

PIWI-Interacting RNAs in Oral Cancer: Paradigm Shift in Prognosis and Diagnosis

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Abstract

Oral squamous cell carcinoma (OSCC) is the most prevalent cancer in Indian subcontinent with high recurrence rate, aggressive metastasis, and poor prognosis. The potential risk-factors for OSCC are tobacco smoking, alcohol intake, and persistent infection of oncogenic human papillomaviruses (HR-HPVs). HPV-positive OSCCs show distinct genetic and epigenetic changes along with distinct clinical, epidemiological and molecular characteristics. Recently, with the accumulation of large amount of genomic and epigenomic data, there is an increasing focus on epigenetic alterations playing key roles in cancer pathogenesis. Non-coding RNAs, especially the small non-coding RNAs (sncRNAs) have gained attention since they have been demonstrated to fine tune transcription via alterations in the epigenetic landscape. There are ample evidences supporting the role of small non-coding RNAs such as miRNAs and piRNAs in development and disease including cancers. PIWI-interacting RNAs (piRNAs), a class of sncRNAs are emerging players involved in transcription silencing. Its altered regulation is associated with the development of variety of tumors including oral carcinogenesis; however, their tumor specific roles are not fully understood. Therefore, identification and comprehensive characterization of oncogenic as well as tumor suppressive pi-RNAs and dissecting their roles in tumorigenesis is of great importance in the field of cancer biology. Furthermore, piRNAs may potentially serve as unique therapeutic targets and/or molecular markers for early detection and effective treatment of OSCC subtypes. In this mini review, we briefly summarize the emerging role of PIWI-RNAs in oral cancer.

Keywords: Head and Neck Squamous Cell Carcinoma (HNSCC); HR-HPVs; Epigenetics; Tobacco smoking; Non-coding RNAs and Piwi RNAs

Introduction

Cancer is a major public health problem with an estimated global cancer burden increasing to 18.1 million new cases and 9.6 million cancer-related deaths [1]. Cancer is a complex disease involving distinct genetic and/or epigenetic alterations in key regulatory genes, modified by environmental cues and variety of other factors that ultimately lead to malignant transformation. Of the several cancers, oral squamous cell carcinoma (OSCC) is the most prevalent and the second most deadliest cancer in India in both sexes [1,2].

OSCC, a highly heterogeneous disorder, mainly occurs due to the exposure to differential risk-factors such as the constant use of tobacco chewing and/or smoking, excess alcohol consumption, and sexual behavior that allow acquisition and persistent of high-risk human papillomaviruses (HR-HPVs) infection [3-10]. These different risk factors effect the outcome of the disease. HPV-positive OSCCs have distinct genetic/epigenetic changes, which can often be correlated with clinic-pathological and epidemiological characteristics. HPV-positive OSCCs are often associated with younger age of onset, better prognosis, less/absent tobacco history, and association with high-risk sexual behaviours when compared with HPV-negative and tobacco induced oral cancers [8-12]. Differential risk-factors which lead to OSCC-associated heterogeneities and genetic/epigenetic alterations effect different intra-oral regions and may contribute to the development of different sub-types of oral cancers. Inter and intra-individual variation in genetic and epigenetic landscape further vary the disease outcome. These are the fundamental challenges surrounding diagnosis, prognosis and therapeutics in OSCC and identifying processes and molecules that govern cancer specific gene expression is of vital importance.

Recent evidences suggest that alteration in the expression of P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs) is frequently linked to cell growth, cell proliferation, cancer progression, differentiation and chemo-radio resistance in many tumors including OSCCs. piRNAs are a class of non-coding RNAs (ncRNAs) that was identified in *Drosophila* in relation to germ stem cells maintenance and spermatogenesis. It is suggested that pi-RNAs are expressed in a tissue-specific manner and are extremely conserved across different phyla with highly conserved roles including retrotransposon silencing, genomic/epigenomic regulation, spermatogenesis and stemness maintenance in germline tissues [8,13,14]. Recent studies have confirmed the significant role of piRNAs in carcinogenesis including that of oral and head and neck cancers (HNCs) [8,15-17] however, there are still several gaps in the understanding of potential oncogenic roles of piRNAs in different subtypes of OSCCs. Therefore, pi-RNAs are emerging as a distinct class biomarkers for early detection, diagnosis, prognosis and progression of OSCCs. In this mini review, we briefly discuss biogenesis of piRNAs, their potential roles in oral and other cancers. We further discussed piRNAs as predictive biomarkers for diagnosis and prognosis of OSCCs.

