Review Article

Relevance of micro-RNAs and their targets as a diagnostic and prognostic marker in oral squamous cell carcinoma

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Abstract

Oral squamous cell carcinoma (OSCC) ranks sixth among all cancers in the world, affecting various sites of the oral cavity with associated several risk factors. High mortality has been associated with the presence of metastasis during the time of diagnosis and an increase in therapeutic relapses. Micro-RNAs (miRNAs) are a group of small non-coding RNAs with salient roles in the initiation and progression of cancer. The tumorigenesis of OSCC is associated with the dysregulation of several miRNAs. MicroRNAs are an area of recent interest, and numerous studies have been reported and are being undertaken to identify their role in diagnostic and prognostic value for oral cancers. Most of the miRNA processing machinery is considered to be either up-/down-regulated in OSCC, but the underlying mechanism of miRNA dysregulation and their activity as either a tumour suppressor or an oncogene in oral carcinogenesis is not yet clear. The article presents a concise review of the available current literature regarding the various miRNAs' signatures in OSCC and their role as diagnostic/prognostic biomarkers.

Keywords: microRNA, oncomiRs, oral squamous cell carcinoma, prognostic biomarkers

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) comprises a group of diverse heterogeneous cancers that develop in different oral anatomical locations, associated with several lifestyles and genetic and environmental factors.[1] OSCC accounts for over 40% of head and neck malignancies and is the sixth most common cancer globally.[2] The prevalence of OSCC varies in different areas of Pacific and Asian countries. The majority of the OSCCs get diagnosed at a later stage with tumour node metastasis, resulting in a poor prognosis for the patient.[3] This could be related to their invasive nature despite the advances made in diagnosis and treatment. The 5-year survival rates remain less than 50%, and 35–55% of the patients develop recurrences within 2 years even with advanced therapeutic approaches.[4]

Micro-RNAs (miRNAs or miR) are considered to be the principal regulators of various cellular processes. They are small, single-stranded, and non-coding RNAs (22 nucleotides) that regulate post-transcriptional gene expression.[5] These small molecules were discovered in 1993 in *Caenorhabditis elegans*.[6] It has been predicted

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that the human genome encompasses about 1000 microRNA (miRNA) genes,[7] and presently, about 2500 known mature miRNA species are present in the human miR-Base.[8,9] Approximately one-third of human genes might be regulated by miRNAs.[10] Numerous miRNA molecules have been identified to play a key role in a variety of physiologic and pathologic processes and are regarded as critical regulators of gene expression in biological systems.[11]

Over the recent years, miRNAs have been immensely studied and have been identified as the potential biomarkers of cancer. Altered expression of miRNAs has been shown to directly dictate various cancer phenotypes and has been implicated in specific clinical outcomes. It is well established that dysregulation of these RNAs either enhances the expression of oncogenes or subdues the expression of tumour suppressor genes.[12] The expression patterns of miRNAs are associated with cancer growth and expansion and can be considered promising predictive biomarkers in the diagnosis and as therapeutic targets.[13] Several miRNAs have been identified to be differentially expressed in OSCC, indicative of the fact that miRNAs may be involved in the pathophysiology of OSCC. Numerous studies have reported the diagnostic and prognostic significance of miRNA in OSCC, and the purpose of this paper is to investigate the role and relevance of miRNAs as diagnostic biomarkers and their therapeutic potential in OSCC.

miRNA BIOLOGY AND BIOGENESIS

These small RNA molecules have been found to regulate endogenous gene expression through translational repression and messenger RNA cleavage. miRNAs as such do not possess the ability to code proteins but partake in biological processes through their target genes.[14] A single miRNA can target numerous mRNA transcripts, have multiple target sites, and regulate numerous genes downstream.[8]

Several mechanisms are involved in miRNA-mediated post-transcriptional regulation of gene expression: viz. site-specific cleavage, enhanced mRNA degradation, and translational inhibition.[15]

The biogenesis of miRNA follows two mechanisms: canonical and non-canonical pathways wherein the canonical pathway functions as the dominant mechanism.

CANONICAL miRNA BIOGENESIS PATHWAY

miRNA genes are transcribed by RNA polymerase II (Pol II) into primary miRNA (pri-miRNA with a hairpin structure) transcripts within the nucleus. Each miRNA located in the same genomic cluster can be transcribed and regulated independently.[16] This is then subsequently processed in the nucleus and cytoplasm by two endonuclease enzymes: RNA polymerase III: DROSHA and Dicer, respectively. Within the nucleus, miRNA gene transcription is catalysed by the microprocessor complex, comprising the RNase III-type enzyme DROSHA (double-stranded RNA-binding protein [RBP]) and DGCR8 (DiGeorge syndrome critical region 8), resulting in a pre-miRNA sequence of approximately 70–100 nucleotide stem-loop precursor miRNAs/pri-miRNA.[17] The transport of pre-miRNAs from the nucleus to the cytoplasm is aided by Exportin-5. The pre-miRNA is then cleaved by Dicer (cytoplasmic ribonuclease) concurrent with TRBP (HIV-1 TAR RNA RBP) and PACT (protein activator of PKR), adjacent to the TL (apical loop) region liberating the double-stranded/duplex miRNA.[18] The miRNA duplex is then unified with Argonaute (AGO2) protein to form the RNA-induced silencing complex (RISC). After maturation, AGO2 unwinds the miRNA duplex separating the mature RISC complex. This active miRNA–RISC complex binds to target mRNAs.[19] The miRNA targets mRNAs through mRNA cleavage, deadenylation or translational repression [Figure 1: Canonical pathway in the biogenesis of miRNA]. The mature miRNA identifies its complementary sequences and regulates gene expression through binding to 3'-untranslated regions (UTRs) of their target mRNAs which are then degraded or translationally inhibited.

