jiang, L., Wang, A., Shi, F., Ye, H., and Zhou, X. (2009b). MicroRNA-222 regulates cell invasion by targeting matrix metalloproteinase 1 (MMP1) and manganese superoxide dismutase 2 (SOD2) in tongue squamous cell carcinoma cell lines. *Cancer Genomics & Proteomics*, Vol.6, No.3, (April 2009), pp. 131-139, ISSN 1109-6535

Liu, Z., Li, G., Wei, S., Niu, J., El-Naggar, A. K., Sturgis, E. M., and Wei, Q. (2010). Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. Cancer, Vol.116, No.20, (October 2010), pp. 4753–4760, ISSN 0008-543X

Lo, A. K. F., To, K.-F., Lo, K. W., Lung, R. W.-M., Hui, J. W. Y., Liao, G., and Hayward, S. D. (2007). Modulation of LMP1 protein expression by EBV-encoded microRNAs. Proceedings of the National Academy of Sciences of the United States of America, Vol.104, No.41, (October 2007), pp. 16164–16169, ISSN 0027-8424

Lo, E. J., Bell, D., Woo, J., Li, G., Hanna, E. Y., El-Naggar, A. K., and Sturgis, E. M. (2010). Human papillomavirus & WHO type I nasopharyngeal carcinoma. Laryngoscope, Vol.120, No. Suppl 4, pp. S185, ISSN 0023-852X

Lodish, H. F., Zhou, B., Liu, G., and Chen, C.-Z. (2008). Micromanagement of the immune system by microRNAs. Nature Reviews. Immunology, Vol.8, No.2, (Feburary 2008), pp. 120–130. ISSN 1474-1733

Long, X.-B., Sun, G.-B., Hu, S., Liang, G.-T., Wang, N., Zhang, X.-H., Cao, P.-P., Zhen, H.-T., Cui, Y.-H., and Liu, Z. (2009). Let-7a microRNA functions as a potential tumor suppressor in human laryngeal cancer. Oncology Reports, Vol.22, No.5, (November 2009), pp. 1189–1195, ISSN 1021-335X

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., et al. (2005). MicroRNA expression profiles classify human cancers. Nature, Vol.435, No.7043, (June 2005), pp. 834–838, ISSN 0028-0836

Lung, R. W.-M., Tong, J. H.-M., Sung, Y.-M., Leung, P.-S., Ng, D. C.-H., Chau, S.-L., Chan, A. W.-H., Ng, E. K.-A., Lo, K.-W., and To K.-F. (2009). Modulation of LMP2A expression by a newly identified Epstein-Barr virus-encoded microRNA miR-BART22. Neoplasia (New York, NY), Vol.11, No.11, pp. 1174–1184, ISSN 1476-5586

Melo, S. A., and Esteller, M. (2011). Dysregulation of microRNAs in cancer: Playing with fire. FEBS Letters, Vol.585, No.13, (July 2011), pp. 2087–2099, ISSN 0014-5793

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A.,

Motsch, N., Pfuhl, T., Mrazek, J., Barth, S., and Grässer, F. A. (2007). Epstein-Barr virus-encoded latent membrane protein 1 (LMP1) induces the expression of the cellular microRNA miR-146a. RNA Biology, Vol.4, No.3, (November 2007), pp. 131–137, ISSN 1547-6286

Muñoz, N., Bosch, F. X., de Sanjosé, S., Herrero, R., Castellsagué, X., Shah, K. V., Snijders, P. J. F., Meijer, C. J. L. M., International Agency for Research on Cancer Multicenter Cervical Cancer Study Group (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England Journal of Medicine, Vol.348, No.6, (February 2003), pp. 518–527, ISSN 0028-4793

Muralidhar, B., Winder, D., Murray, M., Palmer, R., Barbosa-Morais, N., Saini, H., Roberts, I., Pett, M., and Coleman, N. (2011). Functional evidence that Drosha overexpression in cervical squamous cell carcinoma affects cell phenotype and microRNA profiles. The Journal of Pathology, Vol.224, No.4, pp. 496–507, ISSN 0022-3417

