Roles of microRNAs in Regulating Cancer Stemness in Head and Neck Cancers

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Simple Summary: Head and neck squamous cell carcinomas (HNSCCs) are highly heterogeneous human malignancies associated with genetic and environmental factors. In HNSCCs, cancer stem cells (CSCs) provide the plasticity for cancer cell progression, metastasis, therapeutic resistance, and recurrence. During carcinogenesis, microRNAs (miRNAs) play important roles in regulating the maintenance and acquisition of cancer stem cell features. Therefore, in this review, we summarize the roles of miRNAs in regulating the cancer stemness of HNSCCs to provide potential therapeutic applications.

Abstract: Head and neck squamous cell carcinomas (HNSCCs) are epithelial malignancies with 5-year overall survival rates of approximately 40–50%. Emerging evidence indicates that a small population of cells in HNSCC patients, named cancer stem cells (CSCs), play vital roles in the processes of tumor initiation, progression, metastasis, immune evasion, chemo-/radioresistance, and recurrence. The acquisition of stem-like properties of cancer cells further provides cellular plasticity for stress adaptation and contributes to therapeutic resistance, resulting in a worse clinical outcome. Thus, targeting cancer stemness is fundamental for cancer treatment. MicroRNAs (miRNAs) are known to regulate stem cell features in the development and tissue regeneration through a miRNA–target interactive network. In HNSCCs, miRNAs act as tumor suppressors and/or oncogenes to modulate cancer stemness and therapeutic efficacy by regulating the CSC-specific tumor microenvironment (TME) and signaling pathways, such as epithelial-to-mesenchymal transition (EMT), Wnt/β-catenin signaling, and epidermal growth factor receptor (EGFR) or insulin-like growth factor 1 receptor (IGF1R) signaling pathways. Owing to a deeper understanding of disease-relevant miRNAs and advances in in vivo delivery systems, the administration of miRNA-based therapeutics is feasible and safe in humans, with encouraging efficacy results in early-phase clinical trials. In this review, we summarize the present findings to better understand the mechanical actions of miRNAs in maintaining CSCs and acquiring the stem-like features of cancer cells during HNSCC pathogenesis.

Keywords: microRNA; cancer stem cell; stemness; head and neck squamous cell carcinoma
1. Introduction

Cancer is responsible for about 30% of all premature deaths from non-communicable diseases (NCDs) in adults aged approximately 30–69 years [1]. In 2018, there were 18.1 million people who suffered from cancer worldwide, and 9.6 million of them died from cancer (around one in six deaths globally). In addition, 354,864 (2% of all cancer sites) of new cases were of the lip and oral cavity, accounting for 177,384 (1.9% of all cancer sites) deaths [1,2]. Head and neck squamous cell carcinomas (HNSCCs) are epithelial malignancies located in the oral cavity, nasal cavity, pharynx (nasopharynx, oropharynx, and hypopharynx), and larynx [3,4]. HNSCC subtypes include oral SCCs (OSCCs), laryngeal SCCs (LSCCs), nasopharyngeal carcinomas (NPCs), and oropharyngeal SCCs (OPSCCs) [5,6]. It should be noted that with tongue squamous cell carcinomas, an OSCC includes the anterior two-thirds of the tongue (anterior to the circumvallate papillae) and an OPSCC consists of the base (or posterior one-third) of the tongue [7]. The incidence of HNSCC continues to rise and is anticipated to increase by 30% with 1.08 million new cases annually by 2030 [2,8,9].

Risk factors for the malignant incidence of HNSCCs include tobacco use, alcohol abuse, and human papillomavirus (HPV) infection [3,6]. Signs and symptoms can manifest as a lesion in the nose, mouth, or throat; a lump or neck mass; and ear discomfort; and functional abnormalities such as difficulty swallowing and/or chewing are often found in the later stages of these diseases [3]. The tumor, node, and metastasis (TNM) staging system is used for clinical staging and as a basis for treatment choice [10]. Therapeutic approaches include surgery, radiotherapy, chemoradiotherapy, a combination of surgery with radiotherapy or chemotherapy, and a combination of surgery with adjuvant chemotherapy and radiotherapy. Chemoradiotherapy may be taken as adjuvant therapy in advanced stages [11]. Unfortunately, despite several treatment options being available, outcomes of HNSCC treatment remain poor, patients generally develop resistance, and, as a result, the five-year overall survival rates of HNSCC patients are approximately 40–50% [6,12]. Advanced approaches have been developed by applying immunotherapy or combined immunotherapy treatment to treat resistant and recurrent cases [13].

The major obstacle in cancer therapy is tumor heterogeneity. Cancer stem cells (CSCs) are small populations of cancer cells and are well-known for their association with cancer resistance, relapse, tumorigenesis, and poor clinical outcomes in HNSCCs, which has promoted the development of novel and effective therapeutic protocols for better clinical outcomes [5,14]. Therefore, targeting CSCs has become an attractive approach for potential strategies to treat HNSCCs [15,16]. The abnormal activation of signaling casettes, genetic and epigenetic modification, and microRNA (miR or miRNA) regulation are central regulators of CSC malignancy [17–19]. miRNAs work as hub regulators to modulate cell functions by binding to multiple 3′-untranslated regions (3′-UTRs) of target messenger RNAs (mRNAs) and cause the translational inhibition and/or degradation of transcripts [19–21]. Therefore, in this review, we address the roles of miRNAs in regulating the cancer stemness of HNSCCs.

2. CSCs and Cancer Stemness

CSCs are a minor population of cells within tumor tissues with a tumor-initiating capacity [22] and stem-like features, including self-renewal [23,24] and asymmetric cell division (ACD) [25]. Under chemotherapy, the cycling rates of CSCs slow and they enter the G0 phase in order to survive, accounting for therapeutic heterogeneity [26–28]. Cancer patients with higher stem cell signatures present poorly differentiated histological properties and are associated with a worse clinical outcome [29]. CSCs are heterogeneous populations. In colorectal cancer (CRC) tissues, prominin-1 (CD133) is the first molecular marker used to isolate colorectal cancer stem cells (CRCSCs) [30,31]. The epithelial-specific antigen (ESA)(+)/CD44 molecule and Indian blood group (CD44)(+) CRCSCs are associated with tumor recurrence after chemotherapy [32]. Dipeptidyl peptidase 4 (CD26)(+) CRCSCs enriched from CD133(+)CD44(+) cells drive tumor metastasis [33]. Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)(+) CRCSCs are considered to be
responsible for liver metastasis [34]. CD24 molecule (CD24) and activated leukocyte cell adhesion molecule (CD166) surface antigens are often combined with CD44 or CD133 for the identification and separation of CRCSCs [35]. In HNSCC, CSCs are grouped in accordance with the expression of surface markers such as CD44+ and aldehyde dehydrogenase 1+ (ALDH1+). CD44 mediates the adhesion, migration, and metastasis of CSCs [36], while ALDH1 ameliorate oxidative stress under therapeutic regimens such as platinum, taxanes, and oxazaphosphorine [37,38].

Despite the fact that the origins of CSCs have been linked to genetic mutation, epigenetic alterations, and unrestrained signaling pathways for the normal stem cells and progenitor cells [39,40], CSC properties would be induced or maintained by inflammatory mediators. Inflammatory cytokines and chemokines secreted by CSCs, including interleukin (IL)-1, IL-4, IL-6, and IL-8, sustain CSC niches in an autocrine manner [41–44]. Besides, the expression of IL-8 promotes the migratory and tube-forming capacities of endothelial cells [44]. IL-6 is also involved in cancer metastasis [45]. IL-6 activates Janus kinase 1 (JAK1) and phosphorylates programmed death–ligand 1 (PD-L1) and promotes PD-L1 protein stability [46]. CSCs also enhance PD-L1 expression to escape immune surveillance, thereby enriching the CSC subpopulation [47–49]. In addition to secretory proteins, CSCs create an immunosuppressive, pro-tumoral microenvironment by releasing CSC exosomes for cancer progression [50,51].

To target CSCs, researchers have focused on deciphering how cancer cells acquire stemness properties. The major mechanisms involve the expression of genes associated with early development and aberrant intracellular signaling activation. Activation of stemness regulators sustains the stemness properties of HNSCCs, including the MYC proto-oncogene, bHLH transcription factor (MYC), sex-determining region Y-box 2 (SOX2), Nanog homeobox (NANOG), Krüppel-like factor 4 (KLF4), octamer-binding transcription factor 4 (OCT4), high-mobility group AT-hook 2 (HMG2A), cytokines, and epithelial-to-mesenchymal transition (EMT) transcription factors (EMT-TFs) [52–58]. On the other hand, abnormal signaling activation in Notch, Wnt(gingless)/β-catenin, transforming growth factor-β (TGF-β), Janus-activated kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor-κB (NF-κB), and the sonic hedgehog (SHH) pathway maintains cancer stemness [59–64]. Therefore, the rationale for identifying combinatorial therapeutic strategies combating CSC is intriguing.

3. miRNAs

miRNAs are non-coding (nc) RNA components with approximately 21–23 nucleotides that bind to and repress complementary mRNA targets [21,65]. Previously, ncRNAs were only considered to be evolutionary junk, but emerging evidence has indicated that miRNAs have important cellular functional roles and act as post-translational regulators [21,65–67]. miRNAs control around 30% of human genes, and about half of those genes are tumor associated or situated in vulnerable loci [68–70]; other studies have even suggested that miRNAs can regulate the expression of more than 60% of human genes [71,72]. miRNA expression is modulated by several mechanisms, such as transcriptional control, epigenetic modulation, and post-transcriptional regulation [67,73,74]. On the other hand, the biogenesis of miRNAs can mainly be divided into six steps: (1) RNA polymerase II transcribes miRNA genes into primary (pri)-miRNAs in the nucleus [75], (2) intermediate precursor (pre)-miRNA is released by pri-miRNA after being processed by the Drosha/DiGeorge syndrome critical region 8 (DGCR8) complex [75,76], (3) pre-miRNA bonds to exportin-5 (Exp5)/ras-related nuclear protein (Ran)-guanosine 5′-triphosphate (GTP) complex and is transferred to the cytoplasm [77], (4) the Dicer/(HIV-1 transactivating response (TAR)) RNA-binding protein (TRBP)/PACT complex turns pre-miRNA into double-stranded (ds) RNA in the cytoplasm [78–80], (5) the miRNA duplex is released into single strands by helicase [81], and (6) the miRNA-induced silencing complex (RISC) is bound to the 3′-UTRs of target miRNAs via the seed region of miRNA and subsequently triggers inhibition of the translation or degradation of target miRNAs [82]. The seed region of an miRNA (also
known as the seed sequence) is a short, conserved sequence at nucleotides 2–8 at the 5′ end of the miRNA [83–85]. Therefore, the miRNA target prediction tools rely on an algorithm with the thermodynamics-based modeling of RNA, i.e., RNA duplex interactions with comparative sequence analysis to evaluate the seed region matching to the mRNAs [86].

The squamous epithelium covering the oral mucosa and skin depends on epithelial stem cells for tissue renewal [87]. In the oral mucosa, the basal cell layer harbors the self-renewing stem cells and their immediate descendants, the transient amplifying progenitor cells, to produce expanded terminally differentiating cells [88]. The terminally differentiating cells then leave the basal layer and form the outer layers to maintain the oral mucosa integrity. Therefore, stem cells and the proper controls between the phase transition of stem cells and differentiating cells are critical to maintaining tissue homeostasis. Evidence has shown that miRNA expression patterns control the epithelium stem cells’ characteristics. For example, Peng et al. indicated that the miR-103/107 family is highly expressed in the stem-cell-enriched limbal epithelium. The miR-103/107 family regulates and integrates these stem cell characteristics, thereby sustaining tissue maintenance and regeneration [89]. Moreover, studies have also indicated that the epidermal-specific deletion of enzymes responsible for miRNA maturation, such as DICER, Drosha, and DGCR8, severely impairs the homeostasis and morphogenesis of the epidermis [90–93]. These results indicate that miRNA expression is critical for the proper development of the epidermis and oral mucosa. Moreover, emerging evidence also highlights the importance of miRNAs in regulating carcinogenesis and CSCs. Better characterizations of miRNAs in regulating the stemness features of CSCs will contribute to better cancer treatment strategies (Figure 1).

Figure 1. The generations and roles of miRNAs in cancer stem cell (CSC) regulation. In miRNA generation, miRNA genes are commonly transcribed by RNA Pol II in the nucleus. This transcription, which is cleaved by Drosha, produces a multiprotein complex with the DGCR8 protein. Cleavage induces pre-miRNA binding to the nuclear export factor EXO5 and then transported from the nucleus to the cytoplasm. In the cytoplasm, Dicer (another RNase III), which forms a complex with the double-stranded RNA-binding protein TRBP, cuts out the hairpin and produces an RNA duplex with approximately 22 nucleotides. The RNA duplex is dissociated to a single strand via AGO2 mediation, incorporated into the RISC, and binds to 3′-UTRs of target mRNAs via the seed region of miRNA and subsequently triggers mRNA degradation. Therefore, miRNAs can regulate cancer stemness properties and phenotypes by targeting the critical genes that control the activation of signaling pathways, transcriptional factors, and secreted factors. RNA Pol II, RNA polymerase II; pri-miRNAs, primary miRNAs; pre-miRNAs, precursor miRNAs; EXO5, exportin 5; AGO2, argonaute 2; RISC, miRNA-induced silencing complex; mRNA, messenger RNA; miRNA, microRNA (created with Biorender.com (accessed on 30 January 2021)).
4. miRNAs in Regulating Cancer Stemness

CSCs maintain and acquire stemness features through complex mechanisms, including abnormal activation of oncogenes, cytokines, signaling pathways, and EMT-TFs, as mentioned in Section 2 above. Studies indicated that miRNAs that regulate cancer stemness mainly depend on post-translational regulation to modulate activation of those stemness-related factors. Several studies have proven that abnormal miRNA expressions can act as oncogenes, tumor suppressors, or dual-role regulators [94,95]. All of these data highlight the potential for targeting miRNAs to eradicate CSCs, and researchers are working on anti-miRNA drugs and are searching for diagnostic miRNAs [96–98]. miRNAs have been applied as biomarkers to determine cancer prognoses and diagnoses due to their stability [99–101]. Xia et al. indicated that various tumor mutational burden levels had different miRNA expression patterns in HNSCC patients [102], and correlations between miRNA prognostic values as applied to HNSCCs have generated significant interest among researchers [103–105].