NON-CANONICAL miRNA BIOGENESIS PATHWAY

This pathway uses different combinations of the proteins involved in the canonical pathway; viz. DROSHA, Dicer, exportin 5, and AGO2. Non-canonical miRNA biogenesis is grouped into DROSHA/DGCR8-independent and Dicer-independent pathways. Pre-miRNAs produced by the DROSHA/DGCR8-independent pathway are directly exported to the cytoplasm through exportin 1 without DROSHA cleavage.[20]

In the Dicer-independent pathway, miRNAs are processed by DROSHA from endogenous short hairpin RNA (shRNA) transcripts. The pre-miRNAs are of insufficient length to be Dicer substrates and therefore require AGO2 binding to complete their maturation within the cytoplasm. The entire pre-miRNA is loaded into AGO2 and AGO2-dependent slicing of the 3p strand occurs. Then, the 3-5' slicing of the 5p strand completes their maturation.[21]

A single miRNA may target up to several hundred mRNAs as high complementarity is not required for regulation, and the resulting aberrant miRNA expression may affect a horde of transcripts, which can influence cancer-related

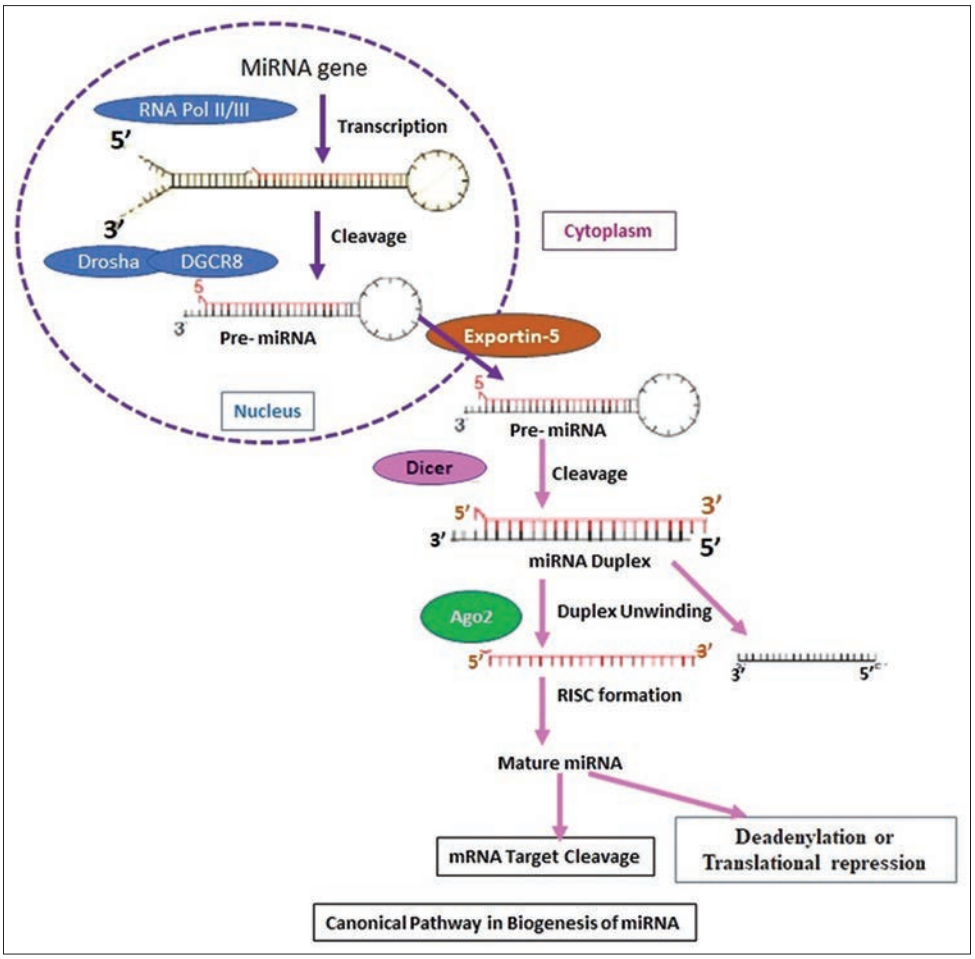

Figure 1: Canonical pathway in the biogenesis of miRNA

signalling pathways.[22] The potential for miRNA-mediated regulation of gene expression is extensive as 60% of mRNAs are considered to be controlled by miRNAs.

miRNAs IN TUMORIGENESIS AND ITS DETECTION IN CANCERS

miRNA has been an area of interest for a variety of cancers. Literature reports have verified that miRNAs have a critical part in cancer biology either as a tumour suppressor or as oncogenes.[23] The majority of encoding sequences of miRNAs have been located in the cancer-related regions of the genome,[24] and a dysregulated miRNA profile plays an imperative role in tumour-associated bio-pathological processes including initiation, promotion, malignant transformation, progression and metastasis.[25] miRNAs play vital roles in morphogenesis, proliferation, differentiation, apoptosis, metastasis and survival of cancer cells. Many studies have demonstrated that certain cancer has a unique mir signature[11] and has been confirmed to be important diagnostic or prognostic markers in various cancers and serve as targets for novel therapeutic strategies.

Microarray analysis of oligonucleotide miRNA and real-time RT-PCR are the most commonly employed techniques for the assessment of miRNA profiling in a large number of cancer-specific cell types.[26] Next-generation sequencing is emerging as a cost-effective technique for analysis. The materials used for profiling include fresh frozen samples, but recent advancements made it possible to obtain reproducible profiles from formalin-fixed paraffin-embedded tissue (FFPE), thus marking the accessibility of archived tumour tissues for research.[27] *In situ* hybridisation (ISH) also allows sensitive detection of miRNAs in tissues, defining miRNA cellular localisation.[28] Newer detection methods, to improve the sensitivity and selectivity of miRNA, rely on signal amplification strategies, such as nanoparticle-based amplification, isothermal exponential amplification, rolling circle amplification (RCA), hybridisation chain reaction (HCR) and a combination of these methods.[29]

miRNAs are consistent and demonstrable in body fluids also (plasma and serum) owing to their protection from RNases[30] and many research studies are being carried

out for the identification of new biomarkers within body fluids due to their non-invasive nature collection and also on the datum that they reflect the physiological/pathological state.

