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The Emerging Role of Micro RNA21 in Oral Cancer

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Oral cancer is one among the leading causes of death in developing countries of south Asia. A very high incidence of oral cancer in India has resulted due to the prevalence of tobacco use both in smokable and Chewable forms. Though molecular level changes that occur in the initiation and progression of oral cancer has been studied, the disease process is still poorly understood unlike other cancers. MicroRNAs are the trending name in cancer research. They are non-coding RNAs that control the genome by their complementarity and affect protein synthesis. Their role in various cancers have been well studied. This paper enlightens the role of MicroRNA21 in oral cancer.

Keywords: Biomarker, Squamous cell carcinoma, non-coding RNA.

Oral cancer is one of the widely prevalent cancer in the developing countries with a worldwide age standardized incidence of 12.9 and 4.5 per 1,00,000 in men and women respectively[1]. Oral squamous cell carcinoma(OSCC) is the most common type of oral cancer that affects the epithelial cells of the mucosa covering the oral cavity and presents with varying degrees of differentiation[2]. Though the pathogenesis of the disease is clear, the prognosis of these lesions is still highly unpredictable. Treatment planning for the cure lies purely on the clinical staging and histopathological grading. However, the stage and grade of these tumors are usually not highly correlative and at times different grades of differentiation can be noted within the same tumor[3]. The survival rates of these patients have been highly compromised due to factors like late diagnosis, disease spread and lack of targeted therapy[4]. Identification of a biomarker to overcome these pitfalls is the need of the hour.

The new era of cancer research has been started with the discovery of certain non-coding RNA molecules named MicroRNAs that regulate gene expression post transcriptionally[5]. Though these RNAs do not code for any protein, they control protein synthesis by base pairing to the target mRNA thereby resulting in either translational repression or complete degradation of the mRNA itself. The partial complementarity of the MicroRNAs to their target transcripts enables them to regulate hundreds of genes simultaneously. Through this enormous modulatory potential, MicroRNAs play important roles in a wide range of physiological processes like cell differentiation, proliferation, motility and apoptosis[6]. Since cancer results due to the deregulation of the above mentioned cellular processes it can be well justified that microRNAs play a very significant role in both tumor initiation and progression as proven by various studies. Thus microRNAs have directed the focus of oral cancer research towards genetic

and epigenetic alterations with a goal to discover specific biomarkers that would equip us with early diagnosis, prediction of Prognosis and targeted therapy.

MicroRNA 21(miR-21) is the most studied oncogenic microRNA in the field of cancer. Mapped on chromosome 7q23.2, it is the most conserved microRNA in mammals[7]. Till date miR-21 is upregulated in all the studied human cancers including head and neck, breast, lung, stomach, colon, pancreas, prostate, thyroid, ovary, cervix and other hematological malignancies like leukemia, and B cell lymphomas. It regulates various genes that are involved in oncogenic pathways[8]. This Review describes the implications of microRNA 21 in the pathogenesis of oral cancer and its potential role in the prognosis of the disease.

Expression of MicroRNA 21 in oral cancer

MicroRNA21 is the most consistently deregulated microRNA in OSCC. A review conducted by Kolokythasin 2011 showed that 7 out of 9 studies demonstrated an upregulation of miR-21 in oral cancer andmiR- 21 was the highest expressed microRNA among 255 miRNAs that were identified in OSCC tissues.[9],[10, 11] We analyzed the expression of miR-21 & miR-375 in 25 cases of OSCC. Our study results revealed a significant upregulation of miR-21 in OSCC with a 17.39-fold elevation compared to its paired normal tissues of the oral cavity. (unpublished data) miR-21 expression in oral cancer tissues is higher in the center of the lesion compared to the tumor margins and is intense in the cytoplasm than the nucleus of the tumor cells.[12] While most of the studies revealed the upregulation of miR-21 in the squamous dysplastic epithelial, Nora *et al* was the first to localize its expression in myofibroblasts, endothelial and salivary acinar cells of the tumor stroma[13]. In addition to tissues miR-21 has also be quantified from oral cytological samples, serum and saliva of oral cancer patients.[14, 15, 16,17]