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Literature Review

The non-coding RNAs (ncRNAs)

A major portion (93%) of the human genome is transcribed, out of which the protein coding transcripts account for only 1-2%, thus a large fraction of the transcripts are non-coding [18]. There are number of reports providing evidence in favor of critical roles of non-coding RNAs (ncRNAs) in embryonic development and various types of diseases including cancers [18]. ncRNAs are categorized into housekeeping and regulatory ncRNAs [19]. The regulatory ncRNAs are further classified into small (<200 nts) and long ncRNAs (>200nts). microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcription initiation RNAs (tiRNAs) and circular RNAs (cRNAs) form the small ncRNA subclass.

PIWI-interacting RNAs: discovery and biogenesis

PIWI-RNAs are 24–32 nucleotides in length and bring about PIWI-dependent transposon silencing. They have been identified as essential players in the process of germline stem cells maintenance and self-renewal. This important sub-class of short ncRNAs are named so, because of their interaction with PIWI (P-element induced *wimpy* testis) proteins. piRNAs were first described in relation to *Su(ste)*-derived small RNAs and their role in male fertility in *Drosophila* [20]. As of now, piRNAs have been implicated in spermiogenesis and germline stem cell maintenance [21,22], epigenetic regulation and transposon silencing [23] and genomic rearrangements [14,24,25].

PIWI proteins are divided into three sub-families namely the Aub, AGO3 and PIWI. PIWI proteins in complex with piRNAs participate

in transposon silencing and spermiogenesis as demonstrated in the PIWI knock-outs that impair sperm development. piRNAs can be derived from 1) transposons; 2) mRNA and 3) lncRNA [26]. The piRNAs derived from transposons are transcribed from both sense and antisense strands thus producing sense and anti-sense piRNAs. The piRNAs derived from lncRNAs are produced from the entire transcript, this is in contrast to the mRNA-derived piRNAs which are produced from 3'UTR [27]. Primary piRNAs are derived from precursor transcripts (up to 200kb in length) which are processed by Dicer ribonuclease [28]. These primary piRNAs are further cleaved into small RNAs which then form complex with PIWI proteins. The 3' to 5' exonuclease incorporates the 3' fragment into the PIWI protein and the Hen1 enzyme methylates the 2'OH group at 3' end. The finished piRNA/PIWI complex thus formed migrates to the nucleus and targets specific gene for silencing. This mechanism of piRNA synthesis and targeting is the primary mechanism and effects transposon silencing via their recruitment of DNA and histone methyltransferase (Figure 1). On the other hand, the “ping-pong mechanism” (Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*; A slicer-mediated mechanism for repeat-associated siRNA 5' end formation in *Drosophila*;) accumulates of the piR in cytoplasm. piRs form complex with Ago proteins which then synthesizes new piRNAs. The newly synthesized piRNAs are loaded onto the Aub protein which functions to synthesize new piRs. This cycle continues and thus is known as the Ping-Pong mechanisms (ppm) of piRNA biogenesis.