4.1. miRNAs as Oncomirs

As oncomirs, miRNAs can act as oncogenic miRNAs that promote biological processes such as proliferation, migration, angiogenesis, invasion, EMT, and stemness [106–111]. Oncomirs regulate cancer stemness through targeting their downstream targets which results in activation of stemness-related factors and signaling pathways. Therefore, oncomirs were shown to enhance tumor initiation and progression by modifying CSC properties such as self-renewal, tumorigenesis, drug resistance, and signaling pathways in cancer [112–115].

Several mechanisms for the oncogenicity of HNSCCs can be affected by miRNA presence. For example, miR-125a enhances the proliferation, migration, invasion, and stemness maintenance in cancer cells via suppressing p53 expression [116]. The overexpression of p53 makes cell viability significantly decrease and induces cell cycle arrest at the G0/G1 phase [116]. miR-134 suppresses E-cadherin expression and promotes OSCC cell progression through targeting programmed cell death 7 (PDCD7) [117]. E-cadherin can suppress cancer stemness by regulating the expressions of pluripotent genes (MYC, NESTIN, POU5F1, and SOX2) via the activation of Wnt/β-catenin signaling [118]. On the other hand, by suppressing the expression of the WW domain-containing oxidoreductase (WWOX) gene, miR-134 can trigger oncogenicity and metastasis in HNSCCs [119]. WWOX is a tumor stemness suppressor that reduces the self-renewal ability of CSCs, differentiation potential, in vivo tumorigenic capability, and multidrug resistance [120]. Consistently, the downregulation of WWOX was indicated to induce EMT, enhance stemness, and increase chemoresistance in breast cancer [121]. miR-1246 confers tumorigenicity and affects cancer stemness in OSCC through suppressing cyclin-G2 (CCNG2) [122] CCNG2 has been shown to suppress EMT by disrupting Wnt/β-catenin signaling [123], which has been proven to be involved in the migration and invasion of OSCCs [124].

Protocadherins are cell–cell adhesion molecules. The loss of protocadherins may contribute to cancer development not only by altering cell–cell adhesion but also by enhancing proliferation and EMT via activating the Wnt signaling pathway [125,126]. With LSCC, Giefing et al. showed that protocadherin 17 (PCDH-17) acts as a tumor suppressor gene [127]. Inhibition of miR-196b can suppress cell proliferation, migration, and invasion abilities but promote apoptosis by targeting PCDH-17 in LSCC cells [128]. Moreover, LSCC patients with low expression of miR-196b and high expression of PCDH-17 were shown to have an increase in the 5-year survival rates [128]. miR-19a and miR-424 inhibit the TGF-β type III receptor (TGFBR3), also known as β-glycan, which results in promoting the EMT of tongue squamous carcinoma cells [108]. Other studies have also indicated that miR-19a promotes migration and EMT in gastric cancer, CRC, and lung cancer [129–131].

Mitogen-activated protein kinase (MAPK) signaling cascades are critical signal pathways related to EMT, which promotes cancer cell progression and metastasis in CSCs [132]. miR-106A-5p facilitates a malignant phenotype by acting as an autophagic suppressor through targeting BTG anti-proliferation factor 3 (BTG3) and activates autophagy-regulating
MAPK signaling in NPC [133]. MYC target 1 (MYCT1), a direct target gene of MYC, is a novel candidate tumor suppressor gene cloned from LSCC [134,135]. MYCT1 protein suppresses miR-629-3p expression by reducing specificity protein 1 (SP1) expression. SP1 is also a TF for miR-629-3p, and its suppression enhances the expression of miR-629-3p’s downstream target, epithelial splicing regulatory protein 2 (ESRP2). Taken together, MYCT1 protein suppresses the EMT of laryngeal cancer via the SP1/miR-629-3p/ESRP2 pathway [136]. Previous studies have shown that oral CSCs switch from expressing the CD44-variant form (CD44v) to expressing the standard form (CD44s) during the acquisition of cisplatin resistance, which results in EMT induction [137]. During the process, CD44s induces miR-629-3p expression, which inhibits apoptotic cell death under cisplatin treatment conditions and promotes cell migration in HNSCCs [138]. Therefore, miR-629-3p serves as a therapeutic target to reverse chemotherapy resistance. Altogether, the miRNAs as oncomirs that regulate the stemness process of HNSCC are summarized in Table 1.

### Table 1. miRNAs as oncomirs that are involved in the stemness of head and neck squamous cell carcinomas (HNSCCs).

| Oncomirs    | Target(s) | Molecular Mechanism                                                                 | Action Mode | Refs            |
|-------------|-----------|-------------------------------------------------------------------------------------|-------------|-----------------|
| miR-125a    | p53       | miR-125a enhances cell proliferation, migration, invasion, and stemness maintenance through suppression of p53. | Gene expression | [116]          |
| miR-134     | PDCD7     | miR-134 reduces E-cadherin expression by suppressing PDCD7. E-cadherin inhibition enhances expressions of pluripotent genes. | Gene expression | [117,118]      |
| miR-134     | WWOX      | miR-134 suppresses WWOX, a tumor stemness suppressor.                               | Suppressor inhibition | [119,120]      |
| miR-1246    | CCNG2     | miR-1246 promotes cancer stemness and tumorigenicity by suppressing CCNG2.         | Suppressor inhibition | [122,123]      |
| miR-196b    | PCDH-17   | miR-196b promotes cell proliferation, migration, and invasion abilities by inhibiting PCDH-17. | Suppressor inhibition | [127,128]      |
| miR-19a,    | TGFBR3    | miR-19a and miR-424 promote EMT by suppressing TGFBR3.                             | Signal transduction | [108]          |
| miR-424     | BTG3      | miR-106A-5p inhibits autophagy and activates MAPK signaling by targeting BTG3.       | Signal transduction | [133]          |
| miR-629-3p  | ESRP2     | miR-629-3p enhances EMT via targeting ESRP2.                                       | EMT process  | [136]          |

EMT, epithelial-to-mesenchymal transition; MAPK, mitogen-activated protein kinase.

### 4.2. miRNAs as Tumor Suppressors

In contrast, tumor suppressor miRNAs were found to suppress activation of stemness factors, thereby decreasing CSC populations and tumor progression. Studies indicated that expressions of tumor suppressor miRNAs were commonly reduced in tumor samples. Conversely, their corresponding oncogenic downstream targets were activated, thereby activating stemness factors and enhancing the ability of cancer cells to acquire stemness features.

#### 4.2.1. miRNAs in HNSCC

The miRNA let-7 family controls normal cellular development and differentiation, and a reduction in let-7 contributes to carcinogenesis via the upregulation of oncogenic downstream targets and stemness properties [99]. Therefore, members of the let-7 family are considered to be tumor suppressors for various cancers [139]. Ten members of the human let-7 family have been identified, i.e., let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98, and miR-202, which share the same seed region sequence [140,141]. Expressions of let-7 family members decrease in HNSCCs patients, and among them, let-7i has been shown to most significantly suppress the expression of the chromatin modifier AT-rich interacting domain 3B (ARID3B). By suppressing let-7i expression, cells enhance ARID3B expression and acquire stemness features by activating embryonic SC (ESC)-specific genes such as POLSF1, NANOG, and SOX2 via histone modifications [142]. The study also
indicated that the EMT factor twist family bHLH transcription factor 1 (Twist1) cooperates with B lymphoma Mo-MLV insertion region 1 (BMI1), suppresses let-7i expression, and contributes to stem-like properties, thus enabling mesenchymal movements [143]. In OSCC of the tongue, ALDH1+ cells with cancer stemness characteristics show decreased expression of let-7a and high expressions of NANOG and POU5F1. let-7a overexpression in ALDH1+ cells further inhibited tumor formation and metastasis in vivo, suggesting that the let-7a gene plays an important role in modulating tumorigenesis stemness of HNSCC cells [144].

Moreover, radioresistance poses a major challenge in HNSCC treatment, in which CSCs are relatively radioresistant owing to different intrinsic and extrinsic factors [145]. Evidence has indicated that miRNAs might regulate not only stemness properties but also radiotherapy response. For example, let-7c contributes to oral cancer stemness and radio/chemoresistance through suppressing CXCL8 (IL-8) [146]. Similarly, CXCL8 was identified as a direct target of miR-203, and the reduction in miR-203 promoted radioresistance by activating IL-8/AKT serine/threonine kinase 1 (AKT) signaling in NPC cells [147]. The low expression of miR-203 was also showed to enhance EMT and result in intrinsic radioresistance of HNSCC, which could enable identification and treatment modification of radioresistant tumors [148]. miR-520b attenuates cell mobility via EMT suppression and suppressed spheroid cell formation, as well as reduced expressions of multiple stemness regulators (Nestin, Twist1, NANOG, OCT4) through targeting suppression of CD44 in HNSCC cells [149]. Moreover, miR-520b also sensitized cells to therapeutic drugs and irradiation through targeting CD44 [149]. CD44 is an adhesion molecule expressed in CSCs, which interacts with a glutamate–cystine transporter and controls the intracellular level of reduced glutathione (GSH). Therefore, CSCs with high CD44 expression show an enhanced capacity for GSH synthesis, resulting in higher reactive oxygen species (ROS) defense and radiotherapy resistance [150,151]. Therefore, miR-520b suppresses CD44 and not only inhibits cancer stemness and multiple malignant properties but also sensitizes cells to chemoradiotherapy [149].

miR-101 acts as a potent tumor suppressor, and its downregulation is associated with oral carcinomas [152]. In HNSCCs, low expressions of miR-101 upregulate the oncogene Zeste homolog 2 (EZH2), which subsequently downregulates another tumor suppressor gene rap1GAP, which promotes HNSCC progression. EZH2 is a histone methyltransferase that belongs to the polycomb repressive complex 2 (PRC2) family that facilitates the trimethylation of H3K27 on the rap1GAP promoter to suppress its activation [153,154]. EZH2 can regulate cancer stemness by mediating the NOTCH1 activator and signaling to promote the initiation and growth of SCs [155]. EZH2 was shown to promote cell migration, invasion, and metastasis, and EMT, thereby enhancing cellular plasticity for oral tongue squamous cell carcinomas [156,157]. The miR-29 family is also significantly downregulated in HNSCC patients [158]. Moreover, miR-29b suppresses DNA methyltransferase 3 beta (DNMT3B), resulting in inhibited EMT and promoted invasiveness of HNSCC cell lines through restoring E-cadherin expression by the demethylation of the promoter region [159].

miR-204-5p is a tumor suppressor in HNSCCs, which inhibits tumor growth, metastasis, and stemness by suppressing the signal transducer and activator of transcription 3 (STAT3) signaling and EMT via targeting SNAI2, SUZ12, HDAC1, and JAK2 [160]. STAT3 is a critical regulator of CSCs because of its relationship with EMT as one of the major proposed mechanisms for generating CSCs. It also plays a critical role in the angiogenesis and regulation of the tumor microenvironment (TME), which provides signals for differentiation or proliferation, especially through its involvement in the inflammatory NF-κB pathway [161]. miR-124 was observed to target STAT3 to repress tumor growth and metastasis in NPCs [162]. miR-365-3p targets the ETS homologous factor (EHF), a keratin 16 (KRT16) transcription factor, thereby suppressing KRT16 expression. The decrease in KRT16 further enhances the lysosomal degradation of β5-integrin and c-Met, leading to inhibition of their downstream Src/STAT3 signaling. In OSCC cells, miR-365-
3p decreases migration, invasion, metastasis, cancer stemness, and chemoresistance via inhibiting Src/STAT3 signaling [163].

4.2.2. miRNAs in OSCC

In OSCC, let-7d was shown to function as a negative regulator of EMT and exhibited chemoresistant properties and silencing of enhanced mesenchymal, stem-like, and chemoresistant traits through suppressing TWIST1 and Snail family transcriptional repressor 1 (SNAI1) expression [164]. miR-98 acts as a tumor suppressor, which reduces tumor cell growth and metastasis through targeting the insulin-like growth factor 1 receptor (IGF1R) in OSCCs [165]. IGF1R is critical in the human embryonic niche for self-renewal and SC expansion and regulates SC maintenance in normal tissue processes [166,167]. Moreover, the IGF1R pathway is critical for EMT induction/maintenance and the expansion of cancer stem-like cells [167–170]. In HNSCCs, Leong et al. indicated increased epidermal growth factor (EGF) receptor (EGFR) and IGF1R expressions and phosphorylation, which increased the activation of downstream pathways in ALDH1+ cells compared to ALDH- cells. Importantly, treatment with EGFR and IGF1R inhibitors reduced SC fractions, implying that the IGF1R is critical for maintaining HNSCC CSCs [171].

miR-139-5p overexpression inhibits OSCC cell proliferation, in vitro mobility of OSCC, and the expression of WNT-responsive MYC, CCND1, and BCL2 through suppressing CXC chemokine receptor 4 (CXCR4) [172]. MYC-related signaling regulates CSC chemotherapeutic resistance and CRC organoids [173]. In addition, miR-139 triggers the apoptosis of an oral cancer cell line, Tca8113 cells, through the Akt signaling pathway [174]. Another study suggested that miR-139-5p suppresses the tumorigenesis process and OSCC cell mobility by targeting homeobox (HOX)-A9 (HOXA9) [175]. HOX genes can encode master regulatory TFs that regulate SCs during development in various cancers; HOX4 and HOX9 were observed to upregulate expression of the SC marker ALDH1 and increase SC self-renewal [176]. Similarly, miR-495 was observed to suppress EMT, proliferation, migration, and invasion and promote the apoptosis of CSCs by inhibiting the HOXC6-mediated TGF-β signaling pathway in OSCCs [177]. Other studies have also indicated that miR-495 significantly inhibits cell proliferation, migration, invasion, and EMT through the miR-495/IGF1/AKT signaling axis or by targeting NOTCH1 in OSCCs [178,179].