Oral Carcinogenesis

Oral carcinogenesis is a multistep process involving sequential genetic events that leads to the disruption of proliferation, differentiation and death of the squamous cells.[18] The etiological factors such as tobacco, alcohol & human papilloma virus(HPV)induce various epigenetic and genetic alterations that imparts genomic instability to the oral epithelial cells.[19] Our previous study revealed that Expression of miR-21 was associated with smoking (unpublished data) and a recent studies have revealed an increased expression of miR-21 in pan masala chewers and alcoholics.[16, 20] Nicotine induced upregulation of miR-21 through the EMT transforming growth factor beta (TGF-â) pathway was demonstrated by Zhang *et al*.[21] Recent cancer statistics shows an increased incidence of oral cancer among nonsmokers and younger individuals.[22] About 70% of oropharyngeal cancers may be caused by HPV.[23] The location of the miR-21 gene is at the fragile site of FRA17B in 17q23.2. This site is considered as one of the loci for integration of the HPV in humans. The elevation of miR-21 in OSCC can be attributed to genetic alterations produced by HPV at the miR-21 genetic loci as a similar association has been proposed by Yin-Hsun Feng in cervical squamous cell carcinomas caused by HPV.[24]

MicroRNA 21 and Tumor formation

MicroRNA-21 serves as an oncogene and modulates tumorigenesis in many cancers.[24] There is a steady increase in expression of miR-21 from normal mucosa to leukoplakia to OSCC[25]. Cervigne *et al* studied the changes of microRNA expression in lesions that progressed from leukoplakia to OSCC. miR-21showed a consistent increase in expression which was associated with the severity of the lesion suggesting its possible role in tumor initiation.[26] In a similar study conducted by Pooja *et al* the miR-21 levels were higher in the serum of OSCC cases compared to oral sub mucous fibrosis patients.[16]Antagonizing miR-21 with specific antisense oligonucleotide in oral cancer cell lines inhibits tumor formation in nude mice.[27] The cyclooxygenase/prostaglandin E2 (PGE2) pathway of inflammation contributes to the development and progression of squamous cell carcinoma of the oral cavity.[28]Qianting He *et al* demonstrated that miR-21 regulates prostaglandin regulatory pathway by targeting Hydroxyprostaglandin Dehydrogenase, and this regulatory mechanism plays a critical role in the initiation of tongue squamous cell carcinoma(TSCC).[29]There is a gradual reduction of miR-21 expression in the plasma of OSCC patients post surgically compared to Pre surgery. This finding equips us with high evidence that miR-21 is related to oral cancer pathogenesis.[30]

Tumor growth, invasion and metastasis

Tumor growth is a complex process

that depends on factors like sustained growth signals and angiogenesis. Antagonizing miR-21 in TSCC(Tca8113) cell lines demonstrated an inhibition of miR-21 expression that led to a suppression of tumor growth, reduced atypia and decreased angiogenesis.[31] Inhibition of miR-21 in OSCC also showed a Phosphatase and tensin homolog (PTEN) mediated S-G2/M cell cycle arrest.[32] A recent study also proved that miR-21 promotes tumor growth by modulating clusterin(CLU) gene in SCC cell lines.[33]

Invasion and metastasis are the two most important prognostic indicators of OSCC. Metastasis is a multi-step process that aids distant spread of the disease. Epithelial-mesenchymal transition(EMT) is a major event in oral carcinogenesis that equips the squamous cells with both invasive and metastatic properties.[34, 35] Wei Liu *et al* demonstrated p38MAPK signal pathway mediated inhibition in proliferation, invasion and migration of head and neck squamous cell carcinoma(HNSCC)cells by suppressing the maturation of miR-21 with Sophocarpine. The study also concluded that the EMT in SCC can be inhibited and reversed by modulating miR-21.[36] Injection of miR-21-3p inhibitor caused abrogation of the invasive abilities of OSCC cell lines.[37] Kawakita A*et al* showed that miR-21 targets DKK gene and promotes invasion of TSCC through the Wnt/â-catenin pathway.[38]