No, J. H., Kim, M.-K., Jeon, Y.-T., Kim, Y.-B., and Song, Y.-S. (2011). Human papillomavirus vaccine: widening the scope for cancer prevention. Molecular Carcinogenesis, Vol.50, No.4, (April 2011), pp. 244–253, ISSN 0899-1987

Noteboom, J., O'Briant, K. C., Allen, A., et al. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America, Vol.105, No.30, (July 2008), pp. 10513–10518, ISSN 0027-8424

Näsman, A., Attner, P., Hammarstedt, L., Du, J., Eriksson, M., Giraud, G., Ahrlund-Richter, S., Marklund, L., Romanitan, M., Lindquist, D., et al. (2009). Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? International Journal of Cancer, Vol.125, No.2, (July 2009), pp. 362–366, ISSN 0020-7136

Nurul-Syakima, A. M., Yoke-Kqueen, C., Sabariah, A. R., Shiran, M. S., Singh, A., and Learn-Han, L. (2011). Differential microRNA expression and identification of putative microRNA targets and pathways in head and neck cancers. Int Journal of Molecular Mediocine, Vol.28, No.3, (September 2011), pp. 327–336, ISSN 1078-0432

Papagiannakopoulos, T., Shapiro, A., and Kosik, K. S. (2008). MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. Cancer Research, Vol.68, No.19, (October 2008), pp. 8164-8172, ISSN 0008-5472

Park, N. J., Zhou, H., Elashoff, D., Henson, B. S., Kastratovic, D. A., Abemayor, E., and Wong, D. T. (2009). Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clinical Cancer Research, Vol.15, No.17, (September 2009), pp 5473–5477, ISSN 1078-0432

Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine 2006; 24 (Suppl 3):S/11–S25.

Parkin, D. M., Bray, F., Ferlay, J., and Pisani, P. (2005). Global cancer statistics, 2002. CA: A Cancer Journal for Clinicans, Vol.55, No.2, (Feburary 2005), pp. 74–108, ISSN 0007-9235

Persson, M., Andrén, Y., Mark, J., Horlings, H. M., Persson, F., and Stenman, G. (2009). Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proceedings of the National Academy of Sciences of the United States of America, Vol.106, No.44, (November 2009), pp. 18740–18744. ISSN 0027-8424

Pfeffer, S., Zavolan, M., Grässer, A.-F., Russo, J.-J, Ju J., John, B., Enright, J.-A., Marks, D., Sander, C., and Tuschi, T. (2004) Identification of virus-encoded microRNAs. Science (New York, NY), Vol.304, No.5671 (April 2004), pp 734–736, ISSN 0036-8075

Place, R. F., Li, L.-C., Pookot, D., Noonan, E. J., and Dahiya, R. (2008). MicroRNA-373 induces expression of genes with complementary promoter sequences. Proceedings of the National Academy of Sciences of the United States of America, Vol.105, No.5, (Feburary 2008), pp. 1608–1613, ISSN 0027-8424

Posner, M. R. (2010). Integrating systemic agents into multimodality treatment of locally advanced head and neck cancer. Annals of Oncology, Vol.21, No. Suppl 7, (October 2010), pp. vii246-vii251, ISSN 0923-7534

Rentoft, M., Fahlén, J., Coates, P. J., Laurell, G., Sjöström, B., Rydén, P., and Nylander, K. (2011). miRNA analysis of formalin-fixed squamous cell carcinomas of the tongue is affected by age of the samples. International Journal of Oncology, Vol.38, No.1, (January 2011), pp. 61–69, ISSN 1019-6439

Ribeiro, K. B., Levi, J. E., Pawlita, M., Koifman, S., Matos, E., Eluf-Neto, J., Wunsch-Filho, V., Curado, M. P., Shangina, O., Zaridze, D., et al. (2011). Low human papillomavirus prevalence in head and neck cancer: results from two large case-control studies in high-incidence regions. International Journal of Epidemiology, Vol.40, No.2, (April 2011), pp. 489–502, ISSN 0300-5771

