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Review Article

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MICRORNAS AS A NOVEL BIOMARKER IN ORAL CANCER

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ABSTRACT

Oral cancer is one of the commonest cancers in the world which spread from oral cavity to neck lymph nodes. However the oral cancer is not easily diagnosed until their advanced stage which is the main cause for the low survival rate of patients. In recent years, the growing interest of researchers to investigate the role of microRNAs (miRNAs) in various biological processes as well as many disease including carcinomas. With the advent of next generation sequencing technique, it has been determined that about 1400 miRNAs act as oncogenes and tumor suppressor genes, these play the pivotal role in developmental processes, growth control and in various human diseases like cancers. With the altered expression of tumor suppressor as well as oncogenic miRNAs, various processes such as pathological and biological have been found in altered form in various studies. Alteration in gene expression helps in the diagnosis of cancers at early stage. In this review we focused on the role and significance of tumor suppressor as well as oncogenic miRNAs as an emerging novel biomarker for oral cancer.

KEYWORDS: MicroRNA, OSCC, Oncogene, Gene expression.

INTRODUCTION

Oral cancer is the sixth most common human cancer in men and women,[1] which signify that three percent of all types of human carcinoma. Oral cancer exhibits precancerous lesions like Leukoplakia & Erythroplakia. It has been found that >300,000 new cases of oral squamous cell carcinoma (OSCC) are diagnosed yearly.[2] Its incidence rate is 650,000 while 350,000 death occur in United State alone yearly.[3,4] In the developed countries, the overall incidence rates are very high with 4.0-6.8 per 100,000 in males and 0.8-4.5 per 100,000 in females.[5]

In India as well as south and Southeast Asian countries all have highest incidence rate of oral cancer. With 90% to 95% of oral squamous cell carcinoma in oral cancer in Indian population[6]. This data shows that Head & Neck Squamous Cell Carcinoma (HNSCC)is the 4th most common cancer in men while 9th most common cancer in women.[7] Oral cancer begins in the mouth or oropharynx. It is particularly dangerous because patients do not notice in the early stage. It frequently prospers without producing pain or symptoms with high recurrence rate. Often oral cancer appears when the cancer metastasized to another location most likely to lymph nodes of the neck due to this, its prognosis at this stage is significantly worse.[8]

Nowadays early detection of oral cancer is attracting a lot of researchers and clinicians towards the identification of altered miRNAs. Many recent studies have been identified that miRNAs are playing a major role in different types of biological and pathological processes, mainly in the progression of cancers and also have been revealed that all types of cancer with their tumor staging and treatments are associated with the altered expression of miRNAs. Consequently miRNAs are considered as an emerging prognostic and diagnostic biomarker in addition to cancer therapeutic marker. There are numerous approaches to know the molecular basis of oral cancer.[9-11] These are microarray technology, methylation microarrays, gene expression microarrays, mitrochondrial array and miRNA arrays. In recent years miRNAs array are in use to investigate oral cancer in solid tissue and biofluids like plasma, serum, saliva[12] and urine.[13] On the basis of miRNAs present in different types of solid tissues and fluids, it is classified as tumor tissue miRNAs and circulating miRNAs, while the biopsy procedure of tumor tissue is painful and higher risk procedure for cancer patients so circulating miRNAs are identified as an non invasive biomarker to detect oral cancer at their early stage.

Risk Factors for Oral Cancer

Use of tobacco and Alcohal consumption are the major risk factor for oral cancer. Drinking and Smoking both are independent factors but they have synergistic effect

and significantly increase the risk of oral cancer.[14,15]

Micro –RNA

MicroRNAs (miRNAs) are small non-coding RNAs which involves in the post transcriptional modification of coding RNA. These non coding small regulatory RNA are evolutionarily conserved and widely spread among different species (June et al., 2011). Lee et al. discovered the first micro RNA in *C. elegans* in 1993 which was known as LIN-4.[16] Molecular markers play significant role in clinical diagnosis due to progression of advance techniques in molecular biology (Hui et al. 2010).[17] When miRNAs expression pattern of cancer and normal tissue were compared by Krutovskikh et al. in 2010, found that it may help in monitoring different types of carcinoma.[18] In many studies MiRNAs are considered as a potential biomarker for prognosis, cancer onset at early stage and categorization of different types of cancer.[19] MiRNAs are stable biomarker because they are resistant to degradation because they get packaged into microvesicle, exosomes, apoptotic bodies or they form the miRNA– Protein complex (Li et al. 2007).[20] These miRNAs are found in significant amount in different fluids such as saliva, peripheral blood, urine and semen (Mitchell et al., 2008; Hanke et al., 2009; Park et al., 2009; Zubakov et al. 2010).[21,22]

