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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 paranasal 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 Cenorhabditis 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 cytoplasm 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 (microRNA 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 cancer (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
We here performed a review on the potential oncogenic microRNA and tumor suppressing microRNA identified in HNSCC.

6.1 Let-7 Family


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 - uagcuauacagacugauugu - 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 - uagcaccauuugaaaucgguu - 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 - uuuggucccuuaacacagcu - 74 (MI0000450)] and miR-133b [Mature sequence: 66 - uuuggucccuuaacacagcu - 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 - agcugguguuguaucggcg - 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 - uaacugucuguagaugug – 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 - uggacggagaacuguaaaggu – 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 - uccuuaaucacggagucug – 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 - agcuacaucggucauccgggu – 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).
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## Table 1. MicroRNA dysregulation in HNSCC

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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 miR-363 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 methods, 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 capillary 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


MicroRNA Dysregulation in Squamous Cell Carcinoma of Head and Neck


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


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 photodynamic 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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