Robbins, K. T., Kumar, P., Harris, J., McCulloch, T., Cmelak, A., Sofferman, R., Levine, P., Weisman, R., Wilson, W., Weymuller, E., et al. (2005). Supradose intra-arterial cisplatin and concurrent radiation therapy for the treatment of stage IV head and neck squamous cell carcinoma is feasible and efficacious in a multi-institutional setting: results of Radiation Therapy Oncology Group Trial 9615. Journal of Clinical Oncology, Vol.23, No.7, (March 2005), pp. 1447–1454, ISSN 0732-183X

Scapoli, L., Palmieri, A., Muzio, Lo, L., Pezzetti, F., Rubini, C., Girardi, A., Farinella, F., Mazzotta, M., and Carinci, F. (2010). MicroRNA expression profiling of oral carcinoma identifies new markers of tumor progression. International Journal of Immunopathology and Pharmacology, Vol.23, No.4, (September 2010), pp. 1229–1234, ISSN 0394-6320

Sengupta, S., den Boon, J. A., Chen, I. H., Newton, M. A., Stanhope, S. A., Cheng, Y. J., Chen, C. J., Hildesheim, A., Sugden, B., and Ahlquist, P. (2008). MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proceedings of the National Academy of Sciences of the United States of America, Vol.105, No.15, (April 2008), pp. 5874-5878, ISSN 0027-8424

Skog, J., Würdinger, T., van Rijn, S., Meijer, D. H., Gainche, L., Sena-Esteves, M., Curry, W. T., Carter, B. S., Krichevsky, A. M., and Breakefield, X. O. (2008). Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature Cell Biology, Vol.10, No.12, (December 2008), pp. 1470–1476, ISSN 1465-7392

Slaby, O., Bienertova-Vasku, J., Svoboda, M., and Vyzula, R. (2011). Genetic polymorphisms and MicroRNAs: new direction in molecular epidemiology of solid cancer. Journal of Cellular and Molecular Medicine, (June 2011), ISSN 1582-1838 (Print)

Spafford, M. F., Koch, W. M., Reed, A. L., Califano, J. A., Xu, L. H., Eisenberger, C. F., Yip, L., Leong, P. L., Wu, L., Liu, S. X., et al. (2001). Detection of head and neck squamous cell carcinoma among exfoliated oral mucosal cells by microsatellite analysis. Clinical Cancer Research, Vol.7, No.3, (March 2001), pp. 607–612, ISSN: 1078-0432

Stefani, G., and Slack, F. J. (2008). Small non-coding RNAs in animal development. Nature Review. Molecular Cell Biology Vol.9, No.3, (March 2008), pp. 219–230. ISSN 1471-0072

Stell, P. M. (1989). Survival times in end-stage head and neck cancer. European Journal of Surgical Oncology, Vol.15, No.5, (October 1898), pp. 407–410, ISSN 0748-7983

Suzuki, H. I., Yamagata, K., Sugimoto, K., Iwamoto, T., Kato, S., and Miyazono, K. (2009). Modulation of microRNA processing by p53. Nature, Vol.460, No. 7254, (July 2009), pp. 529–533, ISSN 0028-0836

Syrjänen, S. (2004). HPV infections and tonsillar carcinoma. Journal of Clinical Pathology, Vol.57, No.5, (May 2004), pp. 449–455. ISSN 0021-9746

Théry, C., Zitvogel, L., and Amigorena, S. (2002). Exosomes: composition, biogenesis and function. Nature Review. Immunology Vol.2, No.8, (August 2002), pp. 569–579, ISSN 1474-1733

Tran, N., McLean, T., Zhang, X., Zhao, C. J., Thomson, J. M., O'Brien, C., and Rose, B. (2007). MicroRNA expression profiles in head and neck cancer cell lines. Biochemical and biophysical research communications, Vol.358, No.1, (June 2007), pp. 12-17, ISSN 0006-291X

Tran, N., Rose, B. R., and O'Brien, C. J. (2007). Role of human papillomavirus in the etiology of head and neck cancer. Head & Neck, Vol.29, No.1, (January 2007), pp. 64–70, ISSN 1043-3074

Turchinovich, A., Weiz, L., Langheinz, A., and Burwinkel, B. (2011). Characterization of extracellular circulating microRNA. Nucleic Acids Research, Vol.1, No.11, (May 2011), pp. 1-11, ISSN 0305-1048