Emerging role of piRs in oral carcinogenesis

Few studies have demonstrated the potential function of pi-

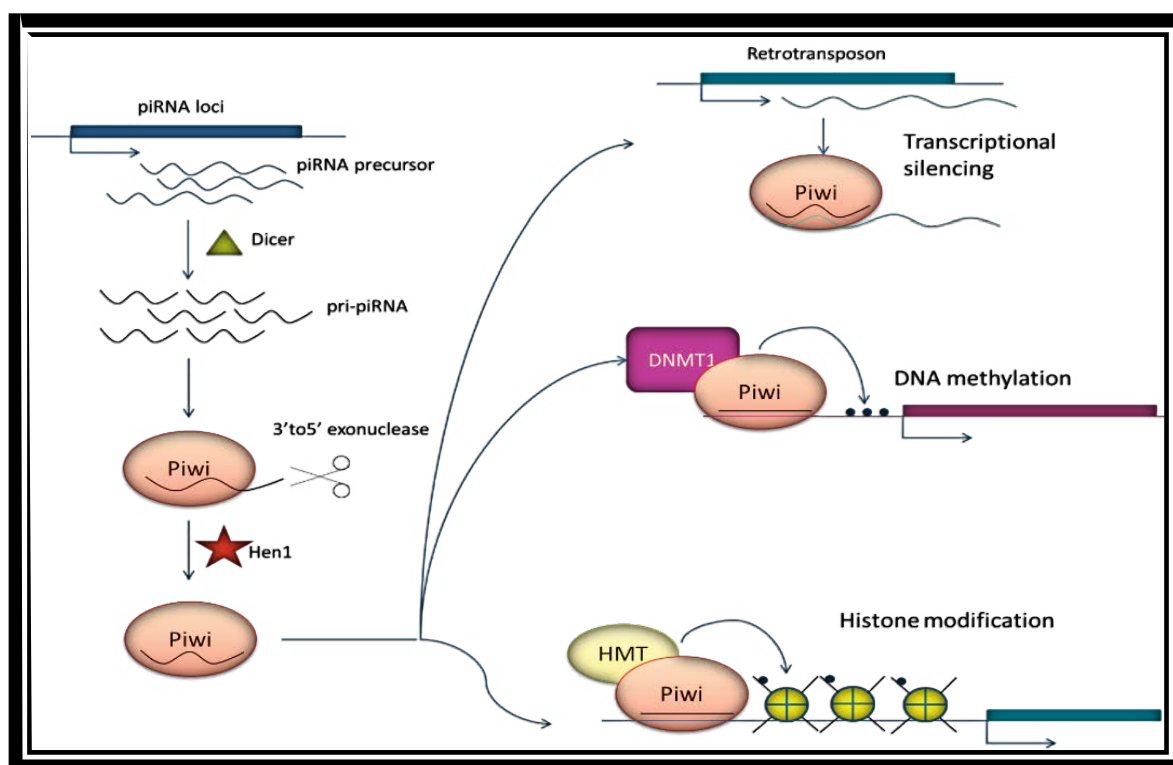


Figure 1: The PIWI-piRNA complex may bring about transcriptional silencing by directly binding to the transcripts and brings about degradation or production of secondary piRNA. The PIWI-piRNA may form complex with DNA (DNMT1) and Histone methyltransferase (EZH2) and bring about DNA methylation and histone methylation, respectively which in turn bring about transcriptional silencing.

RNAs in oral carcinogenesis. Altered expression of pi-RNAs has been associated with oral cancer prognosis and survival [17]. Dysregulation in piRNA-34376 expression correlated with tobacco smoking status in generic oral cancers [29]. Low expression level of piR-NONHSAT069719 is associated with 10q23.3 deletion at *PTEN* gene locus in oesophagus-associated adenocarcinoma [30]. piR-NONHSAT102574 and piR-NONHSAT144936 expression correlated with NOTCH mutations in HNCs [31]. In a recent study, expression profiles of 41 pi-RNAs have been screened in both HPV+ve and HPV-ve OSCC cases. Interestingly, out of 41 pi-RNA panels, 11 were found to be upregulated mainly in HPV type 16/18 infected OSCCs and five piRNA signature (piR-35953, piR-36984, piR-39592, piR-36715 and piR-30506) correlated with poor survival in HPV+ve OSCC patients [32]. Further, altered expression of a panel of piRNAs correlated with smoking-induced oral cancers. These altered piRNAs; NONHSAT108298, NONHSAT113708, NONHSAT067200 and NONHSAT123636 and NONHSAT081250 are associated with TP53 mutations and TP53 mutation-3p deletion

co-occurrence and 3q26, 8q24, and 11q13 amplification leading to advanced tumor stage and reduced oral cancer patient's overall survival [15,17]. The authors have further shown the differential expression pattern of piR-NONHSAT077364, piR-NONHSAT102574, and piR-NONHSAT128479 in both HPV+ve and HPV-ve OSCC cells and dysregulation in the expression level of these piRNAs is associated HPV infection [16,17]. These findings indicate that piRNAs are associated with clinic-pathological characteristics, different subtypes and associated risk factors in OSCC patients. These studies suggest the potential role of piRNA landscape to understand molecular mechanism of distinct variety of oral cancers.

piRs and other cancers

Overexpression and mis-regulation of piRs have been associated with different varieties of human solid tumors such as colorectal cancers (CC), lung cancer (LC), breast cancer (BC), gastric cancer (GC) and head and neck cancer (HNC) etc [14-17,24,25,33-35].