miRNA dysregulation could initiate tumorigenesis and the oncogenic function of miRNAs vary based on cell or tissue type. Deranged miRNA profiles in cancer cells are tumour-type-specific and related to malignant features.[11]

Several mechanisms have been described for tumorigenesis through miRNA. miRNAs act by inhibiting gene expression with the target mRNAs through direct base-pairing. Overexpression, amplification, and loss of epigenetic silencing of a miRNA-targeting tumour suppressor gene/s could inhibit the activity of an antioncogenic pathway. In contrast, the epigenetic silencing of a miRNA that normally represses the expression of oncogenes might lead to increased protein expression with the gain of oncogenic potency.[31] Any defects in the miRNA processing mechanism can contribute to miRNA-mediated oncogenic processes. Commotion in any of the components that regulate miRNA biogenesis (microprocessor complex, Dicer, AGO2, and GW182) could cause variations in miRNAs, capable of causing cancer.[32]

miRNA AND ORAL SQUAMOUS CELL CARCINOMA

Oral tumorigenesis arises through the accrual of both or either genetic/epigenetic alterations in the expression of coding and non-coding RNAs. Oral cancers express a specific miRNA portfolio, that contributes to maintaining the characteristic epithelial features of the cells.

Numerous studies have analysed miRNA expression profiles in OSCC using microarrays or quantitative reverse transcription-polymerase chain reaction (qRT-PCR). miRNAs that are up- or down-regulated are, respectively, denoted as oncogenic or tumour suppressor miRNAs.

OSCC has been found to be associated with numerous miRNAs. A meta-analysis conducted by Chen *et al*.[33] found 432 differentially expressed miRNAs in head and neck squamous cell carcinoma (HNSCC). A total of 67 miRNAs with constant directions reported included 46 (68.7%) to be up-regulated and 21 (31.3%) to be down-regulated. Consistency in the regulation pathway was seen in seven miRNAs (miR-21, miR-7, miR-155, miR-130b, miR-31, miR-223 and miR-34b) to be up-regulated and four down-regulated miRNAs (miR-100, miR-99a, miR-125b and miR-375). The other frequently down-regulated miRNA in OSCC reported include miR-133a, −133b, −137 and − 193a.[34]

Among the many reported, miR-21 is broadly studied and may be considered the most striking biomarker for diagnostic, therapeutic and prognostic application.[35] Overexpression of miR-21 and miR-221 and decreased expression of the let-7 family of miRNAs have been frequently detected in OSCC.[36] Increased miR-21 expression in OSCC has been negatively associated with low levels of TPM1 and PTEN – tumour suppressors mediating apoptotic and cell-cycle events, respectively. miR-21 facilitates the growth of OSCC cells, partly through down-regulating TPM1.[37] The miR-221/miR-222 cluster has been depicted to endorse oral carcinogenesis via the downregulation of PTEN.[38]

An inverse relation between let-7b miRNA and Dicer levels has been detected in OSCC cell lines, with elevated levels of Dicer protein inversed with significantly reduced levels of let-7b. Dicer has been associated with cell-cycle regulation and cell growth and is considered a target of let-7 miRNAs. Combined dicer upregulation with let-7b down-regulation yields the progression of OSCC.[39]

Many miRNAs have been found to be site-specific. Decreased levels of miR-138 and miR-222 can enhance and augment the metastatic potential of tongue OSCC.[40]

miRNA as diagnostic biomarkers

Circulating tumoural miRNAs recently have found their way as prospective biomarkers for early detection. Nakamura *et al*.[10] studied the RNA profiles in ten of the OSCC serum samples via microarrays to identify postulant miRNAs OSCC markers. They identified a panel of six miRNAs as diagnostic markers and found a significant difference in the expression levels of miR-19a, miR-20a, and miR-5100 between OSCC and control groups. miR-19a and miR-20a in OSCC patients were significantly increased, whereas miR-5100 expression was found to be decreased. miR-20a inhibits cancer cell migration, thus, may be considered a prognostic marker.

Liu *et al*.[41] observed a substantial upregulation in the expression of miR-187-5p in 63 OSCC plasma samples compared to 23 healthy controls. The elevated expression of miR-187-5p was markedly reduced in post-tumour resection samples, suggesting that circulating miR-187-5p originated from the tumour cells and miR-187-5p could act as a potential oncogene, promoting migration and proliferation of oral cancer cells and could be used as a predictor of tumour recurrence.

Elevated serum levels of miR-21 are suggested to have a diagnostic and prognostic value in OSCC. Mahmood *et al*.[42] observed a significant overexpression of serum miRNA 21 in OSCC samples, compared to controls and also reported its increased expression proportional to tumour size, invasion and metastasis.

miR-196a/b was detected to be up-regulated at the early developmental stages of oral cancer. miR-196a has been considered an excellent marker for detection specificity whereas miR-196b for detection sensitivity[43,44]

Chen Sun and Jianxia Li[45] investigated the role and clinical significance of miRNA-137, a potential marker for the early diagnosis of OSCC. They observed that miRNA-137 showed a decreased expression in OSCC tissues based on the tumour differentiation and exhibited no correlation with the patient's gender/age, TNM stage, habits and lymph node metastasis.

miRNA as prognostic biomarkers

These small RNAs are also considered to be biomarkers for predicting metastasis and survival rates and can be used to differentiate the stages of OSCC tumours. The patterns of expression of various miRNAs have been found to correlate with clinical stage, lymph node metastasis and patient survival. Higher expression of several oncomiRs and lower expression of tumour suppressor miRNAs have been correlated with poor patient outcomes or overall survival. Few oncogenic miRNAs that have been studied as prognostic markers with their targets in OSCC include miR-21 (via Dickkopf-related protein: DKK2), miR-29a (via upregulating MMP2), miR-196 (non-metastatic cells 4 (NME4)), miR-155 (B-cell CLL/lymphoma 6-BCL6), miR-24 (target F-box and WD-40 domain protein 7 (FBXW7)) and miR-1275 (upregulate insulin-like growth factor 1 receptor: IGF-1R and C-C chemokine receptor type 7: CCR7) promote invasion.[46]

Aberrant expression of miR-21 has also been suggested as an independent predictor of poor survival in patients with tongue OSCC.[37] Yoon *et al*.[47] developed a miRNA-based risk scoring system to identify high-risk early-stage OSCC patients with cancer-specific mortality. They observed that miRNAs-127-3p, 4736 and 655-3p exhibited a prognostic significance related to the AJCC 8 TNM staging system and histologic grading. The high-risk cases showed a 23-fold increased mortality risk with a median time of recurrence of 6 months and a survival time of 11 months. This miRNA scoring system has been evidenced to be reliable for assessing the prognosis of patients with early-stage OSCC.