Biogenesis of miRNA

miRNAs are important key regulatory factors of gene expression, they involve in either repression or degradation of their target messengerRNAs (mRNAs).[23-25] miRNAs represent 1%-3% of the whole mammalian genome.[26] These micro RNAs are found in both introns as well as in exons. They are transcribed by RNA polymerase II and involve in the formation of the primary precursor miRNA (pri-miRNA) which is generated by stem loop precursor. Pri- mi RNA resembles with the messenger RNA in the features like 5' cap and 3'poly AAA tail. In nucleus the stem –loop structure is processed by DROSHA and DiGeorge Syndrome Critical Region 8 (DGCR8) enzyme and produce as a precursor miRNA (pre-miRNA). Then these pre-miRNAs is transported from nucleus to cytoplasm through nuclear transporter known as Exportin-5 with the help of nuclear monomeric G protein (Ran). In the cytoplasm pre-mi RNA is processed by Dicer to produce duplex miRNA complex, after the processing of duplex micro RNA, one strand of miRNA is incorporated into RNA inducing silencing complex (RISC) to either suppress or degrade the target mRNA gene after that Argonaute (Ago) proteins joins with the RISC complex, which plays the important role in transcriptional and post-transcriptional gene silencing of targeted mRNA (Fig.1).[26-2]

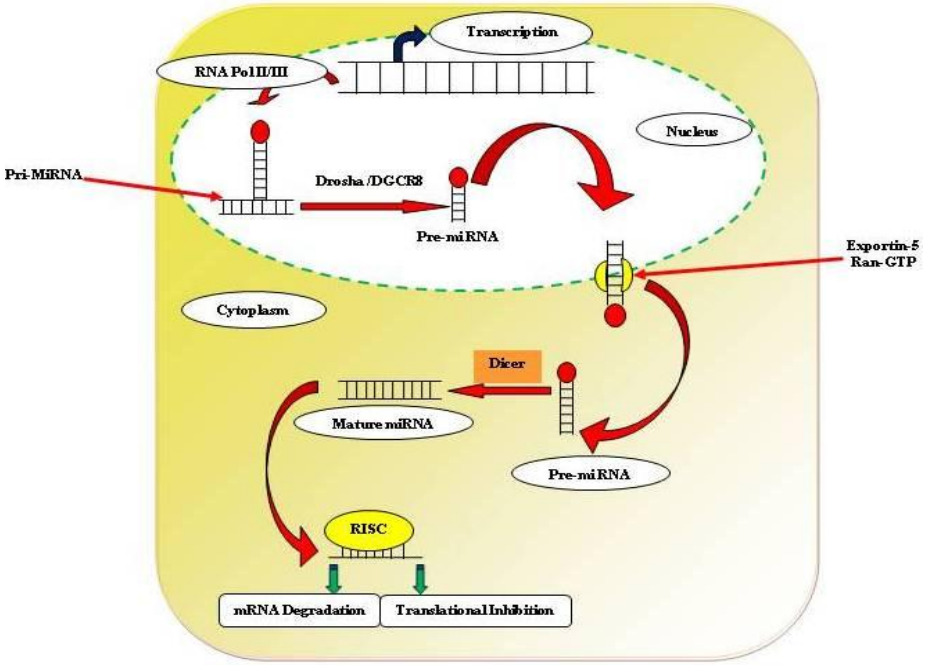

Fig. 1. This figure is Showing microRNA processing and its role in mRNA degradation and Translational Inhibition *(Taken from the review of Ahmad J et al. 2013).*

There are two types of miRNAs on the basis of its occurrence, work as a functional novel biomarkers for the detection of various types of cancer at their early stage, are given bellow.

- **Tumor Tissue miRNA**
- **Circulating miRNA**

Tumor Tissue miRNA

Various studies have been explored that the different expression profile of miRNAs play important role, to identify the tissue origin for tumors whose origin is not known. It is also involve in the diagnosis of subtypes of tumors or the tissues which are poorly differentiated on the basis of tissue specific deregulation of miRNAs.