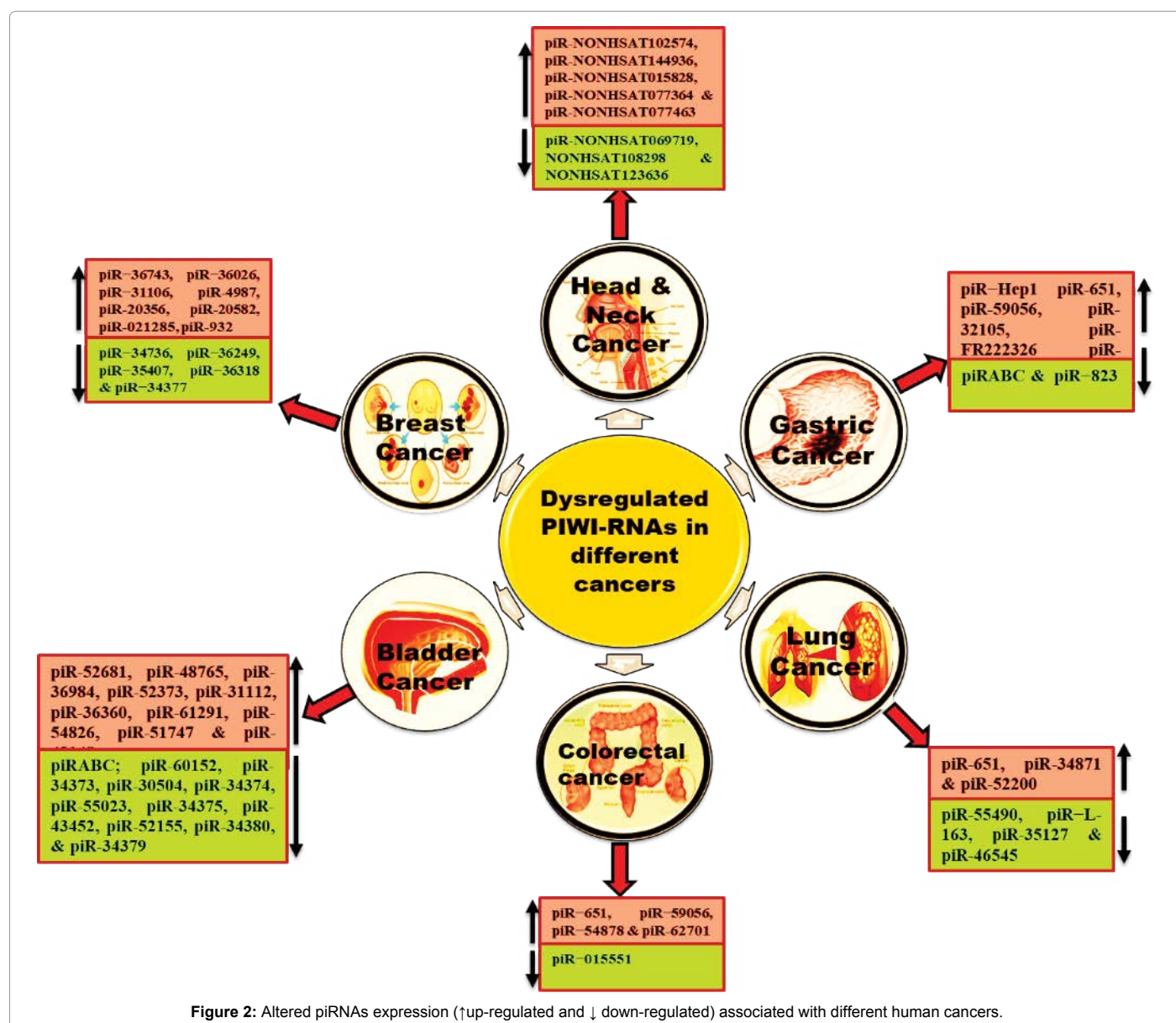


Figure 2: Altered piRNAs expression (↑up-regulated and ↓ down-regulated) associated with different human cancers.