Serum miR-99a was identified by Chen *et al*.[48] to be an independent prognostic indicator for OSCC. They observed that patients with high miR-99a expression had longer overall survival and recurrence-free survival.

Supic *et al*.,[49] in tongue OSCC patients, observed that miR-183 upregulation had shorter overall survival and miR-21 overexpression had a tendency towards poorer survival. miR-1 and miR-133a have been detected at reduced levels in OSCC and mapped to the same chromosomal locus. miR-1 may a play role in the suppression of metastasis by targeting TAGLN2, a gene coding for an actin-binding protein that mediates cell migration and invasion.[50]

The expression of miR-155-5p was found to be elevated in another study carried out in 68 formalin-fixed, paraffin-embedded OSCC specimens, and was found to be associated with higher pathological TNM stage and relapse. miR-155-5p expression was correlated with clinicopathological parameters, angiolymphatic invasion and stage, and it proved to be a prognostic factor for poorer disease-free survival and could be used as a biomarker predicting relapse.[51]

Another study conducted by Liu *et al*.[52] identified ten miRNAs whose expression levels were highly associated with nodal-disease-free survival, out of which increased expression of miR-21 and miR-107 was found to be commonly associated with OSCC progression and prognosis. They also reported a panel of four miRNA (miR-21-5p, miR-107, miR-1247-3p, and miR-181b-3p) as prognostic biomarkers for nodal disease with high accuracy in assessing the lymph node status.

Rajan *et al*.[53] elucidated new miRNA prognostic signatures for oral cancer in 30 tumours and 18 normal samples. They observed that the upregulation of miR-196a, miR-21, miR-1237 and downregulation of miR-204, and miR-144 were associated with poor prognosis of OSCC patients and identified that the expression ratio of mir-196a/miR-204 could be used as a predictor for disease recurrence and patient survival.

Considering the varied expression of miRNA, we have investigated the recent research works carried out in miRNA in OSCC as prognostic or diagnostic markers, available in Pub-Med/Medline and Google Scholar databases with the keywords 'microRNA' OR 'miRNA' AND 'oral squamous cell carcinoma/OSCC' AND 'Prognostic' OR 'Diagnostic biomarker', to retrieve and update related articles published from 2018 to 2022. The abstracts were assessed to validate

the relevance of articles with the title of the review. Table 1 lists the biomarkers which have prognostic and diagnostic significance.

miRNA as a target for OSCC therapy

Resistance to chemotherapy and radiotherapy are the major challenges faced in the management of OSCC patients as a high proportion of OSCC lesions fail to respond to these treatment modalities. miRNA-based cancer therapy has shown a promising future, as they have the ability to simultaneously target multiple effectors of pathways involved in cell proliferation, differentiation and survival.

miR-101 functions as a tumour suppressor in OSCC by hindering tumour cell proliferation and inducing apoptosis by targeting TGF-βR1. Wang *et al*.[76] observed that miR-101 levels were down-regulated in OSCC cell lines and tissues, repressing cell proliferation and with reduced OSCC

Table 1: MiRNA's as prognostic and diagnostic markers

miRNA	Source	Up/down-regulated	Target	Function	Reference
miR-let-7a	Tissue Cell lines	Down	c-Myc	promotes cell growth and invasion, low expression – poor prognosis	[54]
miR-19a,	Serum	Up	TGFBR3	Marker for OSCC epithelial-to-mesenchymal transition and migration	[10]
miR-20a	Serum	Up	STAT3, PTEN	cell proliferation and cancer progression, prognostic marker	[10]
miR-21	Cell lines	Up	AP1, STAT3	Prognostic tool for survival	[55]
	Plasma	Up		circulating miR-21 originates from tumour mass with a positive association with tumour size, local invasion and metastasis	[42]
miR-23a-3p	Tissue Cell lines	Up	FGF2	suppresses cell proliferation prognostic indicator	[56]
miR-24-3p	Saliva	Up	PER1	Diagnostic biomarker, promotes the proliferation of tumour cells	[57]
miR-29b-1-5p	Tissue Cell lines	Up	CDH1	associated with clinical stage, nodal metastasis, poor prognosis, proliferation	[58]
miR-31-5p	Serum	Up	PTEN/AKT	Diagnostic marker	[59]
miR-99a	Serum	Down	-	Poor differentiation and advanced clinical stage – Prognostic marker	[48]
mir-99b-3p	Tissue	Up	-	Better survival	[60]
mir-100-5p				Poor survival	
miR-105	Tissue Cell lines	Up	-	Promotes Progression of cells	[61]
miRNAs-127-3p, 4736, 655-3p	Tissue	Up		Prognostic marker panel for early-stage OSCC	[47]
miR-134	Plasma Saliva	Up	PDCD7 (Programmed Cell Death 7)	Diagnostic marker	[62]
miRNA-137	Tissues Cell lines	Down		Early diagnosis	[45]
miR-139-5p	Tissue Cell lines	Down	CXCR4	poor survival in OSCC	[63]
microRNA-149-3p	Tissue Cell lines	Down	MMP2	Inhibit OSCC cell migration, prognostic biomarker and therapeutic target for OSCC	[64]
miR-155-5p	Tissue	Up	ARID2	proliferation, migration, invasion, EMT of OSCC cells, prognostic marker	[51,65]
miR-183	Tissues	Up		potential marker of clinical stage, a prognostic biomarker of tumour progression	[66]
miR-196a	Tissues	Up		Low expression - favourable Prognostic marker	[44]
miR-196b	Cell lines				
miR-200b-3p	Plasma	Up		Diagnostic biomarker	[67]
miR-204	Tissue	Down	CDC42, RAB22A, EZR	Patient survival, prognostic marker	[53]
miR-222	Tissue Cell lines	Up	CDKN1B	Early diagnostic marker	[68]
miR-423-5p	Saliva	Up	-	OSCC progression and metastasis – diagnostic and prognostic biomarker	[69]
miR-448	Tissue	Up	MPPED2	prognostic marker	[70]
miR-626	Serum	Up	-	Prognostic role, a predictor of survival	[71]
miR-5100					
miR-654-5p	Tissue Cell lines	Up	GRAP	promote proliferation, metastasis, chemoresistance of OSCC, diagnostic marker	[72]
MiR-770	Tissue Cell lines	Up	Sirtuin 7 (Sirt7)	promoted OSCC cell migration and invasion, metastasis, poor patient survival	[73]
MiR-4282	Cell lines	Down	LIN28B	inhibits tumour progression, prognostic significance	[74]
miR6870-5p miR7111-5p	Tissue	Down	SRF	prognostic marker, Early detection of OSCC	[75]