Evaluations of miRNAs do not require large amount tissue biopsy. According to recent researchers, it have been found that miRNAs can be measured in formalin–fixed paraffin–embedded tissues (FFPE) of pappilary thyroid carcinoma,[29] gall bladder cancer, lung cancer,[30] renal tumor,[31] hepatocellular carcinoma[32] including oral cancer.[33]

Circulating miRNA

In 2008, circulating miRNAs have been discovered in mammalian body fluids such as saliva, serum, plasma and sputum[34] and also present in solid tissues. The National Cancer Institute defined miRNAs as biomarkers in blood, or other body fluids, that is used for the diagnosis of healthy or diseased person. Different studies have been described that, circulating miRNAs play as a regulatory roles in near about every physiological and pathological aspect of biology.[35,36] These microRNAs are stable biomarkers and shows variability in expression patterns.[37,38,39] Body fluids contain ribonuclease which can degrade miRNA so extracellular miRNAs evolve the new way to protect from the RNase digestion by packaging into vesicles and different proteins. These cancer miRNAs biomarkers should be diagnostic, prognostic and predictive with high sensitivity, specificity.[35,36]

(a) Oncogenic miRNAs in Oral Squamous Cell Carcinoma

Micro RNA-21 in Oral Cancer

In oral cancer miRNA-21 acts as oncogene and it targets phosphate pensin (PTEN).[40,41] In many studies it has been concluded that up-regulation of micro RNA 21 involves in the low expression of PTEN.[42] and their transcriptional regulator Grhl3.[43] PTEN involves in the inhibition of phosphatidyl inositol-3 kinase pathway (PI3K). When expression of PTEN down-regulate, then PTEN is unable to inhibit the PI3K pathway.[44]

microRNA-31

miRNA-31 shows up-regulation in oral leukoplakia and OSCC. They play oncogenic role in OSCC.[45-49] Liu et al. (2010) suggested that ectopic expression of miR-31 involves in the repression of its target factor, inhibiting hypoxiainducible factor (FIH) expression to activate hypoxianinducible factor(HIF) under normoxic conditions, both in vitro and in vivo.[45] The signal cascading of miRNA-31-FIH-HIF-VEGF affects many biological processes such as cell migration, proliferation and epithelial mesenchymal transition (EMT) in OSCC.[46]

microRNA-134

MiRNA -134 is over-expressed in HNSCC patients when these samples are compared to normal controls.[50]

microRNA-146a

MiRNA-146a is up-regulated in OSCC. Recent studies revealed that down-regulation of IL-1 receptor associated kinase-1(IRAK-1), NUMB and TNF receptor associated factor-6(TRAF-6) is related with oncogenic function of miRNA-146 in OSCC.[49-51]

microRNA–155

MiRNA-155 is up-regulated in OSCC patient.[52] The oncogenic role of miRNA-155 is linked with down-regulation of a tumor suppressor CDC73 in OSCC.[53,54]

(b) Tumor Suppressor MiRNA in Oral Squamous Cell Carcinoma

microRNA-7

miRNA-7 plays the role as tumor suppressor in many human cancer like breast cancer, glioblastoma including oral squamous cell carcinoma. In many studies it have been confirmed that there are many protooncogenes, which work as target genes of miRNA-7 like insulin receptor substrate 1(IRS1), Insulin receptor substrate 2(IRS2), v-raf1 murine leukemia viral oncogene homologue1(RAF1) and p21/CDC42/RAC1 activated kinase1(PAK1).[55,56.57] Jiang et.al. concluded that miRNA-7 is involved in regulation of IGFIR/IRS/PI3K/Akt signaling cascade by regulation of insulin like growth factor 1 receptor at post-transcriptional level in cells of tongue squamous cell carcinoma (TSCC).[58]

microRNA-99a

The target genes of miRNA-99a are IGF1R and mTOR (mammalian target of rapamycin), that plays the crucial role in IGF1R signaling pathway. miRNA-99a is downregulated in OSCC patients. It has been seen in many studies that miR-99a play a major role in lymphovascular invasion.[59,60]

micro RNA-218

It works as a tumor suppressor by the regulation of mTOR in OSCC cases. It has been shown that miRNA-218 is epigenetically silenced in tissue specimens of OSCC.[61]

microRNA- 9

It has been proved that hypermethylation of DNA is associated with decrease expression of MiRNA-9.[62] In oral squamous cell carcinoma and oropharyngeal carcinoma the molecular process such as DNA hypermethylation downregulates the level of miR-9[63] and miRNA-9 targets CXC chemokine receptor 4 (CXCR4) genes and wnt/β–catenin pathway.[64]

microRNA-138

It has been found that miRNA -138 plays a major role in cell proliferation, migration and invasion in HNSCC derived cells. MiRNA -138 has been shown to regulate EMT related molecules like vimentin (VIM), Foslike antigen-1(FOSL-1), zinc finger E-box binding homeobox2 (ZEB2), RhoC and ROCK2.[65,66,67]