Vasudevan, S., Tong, Y., and Steitz, J. A. (2007). Switching from repression to activation: microRNAs can up-regulate translation. Science, Vol.318, No.5858, (December 2007), pp. 1931–1934, ISSN 0036-8075

Volinia, S., Calin, G. A., Liu, C.-G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., et al. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. Proceedings of the National Academy of Sciences of the United States of America, Vol.103, No.7, (February 2006), pp. 2257-2261, ISSN 0027-8424

Wald, A. I., Hoskins, E. E., Wells, S. I., Ferris, R. L., and Khan, S. A. (2011). Alteration of microRNA profiles in squamous cell carcinoma of the head and neck cell lines by human papillomavirus. Head & Neck, Vol.33, No.4, (April 2011), pp. 504–512, ISSN 1043-3074

Wang, X., Tang, S., Le, S.-Y., Lu, R., Rader, J. S., Meyers, C., and Zheng, Z.-M. (2008). Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS ONE, Vol.3, No.7, (July 2008), pp. e2557, ISSN 1932-6203

Wang, X., Wang, H.-K., McCoy, J. P., Banerjee, N. S., Rader, J. S., Broker, T. R., Meyers, C., Chow, L. T., and Zheng, Z.-M. (2009). Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. RNA, Vol.15, No.4, (April 2009), pp. 637–647, ISSN 1355-8382

Wei, W. I., and Sham, J. S. T. (2005). Nasopharyngeal carcinoma. Lancet, Vol.365, No.9476, (May 2005), pp. 2041–2054, ISSN 0140-6736

Wienholds, E., Kloosterman, W. P., Miska, E., Alvarez-Saavedra, E., Berezikov, E., de Bruijn, E., Horvitz, H. R., Kauppinen, S., and Plasterk, R. H. A. (2005). MicroRNA expression in zebrafish embryonic development. Science, Vol.309, No.5732, (July 2005), pp. 310–311, ISSN 0036-8075

Wightman, B., Ha, I., and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell, Vol.75, No.5, (December 1993), pp. 855–862, ISSN 0092-8674

Wong, S. J., Harari, P. M., Garden, A. S., Schwartz, M., Bellm, L., Chen, A., Curran, W. J., Murphy, B. A., and Ang, K. K. (2011). Longitudinal Oncology Registry of Head and Neck Carcinoma (LORHAN): analysis of chemoradiation treatment approaches in the United States. Cancer, Vol.117, No.8, (April 2011), pp. 1679–1686, ISSN 0008-543X

Wong, T.-S., Liu, X.-B., Ho, C.-W., Yuen, A.-P., Ng, W.-M., Wei, W. (2008). Identification of pyruvate kinase type M2 as potential oncoprotein in squamous cell carcinoma of tongue through microRNA profiling. International Journal of Cancer, Vol.123, No.2, (July 2005), pp. 251–257, ISSN 0020-7136

Wong, T.-S., Liu, X.-B., Wong, B. Y.-H., Ng, R. W.-M., Yuen, A. P.-W., and Wei, W. I. (2008). Mature miR-184 as Potential Oncogenic microRNA of Squamous Cell Carcinoma of Tongue. Clinical Cancer Research, Vol.14, No.9, (May 2008), pp. 2588–2592, ISSN 1078-0432

Wong, T.-S., Man, O.-Y., Tsang, C.-M., Tsao, S.-W., Tsang, R. K.-Y., Chan, J. Y.-W., Ho, W.-K., Wei, W. I., and To, V. S.-H. (2011). MicroRNA let-7 suppresses nasopharyngeal carcinoma cells proliferation through downregulating c-Myc expression. Journal of cancer research and clinical oncology, Vol.137, No.3, (March 2011), pp. 415–422, ISSN 0171-5216

Yu, C.-C., Chen, Y.-W., Chiou, G.-Y., Tsai, L.-L., Huang, P.-I., Chang, C.-Y., Tseng, L.-M., Chiou, S.-H., Yen, S.-H., Chou, M.-Y., et al. (2011). MicroRNA let-7a represses chemoresistance and tumourigenicity in head and neck cancer via stem-like properties ablation. Oral Oncology, Vol.47, No.3, (March 2011), pp. 202–210, ISSN 1368-8375