growth and metastasis. miR-101could act as a therapeutic agent.

Kang *et al*.[77] studied the effect of miR198 on cell proliferation and invasion through direct targeting of cyclin-dependent kinase 4 (CDK4). The overexpression of miR-198 was observed to suppress tumour growth and metastasis in OSCC cells and miR-198/CDK4 axis may be considered as a therapeutic target in OSCC.

miRNAs have been increasingly targeted for therapeutic purposes as they can be effectively administered through local and systemic delivery of miRNA inhibitors (anti-miRNA oligonucleotides or miRNA sponges. Ogawa *et al*.[78] identified a locked nucleic acid (LNA)/DNA antisense oligonucleotide against miR-361-3p (LNA-miR-361-3p) which showed a high degree of growth inhibition of OSCC cell lines and miRNA-361-3p can be considered as a potential therapeutic target for oral squamous cell carcinoma.

Zheng *et al*.[79] observed that miR-211 was highly expressed in OSCC tissues and cell lines, and via its potential target BIN1 (bridging integrator-1), it could inhibit the proliferation, migration and invasion abilities of OSCC cells. They also found that the miR-211 inhibitor could decrease the tumorigenic behaviour of OSCC cells by upregulating BIN1 expression and inhibiting the activation of the EGFR/MAPK pathway, thereby evidencing that miR-211 could function as a potential therapeutic target for the OSCC treatment.

miR-18a-5p/Smad2 could prove to be a potential therapeutic target for OSCC.[80] The overexpression of miR-18a-5p could decrease cell viability, migration and the invasion abilities of OSCC cells and inhibit cell apoptosis.

miRNA IN DIAGNOSTICS AND ITS FUTURE DIRECTIONS IN CANCER DIAGNOSIS AND THERAPY

The literature has evidenced the potential of miRNAs as a diagnostic tumour marker and their expression patterns have been linked to clinical outcomes such that miRNAs modulate tumour behaviour, tumour progression and metastasis.[81]

Many studies have reported the role of miRNAs in many oncogenic processes and the feasibility of miRNA-based therapies. Therapeutic miRNA methods are based on miRNA mimics (miRNA-replacement therapy) and anti-miRs (miRNA-silencing therapy). Based on their role, miRNAs can be either silenced with miRNA inhibitors or therapeutically administered into tumour cells via vectors or through direct delivery into the tissues.[82] Oncogenic miRNAs can be silenced using anti-miR oligonucleotides, miRNA sponges, miRNA masking, and small RNA inhibitors.[83] Li *et al*. have demonstrated that intravenous injection of antisense miR-21 oligonucleotides into tongue squamous cell carcinoma murine models have been shown to reduce tumour growth.[37] The miRNA sponge method is based on transcripts with multiple complementary binding sites to specific miRNAs, thereby making enabling it to inhibit an entire family of related miRNAs.[84] The 'SMIR-approach'/inhibitory-miRNA therapy is based on small-molecule inhibitors of miRNAs that are known to interact with RNA, inhibiting miRNA biogenesis or hindering miRNA-target interaction.[85] Wong *et al*.[86] established that miR-184 inhibitor reduced the proliferation rate in three different tongue squamous cell carcinoma cell lines.

Many studies have also validated the role of miRNAs in chemosensitivity and drug resistance for OSCC. The role of miR-23a and miR-214 in chemoresistance and miR-21 in chemosensitivity have been demonstrated by the administration of anti-miR-23a, anti-miR-214, or pre-miR-21 plasmid in the Tca/cisplatin line which reduced the cell viability during cisplatin treatment.[87]

miRNAs are being used in nanotherapeutics for the treatment of cancer. The co-delivery of an anti-miRNA with a chemotherapeutic drug holds the potential as an improved treatment modality. These molecules have a synergistic effect in combinational therapy. Targeted delivery of short RNA therapeutics in combination with chemotherapeutic drugs not only increases its uptake by cancer cells but also inhibits chemoresistance in cancer cells, thereby enhancing their cytotoxic effects.[88]

CONCLUSION

The repository of miRNAs associated with OSCC is ever-increasing. Many studies are ongoing, and there exist numerous unknown and unidentified miRNAs that play a pivotal role in regulating the hallmarks of tumorigenesis. The use of miRNA as biomarkers for the diagnosis of OSCC has been promising, but there still exists a need for further understanding of miRNA biology in OSCC. It is highly necessary to further assess the different roles of miRNAs, which can contribute to early diagnosis, targeted therapy, and prognostic evaluation of oral cancer patients. To determine the clinical value of miRNA expression, the classification of OSCC by site specificity and therapeutic outcome is a rational strategy to pursue

and the biggest challenges for the future will be the creation of cancer-specific miRNA signature panels that can be highly reproducible and independently predictive of clinico-biological features of the tumour to improve diagnosis and treatment. A combination of several miRNAs would also contribute to the development of multi-omic panels for refining precision oncology. With the existing robust technology and high-end bioinformatics, validating the panel of miRNAs associated with OSCC holds a promising avenue for changing its prognosis and improving its clinical outcome.