Micro RNA-133 in Oral Cancer

In many studies it has been found that deregulation of gene expression is associated with the altered expression

of miRNA.[68] It has been found in several studies that miRNA 133a and miRNA-133b are down-regulated in tongue Squamous cell carcinoma when they were compared with control tissue samples. MiRNA-133a and 133b are mainly found in muscle cells but their functions are still not known.[69] MiRNA-133a and MiRNA 133b play the main role as tumor suppressing miRNAs in squamous cell oral carcinoma. In many studies it has been found that micro RNA 133a and MiRNA 133b involves in the intrinsic apoptotic pathway. In the glycolytic pathway Pyruvate Kinase enzyme catalyzes the conversion of pyruvate from phosphoenol pyruvate (PEP). Pyruvate Kinase enzyme has four isoforms: these isoforms are L, R, M1 and M2 and these isoforms are specific for tissue site.[70-73] Pyruvate Kinase L (PKL) gene generates two isotypes via alternative promoters like Type L and Type R and the Pyruvate Kinase M genes involves in the generation of type M1 and type M2. These two isotypes have different sites where they play their important roles, the sites of action of Type M1 is brain, heart and muscle cells. Type M2 is found in proliferating cells like neoplastic cells. Pyruvate Kinase M gene have total 12 exons. At exon 9 and exon 10, Pyruvate Kinase M1 and M2 have difference like Exon 9 and Exon 10 determine sequence that are specific for PKM1 and PKM2.[74,75] When the tumor progression occurs the PKM2 replaces the original tissue specific pyruvate kinase isoforms like Type L, R and M1.Tetrameric form of PKM2 have high affinity with Phosphoenol pyruvate in the normal cell while in the cancerous cell PKM2 is found in the dimeric form which have lower affinity with the Phosphoenol pyruvate. PKM2 inactivates the glycolytic pathway,[76] because it is not associated with the glycolytic complex. In the low glucose and oxygen environment PKM2 enhances the tumor progression and invasion.[77] The oncoproteins such as pp60 kinase and E7 of human pappiloma virus helps in the PKM2 dimerization.[77,78] In many studies it has been concluded that PKM2 upregulates in many cancers like skin,[79] gastric, colorectal[80] and cervical cancers.[81] PKM2 transcript was formed by exon 11 of pyruvate kinase M gene, MiRNA-133a and MiRNA-133b binds on the same exonic segment of gene. Wong et al. (2008) concluded that upregulation of PKM2 expression involves in the downregulation of miRNA-133 in all types of tumors, they found that miRNA-133 targets the oncogene PKM2 in squamous cell oral carcinoma.[8]

CONCLUSION

Recent studies of miRNAs research in the cancer biology, have shed light on the role of miRNAs as novel biomarker. However, these biomarker alterations affect diseases such as Cardiovascular disease, Alzheimer disease and cancer including Oral cancer. miRNAs is the non coding RNA, that regulates their target genes in many processes of cancer like cancer initiation, progression and metastasis, which will definitely help the researchers to do comprehensive mechanistic analysis of miRNAs. Future work should be undoubtedly focused on the miRNAs and their co-interaction associated with their target signaling pathways. More detailed study and better understanding of miRNAs will help the researchers to design miRNAs inhibitors for the creation of effective drug therapeutic against cancer including oral cancer.

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REFERENCES

1. Williams HK. Molecular pathogenesis of oral squamous carcinoma. Molecular Pathology, 2000; 53: 165-172.
2. Parkin DM, Laara E, Muir CS. "Estimates of the worldwide frequency of sixteen major cancer in 1980". International Journal of Cancer, 1988; 41: 184-197.
3. Ragin CC, Modugno F, Gollin S. The epidemiology and risk factor of head and neck cancer: a focus on human papilloma virus. J Dent Res., 2007; 86: 104-114.
4. Parkin DM, Bray F, et al. Global cancer statistics, 2002. CA Cancer J Clin, 2005; 55: 74-108.
5. Ferlay J, Shin HR, Bray F, Forman D, Mathers C. GLOBCON. Cancer incidence and mortality worldwide. IARC cancer base no. 10 [internet] Lyou, France: International agency for research on cancer. 2010. Available from:http://globcon.iarc.fr.
6. Sharma M, Madan M, Manjari M, Bhasin TS, Jain S, Garg S. Prevalence of head and neck squamous cell carcinoma (HNSCC) in our population: the clinicopathological and morphological dicription of 198 cases. International journal of cancer research, 2015; 3: 827-833.
7. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBCON 2008. Int J Cancer, 2010; 127: 2893-2917.
8. Gray L. Woolqar J, Brown J. A functional map of cervical metastasis from oral squamous cell carcinoma. Acta Otolaryngol, 2000; 120: 885-890.
9. Campo-TraperoJ, Cano-Sanchez J, Palacios Sanchez B, Sanchez-Gutierrez JJ, GonzaleZ-Moles MA, Bascones-Martinez A. Update on molecular pathology in oral cancer and precancer. Anticancer Research, 2008; 28: 1197-1205.

