MicroRNAs (miRNA) were studied recently to a large extent in order to find out their role in the development of tumorigenesis. Continuous growth and expansion was observed in the microRNA gene family with the discovery of new members and advances in genome technologies. To date, 2042 microRNA species are known in humans. They are noncoding, highly conserved and double stranded RNA molecules comprising of 18 – 25 nucleotides. miRNAs are capable of regulating the expression of different genes in biological processes like apoptosis, differentiation and proliferation at the post transcriptional level (6). Characteristically, miRNAs or mimics to effectively inhibit cancer.

MicroRNAs and head and neck cancer

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Dysregulation (which can be in the form of overexpression or loss of expression) of miRNA is associated with different types of human cancers, as miRNA affects cell proliferation, differentiation and apoptosis. Expression profiles of miRNA have been reported to be altered for HNC in comparison with normal tissues (7-13). Micro RNAs possess the ability to behave as proto-oncogenes, can affect different tumor suppressors and can act as tumor suppressors as well. Different types of miRNA are expressed differently in HNC expression profiles, some miRNAs were found to be upregulated and others downregulated in HNC cell lines (8,14). Micro RNA let-7g, miRNA-34a, miRNA-23a and miRNA-139 have been shown to be down regulated in oral squamous cell cancer cell lines as detected by RT PCR (15). Tongue squamous cell carcinoma also showed reduced expression of miRNA-139 and increased expression of miRNA-30a (16). Microarray analysis of head and neck cancer cell line reveal reduced expression of miRNA-449 and miRNA-24 and increased expression of miRNA-23a (8). Micro RNA expression analysis through microarray on animal model (hamster) of oral squamous cell carcinoma showed reduced expression of miRNA-26a (17). Major breakthroughs in miRNA biology have considerably improved our understanding of the mechanisms which underlie cancer and there is a rapidly expanding list of miRNAs which are currently being targeted in preclinical studies to inhibit cancer progression, reverse drug resistance and induce apoptosis.

p16 and head and neck cancer

CDKN2A gene produces 2 major proteins: p14(ARF), which binds p53-stabilizing protein MDM2.
and p16(INK4), a CDK inhibitor. HNC is associated with many dynamic alterations in genome and these changes included activation of the proto-oncogenes and/or suppression of the tumor suppressor genes. Cdkn2a/p16 which is located on chromosome 9p21 is a gene involved in tumor suppression. It has been reported that many human cancers are caused because of the functional loss of p16 gene which is mostly due to hypermethylation and less commonly by homozygous deletion and point mutations. Inactivation of p16 accounts for about 80% of molecular abnormalities in HNC and p16 is mostly downregulated in HNC which causes proliferation and tumor formation. Molecular mechanism involved in suppression of tumor formation by p16 gene is that p16 protein binds to CDK4 or CDK6 (cyclin dependent kinases) and thus blocks the proliferation and this process occurs during G1-S phase of cell cycle. P16 gene expression is not found to be associated with age, sex or level of proliferation nevertheless it is associated with location of tumor formation i.e. more decreased expression of p16 in HNC as compared to HNC in pharynx (19, 20). p16 gene was epigenetically silenced in HNC due to factors like hypermethylation of promoter (20).

### NOTCH1 and head and neck cancer

Notch is expressed as a heterodimer on cell surface, consisting of an extracellularly located ligand binding domain that is linked noncovalently with a transmembrane polypeptide. Signals are transduced intracellularly by interaction of Notch with Jagged or Delta-like ligands (DLLs) that induced a conformational change in Notch and exposed a previously protected site to proteolytic cleavage (21). It has previously been studied that proteolytic cleavage of Notch was triggered by different enzymes including ADAM (a disintegrin and metalloproteinase)-family protease and a γ-secretase complex (22, 23).

Ligand binding consequently induced sequentially cleaved form of Notch, processed initially by an ADAM and afterward by a γ-secretase complex. Final cleavage sequestered Notch intracellular domain (NICD) away from cell membrane and consequently nuclear accumulation of NICD was noted. Mechanistically, NICD facilitated positioning of specific coactivators to transcriptionally upregulate expression of target genes.