Author contribution statement

Priya Thomas undertook literature mining and drafted the manuscript, K Auxzilia Preethi and Sushmaa Chandralekha Selvakumar helped with literature mining and revision. Prathiba Ramani helped in the critical review of the manuscript and Durairaj Sekar was responsible for the final validation and proofreading of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marcu LG, Yeoh. A review of risk factors and genetic alterations in head and neck carcinogenesis and implications for current and future approaches to treatment. J Cancer Res Clin Oncol 2009;135:1303-14.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J. Clin 2015;65:5-29.
3. Saikishore R, Velmurugan P, Ranjithkumar D, Latha R, Sathiamoorthi T, Arun A, *et al*. The circular RNA-miRNA axis: A special RNA signature regulatory transcriptome as a potential biomarker for OSCC. Mol Ther Nucleic Acids 2020;22:352-61.
4. Gombos K, Horváth R, Szele E, Juhász K, Gocze K, Somlai K, *et al*. MiRNA expression profiles of oral squamous cell carcinomas. Anticancer Res 2013;33:1511-8.
5. Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 2004;116:281-97.
6. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843-54.
7. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, *et al*. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005;37:766-70.
8. Chen D, Cabay RJ, Jin Y, Wang A, Lu Y, Shah-Khan M, *et al*. MicroRNA deregulations in head and neck squamous cell carcinomas. J Oral Maxillofac Res 2013;4:e2.
9. Kozomara A, Griffiths-Jones S. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42:D68-73.
10. Nakamura K, Hiyake N, Hamada T, Yokoyama S, Mori K, Yamashiro K, *et al*. Circulating microRNA panel as a potential novel biomarker for oral squamous cell carcinoma diagnosis. Cancers 2021;13:449. doi: 10.3390/cancers13030449.
11. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857-66.
12. Nagadia R, Pandit P, Coman WB, Cooper-White J, Punyadeera C. miRNAs in head and neck cancer revisited. Cell Oncol 2013;36:1-7.
13. Lan H, Lu H, Wang X, Jin H. MicroRNAs as potential biomarkers in cancer: Opportunities and challenges. Biomed Res Int 2015;2015:125094. doi: 10.1155/2015/125094.
14. Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: An overview. Methods Mol Biol 2017;1509:1-10.
15. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: Diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012;4:143-59.
16. Song G, Wang L. MiR-433 and miR-127 arise from independent overlapping primary transcripts encoded by the miR-433-127 locus. PLoS One 2008;3:e3574.
17. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature 2004;432:231-5.
18. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004;10:185-91.
19. Kobayashi H, Tomari Y. RISC assembly: Coordination between small RNAs and Argonaute proteins. Biochim Biophys Acta 2016;1859:71-81.
20. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol 2018;9:402. doi: 10.3389/fendo. 2018.00402.
21. Yang JS, Maurin T, Robine N, Rasmussen KD, Jeffrey KL, Chandwani R, *et al*. Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated microRNA biogenesis. Proc Natl Acad Sci USA 2010;107:15163-8.
22. Reddy KB. MicroRNA (miRNA) in cancer. Cancer Cell Int 2015;15:1-6.
23. Lai EC, Tomancak P, Williams RW, Rubin GM. Computational identification of Drosophila microRNA genes. Genome Biol 2003;4:R42. doi: 10.1186/gb-2003-4-7-r42.
24. Calin CA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, *et al*. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999-3004.
25. Baranwal S, Alahari SK. miRNA control of tumor cell invasion and metastasis. Int J Cancer 2010;126:1283-90.
26. Hammond SM. microRNA detection comes of age. Nat Methods 2006;3:12-3.
27. Xi Y, Nakajima G, Gavin E, Morris CG, Kudo K, Hayashi K, *et al*. Systematic analysis of microRNA expression of RNA extracted from fresh frozen and formalin-fixed paraffin-embedded samples. RNA 2007;13:1668-74.
28. Nelson PT, Baldwin DA, Kloosterman WP, Kauppinen S, Plasterk RH, Mourelatos Z. RAKE and LNA-ISH reveal microRNA expression and localization in archival human brain. RNA 2006;12:187-91.
29. Ye J, Xu M, Tian X, Cai S, Zeng S. Research advances in the detection of miRNA. J Pharm Anal 2019;9:217-26.
30. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, *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.
31. Ventura A, Jacks T. MicroRNAs and cancer: Short RNAs go a long way. Cell 2009;136:586-91.
32. Lajer CB, Nielsen FC, Friis-Hansen L, Norrild B, Borup R, Garnæs E, *et al*. Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: A prospective translational study. Br J Cancer 2011;104:830-40.
33. Chen Z, Jin Y, Yu D, Wang A, Mahjabeen I, Wang C, *et al*. Down-regulation of the microRNA-99 family members in head and neck squamous cell carcinoma. Oral Oncol 2012;48:686-91.
34. Wong TS, Liu XB, Chung-Wai Ho A, Po-Wing Yuen A, Wai-Man Ng R, *et al*. Identification of pyruvate kinase type M2 as potential oncoprotein in squamous cell carcinoma of tongue through microRNA profiling. Int J Cancer 2008;123:251-7.