10. Patel V, Leethanakul C, Gutkind JS. New approaches to the understanding of the molecular basis of oral cancer. Critical review in Oral Biology and Medicine, 2001; 12(1): 55-63.
11. Tsantoulis PK, Kastrinakis NG, Tourvas AD, Laskaris G, Gorgoulis VG. Advances in the biology of oral cancer. Oral oncology, 2007; 43: 523-534.
12. Viet CT, Schmidt BL. Understanding oral cancer in the genome era. Head and Neck. 2010; 32(9): 1246-1268.
13. Jia Y, Guan M, Zheng Z, Zhang Q, Tang C, Xu W, Xiao Z, Wang L, Xue Y. miRNA in urine extracellular vesicles as predictor of early-stage diabetic nephropathy. Journal of diabetes research. 2016; Doi 7932 765.
14. Warnakulasuriya S, Sutherland G, Scully C. Tobacco oral cancer and treatment of dependence. Oral oncology. 2005; 41: 244-260.
15. Ogden GR. Alcohol and oral cancer. Alcohol., 2005; 35: 169-173.
16. Tie J, Daiming F. Big roles of micro RNAs in tumorigenesis and tumor development. Histology and Histopathology., 2011; 26: 1353-1361.
17. Hui AB, Lenarduzzi M. Krushel T, Waldron L, Pintilie M, Shi W, Perez Ordonez B, Jurisika I, et al. Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas. Clinical Cancer Research., 2010; 16(4): 1129-39.
18. Krutovskikh VA, Herceq Z. Oncogeneic micro RNAs (OncomiRs) as a new class of cancer biomarkers. Bio Essays. 2010; 32(10): 894-904.
19. Planell-Saguer M, Rodicio MC, Analytical aspects of microRNA in Diagnostics: A review. Analytical Chemica Acta., 2011; 699(2): 134-152.
20. Li J. Smyth P, Flavin R, Cahill S, Denning K, Aherne S, Guenther SM, O'Leary JJ, Sheils O. Comparision of micro RNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cell. BMC Biotechnology. 2007; 7: 36-41.
21. Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, Jocham D, Warnecke JM, Sczakiel G. A robust methodology to study urine micrRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urologic Oncology., 2010; 28(6): 655-661.
22. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, Wang DT. Salivary microRNA: discovery, characterization and clinical utiity for oral cancer detection. Clinical Cancer Research, 2009; 15(17): 5473-5477.
23. Ambross V. The functions of animal microRnas. Nature., 2004; 431(7006): 350-355.
24. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and post transcriptional gene silencing. Cell, 2005; 123(4): 631-640.
25. Mattick JS, Makunin IV. Non-coding RNA. Hum. Mol. Genet., 2006; 15: R17-29.
26. Bartel DP. microRNAs: Genomics, biogenesis, mechanism and function. Cell, 2004; 116: 281-297.
27. Kusenda B, Mraz M, Mayer J, Pospisilova S. MicroRNA biogenesis functionality and cancer relevance. Biomed. Pap., 2006; 1550(2): 205-215.
28. Hock J, Meister G. The Argonaute protein family. Genome Biol, 2008; 9(2): 210.
29. Tetzlaff MT, Liu A, Xu X, Master SR, Baldwin DA, Tobias JW, Livolsi VA, Baloch ZW. Differetial expression of miRNA in papillary thyroid carcinoma compared to multinodular goiter using formalin fixed paraffine embedded tissues. Endocr. Pathol, 2007; 18: 163-173.
30. Barshack I, Lithwick Yanai G, Afek A, Rosenblatt K, Tabibian-Keissar H, Zepeniuk M, Cohen L, Dan H, Zion O et al. MicroRna expression differentiates between primary lung tumors and metastases to the lung. Pathol. Res. Pract, 2010; 206: 578-584.
31. Fridman E, Dotan Z, Barshack I, David MB, Dov A, Tabak S, Zion O, Benjamin S, Benjamin H,, Kuker H, et al Accurate molecular classification of renal tumors using microRNA expression. J. Mol. Diagn., 2010; 12: 687-696.
32. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Moore J. Wrobel MJ Lerner J et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N. Engl. J. Med, 2008; 359: 1995-2004.
33. Courthod G, Franco P, Palermo L, Pisconti S, Numico G. The role of microRNA in head and neck Cancer: Current Knowledge and Perspectives. Molecules, 2014; 19(5): 5704-5716.
34. Huber K Kirchheimer JC, Ermler D, Bell C, Binder BR. Determination of plasma urokinase- type plasminogen activator antigen in patients with primary liver cancer: characterization as tumor-associated antigen and comparision with alpha-fetoprotein. Cancer Res., 1992; 52: 1717-1720.
35. Raymond CK, Robert BS, Garrett-Engeke P, Lim LP, Johnson JM. Simple, quantitative primerextention PCR assay for direct Monitoring of microRNAs and short-interfering RNAs. RNA, 2005; 11: 1737-1744.
36. Barteles CL, Tsongalis GJ. MicroRNA: Novel biomarkers for human cancer. Clin. Chem., 2009; 55(4): 623-31.
37. Chen X, Ba Y, Ma L, CaiX, Yin Y, Wang K GuoJ, Zhang Y, Chen J, Guo X, Li Q, et al. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res., 2008; 18: 997-1006.
38. Lodes MJ, Caraballo M, Suciu D, Munro S Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS ONE, 2009; 4(7): e6229.
39. Hu Z, Chen X, ZhaoY, Tian T, Jin G, ShuY, Chen Y, Xu L, Zen K, Zhang C, Shen H. Serum MicroRNA signatures identified in a genome-wide serum microRNA expression profiling predict