There is a direct piece of evidence suggesting considerably downregulated levels of miR-200a-3p, miR-200b-3p, miR-34b-5p, miR-34c-5p and/or miR-200c-3p in Head and neck paragangliomas that often co-occurred with genomically amplified of NOTCH pathway genes (24). Circumstantial evidence also revealed frequently mutated Notch pathway in 35.2% of oesophageal squamous cell cancer cases. 8 cases were found to be mutated for NOTCH1, 4 mutations in NOTCH2 and 2 mutations in NOTCH3 have also been reported. The mutation and amplified NOTCH1, NOTCH2 and NOTCH3 were detectable in 16.4% of cases (25).

**NOTCH pathway plays an important as tumor sup-**

<table>
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<tr>
<th>Cancer/Cancer cell lines/Cancer cells</th>
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<td>miR-21, miR-200c, miR-34a, miR-375</td>
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<td>Cancer Type</td>
<td>miRNA</td>
<td>Effect</td>
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<td>Head and Neck squamous cell carcinoma (HNSCC)</td>
<td>miR-196a</td>
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<td>Combined up-regulation – † †, Down-regulation = †, Inhibition = ‡, Combined up-regulation = † †</td>
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<td>miR-143</td>
<td>Viability, colony formation, and anchorage-independent growth ‡ ‡, KRAS ‡ ‡</td>
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<td>Oral squamous cell carcinoma (OSCC)</td>
<td>miR-26a/b</td>
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<tr>
<td>Esophageal cancer (EC)</td>
<td>miR-21, miR-143, miR-196a, miR-203, miR-205 and miR-221</td>
<td>Combined up-regulation = † †, Down-regulation = †, Inhibition = ‡, Combined up-regulation = † †</td>
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<tr>
<td>Head and Neck squamous cell carcinoma (HNSCC)</td>
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<td>miR-93 overexpression leads tumour progression, metastasis and poor prognosis (82)</td>
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<tr>
<td>Esophageal squamous cell carcinoma (ESCC)</td>
<td>miR-101 and miR-217</td>
<td>(MALAT1, migration and invasion) †</td>
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<tr>
<td>Head and Neck squamous cell carcinoma (HNSCC)</td>
<td>miR-196a</td>
<td>Annexin A1 (Cell proliferation, migration and invasion and epithelial to mesenchymal transition) †</td>
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<td></td>
</tr>
</tbody>
</table>

This section mainly deals with intricate modulation of NOTCH1 by different miRNAs. miR-34a is highly expressed in normal spleen, adrenal gland, testis and lung tissues and is one of the first and best-studied miRNAs related to carcinogenesis. miR-34a is commonly repressed in tumors like neuroblastoma, colon cancer.
and non-small-cell lung cancer cell lines. Forced expression of miRNA-34a causes reduction in the ability of cell to invade in case of cervical cancer through reduced NOTCH1 expression (34). miRNA-139-5p is also reportedly downregulated in colorectal cancer. miRNA-139-5p inhibited cell proliferation, apoptosis, metastasis and also arrested G0/G1 phase of cell cycle through NOTCH1 repression (35). miRNA-449a quantitatively controlled NOTCH1 by binding to its 3’UTR and inhibiting its expression. Therefore if miRNA-449a expression is reduced it may lead to cancer formation through cell proliferation pathways (36).

**Interplay of Notch and miRNAs in different Cancers**

MITF has been shown to bind and transcriptionally repress miR-222-221, RBPK1-dependently. However, when melanoma cells are in contact with distal Notch ligands expressing differentiated keratinocytes, the activated NICD inhibits MITF positioning at promoter region of miR-222-221. De-repression of miR-222-221 induced invasive phenotype (49). There is evidence of miR-19a induced activation of Notch signaling and considerably enhanced cell proliferation, migration and invasive potential of cancer cells (50). Overexpression of Notch signaling was noted in miR-9 expressing breast cancer MDA-MB-231 cells. There results revealed that metastasizing potential of breast cancer cells can be inhibited by targeting of miR-9 (51).

F-box and WD repeat domain-containing 7 (Fbw7) is reportedly involved in targeting of Notch via proteasomal degradation. However, miR-223 mediated targeting of Fbw7 in drug resistant pancreatic cancer cells stabilized Notch1 protein levels. Targeting of miR-223 significantly enhanced Fbw7 levels and Notch levels were dramatically reduced (52).

However, it has recently been convincingly revealed that apoptotic activity reduced substantially in cancer cells simultaneously treated with antisense against miR-100 and Notch signaling inhibitor (53).