The miR-34 family contains three members, miR-34a, miR-34b, and miR-34c, clustered on two different chromosomal loci on chromosomes 1p36.22 (Mir34a) and 11q23.1 (Mir34b/c) [180,181]. In OSCCs and OPSCCs, miR-34a is described as a regulator of SCs [182]. miR-34a was observed to be downregulated in HNSCC tumors and cell lines [183]. Sun et al. observed that CSC enrichment by a spheroid culture showed significant downregulation of miR-34a expression. Furthermore, the restoration of miR-34a significantly inhibited EMT formation of the CSC phenotype and functionally reduced clonogenic and invasive capacities in HNSCC cell lines [184]. During the EMT process, cancer cells acquire the ability for tumor metastasis, invasion, drug resistance, and recurrence, which are associated with CSC functions. Gregory et al. first indicated that miR-205 and the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) suppressed the EMT by targeting zinc finger E-box binding homeobox 1 (ZEB1) and Smad-interacting protein 1 (SIP1), also known as ZEB2) in breast cancer [185]. Similarly, the miR-200 family was indicated to enhance EMT through a reciprocal feedback loop between the miR-200 family and ZEB1 in HNSCCs [186,187]. Recent emerging evidence has indicated that the EMT process might not simply be divided into a dichotomous system but may actually be an EMT spectrum. The epithelial/mesenchymal (E/M) hybrid status provides plasticity for cells with mixed E and M characteristics [188]. Lu et al. devised a unique model of miRNA-based coupled chimeric modules to elucidate the core regulatory network that underlies the hybrid E/M status. In that model system, two double-negative feedback loops of miR-34/SNAI1 and the miR-200/ZEB mutually regulate the E and M phenotypes and the hybrid phenotype. miR-200/ZEB was indicated to act as the decision-making module for cancer cells to undergo partial or complete EMT [189,190].
miR-22 inhibits phosphatidylinositol 3-kinase (PI3K)/Akt/NF-κB signaling via downregulating activators such as S100A8, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), which implies a tumor suppressor role of miR-22 in tongue squamous cell carcinoma [191]. PI3K is well known as a regulator for stemness-related signaling, including RAS/mitogen-activated protein kinase (MAPK) [192,193], NF-κB [194,195], Wnt/β-catenin [196,197], and TGF-β [198–202]. The NF-κB pathway maintains stemness by regulating many tumor-promoting inflammation-related cytokines, like tumor necrosis factor (TNF)-α [203], IL-1 [204], IL-6 [205,206], monocyte chemoattractant protein 1 (MCP1) [207], cytochrome oxidase subunit 2 (COX2) [203], and inducible nitric oxide synthase (iNOS) [203,208]. Simultaneously, the NF-κB pathway downregulates the expression of matrix metalloproteinases (MMPs) to increase tumor cell invasion [209]. miR-22 also targets the expression of node-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) and suppresses OSCC cell growth, migration, and invasion [210]. The NLRP3 inflammasome was associated with the carcinogenesis and CSC self-renewal activation in HNSCC patients with upregulated expression of BMI1, ALDH1, and CD44 [211]. The overexpression of miR-22 results in reduced cell viability and an increase in the OSCC cell apoptotic rate by targeting the Wnt/β-catenin signaling pathway [212]. Qiu et al. indicated that the downregulation of miR-22 would result in the upregulation of CD147 in tongue squamous cell carcinomas [213]. CD147 is also known as an extracellular MMP inducer, which promotes tumor initiation and progression through NF-κB signaling and also mediates the TGF-β1-induced EMT in HNSCC cells [214,215]. Therefore, CD147 might be a potential prognostic and treatment biomarker for HNSCCs.

4.2.3. miRNAs in LSCC

In LSCC, miR-98 was shown significantly reduced in both clinical specimens and cell lines, and miR-98 directly targeted HMGA2-POSTN signaling and then suppressed cell migration, metastasis, invasion, and EMT-TFs of SNAI1 and Twist1, as well as SC-like features [216]. Moreover, miR-101 inhibited tumorigenesis progression by regulating the Wnt/β-catenin signaling pathway by directly targeting cyclin-dependent kinase 8 (CDK8) in LSCC [217]. CDK8 plays an important role in regulating biological processes at the transcription level in the Wnt/β-catenin signaling pathway, and it is considered a CRC oncogene [218,219].

4.2.4. miRNAs in NPC

miR-139-5p inhibits the proliferation, invasion, and migration of human NPC cells by modulating EMT [220]. EMT enhances cancer cell motility and dissemination, which led to the concept of migrating CSCs as the basis of metastasis [221]. Findings have demonstrated a direct molecular link between EMT and stemness, where EMT activators such as Twist1 can co-induce EMT and stemness properties, thereby linking the EMT and CSC concepts [188]. EMT plays an important role in tumor metastasis and recurrence, and thus it is closely related to CSC functions [222,223]. Moreover, miR-139-5p reduces cisplatin resistance in NPC cells [220].

miR-488-3p activates the p53 pathway through suppressing zinc finger and BTB domain-containing protein 2 (ZBTB2), a reader of unmethylated DNA that regulates embryonic stem cell differentiation, thereby inhibiting proliferation and inducing apoptosis in esophageal SCCs [224,225]. p53 is able to suppress CD44, which is a CSC marker and suppresses cellular plasticity [226]. In NPCs, miR-372 promotes radiosensitivity by activating the p53 signaling pathway via the inhibition of PDZ-binding kinase (Pbk) [227]. Moreover, p53 represses EMT by mediating miR-200c expression, which causes the inhibition of the translation of ZEB1 and BMI1 [228]. By downregulating ATF3 expression, miRNA-488 suppresses cell invasion and EMT in tongue squamous cell carcinoma cells [229]. Taken together, miRNAs as tumor suppressors that regulate the stemness process are summarized in Table 2.
Table 2. miRNAs as tumor suppressors that are involved in the stemness of head and neck squamous cell carcinomas (HNSCCs).

| Suppressors miRNAs | Target(s)                  | Molecular Mechanism                                                                 | Action Mode            | Refs                  |
|-------------------|---------------------------|-------------------------------------------------------------------------------------|------------------------|-----------------------|
| **HNSCC**         |                           |                                                                                     |                        |                       |
| *let-7i*          | ARID3B                    | *let-7i* inhibition enhances ARID3B expression and activates the expression of POL5F1, NANOG, and SOX2. Upregulation of *let-7i* in ALDH1^+ cell suppresses tumor formation and metastasis. | Gene expression        | [142]                 |
| *let-7a*          | NANOG, POL5F1             |                                                                                     | Gene expression        | [144]                 |
| *let-7c*          | CXCL8                     | *let-7c* inhibition enhances stemness and radio-/chemoresistance by suppressing CXCL8. | Signal transduction    | [146]                 |
| *miR-203*         | CXCL8                     | *miR-203* reduction promotes EMT and activates IL-8/AKT signaling to trigger radioresistance. | EMT process/Signal transduction | [147]                 |
| *miR-520b*        | CD44                      | *miR-520b* inhibits EMT and the expression of stemness regulators and sensitizes cells to chemoradiotherapy through suppression of CD44. | EMT process/Gene expression | [149]                 |
| *miR-101*         | EZH2                      | *miR-101* inhibits EZH2 and suppresses metastasis and EMT.                           | EMT process/Signal transduction | [153,156,157]         |
| *miR-101*         | CDK8                      | *miR-101* inhibits CDK8 expression and subsequently suppresses Wnt/β-catenin signaling and tumorigenesis. | Signal transduction    | [217]                 |
| *miR-29b*         | DNMT3B                    | *miR-29* suppresses DNMT3B, resulting in inhibition of EMT.                          | EMT process            | [159]                 |
| *miR-204-5p*      | SNAI2, SUZ12, HDAC1,      | *miR-204-5p* inhibits stemness by suppressing STAT3 signaling and EMT via targeting SNAI2, SUZ12, HDAC1, and JAK2. | EMT process/Signal transduction | [160]                 |
|                   | and JAK2                  |                                                                                     |                        |                       |
| *miR-124*         | STAT3                     | *miR-124* inhibits tumor growth and metastasis by suppressing STAT3. *miR-365-3p* decreases metastasis, stemness, and chemoresistance by suppressing EHF, which inhibits Src/STAT3 signaling. | Signal transduction    | [162]                 |
| *miR-365-3p*      | EHF                       |                                                                                     | Signal transduction    | [163]                 |
|                   |                           |                                                                                     |                        |                       |
| **OSCC**          |                           |                                                                                     |                        |                       |
| *let-7d*          | TWIST1, SNAI1             | *let-7d* suppresses EMT.                                                            | EMT process            | [164]                 |
| *miR-98*          | IGF1R                     | *miR-98* reduces self-renewal by suppressing IGF1R.                                 | Signal transduction    | [165]                 |
| *miR-139-5p*      | CXCR4                     | *miR-139-5p* inhibits cell proliferation and the expression of WNT-responsive MYC, CCND1, and BCL2 via inhibiting CXCR4. | Signal transduction    | [172]                 |
| *miR-139-5p*      | HOXA9                     | *miR-139-5p* inhibits HOXA9. HOXA9 can increase stem cell self-renewal.            | Gene expression/Signal transduction | [175,176]            |
| *miR-495*         | HOXC6-mediated TGF-β signaling pathway | *miR-495* inhibits the HOXC6-mediated TGF-β signaling pathway and then suppresses EMT, proliferation, migration, and invasion. | Signal transduction    | [177]                 |
| *miR-495*         | IGF1/AKT signaling axis and NOTCH1 | *miR-495* inhibits cell proliferation migration, invasion, and EMT through targeting the IGF1/AKT signaling axis or NOTCH1. | Signal transduction    | [178,179]            |
| *miR-34a*         | EMT                       | *miR-34a* inhibits EMT formation. The *miR-200* family suppresses EMT by targeting ZEB1/2. | EMT process            | [184]                 |
| *miR-200 family*  | ZEB1/2                    |                                                                                     | EMT process            | [186,187]            |
Table 2. Cont.

| Suppressors miRNAs | Target(s) | Molecular Mechanism | Action Mode | Refs |
|--------------------|-----------|---------------------|-------------|------|
| miR-22             | KAT6B     | miR-22 inhibits activators of PI3K/Akt/NF-κB signaling. | Signal transduction | [191] |
| miR-22             | NLRP3     | miR-22 inhibits NLRP3 which suppresses expressions of BMI1, ALDH1, and CD44. | Signal transduction | [210,211] |
| miR-22             | CD147     | miR-22 inhibits CD147, which suppresses tumor initiation and progression through NF-κB signaling and mediates TGF-β1-induced EMT. | EMT process/Signal transduction | [213–215] |

**LSCC**

| miR-98             | HMGA2-POSTN signaling | miR-98 inhibits HMGA2-POSTN signaling, which suppresses metastasis and EMT-TFs. | EMT process/Signal transduction | [216] |
| miR-101            | CDK8      | miR-101 inhibits CDK8 expression, which suppresses Wnt/β-catenin signaling and tumorigenesis. | Signal transduction | [217] |

**NPC**

| miR-139-5p         | EMT       | miR-139-5p suppresses EMT and inhibits proliferation, invasion, migration, and cisplatin resistance. | EMT process | [220] |
| miR-488-3p         | ZBTB2     | miR-488-3p activates the p53 pathway by suppressing ZBTB2 to inhibit proliferation and induce apoptosis. | Signal transduction | [224,225] |
| miR-372            | PBK       | miR-372 activates the p53 signaling pathway via repressing PBK to promote radiosensitivity. | Signal transduction | [227] |

EMT, epithelial-to-mesenchymal transition; TFs, transcription factors; OSCC, oral squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma; NPC, nasopharyngeal carcinoma; IL-8, interleukin 8; EHF, ETS homologous factor; STAT3, signal transducer and activator of transcription 3; IGF1, insulin-like growth factor 1; AKT, AKT serine/threonine kinase 1; TGF-β, transforming growth factor-β; HMGA2, high-mobility group AT-hook 2; NF-κB, nuclear factor-κB.

4.3. miRNAs as Pleiotropic Functions

Some miRNAs play dual roles in oncogenes and tumor suppression, depending on the specific cell/tissue context. This reflects the complexity of the miRNA–target regulatory network. For example, *miR-107* was observed to antagonize and degrade *let-7*. *miR-107* suppressed *let-7* expression and activated downstream oncoprotein expressions such as HMGA2 and RAS and enhanced the tumorigenic and metastatic potential of cancer cells [230,231]. In HNSCCs, a *miR-107* increment was found in patients with lymph node metastasis, suggesting an oncogenic role for *miR-107* [232]; however, *miR-107* was indicated to suppress the proliferation, invasion, and colony formation of cells in LSCCs via inhibiting the voltage-gated calcium channel subunit α2δ1 (encoded by CACNA2D1) [233]. In non-small-cell lung cancer (NSCLC), α2δ1 also enhances radioresistance in cancer stem-like cells by enhancing the efficiency of DNA damage repair [234]. Those results indicate the pleiotropic functions of *miR107* in HNSCCs.

miRNAs also mediated the regulation of cytokines/chemokines and the TME that modulates the CSC signaling pathway and sustains the CSC niche for acquiring and maintaining CSC features. For example, downregulation of *miR-9*, *miR-542-3p*, and *miR-34a*, and significant upregulation of *miR-21* were shown in CD44-positive CSCs with increased IL-6 and IL-8 expressions via targeting of the CD44v6/NANOG/phosphatase and tensin homolog (PTEN) axis in oral cancer [235]. *miR-9* acts as a tumor suppressor microRNA in HNSCC, and its role seems to be mediated through CXCR4 suppression [236]. Studies have indicated that *miR-9* overexpression results in decreased cellular proliferation and inhibited colony formation in soft agars when targeting CXCR4 in HNSCC cells [236]. Conversely, another study has also indicated that *miR-9* was expressed at high levels in...
patients with recurrent HNSCCs [237]. Similar results were also shown for breast cancer with miR-9 acting as a tumor suppressor in breast cancer proliferation in the early stage of breast cancer, while, with a higher malignancy, miR-9 plays an opposite role in the metastatic process [238]. Thus, miR-9 was suggested to have dual roles in carcinogenesis. Taken together, miRNAs with pleiotropic functions in HNSCCs are summarized in Table 3.

**Table 3.** miRNAs with pleiotropic functions that are involved in the stemness of head and neck squamous cell carcinoma (HNSCCs).

| Pleiotropic miRNAs | Target(s) | Molecular Mechanism                                                                 | Action Mode                          | Refs |
|--------------------|-----------|-------------------------------------------------------------------------------------|--------------------------------------|------|
| miR-107            | let-7     | miR-107 suppresses let-7 expression and activates downstream oncoprotein expressions for enhancing tumorigenic and metastasis. | Gene expression/Signal transduction | [230, 231] |
| miR-107            | CACNA2D1  | miR-107 suppresses proliferation, invasion, and colony formation of LSCC cells via inhibiting CACNA2D1. | Signal transduction                  | [233] |
| miR-9              | CD44v6/NANOG/PTEN axis | miR-9 inhibits the CD44v6/NANOG/PTEN axis for suppressing IL-6 and IL-8 signaling. | Signal transduction                  | [235] |
| miR-9              | CXCR4     | miR-9 decreases proliferation and colony formation by targeting CXCR4.               | Signal transduction                  | [236] |

LSCC, laryngeal squamous cell carcinoma; NANOG, Nanog homeobox; PTEN, phosphatase and tensin homolog.