survival of non-small cell lung cancer. J Clin. Oncol, 2010; 28: 1721-1726.
40. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacb ST, Patel T. microRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology, 2007; 133: 647-658.
41. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4(PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J.Biol. Chem., 2008; 283(2): 1026-1033.
42. Li J, Huang H, Sun L, Yang M, Pan C, Chen W, Wu D. Lin Z. Zheng C, Yao Y, et al. miR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. Clin. Cancer Res., 2009; 15(12): 3998-4008.
43. Darido C, Georgy SR, Wilanowski TT, Dworkin S, Auden A, Zhao Q, Rank G, Srivastava S, Finlay MJ, Papenfuss AT, et al. Targeting of the tumor suppressor GRHL3 by a miR-21 dependent proto-oncogenic network result in PTEN loss and tumorigenesis. Cancer Cell, 2001; 20: 635-648.
44. Liu CJ, Tsai MM, Hung PS, Kao SY, Liu TY, Wu KJ, Chiou SH, Lin SC, Chsng KW. MiR-31 ablates expression of the HIF regulatory factor FIH activate the HIF pathway in head and neck carcinoma. Cancer Res., 2010; 70(4): 1635-1644.
45. Liu CJ, Tsai MM, Hung PS, Kao SY, Liu TY, Wu KJ, Chiou SH, Lin SC, Chsng KW. MiR-31 ablates expression of the HIF regulatory factor FIH activate the HIF pathway in head and neck carcinoma. Cancer Res., 2010; 70(4): 1635-1644.
46. Xiao W, Bao Z-X, Zhang CY, Shi LJ, Zhou ZT, Jiang WW. Upregulation of miR-32 is negatively associated with recurement/newly formed oral leukoplakia. PLoS ONE, 2012; 7(6): e38648.
47. Ouiang SB, Wang J, Huang ZK, Liao L. Expression of microRNA-31 and its clinicopathologic significance in oral squamous cell carcinoma. Zhonghua Kou Qiang Yi Xue Za Zhi, 2013; 48(8): 481-484.
48. Garofalo M, Di Leva G, Romano G, Suh SS, Ngankeu A, Taccioli C, Pichiorri F, Alder H, Secchiero P, et al. miR-221 & 222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP# downregulation. Cancer Cell., 2009; 16(6): 498-509.
49. Hung PS, Liu CJ, Chou CS, Kao SY, Yang CC, Chang KW, Chiu TH, Lin SC. miR-146a enhances the oncogenicity of oral carcinoma by concominant targeting of the ITAK!, TRAF6 and NUMB genes. PLoS ONE, 2013; 8(11): e79926.
50. Liu CJ, Shen WG, Peng SY, Cheng HW, Kao SY, Lin SC, Chang KW. MiR-134 induces oncogenecity and metastasis in head and neck carcinoma through targeting WWWOX gene. International Journal of Cancer. 2014; 134(4): 811-821.
51. Hung PS, Chang KW, Kao SY, Chu TH, Liu CJ, Lin SC. Association between the rs2910164 polymorphism in pre-mir-146 and oral carcinoma progression. Oral Oncology, 2012; 48(5): 404-408.