**Association of microRNAs with p16**

Micro RNA-24 has been known to be upregulated in human cancers of breast, oral, prostate, hepatic and lung, supporting the significance of miRNA-24 in cell proliferation (37-40). Cell proliferation of primary and cancer cells is controlled by p16 expression and inturn its expression is controlled by microRNA-24 (41). Another family of microRNAs known as let-7 is involved in many cancers via different molecular pathways. One of them, has-let-7g, plays an important role in inhibiting hepatocellular carcinoma via two pathways. One by inhibiting onco gene c-Myc and second is the activation of tumor suppressor gene p16. Increased expression of miRNA-23a and miRNA-30a increased expression of p16. However increased miRNA-26a directly correlated with p16 expression but surprisingly inhibition of miRNA-26a did not reduce p16 levels (42).

Hotair (Hox transcript antisense intergenic RNA) is a 2158-bp IncRNA located in the Hoxc gene cluster and reportedly upregulated in hepatocellular carcinoma. Intriguingly, p16 expression was notably enhanced in Hotair silenced cells (43).

Very little is known about miRNA regulation of Notch signaling in head and neck cancer, however, certain hints have emerged from renal cell carcinoma and hepatocellular carcinoma.

**Clinical Trials of Notch Signaling Inhibitors**

Confusion of information suggested testing of clinical efficacy of different Notch signaling inhibitors. RO4929097, a gamma secretase inhibitor (GSI) of Notch signaling has been noted to be ineffective against platinum resistant ovarian cancer (44). RO4929097 was also clinically insufficient against metastatic melanoma in this phase II clinical trial (45). Patients with advanced solid tumors combinatorially treated with RO4929097 and Gemcitabine revealed a prolonged stable disease in 3 patients and notable response in 1 patient with nasopharyngeal carcinoma (46). Initial testing of MK-0752 was disappointing as evidenced by different reports. Toxicity management of Notch inhibitor MK-0752 in combination with dalotuzumab was challenging in advanced solid tumors and all of 12 evaluable patients experienced disease progression in the first radiological evaluation (47).

Anti-DLL4 agents are also being tested for efficacy however no recommended phase II schedules or doses have been optimized. Reportedly, complications associated with continuous antibody mediated Notch inhibition included development of neovascular tuft formation and angiomas (48). MEDI0639, a DLL4 (IgG1α) monoclonal antibody that abrogated protein-protein interaction of DLL4 and Notch 1, is being evaluated for efficiency in patients with solid tumors (48). DLL4 antibody (Demcizumab or OMP-21M18), is another monoclonal being tested for clinical efficacy in ovarian, fallopian and peritoneal cancer, non-small-cell lung cancer, colorectal and pancreatic cancer (48).

It is interesting to mention that tumor suppressor miRNAs are frequently downregulated in cancer cells having deregulated Notch signaling. Different tumor suppressor miRNAs have shown considerable efficacy as tumor growth inhibitors in xenografted mice. Moreover, miR-34a is a tumor suppressor miRNA which has entered into clinical trials. Future studies must converge on combinatorial treatment using Notch signaling inhibitors with tumor suppressor miRNAs in preclinical studies.

**Conclusion**

Wealth of information is emphasizing on contributory role of miRNAs in cancer development, progression and metastasis (54, 55, 59, 60). Tumor suppressor miRNAs have attracted considerable attention and various strategies are currently being used to effectively deliver tumor suppressor miRNAs to target site (56, 57, 58). Head and neck cancer biology has undergone substantial broadening and confluence of information suggested wide ranging oncogenic and tumor suppressor proteins which contribute in cancer development and progression. miRNAs have also occupied central stage in molecular oncology and researchers have started to develop deeper understanding of the misrepresentation of miRNAs in head and neck cancer.
However, population specific data is still insufficient and future studies emphasizing on intra/inter ethnic variability in different genes in head and neck cancer will bridge the existing gaps. Notch and miRNA interplay needs closer analysis because of context and cell type specific behavior. Different tumor suppressor and oncogenic miRNAs have been evaluated in xenografted mice and comprehensive knowledge of different intra-cellular signaling cascades and interplay with miRNAs will be helpful in identification of true potential of therapeutics with minimal off target effects and significant clinical outcome.

Other articles in this theme issue include references (87-98).

References


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