5. Conclusions

miRNAs can function as cancer suppressors or oncogenes, or even exhibit dual roles during cancer development, depending on the different cancer types or tumorigenesis stage. miRNAs are critical to tumor initiation, progression, metastasis, EMT, and chemoresistance via regulating CSC functions. miRNAs regulate important EMT-TFs and signaling pathways and modulate the TME to sustain and enhance cancer stemness. Therefore, targeting CSCs through miRNA manipulation provides a therapeutic opportunity for managing metastatic diseases. Moreover, with an understanding of miRNAs during tumorigenesis, we can take advantage of miRNA stability and use it as a diagnostic marker for primary diagnoses and patient follow-ups. We can also monitor miRNA changes to predict therapeutic responses as a non-invasive detection method. Recent studies have indicated that exosomal miRNAs can be better sources of biomarkers due to their advantages in terms of their quantity, quality, and stability [239]. Ludwig et al. indicated that miR-205-5p was exclusively expressed in HPV(+) exosomes, whereas miR-1972 was only detected in HPV(−) exosomes. These miRNAs emerge as potential discriminating HPV-associated biomarkers [240]. Intriguingly, human papillomavirus 16 (HPV16) infection has been indicated to enhance CSC properties, including ALDH1 activity, migration/invasion, and CSC-related factor expression, and enhances tumor growth OSCC cells [241]. Whether tumor-derived exosomes (TEX)-miRNAs are also involved in regulating the recipient cell stemness is unclear. In contrast to the extensive studies for cellular miRNAs in regulating cancer stemness, TEX-miRNA knowledge is relatively limited.

Moreover, Huang et al. indicated that only 5.63% of miRNAs were detected in both cells and TEX, which implies that cells can selectively pack certain miRNAs into exosomes in OSCC cells [242]. Meanwhile, exosomes can be released by various cell types, such as cancer-associated fibroblasts (CAFs) [243], dendritic cells [244], B cells [245], T cells [246], and tumor cells [247]. For example, Li et al. indicated that miR-34a-5p was significantly reduced in CAF-derived exosomes in OSCC patients. CAF transfers miR-34a-5p-devoid exosomes to OSCC cells and results in promoting the proliferation and motility of OSCC cells by upregulating the downstream target AXL (encoding AXL receptor tyrosine kinase). Therefore, the miR-34a-5p/AXL axis promotes the proliferation, metastasis, and EMT
of oral cancer cells through the AKT/glycogen synthase kinase (GSK)-3β (GSK-3β)/β-catenin/SNAI1 signaling cascade [248]. Consistently, the cellular miR-34a significantly inhibited EMT formation of the CSC phenotype in HNSCC cell lines [184]. Hence, the sources and biological functions of exosomal miRNAs warrant further research before using them for screening and surveillance.

Among the tumor suppressor microRNAs of HNSCCs, miR-34 is the only one that has been used in a clinical trial applied to treat primary liver cancer, small-cell lung carcinomas, lymphomas, multiple myelomas, and renal cell carcinomas. In 2013, the first microRNA-associated therapeutic drug was tested in a clinical trial (NCT01829971), MRX34, a special amphoteric lipid nanoparticle filled with miR-34 mimics. Although this phase I study provided a dose-dependent modulation of relevant target genes that provide a proof-of-concept for MRX34 application for cancer therapy, severe adverse events were reported in five patients in terms of experiencing serious immune responses [249,250]. Hence, leading up to the MRX34 phase 2 clinical trials, NCT02862145 for melanomas has been withdrawn [251]. Other clinical trials have mainly focused on observational studies to explore the prognostic value of miRNAs in HNSCCs, for example, the prognostic value of miR-29b in the tissue, blood, and saliva in oral squamous cell carcinomas (NCT02009852). The miR-29 family has been used to investigate Twist1-mediated cancer metastasis in HNSCCs (NCT01927354) (Table 4). Further research is warranted to determine the molecular functions and mechanisms of cellular or exosomal miRNAs, as well as their potential as miRNA-based diagnostics and therapeutics for HNSCCs.

Table 4. List of miRNA-related clinical trials in head and neck squamous cell carcinomas (HNSCCs).

| miRNAs        | Clinical Trials                                                                 | Trial ID          |
|---------------|----------------------------------------------------------------------------------|-------------------|
| miR-29b       | Observational study to explore the prognostic value of miR-29b in tissue, blood, and saliva in OSCC | NCT02009852      |
| miR-29 family | Observational study to investigate the role of miR-29 family in Twist1-mediated cancer metastasis in HNSCC | NCT01927354      |

OSCC, oral squamous cell carcinoma.

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References
1. WHO. WHO Report on Cancer: Setting Priorities, Investing Wisely and Providing Care for All; World Health Organization: Geneva, Switzerland, 2020.
2. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424. [CrossRef]
3. Simon, S. Cancer Facts & Figures 2019; American Cancer Society: Atlanta, GA, USA, 2019; pp. 1–71.
30. O'Brien, C.A.; Pollett, A.; Gallinger, S.; Dick, J.E. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nat. Cell Biol.* 2006, 445, 106–110. [CrossRef]

31. Ricci-Vitiani, L.; Lombardi, D.G.; Pilozzi, E.; Biffoni, M.; Todaro, M.; Peschle, C.; De Maria, R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007, 445, 111–115. [CrossRef]

32. Dylla, S.J.; Beviglia, L.; Park, I.-K.; Chartier, C.; Raval, J.; Ngan, L.; Pickell, K.; Aguilar, J.; Lazetic, S.; Smith-Berdan, S.; et al. Colorectal Cancer Stem Cells Are Enriched in Xenogeneic Tumors Following Chemotherapy. *PLoS ONE* 2008, 3, e2428. [CrossRef]

33. Pang, R.; Law, W.L.; Chu, A.C.; Poon, J.T.; Lam, C.S.; Chow, A.K.; Ng, L.; Cheung, L.W.; Lan, X.R.; Lan, H.Y.; et al. A Subpopulation of CD26+ Cancer Stem Cells with Metastatic Capacity in Human Colorectal Cancer. *Cell Stem Cell* 2010, 6, 603–615. [CrossRef]

34. De Sousa e Melo, F.; Kurtova, A.V.; Harnoss, J.M.; Kljavin, N.; Hoeck, J.D.; Hung, J.; Anderson, J.E.; Storm, E.E.; Modrusan, Z.; Koeppen, H.; et al. A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* 2017, 543, 676–680. [CrossRef] [PubMed]

35. Todaro, M.; Francipane, M.G.; Medema, J.P.; Stassi, G. Colon Cancer Stem Cells: Promise of Targeted Therapy. *Gastroenterology* 2010, 138, 2151–2162. [CrossRef] [PubMed]

36. Allegra, E.; Trapasso, S. Role of CD44 as a marker of cancer stem cells in head and neck cancer. *Biol. Targets Ther.* 2012, 6, 379–383. [CrossRef]

37. Landen, C.N.; Goodman, B.; Katre, A.A.; Steg, A.D.; Miller, L.D.; Mejia, P.V.; Jennings, N.B.; Gershenson, D.M.; et al. Targeting Aldehyde Dehydrogenase Cancer Stem Cells in Ovarian Cancer. *Mol. Cancer Ther.* 2010, 9, 3186–3199. [CrossRef] [PubMed]

38. Singh, S.; Brocker, C.; Koppaka, V.; Chen, Y.; Jackson, B.C.; Matsumoto, A.; Thompson, D.C.; Vasiiliou, V. Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. *Free Radic. Biol. Med.* 2013, 56, 89–101. [CrossRef] [PubMed]

39. Pardal, R.; Clarke, M.F.; Morrison, S.J. Applying the principles of stem-cell biology to cancer. *Nat. Rev. Cancer* 2003, 3, 895–902. [CrossRef]

40. Reiman, J.M.; Knutson, K.L.; Davis, A.; Malik, F.; Henry, N.L.; Ithimakin, S.; Quraishi, A.A.; Tawakkol, N.; D’Angelo, R.; Paulson, D.M.; et al. Targeting Aldehyde Dehydrogenase Cancer Stem Cells in Ovarian Cancer. *Mol. Cancer Ther.* 2012, 11, 6125–6129. [CrossRef] [PubMed]

41. Korkaya, H.; Liu, S.; Wicha, M.S. Regulation of Cancer Stem Cells by Cytokine Networks: Attacking Cancer’s Inflammatory Roots: Figure 1. *Clin. Cancer Res.* 2011, 17, 6125–6129. [CrossRef]

42. Todaro, M.; Alea, M.P.; Di Stefano, A.B.; Canmareri, P.; Vermeulen, L.; Iovino, F.; Tripodo, C.; Russo, A.; Gulotta, G.; Medema, J.P.; et al. Colon Cancer Stem Cells Dictate Tumor Growth and Resist Cell Death by Production of Interleukin-4. *Cell Stem Cell* 2007, 1, 389–402. [CrossRef]

43. Samanta, D.; Gilkes, D.M.; Chaturvedi, P.; Xiang, L.; Semenza, G.L. Hypoxia-inducible factors are required for chemotherapeutic resistance of breast cancer stem cells. *Proc. Natl. Acad. Sci. USA* 2014, 111, E5429–E5438. [CrossRef]

44. Hwang, W.; Yang, M.; Tsai, M.; Lan, H.-Y.; Cheng, W.-C.; Huang, S.-C.; Yang, M.-H. Tumor stem-like cell-derived exosomal RNAs prime gastric cancer cell migration. *Mol. Cancer* 2019, 18, 1–16. [CrossRef]

45. Hsu, J.-M.; Xia, W.; Hsu, Y.-H.; Chan, L.-C.; Lee, H.-H.; Cha, J.-H.; Wang, H.-L.; Yang, W.-H.; Yen, E.-Y.; Chang, W.-C.; et al. IL-6/JAK1 pathway drives PD-L1 Y112 phosphorylation to promote cancer immune evasion. *J. Clin. Investig.* 2019, 129, 3324–3338. [CrossRef] [PubMed]

46. Reiman, J.M.; Knutson, K.L.; Radisky, D.C. Immune Promotion of Epithelial-mesenchymal Transition and Generation of Breast Cancer Stem Cells: Figure 1. *Cancer Res.* 2010, 70, 3005–3008. [CrossRef]

47. Hsu, J.-M.; Xia, W.; Hsu, Y.-H.; Chan, L.-C.; Yu, W.-H.; Cha, J.-H.; Chen, C.-T.; Liao, H.-W.; Kuo, C.-W.; Khooh, K.-H.; et al. STT3-dependent PD-L1 accumulation on cancer stem cells promotes immune evasion. *Nat. Commun.* 2018, 9, 1–17. [CrossRef]

48. Liao, T.-T.; Lin, C.-C.; Jiang, J.-K.; Yang, S.-H.; Teng, H.-W.; Yang, M.-H. Harnessing stemness and PD-L1 expression by AT-rich interaction domain-containing protein 3B in colorectal cancer. *Theranostics* 2020, 10, 6095–6112. [CrossRef]

49. Zhang, X.; Shi, H.; Yuan, X.; Jiang, P.; Qian, H.; Xu, W. Tumor-derived exosomes induce N2 polarization of neutrophils to promote gastric cancer cell migration. *Mol. Cancer* 2018, 17, 1–16. [CrossRef]

50. Hwang, W.-L.; Lan, H.-Y.; Cheng, W.-C.; Huang, S.-C.; Yang, M.-H. Tumor stem-like cell-derived exosomal RNAs prime neutrophils for facilitating tumorgenesis of colon cancer. *J. Hematol. Oncol.* 2019, 12, 1–17. [CrossRef] [PubMed]

51. Takahashi, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* 2006, 126, 663–676. [CrossRef]

52. Maherali, N.; Sridharan, R.; Xie, W.; Utikal, J.; Eminli, S.; Arnold, K.; Stadtfeld, M.; Yachecho, R.; Tchieu, J.; Jaenisch, R.; et al. Directly Reprogrammed Fibroblasts Show Global Epigenetic Remodeling and Widespread Tissue Contribution. *Cell Stem Cell* 2007, 1, 55–70. [CrossRef] [PubMed]

53. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. *Nat. Cell Biol.* 2007, 448, 313–317. [CrossRef] [PubMed]