52. Ni Y-H, Huang X-F, Wang Z-Y, Han W, Deng RZ, Mou YB, Ding L, Hou YY, Hu QG. Up regulation of a potential prognostic biomarker, miR-155, enhances cell proliferation in patients with oral squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 2014; 117(2): 227-233.
53. Shi LJ, Zhang CY, Zhou ZT et al. MicroRNA-155 in oral squamous cell carcinoma: overexpression, localization and prognostic potential. Head & Neck, 2015; 37(7): 970-6.
54. Rather MI, Nagashri MN, Swami SS, Gopinath KS, Kumar A. Oncogenic microRNA-155 down-regulates tumor suppressor CDC73 and Promotes oral squamous cell carcinoma cell proliferation implication for cancer therapeutics. Journal of Biological Chemistry, 2013; 288(1): 608-618.
55. Kefas B, Goldlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, Lee J, Fine H, Chiocca EA, Lawler S. Purow B. microRNA-7 inhibits the epidermal growth factor receptor and the akt pathway and is down-regulated in glioblastome. Cancer Research, 2008; 68(10): 3566-3572.
56. Reddy SD, Ohshiro K, Rayala SK, Kumar R. MicroRNA-7, ahomeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions. Cancer Research, 2008; 68(20): 8195-200.
57. Webster RJ, Giles KM, Pricr KJ, Zhang PM, Mattick JS, Leedman PJ. Regulation of epidermal growth factor receptor signaling in huma cancer cells by MICROrna-7. The Journal of Biological Chemistry, 2009; 284(9): 5731-5741.
58. Jiang L, Liu X, Chen Z, Jin Y, Heidbreder CE, Kolokythas A, Wang A, Dia Y, Zhou X. MicroRNA-7 targets IGF1R (insulin-like growth factor 1 receptor) in tongue squamous cell carcinoma cells. Biochemical jornal, 2010; 432(1): 199-205.
59. Yen Y-C, Shiah SG, Chu H-C, Hsu YM, Hsiao JR, Chang JY, Hung WC, Liao CT, Cheng AJ, Lu YC, Chen YW. Reciprocal regulation of microRNA-99a and insuli-like growth factor I receptor signaling in oral squamous cell carcinoma cells. Molecular Cancer., 2014; 13: 6.
60. Yan B, Fu Q, Lai L, Tao X, Fei Y, Shen J, Chen Z, Wang Q. Down regulation of microRNA 99a in oral squamous cell carcinomas contributes to the growth and survival of oral cancer cells. Molecular Medicine Reports, 2012; 6(3): 675-681.
61. Uesugi A, Kozaki KI, Tsuruta T, Furuta M, Morita K, Imoto I, Omura K, Inazawa J. The tumor suppressive microRNA miR-218 targets the mTOR component rictor and inhibits AKT phosphorylation in oral cancer. Cancer Research, 2011; 71(17): 5765-5778.
62. Minor J, WangX, Zhang F, Sing J, Jimeno A, Wang XJ, Lu X, Gross N, Kulesz-Martin M, Wang D, Lu SL. Methylation of microRNA-9 ia a specific and