35. Li X, Zhang Y, Zhang Y, Ding J, Wu K, Fan D. Survival prediction of gastric cancer by a seven microRNA signature. Gut 2010;59:579-85.
36. Zheng J, Xue H, Wang T, Jiang Y, Liu B, Li J, *et al*. miR-21 downregulates the tumor suppressor P12CDK2AP1 and stimulates cell proliferation and invasion. J Cell Biochem 2011;112:872-80.
37. Li J, Huang H, Sun L, Yang M, Pan C, Chen W, *et al*. MiR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. Clin Cancer Res 2009;15:3998-40.
38. Zhou L, Jiang F, Chen X, Liu Z, Ouyang Y, Zhao W, *et al*. Downregulation of miR-221/222 by a microRNA sponge promotes apoptosis in oral squamous cell carcinoma cells through upregulation of PTEN. Oncol Lett 2016;12:4419-26.
39. Gorenchtein M, Poh CF, Saini R, Garnis C. MicroRNAs in an oral cancer context-from basic biology to clinical utility. J Dent Res 2012;91:440-6.
40. Jiang L, Liu X, Jiang X, Yin Q, Hao Y, Xiao L. MiR-223 promotes oral squamous cell carcinoma proliferation and migration by regulating FBXW7. Cancer Biomark 2019;24:325-34.
41. Liu CJ, Lin JS, Cheng HW, Hsu YH, Cheng CY, Lin SC. Plasma miR-187* is a potential biomarker for oral carcinoma. Clin Oral Investig 2017;21:1131-8.
42. Mahmood N, Hanif M, Ahmed A, Jamal Q, Mushtaq S, Khan A, *et al*. Circulating miR-21 as a prognostic and predictive biomarker in oral squamous cell carcinoma. Pak J Med Sci 2019;35:1408-12.
43. Lu YC, Chang JT, Huang YC, Huang CC, Chen WH, Lee LY, *et al*. Combined determination of circulating miR-196a and miR-196b levels produces high sensitivity and specificity for early detection of oral cancer. Clin Biochem 2015;48:115-21.
44. Dioguardi M, Cantore S, Sovereto D, La Femina L, Caloro GA, Spirito F, *et al*. Potential Role of miR-196a and miR-196b as prognostic biomarkers of survival in head and neck squamous cell carcinoma: A systematic review, meta-analysis and trial sequential analysis. Life 2022;12:1269. doi: 10.3390/life12081269.
45. Sun C, Li J. Expression of MiRNA-137 in oral squamous cell carcinoma and its clinical significance. J BUON 2018;23:167-72.
46. Siriwardena SB, Tsunematsu T, Qi G, Ishimaru N, Kudo Y. Invasion-related factors as potential diagnostic and therapeutic targets in oral squamous cell carcinoma—A review. Int J Mol Sci 2018;19:1462. doi: 10.3390/ijms19051462.
47. Yoon AJ, Wang S, Kutler DI, Carvajal RD, Philipone E, Wang T, *et al*. MicroRNA-based risk scoring system to identify early-stage oral squamous cell carcinoma patients at high-risk for cancer-specific mortality. Head Neck 2020;42:1699-712.
48. Chen L, Hu J, Pan L, Yin X, Wang Q, Chen H. Diagnostic and prognostic value of serum miR-99a expression in oral squamous cell carcinoma. Cancer Biomark 2018;23:333-9.
49. Supic G, Zeljic K, Rankov AD, Kozomara R, Nikolic A, Radojkovic D, *et al*. miR-183 and miR-21 expression as biomarkers of progression and survival in tongue carcinoma patients. Clin Oral Invest 2018;22:401-9.
50. Nohata N, Sone Y, Hanazawa T, Fuse M, Kikkawa N, Yoshino H, *et al*. miR-1 as a tumor suppressive microRNA targeting TAGLN2 in head and neck squamous cell carcinoma. Oncotarget 2011;2:29-42.
51. Kim H, Yang JM, Ahn SH, Jeong WJ, Chung JH, Paik JH. Potential oncogenic role and prognostic implication of microRNA-155-5p in oral squamous cell carcinoma. Anticancer Res 2018;38:5193-200.
52. Liu KY, Zhu SY, Brooks D, Bowlby R, Durham JS, Ma Y, *et al*. Tumor microRNA profile and prognostic value for lymph node metastasis in oral squamous cell carcinoma patients. Oncotarget 2020;11:2204-15.
53. Rajan C, Roshan VG, Khan I, Manasa VG, Himal I, Kattoor J, *et al*. MiRNA expression profiling and emergence of new prognostic signature for oral squamous cell carcinoma. Sci Rep 2021;11:1-2. doi: 10.1038/s41598-021-86316-w.
54. Luo C, Zhang J, Zhang Y, Zhang X, Chen Y, Fan W. Low expression of miR-let-7a promotes cell growth and invasion through the regulation of c-Myc in oral squamous cell carcinoma. Cell Cycle 2020;19:1983-93.
55. Hunsaker M, Barba G, Kingsley K, Howard KM. Differential microRNA expression of miR-21 and miR-155 within oral cancer extracellular vesicles in response to melatonin. Dent J 2019;7:48. doi: 10.3390/dj7020048.
56. Chen F, Qi S, Zhang X, Wu J, Yang X, Wang R. miR-23a-3p suppresses cell proliferation in oral squamous cell carcinomas by targeting FGF2 and correlates with a better prognosis: miR-23a-3p inhibits OSCC growth by targeting FGF2. Pathol Res Pract 2019;215:660-7.
57. He L, Ping F, Fan Z, Zhang C, Deng M, Cheng B, *et al*. Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomed Pharmacother 2020;121:109553. doi: 10.1016/j.biopha. 2019.109553.
58. Kurihara-Shimomura M, Sasahira T, Shimomura H, Nakashima C, Kirita T. The oncogenic activity of miR-29b-1-5p induces the epithelial-mesenchymal transition in oral squamous cell carcinoma. J Clin Med 2019;8:273. doi: 10.3390/jcm8020273.
59. Lu Z, He Q, Liang J, Li W, Su Q, Chen Z, *et al*. miR-31-5p is a potential circulating biomarker and therapeutic target for oral cancer. Mol Ther Nucleic Acids 2019;16:471-80.
60. Jakob M, Mattes LM, Küffer S, Unger K, Hess J, Bertlich M, *et al*. MicroRNA expression patterns in oral squamous cell carcinoma: hsa-mir-99b-3p and hsa-mir-100-5p as novel prognostic markers for oral cancer. Head Neck 2019;41:3499-515.
61. Zhang HY, Ma JH. miR-105 promotes the progression and predicts the prognosis for oral squamous cell carcinoma (OSCC). Cancer Manag Res 2020;12:11491-9.
62. Peng SY, Tu HF, Yang CC, Wu CH, Liu CJ, Chang KW, *et al*. miR-134 targets PDCD7 to reduce E-cadherin expression and enhance oral cancer progression. Int J Cancer 2018;143:2892-904.
63. Jiang Q, Cao Y, Qiu Y, Li C, Liu L, Xu G. Progression of squamous cell carcinoma is regulated by miR-139-5p/CXCR4. Front. Biosci 2020;25:1732-45.
64. Shen Q, Xiong P, Yang D, Chen L. Downregulated microRNA-149-3p triggers malignant development and predicts worse prognosis in oral squamous cell carcinoma. Arch Oral Biol 2022;134:105336. doi: 10.1016/j.archoralbio. 2021.105336.
65. Wu M, Duan Q, Liu X, Zhang P, Fu Y, Zhang Z, *et al*. MiR-155-5p promotes oral cancer progression by targeting chromatin remodeling gene ARID2. Biomed Pharmacother 2020;122:109696. doi: 10.1016/j.biopha. 2019.109696.
66. Supic G, Zeljic K, Rankov AD, Kozomara R, Nikolic A, Radojkovic D, *et al*. miR-183 and miR-21 expression as biomarkers of progression and survival in tongue carcinoma patients. Clinical Oral Investigations 2018;22:401-9.
67. Sun G, Cao Y, Wang P, Song H, Bie T, Li M, *et al*. miR-200b-3p in plasma is a potential diagnostic biomarker in oral squamous cell carcinoma. Biomarkers 2018;23:137-41.
68. Chen Q, Wang W, Wang Y. MiR-222 regulates the progression of oral squamous cell carcinoma by targeting CDKN1B. Am J Transl Res 2022;14:5215-7.
69. Romani C, Salviato E, Paderno A, Zanotti L, Ravaggi A, Deganello A, *et al*. Genome-wide study of salivary miRNAs identifies miR-423-5p as promising diagnostic and prognostic biomarker in oral squamous cell carcinoma. Theranostics 2021;11:2987-9.
70. Wei H, Yu K, Liu Y, Li L, Wang G. Tumor expression of miR-448 is a prognostic marker in oral squamous cell carcinoma. BMC Cancer 2020;20:1-6.
71. Shi J, Bao X, Liu Z, Zhang Z, Chen W, Xu Q. Serum miR-626 and miR-5100 are promising prognosis predictors for oral squamous cell carcinoma. Theranostics 2019;9:920-31.
72. Lu M, Wang C, Chen W, Mao C, Wang J. miR-654-5p targets GRAP to promote proliferation, metastasis, and chemoresistance of oral squamous cell carcinoma through Ras/MAPK signaling. DNA Cell Biol 2018;37:381-8.
73. Jia B, Zhang S, Wu S, Zhu Q, Li W. MiR-770 promotes oral squamous cell carcinoma migration and invasion by regulating the Sirt7/Smad4 pathway. IUBMB Life 2021;73:264-72.
74. Zhang Y, Zhang Z, Huang W, Zeng J. MiR-4282 inhibits tumor