54. Li, W.; Wang, Z.; Zha, L.; Kong, D.; Liao, G.; Li, H. HMG A2 regulates epithelial-mesenchymal transition and the acquisition of tumor stem cell properties through TWIST1 in gastric cancer. *Oncol. Rep.* 2016, 37, 185–192. [CrossRef] [PubMed]
56. Lee, S.H.; Hong, H.S.; Liu, Z.X.; Kim, R.H.; Kang, M.K.; Park, N.-H.; Shin, K.-H. TNFα enhances cancer stem cell-like phenotype via Notch-Hes1 activation in oral squamous cell carcinoma cells. Biochem. Biophys. Res. Commun. 2012, 424, 58–64. [CrossRef] [PubMed]
57. Kim, S.-Y.; Kang, J.W.; Song, X.; Kim, B.K.; Yoo, Y.D.; Kwon, Y.T.; Lee, Y.J. Role of the IL-6-JAK1-STAT3-Oct-4 pathway in the conversion of non-stem cancer cells into cancer stem-like cells. Cell. Signal. 2013, 25, 961–969. [CrossRef] [PubMed]
58. Liao, T.-T.; Yang, M.-H. Revisiting epithelial-mesenchymal transition in cancer metastasis: The connection between epithelial plasticity and stemness. Mol. Oncol. 2017, 11, 792–804. [CrossRef]
59. Venkatesh, V.; Nataraj, R.; Thangaraj, G.S.; Karthikeyan, M.; Gnanasekaran, A.; Kaginelli, S.B.; Kuppanna, G.; Kallappa, C.G.; Basalingappa, K.M. Targeting Notch signalling pathway of cancer stem cells. Stem Cell Investig. 2018, 5, 5. [CrossRef] [PubMed]
60. Pelullo, M.; Zema, S.; Nardozza, F.; Checquolo, S.; Scerpanti, I.; Bellavia, D. Wnt, Notch, and TGF-β Pathways Impinge on Hedgehog Signaling Complexity: An Open Window on Cancer. Front. Genet. 2019, 10, 711. [CrossRef]
61. Vermeulen, L.; Melo, F.D.S.E.; Van Der Heijden, M.; Cameron, K.; De Jong, J.H.; Borovski, T.; Tuynnan, J.B.; Todaro, M.; Merz, C.; Rodermond, H.; et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat. Cell Biol. 2010, 12, 468–476. [CrossRef]
62. Dierks, C.; Beigi, R.; Guo, G.-R.; Zirlik, K.; Stegert, M.R.; Manley, P.; Trussell, C.; Schmitt-Graeff, A.; Landwerlin, K.; Veelken, H.; et al. Expansion of Bcr-Abl-Positive Leukemic Stem Cells Depends on Hedgehog Pathway Activation. Cancer Cell 2008, 14, 238–249. [CrossRef]
63. Zhang, F.; Wang, D. The Pattern of microRNA Binding Site Distribution. Genes 2019, 10, 404–416. [CrossRef]
64. A Broderick, J.; Zamore, P.D. MicroRNA therapeutics. Gene Ther. 2011, 18, 1104–1110. [CrossRef] [PubMed]
65. Wright, M.W.; Bruford, E.A. Naming ‘junk’: Human non-protein coding RNA (ncRNA) gene nomenclature. Hum. Genom. 2011, 5, 1–9. [CrossRef]
66. Wang, Y.; Medvid, R.; Melton, C.; Jaenisch, R.; Blaloch, R. DGC8R is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. Nat. Genet. 2007, 39, 380–385. [CrossRef] [PubMed]
67. Lewis, B.P.; Burge, C.B.; Bartel, D.P. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human microRNAs Are Conserved Targets of microRNAs. EMBO J. 2003, 22, 3415–3420. [CrossRef] [PubMed]
68. Friedman, R.C.; Farh, K.K.-H.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009, 19, 20–25. [CrossRef] [PubMed]
69. Calin, G.A.; Sevignani, C.; Dumitru, C.D.; Hyslop, T.; Noch, E.; Yendamuri, S.; Shimizu, M.; Rattan, S.; Bullrich, F.; Negrini, M.; et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. USA 2004, 101, 1752–1757. [CrossRef] [PubMed]
70. Zhang, F.; Wang, D. The Pattern of microRNA Binding Site Distribution. Genes 2017, 8, 296. [CrossRef] [PubMed]
71. Friedman, R.C.; Farh, K.K.-H.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009, 19, 92–105. [CrossRef] [PubMed]
72. Lujambio, A.; Ropero, S.; Ballestar, E.; Fraga, M.F.; Cerrato, C.; Setien, F.; Casado, S.; Suarez-Gauthier, A.; Sanchez-Cespedes, M.; Gitt, A.; et al. Genetic Unmasking of an Epigenetically Silenced microRNA in Human Cancer Cells. Cell. Biol. 2008, 225, 522–532. [CrossRef] [PubMed]
73. Vermeulen, L.; Melo, F.D.S.E.; Van Der Heijden, M.; Cameron, K.; De Jong, J.H.; Borovski, T.; Tuynnan, J.B.; Todaro, M.; Merz, C.; Rodermond, H.; et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat. Cell Biol. 2010, 12, 468–476. [CrossRef]
74. Obernosterer, G.; Leuschner, P.J.; Alenius, M.; Martinez, J. Post-transcriptional regulation of microRNA expression. RNA 2006, 12, 580–587. [CrossRef] [PubMed]
75. Zhang, F.; Wang, D. The Pattern of microRNA Binding Site Distribution. Genes 2017, 8, 296. [CrossRef] [PubMed]
76. Friedman, R.C.; Farh, K.K.-H.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009, 19, 92–105. [CrossRef] [PubMed]
77. Lujambio, A.; Ropero, S.; Ballestar, E.; Fraga, M.F.; Cerrato, C.; Setien, F.; Casado, S.; Suarez-Gauthier, A.; Sanchez-Cespedes, M.; Gitt, A.; et al. Genetic Unmasking of an Epigenetically Silenced microRNA in Human Cancer Cells. Cell. Biol. 2008, 225, 522–532. [CrossRef] [PubMed]
78. Lee, Y.; Hur, I.; Park, S.-Y.; Kim, Y.-K.; Suh, M.R.; Kim, V.N. The role of PACT in the RNA silencing pathway. EMBO J. 2006, 25, 522–532. [CrossRef] [PubMed]
79. Lee, Y.; Hur, I.; Park, S.-Y.; Kim, Y.-K.; Suh, M.R.; Kim, V.N. The role of PACT in the RNA silencing pathway. EMBO J. 2006, 25, 522–532. [CrossRef] [PubMed]
80. Lee, Y.; Hur, I.; Park, S.-Y.; Kim, Y.-K.; Suh, M.R.; Kim, V.N. The role of PACT in the RNA silencing pathway. EMBO J. 2006, 25, 522–532. [CrossRef] [PubMed]
81. Fukuda, T.; Yamagata, K.; Fujiyama, S.; Matsumoto, T.; Koshida, I.; Yoshimura, K.; Miha, M.; Naitou, M.; Endoh, H.; Nakamura, T.; et al. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. Nat. Cell Biol. 2007, 9, 604–611. [CrossRef] [PubMed]
82. Gregory, R.I.; Chendrimada, T.P.; Cooch, N.; Shiekhattar, R. Human RISC Couples MicroRNA Biogenesis and Posttranscriptional Gene Silencing. Cell 2005, 123, 631–640. [CrossRef] [PubMed]
83. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75, 843–854. [CrossRef]

84. Wightman, B.; Ha, I.; Ruvkun, G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* 1993, 75, 855–862. [CrossRef]

85. Wightman, B.; Burglin, T.R.; Gatto, J.; Arasu, P.; Ruvkun, G. Negative regulatory sequences in the lin-14 3′-untranslated region are necessary to generate a temporal switch during Caenorhabditis elegans development. *Genes Dev.* 1991, 5, 1813–1824. [CrossRef] [PubMed]

86. Lewis, B.P.; Shih, I.-H.; Jones-Rhoades, M.W.; Bartel, D.P.; Burge, C.B. Prediction of Mammalian MicroRNA Targets. *Cell* 2003, 115, 797–798. [CrossRef]

87. Lisanti, P.; Gao, Y.; Talal, B.; Xiao, Y.; Li, Y.; Zhang, J.; Pan, J.; Zhang, Y.; Lu, J.; et al. Oral epithelial stem cells—Implications in normal development and cancer metastasis. *Exp. Cell Res.* 2014, 325, 111–129. [CrossRef]

88. Papagerakis, S.; Pannone, G.; Zheng, L.; About, I.; Taqi, N.; Nguyen, N.P.; Matossian, M.; McAlpin, B.; Santoro, A.; McHugh, J.; et al. Oral epithelial stem cells—which cell are the cancer stem cells? *Stem Cells* 2015, 33, 1642–1656. [CrossRef]

89. Peng, H.; Park, J.K.; Katsnelson, J.; Kaplan, N.; Yang, W.; Getsios, S.; Lavker, R.M. microRNA-103/107 Family Regulates Multiple Epithelial Stem Cell Characteristics. *Stem Cells* 2015, 33, 1642–1656. [CrossRef]

90. Andl, T.; Murchison, E.P.; Li, F.; Zhang, Y.; Yunta-Gonzalez, M.; Tobias, J.W.; Andl, C.D.; Seykora, J.T.; Hannon, G.J.; Millar, S.E. The miRNA-Processing Enzyme Dicer Is Essential for the Morphogenesis and Maintenance of Hair Follicles. *Curr. Biol.* 2006, 16, 1041–1049. [CrossRef]

91. Yi, R.; Pasolli, H.A.; Landthaler, M.; Hafner, M.; Ojo, T.; Sheridan, R.; Sander, C.; O’Carroll, D.; Stoffel, M.; Tuschl, T.; et al. Dicer-dependent microRNA biogenesis is essential for skin development. *Proc. Natl. Acad. Sci. USA* 2008, 106, 498–502. [CrossRef]

92. Yi, R.; O’Carroll, D.; A Pasolli, H.; Zhang, Z.; Dietrich, F.S.; Tarakhovsky, A.; Fuchs, E. Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. *Nat. Genet.* 2006, 38, 356–362. [CrossRef]

93. Otsuka-Tanaka, Y.; Ommen, S.; Kawasaki, M.; Kawasaki, K.; Imam, N.; Jalani-Ghazani, F.; Hindges, R.; Sharpe, P.T.; Ohazama, A. Oral Lining Mucosa Development Depends on Mesenchymal microRNAs. *J. Dent. Res.* 2012, 91, 229–234. [CrossRef]

94. Calin, G.A.; Croce, C.M. MicroRNA Signatures in Human Cancers. *Nat. Rev. Cancer* 2006, 6, 857–866. [CrossRef]

95. Khan, A.Q.; Ahmed, E.I.; Elarre, N.R.; Junejo, K.; Steinhoff, M.; Uddin, S. Role of miRNA-Regulated Cancer Stem Cells in the Pathogenesis of Human Malignancies. *Cells* 2019, 8, 840. [CrossRef] [PubMed]

96. Esau, C.C. Inhibition of microRNA with antisense oligonucleotides. *Methods* 2008, 44, 55–60. [CrossRef] [PubMed]

97. Mercatelli, N.; Coppola, V.; Bonci, D.; Miele, F.; Costantini, A.; Guadagnoli, M.; Bonanno, E.; Muto, G.; Frazier, G.V.; De Maria, R.; et al. The Inhibition of the Highly Expressed Mir-221 and Mir-222 Impairs the Growth of Prostate Carcinoma Xenografts in Mice. *PLoS ONE* 2008, 3, e4029. [CrossRef] [PubMed]

98. Van Roosbroeck, K.; Fanini, F.; Setoyama, T.; Ivan, C.; Rodriguez-Aguayo, C.; Fuentes-Mattei, E.; Xiao, L.; Vannini, I.; Redis, R.S.; D’Abundo, L.; et al. Combining Anti-Mir-155 with Chemotherapy for the Treatment of Lung Cancers. *Clin. Cancer Res.* 2016, 23, 2891–2904. [CrossRef] [PubMed]

99. Chirnhe, E.; Oberg, K.C.; Ioffe, Y.J.; Unternaehrer, J.J. Let-7as biomarker, prognostic indicator, and therapy for precision medicine in cancer. *Clin. Transl. Med.* 2019, 8, 24. [CrossRef]

100. Zhao, Q.; Zheng, X.; Guo, H.; Xue, X.; Zhang, Y.; Niu, M.; Cui, J.; Liu, H.; Luo, H.; Yang, D.; et al. Serum Exosomal miR-941 as a promising Oncogenic Biomarker for Laryngeal Squamous Cell Carcinoma. *J. Cancer* 2020, 11, 5329–5344. [CrossRef]

101. Troschel, F.M.; Böhly, N.; Borrmann, K.; Braun, T.; Schwickert, A.; Kiesel, L.; Eich, H.T.; Götte, M.; Greve, B. miR-142-3p attenuates tumor growth and metastasis in laryngeal squamous cell carcinoma. *Oral Oncol.* 2016, 55, 248–254. [CrossRef] [PubMed]

102. Ganci, F.; Lacorte, A.; Manciocco, V.; Sperduti, I.; Battaglia, P.; Covello, R.; Muti, P.; Strano, S.; Spriano, G.; Fontemaggi, G.; et al. microRNA expression as predictor of local recurrence risk in oral squamous cell carcinoma. *Head Neck* 2018, 40, 1686480. [CrossRef]

103. Islam, M.; Datta, J.; Lang, J.C.; Teknos, T.N. Down regulation of RhoC by microRNA-138 results in de-activation of FAK, Src and Erk1/2 signaling pathway in head and neck squamous cell carcinoma. *Oral Oncol.* 2014, 50, 448–456. [CrossRef] [PubMed]

104. Peng, H.; Park, J.K.; Katsnelson, J.; Kaplan, N.; Yang, W.; Getsios, S.; Lavker, R.M. microRNA-103/107 Family Regulates Multiple Epithelial Stem Cell Characteristics. *Stem Cells* 2015, 33, 1642–1656. [CrossRef]

105. Li, D.; Liu, K.; Li, Z.; Wang, J.; Wang, X. miR-19a and miR-424 target TGFBR3 to promote epithelial-to-mesenchymal transition and migration of tongue squamous cell carcinoma cells. *Cell Adhes. Migr.* 2017, 12, 236–246. [CrossRef] [PubMed]
Cancers 2021, 13, 1742

109. Chen, S.; Zhang, J.; Sun, L.; Li, X.; Bai, J.; Zhang, H.; Li, T. miR-611 promotes the proliferation, migration and invasion of tongue squamous cell carcinoma cells by targeting FOXN3. *Oral Dis.* 2019, 25, 1906–1918. [CrossRef]

110. Yao, X.; Xie, L.; Zeng, Y. MiR-9 Promotes Angiogenesis via Targeting on Sphingosine-1-Phosphate Receptor 1. *Front. Cell Dev. Biol.* 2020, 8, 755. [CrossRef]

111. Lages, E. MicroRNAs: Molecular features and role in cancer. *Front. Biosci.* 2012, 17, 2508–2540. [CrossRef]

112. Xu, W.; Ji, J.; Xu, Y.; Liu, Y.; Shi, L.; Liu, Y.; Lu, X.; Zhao, Y.; Luo, F.; Wang, B.; et al. MicroRNA-191, by promoting the EMT and increasing CSC-like properties, is involved in neoplastic and metastatic properties of transformed human bronchial epithelial cells. *Mol. Carcinog.* 2014, 54, E148–E161. [CrossRef]

113. Ceppi, P.; E Peter, M. MicroRNAs regulate both epithelial-to-mesenchymal transition and cancer stem cells. *Oncogene* 2013, 33, 269–278. [CrossRef][PubMed]

114. Kim, J.; Yao, F.; Xiao, Z.; Sun, Y.; Ma, L. MicroRNAs and metastasis: Small RNAs play big roles. *Cancer Metastasis Rev.* 2017, 37, 5–15. [CrossRef][PubMed]

115. Song, S.J.; Ito, K.; Ala, U.; Webster, K.; Sun, S.M.; Jongen-Lavrencic, M.; Manova-Todorova, K.; Teruya-Feldstein, J.; Avigan, D.E.; et al. The Oncogenic MicroRNA miR-22 Targets the TET2 Tumor Suppressor to Promote Hematopoietic Stem Cell Self-Renewal and Transformation. *Cell Stem Cell* 2013, 13, 87–101. [CrossRef][PubMed]