Programmed cell death -4 (PDCD4) gene plays a pivotal role in transcription and translation of proteins involved in neoplastic transformation. It is down regulated in oral cancer and is associated with nodal metastasis and invasion. miR-21 binds to the 3'UTR of PDCD4, causing its down-regulation thereby promoting proliferation, invasion and metastasis.[39]The primary metastatic sites of OSCC include the cervical lymph nodes. miR-21 promotes metastasis in OSCC by targeting slug transcription factors and its expression directly correlates with lymph node metastasis in TSCC. Additionally, miR-21 is proven to impart anchorage independent growth to oral cancer cells which is very crucial for metastasis. [40, 27]

In contrary to all the studies conducted Chih-Yu showed a downregulation of miR-21 in OSCC cell lines. The study also tagged miR-21 as a tumor suppressive gene as its suppression enhanced tumorigenicityand metastasis in OSCC.[41]

Apoptosis

MicroRNA 21 plays a crucial role in the apoptosis of oral cancer cells. Pivotal apoptotic genes such as PDCD4, PTEN and B cell lymphoma-2 (Bcl-2) are its direct targets.[42] PTEN and Tropomysin-1 (TPM1) are tumor suppressor genes that are frequently mutated in various human cancers. Díez-Pérez *et al* reported an 77.8% reduction of PTEN gene expression in oral cancer compared to the normal oral tissues.[19] TPM1 induces apoptosis in cancer cells. The expression of TPM1 and PTEN is inversely correlated with miR-21 levels in OSCC and suppression of miR-21 with ASO induces apoptosis in TSCC cell lines.[27] miR-21 upregulates Bcl-2 and Prevents apoptosis in lung, colon and pancreatic cancer. However, there is no evidence about the effect of miR-21 on Bcl-2 in oral cancer. Studies reveal that miR-21 controls apoptosis in cancer cells via the caspase-dependent pathway by blocking the release of cytochrome-c enzyme from mitochondria into the cytosol.[42,43] These data indicate that increased expression of miR-21 leads to evasion of apoptosis thereby enhancing cell survival in OSCC

MicroRNA-21 and OSCC prognosis

MicroRNA 21 imparts an enormous prognostic impact on OSCC. It is negatively correlated with the prognosis of oral cancer which can be proven by the fact that its expression increases with the clinical stage, histopathological grade and metastasis of the tumor. Overexpression of miR-21 exhibits poor survival tendencies in TSCC patients and a recent meta-analysis concluded that mir-21 can predict poor prognosis in oral cancer.[27,44]

Neural invasion of the cancer cells is considered as a poor prognostic indicator of the disease. En-Hao-Yu *et al* (2017) demonstrated an association between miR-21 expression and perineural invasion in OSCC and also proposed that miR-21 could promote the invasion of tumor cells into the nerve bundles. However, the probable molecular pathways that enable such invasion is not clear.[12]In addition to imparting tumerigenicity to cancer cells, expression of miR-21 in stromal cells and its role in EMT are two independent factors that support unfavorable prognosis in OSCC.[13,36]

Resistance to chemotherapy and radiotherapy is a serious obstacle in cancer therapy that worsens the prognosis. Response

to chemo radiation is poor in patients with high miR-21expression and miR-21 dysregulation imparts chemo resistance to cisplatin in OSCC by targeting the PTEN and PDCD4 genes.[20,45] miR-21 inhibiting therapy would become a promising mode of improving the therapeutic effects of OSCC in future.

CONCLUSION

Having reviewed the comprehensive role played by miR-21 in all the stages of oral carcinogenesis, miR-21 can be labelled as a potential biomarker for both the diagnosis and prognosis of the disease. Its expression pattern from oral potentially malignant conditions to oral cancer promotes its role as an early disease marker for OSCC. Though studies have highlighted its role as a therapeutic agent and chemo modulator for OSCC, future avenues of research should focus to explore novel therapeutic approaches that manipulate miR-21 expression in an aid to cure oral cancer. To conclude miR-21 can serve as a novel diagnostic, prognostic and therapeutic marker for oral cancer.

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