progression through down-regulation of ZBTB2 by targeting LIN28B in oral squamous cell carcinoma. J Cell Physiol 2020;235:8035-47.
75. Saraei M, Garajei A, Jamshidian F. miR-7111-5p and miR6870-5p may be potential biomarkers for oral squamous cell carcinoma. Turk J Oncol 2021;36:252-8.
76. Wang Y, Jia RZ, Diao S, He J, Jia L. MiRNA-101 targets TGF-βR1 to retard the progression of oral squamous cell carcinoma. Oncology Research 2020;28:203-12.
77. Kang Y, Zhang Y, Sun Y. MicroRNA-198 suppresses tumour growth and metastasis in oral squamous cell carcinoma by targeting CDK4. Int J Oncol 2021;59:1-3. doi: 10.3892/ijo. 2021.5219.
78. Ogawa H, Nakashiro KI, Tokuzen N, Kuribayashi N, Goda H, Uchida D. MicroRNA-361-3p is a potent therapeutic target for oral squamous cell carcinoma. Cancer Sci 2020;111:1645-51.
79. Zheng J, Wang J, Jia Y, Liu T, Duan Y, Liang X, Liu L. microRNA-211 promotes proliferation, migration, and invasion ability of oral squamous cell carcinoma cells via targeting the bridging integrator 1 protein. J Cell Biochem 2019;120:4644-53.
80. Huang C, Song H, Lai L. The role and mechanism of microRNA-18a-5p in oral squamous cell carcinoma. Mol Med Rep 2019;20:1637-44.
81. Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. J Pathol 2011;223:102-15.
82. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704-14.
83. Mollaei H, Safaralizadeh R, Rostami Z. MicroRNA replacement therapy in cancer. J Cell Physiol 2019;234:12369-84.
84. Ebert MS, Sharp PA. MicroRNA sponges: Progress and possibilities. RNA 2010;16:2043-50.
85. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A. Small-molecule inhibitors of microRNA miR-21 function. Angew Chemie Int Ed 2008;47:7482-4.
86. Wong TS, Liu XB, Wong BYH, Ng RWM, Yuen APW, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res 2008;14:2588-92.
87. Yu ZW, Zhong LP, Ji T, Zhang P, Chen WT, Zhang CP. MicroRNAs contribute to the chemoresistance of cisplatin in tongue squamous cell carcinoma lines. Oral Oncol 2010;46:317-22.
88. Ganju A, Khan S, Hafeez BB, Behrman SW, Yallapu MM, Chauhan SC, *et al*. miRNA nanotherapeutics for cancer. Drug Discovery Today 2017;22:424-32.