116. Chen, J.; Ouyang, H.; An, X.; Liu, S. miR-125a is upregulated in cancer stem-like cells derived from T001 and is responsible for maintaining stemness by inhibiting p53. *Oncol. Lett.* 2018, 17, 87–94. [CrossRef][PubMed]

117. Peng, S.-Y.; Tu, H.-F.; Yang, C.-C.; Wu, C.-H.; Liu, C.-J.; Chang, K.-W.; Lin, S.-C. MiR-134-targetsPDCD7to reduce E-cadherin expression and enhance oral cancer cell progression. *Int. J. Cancer* 2014, 139, 2892–2904. [CrossRef]

118. Farmakovskyaya, M.; Khromova, N.; Rybko, V.; Dugina, V.; Kopnin, B.; Kopnin, P. E-Cadherin repression increases amount of cancer stem cells in human A549 lung adenocarcinoma and stimulates tumor growth. *Cell Cycle* 2016, 15, 1084–1092. [CrossRef]

119. Liu, C.-J.; Shen, W.G.; Peng, S.-Y.; Cheng, H.-W.; Kao, S.-Y.; Lin, S.-C.; Chang, K.-W. miR-134induces oncogenicity and metastasis in head and neck carcinoma through targetingWWOXgene. *Int. J. Cancer* 2013, 134, 811–821. [CrossRef]

120. Yan, H.C.; Xu, J.; Fang, L.S.; Qiu, Y.Y.; Lin, X.M.; Huang, H.X.; Han, Q.Y. Ectopic expression of the WWOX gene suppresses stemness of human ovarian cancer stem cells. *Onco. Lett.* 2015, 9, 1614–1620. [CrossRef]

121. Li, J.; Liu, J.; Li, P.; Zhou, C.; Liu, P. The downregulation of WWOX induces epithelial–mesenchymal transition and enhances stemness and chemoresistance in breast cancer. *Exp. Biol. Med.* 2018, 243, 1066–1073. [CrossRef]

122. Lin, S.-S.; Peng, C.-Y.; Liao, Y.-W.; Chou, M.-Y.; Hsieh, P.-L.; Yu, C.-C. miR-1246 Targets CCNG2 to Enhance Cancer Stemness and Chemoresistance in Oral Carcinomas. *Cancers* 2018, 10, 272. [CrossRef]

123. Bernaudo, S.; Salem, M.; Qi, X.; Zhou, W.; Zhang, C.; Yang, W.; Rosman, D.; Deng, Z.; Ye, G.; Yang, B.B.; et al. Cyclin G2 inhibits epithelial-to-mesenchymal transition by disrupting Wnt/β-catenin signaling. *Onco gene* 2016, 35, 4816–4827. [CrossRef][PubMed]

124. Iwai, S.; Yonekawa, A.; Harada, C.; Hamada, M.; Katagiri, W.; Nakazawa, M.; Yura, Y. Involvement of the Wnt-β-catenin pathway in invasion and migration of oral squamous carcinoma cells. *Int. J. Oncol.* 2013, 34, 1095–1103. [CrossRef]

125. Mah, K.M.; Weiner, J.A. Regulation of Wnt signaling by protocadherins. *Semin. Cell Dev. Biol.* 2017, 69, 158–171. [CrossRef][PubMed]

126. DiMeo, T.A.; Anderson, K.; Phadke, P.; Feng, C.; Perou, C.M.; Nabcr, S.; Kuperwasser, C. A Novel Lung Metastasis Signature Links Wnt Signaling with Cancer Cell Self-Renewal and Epithelial-Mesenchymal Transition in Basal-like Breast Cancer. *Cancer Res.* 2009, 69, 5364–5373. [CrossRef][PubMed]

127. Giefing, M.; Zemke, N.; Brauze, D.; Kostrzewska-Poczekaj, M.; Luczak, M.; Szaumkessel, M.; Pelinska, K.; Kiwerska, K.; Tönnies, H.; Grenman, R.; et al. High resolution ArrayCGH and expression profiling identifies PTPRD and PCDH17/PCH68 as tumor suppressor gene candidates in laryngeal squamous cell carcinoma. *Oncogene* 2010, 29, 17286–7296. [CrossRef]

128. Lu, W.; Xu, Z.; Zhang, M.; Zuo, Y. MiR-19a promotes epithelial-mesenchymal transition through PI3K/AKT pathway in gastric cancer. *Int. J. Clin. Exp. Pathol.* 2014, 7, 2726–2729. [CrossRef]

129. Huang, L.; Wang, X.; Wen, C.; Yang, X.; Song, M.; Chen, J.; Wang, C.; Zhang, B.; Wang, L.; Iwamoto, A.; et al. Hsa-miR-19a is associated with lymph metastasis and mediates the TNF-α induced epithelial-to-mesenchymal transition in colorectal cancer. *Sci. Rep.* 2015, 5, 13350. [CrossRef][PubMed]

130. Li, J.; Yang, S.; Yan, W.; Yang, J.; Qin, Y.-J.; Lin, X.-L.; Xie, R.-Y.; Wang, S.-C.; Jin, W.; Gao, F.; et al. MicroRNA-19 triggers epithelial–mesenchymal transition of lung cancer cells accompanied by growth inhibition. *Lab. Invest.* 2015, 95, 1056–1070. [CrossRef][PubMed]

131. Xu, M.; Wang, S.; Wang, Y.; Wu, H.; Frank, J.A.; Zhang, Z.; Luo, J. Role of p38y MAPK in regulation of EMT and cancer stem cells. *Biochim. Biophys. Acta Mol. Basis Dis.* 2018, 1864, 3605–3617. [CrossRef]

132. Zhu, Q.; Zhang, Q.; Gu, M.; Zhang, K.; Xia, T.; Zhang, C.; Chen, W.; Yin, H.; Yao, H.; Fan, Y.; et al. miR106A-5p upregulation suppresses autophagy and accelerates malignant phenotype in nasopharyngeal carcinoma. *Autophagy* 2020, 10, 1–17. [CrossRef]

133. Fu, S.; Fu, Y.; Chen, F.; Hu, Y.; Quan, B.; Zhang, J. Overexpression of MYCT1 Inhibits Proliferation and Induces Apoptosis in Human Acute Myeloid Leukemia HL-60 and KG-1a Cells in vitro and in vivo. *Front. Pharmacol.* 2018, 9, 1045. [CrossRef][PubMed]
135. Fu, S.; Guo, Y.; Chen, H.; Xu, Z.-M.; Qiu, G.-B.; Zhong, M.; Sun, K.-L.; Fu, W.-N. MYCT1-TV, A Novel MYCT1 Transcript, Is Regulated by c-Myc and May Participate in Laryngeal Carcinogenesis. *Plas ONE* 2011, 6, e25648. [CrossRef] [PubMed]

136. Yee, P.-J.; Sun, Y.-Y.; Li, Y.-H.; Xu, Z.-M.; Fu, W.-N. MYCT1 inhibits the EMT and migration of laryngeal cancer cells via the SP1/miR-629-3p/ESRP2 pathway. *Cell. Signal.* 2020, 74, 109709. [CrossRef] [PubMed]

137. Miyazaki, H.; Takahashi, R.-U.; Prieto-Vila, M.; Kawamura, Y.; Kondo, S.; Shirota, T.; Ochiya, T. CD44 exerts a functional role during EMT induction in cisplatin-resistant head and neck cancer cells. *Oncotarget* 2018, 9, 10029–10041. [CrossRef]

138. Chikuda, J.; Otsuka, K.; Shimomura, I.; Ito, K.; Miyazaki, H.; Takahashi, R.-U.; Nagasaki, M.; Mukudai, Y.; Ochiya, T.; Shimane, T.; et al. CD44a Induces miR-629-3p Expression in Association with Cisplatin Resistance in Head and Neck Cancer Cells. *Cancers* 2020, 12, 856. [CrossRef]

139. Büüssing, I.; Slack, F.J.; Grosshans, H. Let-7 microRNAs in development, stem cells and cancer. *Trends Mol. Med.* 2008, 14, 400–409. [CrossRef] [PubMed]

140. Roush, S.; Slack, F.J. The let-7 family of microRNAs. *Trends Cell Biol.* 2008, 18, 505–516. [CrossRef]

141. Wang, X.; Cao, L.; Wang, Y.; Wang, X.; Liu, N.; You, Y. Regulation of let-7 and its target oncogenes (Review). *Oncol. Lett.* 2012, 3, 955–960. [CrossRef]

142. Liao, T.-T.; Hsu, W.-H.; Ho, C.-H.; Hwang, W.-L.; Lan, H.-Y.; Lo, T.; Chang, C.-C.; Tai, S.-K.; Yang, M.-H. Let-7 Modulates Chromatin Configuration and Target Gene Repression through Regulation of the ARID3B Complex. *Cell Rep.* 2016, 14, 520–533. [CrossRef] [PubMed]

143. Yang, W.-H.; Lan, H.-Y.; Huang, C.-H.; Tai, S.-K.; Tseng, C.-H.; Kao, S.-Y.; Wu, K.-J.; Hung, M.-C.; Yang, M.-H. RAC1 activation mediates Twist1-induced cancer cell migration. *Nat. Cell Biol.* 2012, 14, 366–374. [CrossRef] [PubMed]

144. Yue, P.-J.; Sun, Y.-Y.; Li, Y.-H.; Xu, Z.-M.; Fu, W.-N. MYCT1 inhibits the EMT and migration of laryngeal cancer cells via the SP1/miR-629-3p/ESRP2 pathway. *Cell. Signal.* 2020, 74, 109709. [CrossRef] [PubMed]

145. Hittelman, W.N.; Liao, Y.; Wang, L.; Milas, L. Are cancer stem cells radioresistant? *Future Oncol.* 2010, 6, 1563–1576. [CrossRef] [PubMed]

146. Peng, C.-Y.; Wang, T.-Y.; Lee, S.-S.; Hsieh, P.-L.; Liao, Y.-W.; Tsai, L.-L.; Fang, C.-Y.; Yu, C.-C.; Hsieh, C.-S. Let-7c restores radiosensitivity and chemosensitivity and impairs stemness in oral cancer cells through inhibiting interleukin-8. *J. Oral Pathol. Med.* 2018, 47, 590–597. [CrossRef]

147. De Jong, M.C.; Hoeve, J.J.T.; Grupp, H.; Stokkel, M.P.; Oken, C.; Verbeek, E.; Verheij, M.; Begg, A.C. Retreatment microRNA Expression Impacting on Epithelial-to-Mesenchymal Transition Predicts Intrinsic Radioresensitivity in Head and Neck Cancer Cell Lines and Patients. *Clin. Cancer Res.* 2015, 21, 5630–5638. [CrossRef]

148. Dioguardi, M.; Caloro, G.A.; Laino, L.; Alovisi, M.; Sovereto, D.; Crincoli, V.; Aiuto, R.; Coccia, E.; Troiano, G.; Muzzio, L.L. Circulating miR-21 as a Potential Biomarker for the Diagnosis of Oral Cancer: A Systematic Review with Meta-Analysis. *Cancers* 2020, 12, 936. [CrossRef]

149. Lu, Y.-C.; Cheng, A.-J.; Lee, L.-Y.; You, G.-R.; Li, Y.-L.; Chen, H.-Y.; Chang, J.T. MiR-520b as a novel molecular target for suppressing stemness phenotype of head-neck cancer by inhibiting interleukin-8. *J. Oral Pathol. Med.* 2018, 47, 590–597. [CrossRef]

150. Diehn, M.; Cho, R.W.; Lobos, N.A.; Kalisky, T.; Dorie, M.J.; Kulp, A.N.; Qian, D.; Lam, J.S.; Ailles, L.E.; Wong, M.; et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nat. Cell Biol.* 2009, 458, 780–783. [CrossRef]

151. Ishimoto, T.; Nagano, O.; Yae, T.; Tamada, M.; Motohara, T.; Oshima, H.; Oshima, M.; Ikeda, T.; Asaba, R.; Yagi, H.; et al. CD44 Variant Regulates Redox Status in Cancer Cells by Stabilizing the xCT Subunit of System xc. *Oncol. Lett.* 2012, 3, 955–960. [CrossRef]

152. Banerjee, R.; Mani, R.-S.; Russo, N.; Scanlon, C.S.; Tsodikov, A.; Jing, X.; Cao, Q.; Palanisamy, N.; Metwally, T.; Inglehart, R.C.; et al. The tumor suppressor gene rap1GAP is silenced by miR-101-mediated EZH2 overexpression in invasive squamous cell carcinoma. *Oncogene* 2011, 30, 4339–4349. [CrossRef]

153. Simon, J.A.; Lange, C.A. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat. Res. Mol. Mech. Mutagen.* 2008, 647, 21–29. [CrossRef]

154. Gonzalez, M.E.; Moore, H.M.; Li, X.; Toy, K.A.; Huang, W.; Sabel, M.S.; Kidwell, K.M.; Kleer, C.G. EZH2 expands breast stem cells through activation of NOTCH1 signaling. *Proc. Natl. Acad. Sci. USA* 2011, 111, 3098–3103. [CrossRef]

155. Wang, C.; Liu, X.; Chen, Z.; Huang, H.; Jin, Y.; Kolokythas, A.; Wang, A.; Dai, Y.; Wong, D.T.W.; Zhou, X. Polycomb group protein EZH2-mediated E-cadherin repression promotes metastasis of oral tongue squamous cell carcinoma. *Mol. Carcinog.* 2011, 52, 229–236. [CrossRef]

156. Luo, H.; Jiang, Y.; Ma, S.; Chang, H.; Yi, C.; Cao, H.; Gao, Y.; Guo, H.; Hou, J.; Yan, J.; et al. EZH2 promotes invasion and metastasis of laryngeal squamous cells carcinoma via epithelial-mesenchymal transition through H3K27me3. *Biochem. Biophys. Res. Commun.* 2016, 479, 253–259. [CrossRef] [PubMed]

157. Kinoshita, T.; Nohata, N.; Hanazawa, T.; Kikkawa, N.; Yamamoto, N.; Yoshino, H.; Iesako, T.; Enokiida, H.; Nakagawa, M.; Okamoto, Y.; et al. Tumour-suppressive microRNA-29s inhibit cancer cell migration and invasion by targeting laminin-integrin signalling in head and neck squamous cell carcinoma. *Br. J. Cancer* 2013, 109, 2636–2645. [CrossRef] [PubMed]
Cancers 2021, 13, 1742