sensitive biomarker for oral and oropharyngeal squamou cell carcinomas. Oral Oncology, 2012; 48(1): 73-78.
63. Minor J, WangX, Zhang F, Sing J, Jimeno A, Wang XJ, Lu X, Gross N, Kulesz-Martin M, Wang D, Lu SL. Methylation of microRNA-9 ia a specific and sensitive biomarker for oral and oropharyngeal squamou cell carcinomas. Oral Oncology, 2012; 48(1): 73-78.
64. Yu T, Liu K, Wu Y, Fan J, Chen J, Li C, Yang Q, Wang Z. MicroRNA-9 inhibits the proliferation of oral squamous cell carcinoma cells by suppressing expression of CXCR4 via the Wnt/ catenin signaling pathway. Oncogene, 2013; 33: 5017-5027.
65. Jin Y, Wang C, Liu X, Mu W, Chen Z, Yu D, Wang A, Dai Y, Zhou X. Molecular characterization of the MicroRNA-138-For-like antigen 1 (FOSL1) regulatory module in squamous cell carcina. The Jouranal of Biological Chemistry, 2011; 286(46): 40104-40109.
66. Liu X, Wang C, Chen Z, Jin Y, Wang Y, Kolokythas A, Dai Y Zhou X. MicroRNA-138 suppresses epithelial mesenchymal transition in squamous cell carcinoma cell lines. Biochemical Journal, 2011; 440(1): 23-31.
67. Jiang L, Liu X, Kolokythas A, Yu J, Wang A, Heidbreder CE, Shi F, Zhou X. Down regulation of the Rho GTPase signaling pathway is involved in the microRNA-138 mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma. Int. Journal Cancer, 2010; 127(3): 23-31.
68. Jiang L, Liu X, Kolokythas A, Yu J, Wang A, Heidbder CE, Shi F, Zhou X. 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, 2010; 127(3): 505-512.
69. Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. Cancer Res., 2005; 65: 3509-12.
70. McCarthy JJ, Esser K. microRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. J. Appl Physiol, 2007; 102(1): 306-13.
71. Tanaka T, Harano Y, Sue F Morimura H. Crystalliztion, characterization and metabolic regulation of two types of pyruvate kinase isolated from rat tissue. J Biochem, 1967; 62: 71-91.
72. Imamura k, Taniuchi K, Tanaka T. Multimolecular forms of pyruvate kinase II. Purification of M2-type pyruvate kinase form Yoshida as cites hepatoma 130 cells and comparative studies on the enzymological and immunological properties of the three types of pyruvate kinases. L, M1 and M2. J. Biochem, 1972; 72: 1001-15.
73. Nakashima K, Miwa S, Oda S, Tanaka T, Immura K. Electrophoretic and kinetic studies of mutant erythrocyte pyruvate kinase. Blood, 1974; 43: 537-48.
74. Noguchi T, Yamada K, Iamagata K, Takenaka M, Nakajima H, Imai E, Wang Z, Tanaka T. Expression of lover type pyruate kinase is insulinoma cells: involvement of LF-B1 (HNF1).Biochem Biopsy Res Commun, 1991; 181: 259-64.
75. Noguchi T, Inoue H, Tanaka T. The M1 and M2-Type isoenzyme of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. JBiol Chem, 1986; 261: 13807-12.
76. Imakenaka M, Noguchi T, Inoue H, Yamada K, Mastsuda T, Tanaka T. Rat pyruate kinase M gene. Its complete structure and characterization of the 50-flanking region. J Biol Chem, 1989; 264: 2363-7.
77. Koss K, Harrison RF, Gregory J, Darnton SJ, Anderson MR, Jankowski JA. The metabolic Marker tumor pyruvate kinase type M2 (tumor M2-PK) shows increased expression along the meataplasia-dysplasia-adenocarcinoma sequence in Barrett's oesophagous. J Clin Pathol, 2004; 57: 1156-9.
78. Eigenbrodt E, Kallinowski F, Ott M, Mazurek S, Vaupel P. Pyruvate kinase and the interaction of amino acid and carbohydrate metabolism in solid tumors. Anticancer Res., 1998; 18: 3267-74.
79. Zwersckke W, Mazurek S, Massimi P, Banks L, Eigenbrodt E, Jansen-Durr P. Modulation of type M2 pyruvate kinase activity by the human pappilloma virus type 16 E7 oncoprotein Proc Natl Acad Sci USA, 1999; 96: 1291-6.
80. Ugurel S, Bell N, Sucker A, Zimpfer A, Rittgen W, Schadendorf D. Tumor type M2 pyruvate kinase (TuM2-PK) as a novel plasma tumor marker in melanoma. Int J Cancer, 2005; 117: 825-30.
81. Zhang B, Chen JY, Chen DD, Wang GB, Shen P. Tumor type M2 pyruvate kinase expression in gastric cancer, colorectal cancer and controls. World J Gastroenterol. 2004; 10: 1643-6.
82. Kaura B, Bagga R, Patel FD. Evaluation of the pyruvate kinase isoenzyme tumor (Tu M2-PK) as a tumor marker for cervical carcinoma. J Obstet Gynaecol Res., 2004; 30: 193-6.
83. Wong TS, Liu XB, Chung-Wai Ho A, Po- Wing Yuen A, Wai –Man Ng R, Ignace Wei W. Identification of pyruvate kinase type M2 as potential oncoprotein in squamous cell carcinoma of tongue through micro RNA profiling. Int. J. Cancer, 2008; 123: 251-257.