Yu, Z., Li, Z., Jolicoeur, N., Zhang, L., Fortin, Y., Wang, E., Wu, M., and Shen, S.-H. (2007). Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids Research, Vol.35, No.13, (June 2007), pp. 4535–4541, ISSN 0305-1048

Zhang, L., Deng, T., Li, X., Liu, H., Zhou, H., Ma, J., Wu, M., Zhou, M., Shen, S., Li, X., et al. (2010). microRNA-141 is involved in a nasopharyngeal carcinoma-related genes network. Carcinogenesis, Vol.31, No.4, (April 2010), pp. 559–566, ISSN 0143-3334

Zhang, S., Hao, J., Xie, F., Hu, X., Liu, C., Tong, J., Zhou, J., Wu, J., and Shao, C. (2011). Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development. Carcinogenesis, Vol., (July 2011), ISSN 0143-3334

Zhang, X., Cairns, M., Rose, B., O'Brien, C., Shannon, K., Clark, J., Gamble, J., and Tran, N. (2009). Alterations in microRNA processing and expression in pleomorphic adenomas of the salivary gland. International Journal of Cancer, Vol.124, No.12, pp. 2855–2863, ISSN 0020-7136

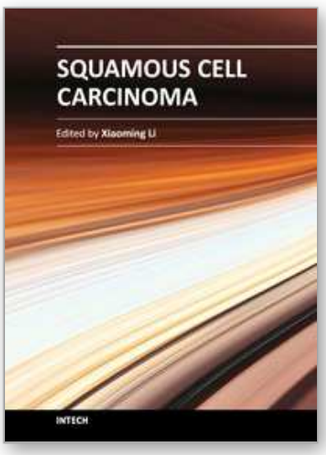
Zhang, X., Yang, H., Lee, J. J., Kim, E., Lippman, S. M., Khuri, F. R., Spitz, M. R., Lotan, R., Hong, W. K., and Wu, X. (2010). MicroRNA-related genetic variations as predictors

for risk of second primary tumor and/or recurrence in patients with early-stage head and neck cancer. Carcinogenesis, Vol.31, No.12, (December 2010), pp. 2118–2123, ISSN 0143-3334

Zheng, Z.-M., and Wang, X. (2011). Regulation of cellular microRNA expression by human papillomaviruses. Biochimica et biophysica acta, (2011), ISSN 0006-3002

Zidar, N., Boštjančič, E., Gale, N., Kojc, N., Poljak, M., Glavač, D., and Cardesa, A. (2011). Down-regulation of microRNAs of the miR-200 family and miR-205, and an altered expression of classic and desmosomal cadherins in spindle cell carcinoma of the head and neck--hallmark of epithelial-mesenchymal transition. Hum Pathology, Vol.42, No.4, (April 2011), pp. 482–488, ISSN 0046-8177

Zubakov, D., Boersma, A. W. M., Choi, Y., van Kuijk, P. F., Wiemer, E. A. C., and Kayser, M. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. International Journal of Legal Medicine, Vol.124, No.3, (May 2010), pp. 217–226, ISSN 0937-9827



Squamous Cell Carcinoma
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This book points to some new areas for investigation on squamous cell carcinoma (SCC). Firstly, the features and management of some specific SCC is discussed to give the readers the general principles in dealing with these uncommon and sophisticated conditions. Some new concepts in adjuvant therapy including neoadjuvant therapy and gold nanoparticle-based photo dynamic therapy are introduced. Secondly, a detailed discussion of molecular aspects of tumor invasion and progression in SCC is provided with the emphasis on the roles of some important factors. The role of tumor microenvironment in head and neck SCC is specifically discussed. Thirdly, the roles of cancer stem cells (CSC) in cancer therapy of SCC are described. Molecular mechanisms involving therapeutic resistance and new therapeutic strategies targeting CSC are discussed in detail. Finally, other aspects concerning SCC are included, which involve the assessment, genetic manipulation and its possible clinical implications for the treatment of SCC.

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