159. Chen, L.-H.; Hsu, W.-L.; Tseng, Y.-J.; Liu, D.-W.; Weng, C.-F. Involvement of DNMT 3B promotes epithelial-mesenchymal transition and gene expression profile of invasive head and neck squamous cell carcinomas cell lines. BMC Cancer 2016, 16, 431. [CrossRef]

160. Zhuang, Z.; Yu, P.; Xie, N.; Wu, Y.; Liu, H.; Zhang, M.; Tao, Y.; Wang, W.; Yin, H.; Zou, B.; et al. MicroRNA-204-5p is a tumor suppressor and potential therapeutic target in head and neck squamous cell carcinoma. Theranostics 2020, 10, 1433–1453. [CrossRef] [PubMed]

161. Malaguarnera, R.; Belfiore, A. The Emerging Role of Insulin and Insulin-Like Growth Factor Signaling in Cancer Stem Cells. Front. Endocrinol. 2015, 6, 59. [CrossRef] [PubMed]

162. Li, X.; Zhao, Z.; Li, M.; Liu, M.; Bahena, A.; Zhang, Y.; Zhang, Y.; Nambiar, C.; Liu, G. Sulforaphane promotes apoptosis, and inhibits proliferation and self-renewal of nasopharyngeal cancer cells by targeting miRNA-124-3p. Biomed. Pharmacother. 2018, 103, 473–481. [CrossRef]

163. Huang, W.-C.; Jang, T.-H.; Tung, S.-L.; Yen, T.-C.; Chan, S.-H.; Wang, L.-H. A novel miR-365-3p/EHF/keratin 16 axis promotes oral squamous cell carcinoma metastasis, cancer stemness and drug resistance via enhancing β3-integrin/c-met signaling pathway. J. Exp. Clin. Cancer Res. 2019, 38, 1–17. [CrossRef] [PubMed]

164. Yu, C.-C.; Chang, C.-J.; Hsu, C.-C.; Tsai, L.-L.; Lu, S.-W.; Huang, H.-S.; Wang, J.-J.; Chou, M.-Y.; Hu, F.-W. Let-7d functions as novel regulator of epithelial-mesenchymal transition and chemoresistant property in oral cancer. Oncol. Rep. 2011, 26, 1003–1010. [CrossRef] [PubMed]

165. Du, Y.; Li, Y.; Lv, H.; Zhou, S.; Sun, Z.; Wang, M. miR-98 suppresses tumor cell growth and metastasis by targeting IGF1R in oral squamous carcinoma cell. Int. J. Clin. Exp. Pathol. 2013, 6, 12252–12259. [PubMed]

166. Bendall, S.C.; Stewart, M.H.; Menendez, P.; George, D.; Vijayaragavan, K.; Werbowetski-Ogilvie, T.; Ramos-Mejia, V.; Rouleau, A.; Yang, J.; Bossé, M.; et al. IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. Nat. Cell Biol. 2007, 448, 1015–1021. [CrossRef] [PubMed]

167. Farabaugh, S.M.; Boone, D.N.; Lee, A.V. Role of IGF1R in Breast Cancer Subtypes, Stemness, and Lineage Differentiation. Front. Endocrinol. 2013, 4, 69. [CrossRef] [PubMed]

168. Xu, C.; Xie, D.; Yu, S.-C.; Yang, X.-J.; He, L.-R.; Yang, J.; Ping, Y.-F.; Wang, B.; Yang, L.; Xu, S.-L.; et al. β-Catenin/POU5F1/SOX2 Transcription Factor Complex Mediates IGF-I Receptor Signaling and Predicts Poor Prognosis in Lung Adenocarcinoma. Cancer Res. 2013, 73, 3181–3189. [CrossRef]

169. Malaguarnera, R.; Belfiore, A. The Emerging Role of Insulin and Insulin-Like Growth Factor Signaling in Cancer Stem Cells. Front. Endocrinol. 2014, 5, 10. [CrossRef] [PubMed]

170. Wang, X.-H.; Wu, H.-Y.; Gao, J.; Wang, X.-H.; Gao, T.-H.; Zhang, M.; Tao, Y.; Wang, W.; Yin, H.; Zou, B.; et al. MicroRNA-204-5p is a tumor suppressor and potential therapeutic target in head and neck squamous cell carcinoma. Theranostics 2020, 10, 1433–1453. [CrossRef] [PubMed]

171. Zhang, L.; Liao, Y.; Tang, M.; Bahena, A.; Zhang, Y.; Zhang, Y.; Nambiar, C.; Liu, G. Sulforaphane promotes apoptosis, and inhibits proliferation and self-renewal of nasopharyngeal cancer cells by targeting miRNA-124-3p. Biomed. Pharmacother. 2018, 103, 473–481. [CrossRef]

172. Leong, H.S.; Chong, F.T.; Sew, P.H.; Lau, D.P.; Wong, B.H.; Teh, B.-T.; Tan, D.S.; Iyer, N.G. Targeting Cancer Stem Cell Plasticity Through Modulation of Epidermal Growth Factor and Insulin-Like Growth Factor Receptor Signaling in Head and Neck Squamous Cell Cancer. Stem Cells Transl. Med. 2014, 3, 1055–1065. [CrossRef]

173. 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, 1732–1745. [CrossRef]

174. Farabaugh, S.M.; Boone, D.N.; Lee, A.V. Role of IGF1R in Breast Cancer Subtypes, Stemness, and Lineage Differentiation. Front. Endocrinol. 2013, 4, 69. [CrossRef] [PubMed]

175. Elbadawy, M.; Usui, T.; Yamawaki, H.; Sasaki, K. Emerging Roles of C-Myc in Cancer Stem Cell-Related Signaling and Resistance to Cancer Chemotherapy: A Potential Therapeutic Target against Colorectal Cancer. J. Int. Mol. Sci. 2019, 20, 2340. [CrossRef] [PubMed]

176. Wang, K.; Jin, J.; Ma, T.; Zhai, H. MicroRNA-139-5p inhibits the tumorigenesis and progression of oral squamous carcinoma cells by targeting HOXA9. J. Cell. Mol. Med. 2017, 21, 3730–3740. [CrossRef] [PubMed]

177. Bhatlekar, S.; Viswanathan, V.; Fields, J.Z.; Boman, B.M. Overexpression of HOXA4 and HOXA9 genes promotes self-renewal and contributes to colon cancer stem cell overpopulation. J. Cell. Physiol. 2018, 233, 727–735. [CrossRef]

178. You, X.; Zhou, Z.; Chen, W.; Wei, X.; Zhou, H.; Luo, W. MicroRNA-495 confers inhibitory effects on cancer stem cells in oral squamous cell carcinoma through the HOXC6-mediated TGF-β signaling pathway. Stem Cell Res. Ther. 2020, 11, 1–14. [CrossRef] [PubMed]

179. Wang, Y.; Jia, L.; Wang, B.; Diao, S.; Jia, R.; Shang, J. MiR-495/IGF-1/akt Signaling as a Novel Axis Is Involved in the Epithelial-to-Mesenchymal Transition of Oral Squamous Cell Carcinoma. J. Oral Maxillofac. Surg. 2019, 77, 1009–1021. [CrossRef] [PubMed]

180. Lv, L.; Wang, Q.; Yang, Y.; Ji, H. MicroRNA-495 targets Notch1 to prohibit cell proliferation and invasion in oral squamous cell carcinoma. Mol. Med. Rep. 2018, 19, 693–702. [CrossRef]

181. Zhang, L.; Xiao, W.; Tang, L. MicroRNA-34 family: A potential tumor suppressor and therapeutic candidate in cancer. J. Exp. Clin. Cancer Res. 2019, 38, 1–13. [CrossRef]

182. Kim, J.S.; Kim, E.J.; Lee, S.; Tan, X.; Liu, X.; Park, S.; Kang, K.; Yoon, J.-S.; Ko, Y.H.; Kurie, J.M.; et al. MiR-34a and miR-34b/c have distinct effects on the suppression of lung adenocarcinomas. Exp. Mol. Med. 2019, 51, 1–10. [CrossRef]

183. Brito, B.D.L.; Lourenço, S.V.; Damascena, A.S.; Kowalski, L.P.; Soares, F.A.; Coutinho-Camilo, C.M. Expression of stem cell-regulating miRNAs in oral cavity and oropharynx squamous cell carcinoma. J. Oral Pathol. Med. 2016, 45, 647–654. [CrossRef]
183. Kumar, B.; Yadav, A.; Lang, J.; Teknos, T.N.; Kumar, P. Dysregulation of MicroRNA-34a Expression in Head and Neck Squamous Cell Carcinoma Promotes Tumor Growth and Tumor Angiogenesis. *PLoS ONE* **2012**, *7*, e37601. [CrossRef]

184. Sun, Z.; Hu, W.; Xu, J.; Kaufmann, A.M.; Albers, A.E. MicroRNA-34a regulates epithelial-mesenchymal transition and cancer stem cell phenotype of head and neck squamous cell carcinoma in vitro. *Int. J. Oncol.* **2015**, *47*, 1339–1350. [CrossRef]

185. Gregory, P.A.; Bert, A.G.; Paterson, E.L.; Barry, S.C.; Tsykin, A.; Farshid, G.; Vadas, M.A.; Khew-Goodall, Y.; Goodall, G.J. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* **2008**, *10*, 593–601. [CrossRef] [PubMed]

186. Arunkumar, G.; Rao, A.K.D.M.; Manikandan, M.; Rao, H.P.S.; Subbiah, S.; Ilangovan, R.; Murugan, A.K.; Munirajan, A.K. Dysregulation of miR-200 family microRNAs and epithelial-mesenchymal transition markers in oral squamous cell carcinoma. *Oncol. Lett.* **2017**, *15*, 649–657. [CrossRef] [PubMed]

187. Tamagawa, S.; Beder, L.B.; Hotomi, M.; Gunduz, M.; Yata, K.; Grenman, R.; Yamanaka, N. Role of miR-200c/miR-141 in the regulation of epithelial-mesenchymal transition and migration in head and neck squamous cell carcinoma. *Int. J. Mol. Med.* **2014**, *33*, 879–886. [CrossRef] [PubMed]

188. Liao, T.-T.; Yang, M.-H. Hybrid Epithelial/Mesenchymal State in Cancer Metastasis: Clinical Significance and Regulatory Mechanisms. *Cells* **2020**, *9*, 623. [CrossRef]

189. He, P.; Qiu, K.; Jia, Y. Modeling of mesenchymal hybrid epithelial state and phenotypic transitions in EMT and MET processes of cancer cells. *Sci. Rep.* **2018**, *8*, 14323. [CrossRef] [PubMed]

190. Lu, M.; Jolly, M.K.; Levine, H.; Onuchic, J.N.; Ben-Jacob, E. MicroRNA-based regulation of epithelial-hybrid-mesenchymal fate determination. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 18144–18149. [CrossRef]

191. Gu, Y.; Liu, H.; Kong, F.; Ye, J.; Jia, X.; Zhang, Z.; Li, N.; Yin, J.; Zheng, G.; He, Z. miR-22/KAT6B axis is a chemotherapeutic determiner via regulation of PI3k-Akt-NF-kB pathway in tongue squamous cell carcinoma. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 164. [CrossRef]

192. Mendoza, M.C.; Er, E.E.; Blenis, J. The Ras-ERK and PI3K-mTOR pathways: Cross-talk and compensation. *Trends Biochem. Sci.* **2011**, *36*, 320–328. [CrossRef]

193. Castellano, E.; Molina-Arcas, M.; Krygowska, A.A.; East, P.; Warne, P.; Nicol, A.; Downward, J. RAS signalling through PI3-Kinase controls cell migration via modulation of Reelin expression. *Nat. Commun.* **2016**, *7*, 11245. [CrossRef]

194. Park, S.; Zhao, D.; Hatanpaa, K.J.; Mickey, B.E.; Saha, D.; Boothman, D.A.; Story, M.D.; Wong, E.T.; Burton, S.; Georgescu, M.-M.; et al. RIP1 Activates PI3K-Akt via a Dual Mechanism Involving NF-κB-Mediated Inhibition of the mTOR-S6K-IRS1 Negative Feedback Loop and Down-regulation of PTEN. *Cancer Res.* **2009**, *69*, 4107–4111. [CrossRef]

195. Oeckinghaus, A.; Hayden, M.S.; Ghosh, S. Crosstalk in NF-κB signaling pathways. *Nat. Immunol.* **2011**, *12*, 695–708. [CrossRef]

196. Inoki, K.; Ouyang, H.; Zhu, T.; Lindvall, C.; Wang, Y.; Zhang, X.; Yang, Q.; Bennett, C.; Harada, Y.; Stankunas, K.; et al. TSC2 Integrates Wnt and Energy Signals via a Coordinated Phosphorylation by AMPK and GSK3 to Regulate Cell Growth. *Cell* **2006**, *126*, 955–968. [CrossRef]

197. Fang, D.; Hawke, D.; Zheng, Y.; Xia, Y.; Meisenhelder, J.; Nika, H.; Mills, G.B.; Kobayashi, R.; Hunter, T.; Lu, Z. Phosphorylation of β-Catenin by AKT Promotes β-Catenin Transcriptional Activity. *J. Biol. Chem.* **2007**, *282*, 11221–11229. [CrossRef] [PubMed]

198. Zhang, L.; Zhou, F.; Drabsh, Y.; Gao, R.; Snaar-Jagalska, B.E.; Mickanin, C.; Huang, H.; Sheppard, K.-A.; Porter, J.A.; Lu, C.X.; et al. USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-β type I receptor. *Nat. Cell Biol.* **2012**, *14*, 717–726. [CrossRef] [PubMed]

199. Zhang, L.; Zhou, F.; Dijke, P.T. Signaling interplay between transforming growth factor-β receptor and PI3K/AKT pathways in cancer. *Trends Biochem. Sci.* **2013**, *38*, 612–620. [CrossRef]

200. Budi, E.H.; Muthusamy, B.P.; Derynck, R. The insulin response integrates increased TGF-β signaling through Akt-induced enhancement of cell surface delivery of TGF-β receptors. *Sci. Signal.* **2015**, *8*, ra96. [CrossRef] [PubMed]