piRNA expression profiling revealed that piR-823 and piR-1245 overexpression is associated with aggressive colorectal cancers (CC) and inhibition of piR-823 leads to cell cycle arrest, apoptosis and reduced cell proliferation in CC cells [14,35]. Microarray screening analysis in lung cancer tissues identified 4 piRNAs that were deregulated, two of them (piR-34871 and piR-52200) were up-regulated and the other two (piR-35127 and piR-46545) down-regulated [34]. A study involving genotypic screening of SNP-containing pi-RNAs in Connecticut-based population (441 breast cancer cases and 479 controls) identified SNP rs1326306 G>T in piR-021285 and correlated with occurrence of SNP and increased chances of breast cancer [25]. The authors further performed transfections with wildtype and variant piR-021285 in BC cells to study methylation status at cancer-associated genes. The screen identified disrupted methylation at 5'UTR and 1st exon of *ARHGAP11A* gene in variant mimic-transfected cell lines [25]. Another study involving meta-analysis of piRNA expression data from TCGA (The Cancer Genome Atlas) identified 20 highly mis-expressed piRNAs in BC patients [24]. piR-36712 was found to be down-regulated in BC and was further correlated with poor clinical outcome in BC patients [24]. Global piRNA expression analysis in 358 non-malignant stomach tissues and gastric adenocarcinoma samples led to the identification of a number of deregulated piRs [33]. Out of the 312 piRNAs which were expressed, 156 (50%) piRNAs were found to show altered expression. Among these, 93 piRNAs were over-expressed and 7 were down-regulated in GC samples. When correlation was made between piRNA expression and survival, piR-FR222326 was significantly correlated with GC patients survival [33]. In a similar study, global piRNA expression profiling identified, 106 piRNAs that were over-expressed and 91 piRNAs that were down-regulated in bladder cancer [36]. Together, these findings established a significant association between pi-RNA expression and clinico-pathological symptoms associated with different types of cancers. Although, it is difficult to pin-point whether deregulated or mis-expressed piRNAs lead to pathogenesis or are a mere downstream effect in a cascade of altered signalling pathways.

piRNAs in prognosis, diagnosis and therapy

Growing number of studies have provided evidence that piRNAs are highly cancer-specific, have differential expression profiles in relation to cancers and their subtypes. Due to their higher sensitivity and specificity in cancer detection [37] piRNAs can serve as potential cancer biomarkers. Recent investigations suggest that understanding the potential role of PIWI-piRNA signalling in modifying the epigenetic landscape in cancers and their subtypes, will be an exciting field of research. Furthermore, selective silencing of oncogenic piRNA for treatment of cancer could be applied for therapeutic manipulation. The progress in identifying high throughput techniques for identification of piRNAs as prognostic and diagnostic markers has been a rapid. Apart from piRNAs as molecular and clinical biomarkers, PIWI proteins can be used as therapeutic tool in cancer biology research. Interestingly, piRNAs are highly stable and abundantly expressed in plasma, blood and saliva. piRNAs have also been identified in circulating body fluid in patients with colorectal cancer, breast cancer, prostate cancer, and pancreatic [38-41]. A comprehensive analysis of these small functional molecules in circulating body fluid such as saliva of oral cancer patients will facilitate for discovery of salivary piRNAs as non-invasive biomarkers for early detection and diagnosis of OSCC.

Discussion

Existing studies have demonstrated the significant role of piRNAs in cancer and other diseases, but it remains to be unidentified whether

they participate in cancer stem cells (CSCs) formation. Although, the role of piRNAs in germline stem cell maintenance and genome stability is well established. There is a recent report which show a correlation between PIWIL1 and PIWIL2 gene expression and expression of stemness markers OCT4 and SOX2 in colon cancer [42]. Hence, it is also exciting to identify the potential role of piRNAs and their interactions in tumor-specific CSCs, since existence of CSCs has been shown in almost all human malignancies and appear to be involved in tumor initiation, recurrence, aggressive metastasis, treatment resistant and cancer relapse. Targeted therapeutic approaches against CSCs will unlock novel options for investigation of possible role of piRs in regulation of cancer stemness as future innovative approaches.

Concluding Remarks

Altogether, identification of aberrantly expressed piRNAs in OSCC and other cancers may help in identifying an emerging class of diagnostic and prognostic cancer biomarkers and will also improve our understanding for the role of piRNAs in cancer pathogenesis. Specific targeting of differentially expressed oncogenic piRs in cancer and cancer stem cells will improve drug efficacy, treatment sensitivity and help to identify new therapeutic drugs and may facilitate personalized therapies for OSCC patients.