201. Hamidi, A.; Song, J.; Thakur, N.; Itoh, S.; Marcusson, A.; Bergh, A.; Heldin, C.-H.; Landström, M. TGF-β promotes PI3K-AKT signaling and prostate cancer cell migration through the TRAF6-mediated ubiquitylation of p85α. *Sci. Signal.* **2017**, *10*, eaal4186. [CrossRef]

202. Madsen, R.R. PI3K in stemness regulation: From development to cancer. *Biochem. Soc. Trans.* **2020**, *48*, 301–315. [CrossRef] [PubMed]

203. Natarajan, K.; Abraham, P.; Kota, R.; Isaac, B. NF-κB-iNOS-COX2-TNF α inflammatory signaling pathway plays an important role in methotrexate induced small intestinal injury in rats. *Food Chem. Toxicol.* **2018**, *118*, 766–783. [CrossRef] [PubMed]

204. Yamamoto, K.; Arakawa, T.; Ueda, N.; Yamamoto, S. Transcriptional Roles of Nuclear Factor κB and Nuclear Factor-Interleukin-6 in the Tumor Necrosis Factor α-Dependent Induction of Cyclooxygenase-2 in MC3T3-E1 Cells. *J. Biol. Chem.* **1995**, *270*, 31315–31320. [CrossRef] [PubMed]

205. Yang, H.; Qi, H.; Ren, J.; Cui, J.; Li, Z.; Waldum, H.L.; Cui, G. Involvement of NF-κB/IL-6 Pathway in the Processing of Colorectal Carcinogenesis in Colitis Mice. *Int. J. Inflamm.* **2014**, *2014*, 1–7. [CrossRef]

206. Greten, F.R.; Arkan, M.C.; Bollrath, J.; Hsu, L.-C.; Goede, J.; Miething, C.; Göktuna, S.I.; Neuenhahn, M.; Fierer, J.; Paxian, S.; et al. NF-κB Is a Negative Regulator of IL-1β Secretion as Revealed by Genetic and Pharmacological Inhibition of Iκκβ. *Cell* **2007**, *130*, 918–931. [CrossRef] [PubMed]

207. Rovin, B.H.; Dickerson, J.A.; Tan, L.C.; Hebert, C.A. Activation of nuclear factor-κB correlates with MCP-1 expression by human mesangial cells. *Kidney Int.* **1995**, *48*, 1263–1271. [CrossRef] [PubMed]
208. Yamamoto, Y.; Gaynor, R.B. IkB kinases: Key regulators of the NF-κB pathway. *Trends Biochem. Sci.* 2004, 29, 72–79. [CrossRef]

209. Rinkenbaugh, A.L.; Baldwin, A.S. The NF-κB Pathway and Cancer Stem Cells. *Cells* 2016, 5, 16. [CrossRef]

210. Feng, X.; Luo, Q.; Wang, H.; Zhang, H.; Chen, F. MicroRNA-22 suppresses cell proliferation, migration and invasion in oral squamous cell carcinoma by targeting NLRP3. *J. Cell. Physiol.* 2018, 233, 6705–6713. [CrossRef]

211. Huang, C.-F.; Chen, L.; Li, Y.-C.; Wu, L.; Yu, G.-T.; Zhang, W.-F.; Sun, Z.-J. NLRP3 inflammasome activation promotes inflammation-induced carcinogenesis in head and neck squamous cell carcinoma. *J. Exp. Clin. Cancer Res.* 2017, 36, 1–13. [CrossRef]

212. Zhang, C.; Hao, Y.; Sun, Y.; Liu, P. Quercetin suppresses the tumorigenesis of oral squamous cell carcinoma by regulating microRNA-22/WNT1/β-catenin axis. *J. Pharmacol. Sci.* 2019, 140, 128–136. [CrossRef]

213. Qiu, K.; Huang, Z.; Huang, Z.; He, Z.; You, S. miR-22 regulates cell invasion, migration and proliferation in vitro through inhibiting CD147 expression in tongue squamous cell carcinoma. *Arch. Oral Biol.* 2016, 66, 92–97. [CrossRef] [PubMed]

214. Yu, B.; Zhang, Y.; Wu, K.; Wang, L.; Jiang, Y.; Chen, W.; Yan, M. CD147 promotes progression of head and neck squamous cell carcinoma via NF-kappa B signaling. *J. Cell. Mol. Med.* 2019, 23, 954–966. [CrossRef] [PubMed]

215. Suzuki, S.; Toyosma, S.; Tsuji, T.; Kawasaki, Y.; Yamada, T. CD147 mediates transforming growth factor-β1-induced epithelial-mesenchymal transition and cell invasion in squamous cell carcinoma of the tongue. *Exp. Ther. Med.* 2019, 17, 2855–2860. [CrossRef]

216. Zhu, M.; Zhang, C.; Chen, D.; Chen, S.; Zheng, H. MicroRNA-98-HMGA2-POSTN signal pathway reverses epithelial-to-mesenchymal transition in laryngeal squamous cell carcinoma. *Biomed. Pharmacother.* 2019, 117, 108998. [CrossRef] [PubMed]

217. Li, M.; Tian, L.; Ren, H.; Chen, X.; Wang, Y.; Ge, J.; Wu, S.; Sun, Y.; Liu, M.; Xiao, H. MicroRNA-101 is a potential prognostic indicator of laryngeal squamous cell carcinoma and modulates CDK8. *J. Transl. Med.* 2015, 13, 271. [CrossRef] [PubMed]

218. Firestein, R.; Bass, A.J.; Kim, S.Y.; Dunn, I.F.; Silver, S.J.; Guney, I.; Freed, E.; Ligon, A.H.; Vena, N.; Ogino, S.; et al. CDK8 is a colorectal cancer oncogene that regulates β-catenin activity. *Nature 2008, 455, 547–551.* [CrossRef] [PubMed]

219. He, S.-B.; Yuan, Y.; Wang, L.; Yu, M.-J.; Zhu, Y.-B.; Zhu, X.-G. Effects of cyclin-dependent kinase 8 specific siRNA on the proliferation and apoptosis of colon cancer cells. *J. Exp. Clin. Cancer Res.* 2011, 30, 109. [CrossRef]

220. Shao, Q.; Zhang, P.; Ma, Y.; Lu, Z.; Meng, J.; Li, H.; Wang, X.; Chen, D.; Zhang, M.; Han, Y.; et al. MicroRNA-139-5p affects cisplatin sensitivity in human nasopharyngeal carcinoma cells by regulating the epithelial-to-mesenchymal transition. *Gene 2018, 652, 48–58.* [CrossRef]

221. Brabletz, T.; Jung, A.; Spaderna, S.; Hlubek, F.; Kirchner, T. Migrating cancer stem cells—an integrated concept of malignant tumour progression. *Nat. Rev. Cancer 2005, 5, 744–749.* [CrossRef] [PubMed]

222. Mani, S.A.; Guo, W.; Liao, M.J.; Eaton, E.N.; Ayyanan, A.; Zhou, A.Y.; Brooks, M.; Reinhard, F.; Zhang, C.C.; Shipitsin, M.; et al. The Epithelial-Mesenchymal Transition Generates Cells with Properties of Stem Cells. *Cell 2008, 133, 704–715.* [CrossRef]

223. Polyak, K.; Weinberg, R.A. Transitions between epithelial and mesenchymal states: Acquisition of malignant and stem cell traits. *Nat. Rev. Cancer 2009, 9, 265–273.* [CrossRef] [PubMed]

224. Karemker, I.D.; Vermeulen, M. ZBTB 2 reads unmethylated CpG island promoters and regulates embryonic stem cell differentiation. *EMBO Rep. 2018, 19, e44993.* [CrossRef]

225. Yang, Y.; Li, H.; He, Z.; Xie, D.; Ni, J.; Lin, X. MicroRNA-488-3p inhibits proliferation and induces apoptosis by targeting ZBTB2 in esophageal squamous cell carcinoma. *J. Cell. Biochem.* 2019, 120, 18702–18713. [CrossRef] [PubMed]

226. Godar, S.; Ince, T.A.; Bell, G.W.; Feldser, D.; Donaher, J.L.; Bergh, J.; Liu, A.; Miu, K.; Watnick, R.S.; Reinhardt, F.; et al. Growth-Inhibitory and Tumor-Suppressive Functions of p53 Depend on Its Repression of CD44 Expression. *Cell 2008, 134, 62–73.* [CrossRef]

227. Wang, Z.; Mao, J.-W.; Liu, G.-Y.; Wang, F.-G.; Ju, Z.-S.; Zhou, N.; Wang, R.-Y. MicroRNA-372 enhances radiosensitivity while inhibiting cell invasion and metastasis in nasopharyngeal carcinoma through activating the PKB-dependent p53 signaling pathway. *Cancer Med. 2019, 8, 712–728.* [CrossRef]

228. Chang, C.-J.; Chao, C.-H.; Xia, W.; Yang, J.-Y.; Xiong, Y.; Li, C.-W.; Yu, W.-H.; Rehman, S.K.; Hsu, J.L.; Lee, H.-H.; et al. p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. *Nat. Cell Biol. 2011, 13, 317–323.* [CrossRef]

229. Shi, B.; Yan, W.; Liu, G.; Guo, Y. MicroRNA-488 inhibits tongue squamous carcinoma cell invasion and EMT by directly targeting ATF3. *Cell. Mol. Biol. Lett. 2018, 23, 28.* [CrossRef]

230. Su, J.-L.; Chen, P.-S.; Johansson, G.; Kuo, M.-L. Function and Regulation of Let-7 Family microRNAs. *MicroRNA 2012, 1, 34–39.* [CrossRef]

231. Chen, P.-S.; Su, J.-L.; Cha, S.-T.; Tarn, W.-Y.; Wang, M.-Y.; Hsu, H.-C.; Lin, M.-T.; Chu, C.-Y.; Hua, K.-T.; Chen, C.-N.; et al. miR-107 promotes tumour progression by targeting the let-7 microRNA in mice and humans. *J. Clin. Investig. 2011, 121, 3442–3455.* [CrossRef]

232. González-Arriagada, W.A.; Olivero, P.; Rodriguez, B.; Lozano-Burgos, C.; De Oliveira, C.E.; Coletta, R.D. Clinicopathological significance of miR-26, miR-107, miR-125b, and miR-203 in head and neck carcinomas. *Oral Dis. 2018, 24, 930–939.* [CrossRef]

233. Huang, C.; Wang, Z.; Zhang, K.; Dong, Y.; Zhang, A.; Lu, C.; Liu, L. MicroRNA-107 inhibits proliferation and invasion of laryngeal squamous cell carcinoma cells by targeting CACNA2D1 in vitro. *Anti-Cancer Drugs 2020, 31, 260–271.* [CrossRef]

234. Sui, X.; Geng, J.-H.; Li, Y.-H.; Zhu, G.-Y.; Wang, W.-H. Calcium channel α2δ1 subunit (CACNA2D1) enhances radioresistance in cancer stem-like cells in non-small cell lung cancer cells. *Cancer Manag. Res. 2018, 10, 5009–5018.* [CrossRef]
235. Patel, S.; Rawal, R. Role of miRNA dynamics and cytokine profile in governing CD44v6/Nanog/PTEN axis in oral cancer: Modulating the master regulators. *Tumor Biol.* 2016, 37, 14565–14575. [CrossRef]

236. Hersi, H.M.; Raulf, N.; Gaken, J.; Folarin, N.; Tavassoli, M. Micro RNA-9 inhibits growth and invasion of head and neck cancer cells and is a predictive biomarker of response to plerixafor, an inhibitor of its target CXCR 4. *Mol. Oncol.* 2018, 12, 2023–2041. [CrossRef]

237. Citron, F.; Armenia, J.; Franchin, G.; Polesel, J.; Talamini, R.; D’Andrea, S.; Sulfaro, S.; Croce, C.M.; Klement, W.; Otasek, D.; et al. An Integrated Approach Identifies Mediators of Local Recurrence in Head and Neck Squamous Carcinoma. *Clin. Cancer Res.* 2017, 23, 3769–3780. [CrossRef] [PubMed]

238. Li, X.; Zeng, Z.; Wang, J.; Wu, Y.; Chen, W.; Zheng, L.; Xi, T.; Wang, A.; Lu, Y. MicroRNA-9 and breast cancer. *Biomed. Pharmacother.* 2020, 122, 109687. [CrossRef]

239. Kamal, N.N.S.B.N.M.; Shahidan, W.N.S. Non-Exosomal and Exosomal Circulatory Micrornas: Which Are More Valid as Biomarkers? *Front. Pharmacol.* 2020, 10, 1500. [CrossRef] [PubMed]

240. Ludwig, S.; Sharma, P.; Wise, P.; Sposto, R.; Hollingshead, D.; Lamb, J.; Lang, S.; Fabbri, M.; Whiteside, T.L. mRNA and miRNA Profiles of Exosomes from Cultured Tumor Cells Reveal Biomarkers Specific for HPV16-Positive and HPV16-Negative Head and Neck Cancer. *Int. J. Mol. Sci.* 2020, 21, 8570. [CrossRef] [PubMed]

241. Lee, S.H.; Lee, C.-R.; Rigas, N.K.; Kim, R.H.; Kang, M.K.; Park, N.-H.; Shin, K.-H. Human papillomavirus 16 (HPV16) enhances tumor growth and cancer stemness of HPV-negative oral/oropharyngeal squamous cell carcinoma cells via miR-181 regulation. *Papillomavirus Res.* 2015, 1, 116–125. [CrossRef]

242. Huang, Q.; Yang, J.; Zheng, J.; Hsueh, C.; Guo, Y.; Zhou, L. Characterization of selective exosomal microRNA expression profile derived from laryngeal squamous cell carcinoma detected by next generation sequencing. *Oncol. Rep.* 2018, 40, 2584–2594. [CrossRef]

243. Whiteside, T.L. Tumor-Derived Exosomes and Their Role in Cancer Progression. *Int. Rev. Cytol.* 2016, 74, 103–141. [CrossRef]

244. Li, Y.-Y.; Tao, Y.-W.; Gao, S.; Li, P.; Zheng, J.-M.; Zhang, S.-E.; Liang, J.; Zhang, Y. Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. *EBioMedicine* 2018, 36, 209–220. [CrossRef]

245. Calin, G.A. miRNAs in oncogenesis and tumor suppression. In *International Review of Cell and Molecular Biology, MiRNAs in Differentiation and Development*, 1st ed.; Galluzzi, L., Vitale, I., Eds.; Academic Press: Cambridge, MA, USA, 2017; Volume 333.