The downstream global effects of piRNAs including the whole genome methylation status and thus the transcriptional status will help us understand the pleiotropic symptoms of cancers. Variant piRNA would further add to the tumor heterogeneity and increase intra- and inter-individual variations. Genomic instability and increase in the number of genomic rearrangements (deletions, insertions and translocations) associated with cancers may be accounted for by the altered behaviours of piRNAs. In future, additional studies should aim to identify more specific piRNAs and their mechanistic role in OSCC pathogenesis.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394-424.
2. Castilho RM, Squarize CH, Almeida LO (2017) Epigenetic modifications and head and neck cancer: Implications for tumor progression and resistance to therapy. *Int J Mol Sci* 18: E1506.
3. Maier H, Dietz A, Gewelke U, Heller WD, Weidauer H (1992) Tobacco and alcohol and the risk of head and neck cancer. *Clin Invest* 70: 320-327.
4. Riedel F, Goessler U, Hormann K (2003) Alcohol-related diseases of the mouth and throat. *Best Pract Res Clin Gastroenterol* 17: 543-555.
5. Hashibe M, Boffetta P, Zaridze D, Shangina O, Szeszenia-Dabrowska N, et al. (2007) Contribution of tobacco and alcohol to the high rates of squamous cell carcinoma of the supraglottis and glottis in Central Europe. *Am J Epidemiol* 165: 814-820.
6. Vidal L, Gillison ML (2008) Human papillomavirus in HNSCC: recognition of a distinct disease type. *Hematol Oncol Clin North Am* 22: 1125-1142.
7. Gillison ML, Castellsague X, Chaturvedi A, Goodman MT, Snijders P, et al. (2013) Eurogin Roadmap: comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. *Int J Cancer* 134: 497-507.
8. Gupta S, Kumar P, Das BC (2018) HPV: Molecular pathways and targets. *Curr Probl Cancer* 42: 161-174.
9. Gupta S, Kumar P, Kaur H, Sharma N, Saluja D, et al. (2018) Constitutive activation and overexpression of NF-kappaB/c-Rel in conjunction with p50 contribute to aggressive tongue tumorigenesis. *Oncotarget* 9: 33011-33029.
10. Gupta S, Kumar P, Kaur H, Sharma N, Saluja D, et al. (2015) Selective participation of c-Jun with Fra-2/c-Fos promotes aggressive tumor phenotypes and poor prognosis in tongue cancer. *Sci Rep* 5: 16811.

11. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, et al. (2007) Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 356: 1944-1956.
12. Mishra A, Bharti AC, Varghese P, Saluja D, Das BC (2006) Differential expression and activation of NF-kappaB family proteins during oral carcinogenesis: Role of high-risk human papillomavirus infection. *Int J Cancer* 119: 2840-2850.
13. Bamezai S, Rawat VP, Buske C (2012) Concise review: The Piwi-piRNA axis: pivotal beyond transposon silencing. *Stem Cells* 30: 2603-2611.
14. Yin Y, Shen Q, Tao R, Chang W, Li R, et al. (2018) Wee1 inhibition can suppress tumor proliferation and sensitize p53 mutant colonic cancer cells to the anticancer effect of irinotecan. *Mol Med Rep* 17: 3344-3349.
15. Gross AM, Orosco RK, Shen JP, Egloff AM, Carter H, et al. (2014) Multi-tiered genomic analysis of head and neck cancer ties TP53 mutation to 3p loss. *Nat Genet* 46: 939-943.
16. Krishnan AR, Korrapati A, Zou AE, Qu Y, Wang XQ, et al. (2017) Smoking status regulates a novel panel of PIWI-interacting RNAs in head and neck squamous cell carcinoma. *Oral Oncol* 65: 68-75.
17. Krishnan AR, Qu Y, Li PX, Zou AE, Califano JA, et al. (2018) Computational methods reveal novel functionalities of PIWI-interacting RNAs in human papillomavirus-induced head and neck squamous cell carcinoma. *Oncotarget* 9: 4614-4624.
18. Beermann J, Piccoli MT, Viereck J, Thum T (2016) Non-coding RNAs in development and disease: Background, mechanisms and therapeutic approaches. *Physiol Rev* 96: 1297-1325.
19. Morey C, Avner P (2004) Employment opportunities for non-coding RNAs. *FEBS Lett* 567: 27-34.
20. Aravin AA, Naumova NM, Tulin AV, Vagin VV, Rozovsky YM, et al. (2001) Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr Biol* 11: 1017-1027.
21. Chuma S, Nakano T (2013) piRNA and spermatogenesis in mice. *Philos Trans R Soc Lond B Biol Sci* 368: 20110338.
22. Pillai RS, Chuma S (2012) piRNAs and their involvement in male germline development in mice. *Dev Growth Differ* 54: 78-92.
23. Senti KA, Jurczak D, Sachidanandam R, Brennecke J (2015) piRNA-guided slicing of transposon transcripts enforces their transcriptional silencing via specifying the nuclear piRNA repertoire. *Genes Dev* 29: 1747-1762.
24. Tan L, Mai D, Zhang B, Jiang X, Zhang J, et al. (2019) PIWI-interacting RNA-36712 restrains breast cancer progression and chemoresistance by interaction with SEPW1 pseudogene SEPW1P RNA. *Mol Cancer* 18: 9.
25. Fu A, Jacobs DI, Hoffman AE, Zheng T, Zhu Y (2015) PIWI-interacting RNA 021285 is involved in breast tumorigenesis possibly by remodeling the cancer epigenome. *Carcinogenesis* 36: 1094-1102.
26. Weick EM, Miska EA (2014) piRNAs: From biogenesis to function. *Development* 141: 3458-3471.
27. Robine N, Lau NC, Balla S, Jin ZG, Okamura K, et al. (2009) A broadly conserved pathway generates 3' UTR-directed primary piRNAs. *Current Biology* 19: 2066-2076.
28. Le Thomas A, Toth KF, Aravin AA (2014) To be or not to be a piRNA: Genomic origin and processing of piRNAs. *Genome Biol* 15: 204.
29. Maccani MA, Knopik VS (2012) Cigarette smoke exposure-associated alterations to non-coding RNA. *Front Genet* 3: 53.
30. Kulke MH, Odze RD, Thakore KS, Thomas G, Wang H, et al. (2001) Allelic loss of 10q23, the PTEN tumour suppressor gene locus, in Barrett's oesophagus-associated adenocarcinoma. *British Journal of Cancer* 84: 748-753.
31. Lobry C, Oh P, Aifantis I (2011) Oncogenic and tumor suppressor functions of Notch in cancer: It's NOTCH what you think. *J Exp Med* 208: 1931-1935.
32. Firmino N, Martinez VD, Rowbotham DA, Enfield KSS, Bennewith KL, et al. (2016) HPV status is associated with altered PIWI-interacting RNA expression pattern in head and neck cancer. *Oral Oncol* 55: 43-48.
33. Martinez VD, Enfield KS, Rowbotham DA, Lam WL (2016) An atlas of gastric PIWI-interacting RNA transcriptomes and their utility for identifying signatures of gastric cancer recurrence. *Gastric Cancer* 19: 660-665.
34. Reeves ME, Firek M, Jliedi A, Amaar YG (2017) Identification and characterization of RASSF1C piRNA target genes in lung cancer cells. *Oncotarget* 8: 34268-34282.
35. Weng W, Li H, Goel A (2018) Piwi-interacting RNAs (piRNAs) and cancer: Emerging biological concepts and potential clinical implications. *Biochim Biophys Acta Rev Cancer* 1871: 160-169.
36. Chu HY, Hui GY, Yuan L, Shi D, Wang YB, et al. (2015) Identification of novel piRNAs in bladder cancer. *Cancer Lett* 356: 561-567.
37. Cui L, Lou Y, Zhang X, Zhou H, Deng H, et al. (2011) Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using piRNAs as markers. *Clin Biochem* 44: 1050-1057.
38. Bahn JH, Zhang Q, Li F, Chan TM, Lin X, et al. (2015) The landscape of MicroRNA, Piwi-Interacting RNA, and circular RNA in human saliva. *Clin Chem* 61: 221-230.
39. Ogawa Y, Taketomi Y, Murakami M, Tsujimoto M, Yanoshita R (2013) Small RNA transcriptomes of two types of exosomes in human whole saliva determined by next generation sequencing. *Biol Pharm Bull* 36: 66-75.
40. Schafer CA, Schafer JJ, Yakob M, Lima P, Camargo P, et al. (2014) Saliva diagnostics: Utilizing oral fluids to determine health status. *Monogr Oral Sci* 24: 88-98.
41. Spielmann N, Ilsley D, Gu J, Lea K, Brockman J, et al. (2012) The human salivary RNA transcriptome revealed by massively parallel sequencing. *Clin Chem* 58: 1314-1321.
42. Litwin M, Dubis J, Arczynska K, Piotrowska A, Frydlewicz A, et al. (2015) Correlation of HIWI and HILI expression with cancer stem cell markers in colorectal cancer. *Anticancer Res* 35: 3317-3324.