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p53 and Cell Fate: Sensitizing Head and Neck Cancer Stem Cells to Chemotherapy

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Abstract

Head and neck cancers are deadly diseases that are diagnosed annually in approximately half a million individuals worldwide. Growing evidence supporting a role for cancer stem cells (CSCs) in the pathobiology of head and neck cancers has led to increasing interest in identifying therapeutics to target these cells. Apart from the canonical tumor-suppressor functions of p53, emerging research supports a significant role for this protein in physiological stem cell and CSC maintenance and reprogramming. Therefore, p53 has become a promising target to sensitize head and neck CSCs to chemotherapy. In this review, we highlight the role of p53 in stem cell maintenance and discuss potential implications of targeting p53 to treat patients with head and neck cancers.

Keywords

head and neck cancer; p53; cancer stem cells; differentiation; self-renewal

I. INTRODUCTION

The tumor suppressor *TP53* is the most commonly mutated gene in cancer. Known as the “guardian of the genome,” it can be considered a master regulator of cell fate. Although p53-null mice are viable, they have early-onset sporadic cancers.¹ In addition, individuals with Li-Fraumeni syndrome, which harbors p53 germ-line mutations, frequently develop cancers.² Given the major impact of p53 in cancer biology, most investigators have focused on the role of p53 as a tumor suppressor. Responses such as apoptosis, cell cycle arrest, and senescence have been the principal focus of p53 studies. Nonetheless, p53 has been found to be involved in normal cellular and developmental processes, including functions in multiple

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stages of embryonic development.^{3,4} Notably, strong evidence shows involvement of p53 in stem cell self-renewal and differentiation.^{5,6}

Two emerging roles for p53 include its ability to inhibit cancer stem cell (CSC) formation and regulate the CSC state. CSCs share many properties with adult stem cells. For example, they have the ability to self-renew and differentiate into committed progenitor cells with limited self-renewal potential.⁷ CSCs are known to be resistant to standard chemotherapy and radiation therapies and have increased tumorigenic capacity, often playing a part in tumor recurrence and metastasis *in vivo*.⁸ This review highlights some of the functions of p53 that are relevant to CSC maintenance (e.g., self-renewal and differentiation) and the potential implications of targeting p53 to sensitize CSCs to conventional chemotherapy in head and neck malignancies.

II. p53: GUARDIAN OF THE GENOME

The transcription factor p53 regulates expression of a large number of protein-coding genes and microRNAs (miRNAs) that mediate its downstream response. In its role as guardian of the genome, p53 is found to be mutated in > 50% of sporadic human cancers and thought to be functionally inactivated in a significant portion of remaining cancers.^{9–11} It can transiently halt the cell cycle to allow DNA damage repair or irreversibly block proliferation through senescence (or programmed cell death) to eliminate cells with damaged or mutated DNA. Functioning primarily as a transcription factor, this protein is involved in preserving genome integrity.

In normal conditions, p53 is under tight regulation, and its abundance is kept low in nonstressed cells. However, after genotoxic insult, p53 is protected from degradation via several post-translational modifications that allow its rapid accumulation in the cell and subsequent transcriptional activation.¹² Mouse double minute 2 (MDM2) is a major negative regulator of p53 and an E3 ubiquitin ligase that functions cooperatively with MDMX to target p53 for proteasomal degradation.¹³ Levels of p53, MDM2, and MDMX are tightly regulated to sense and respond to stress.¹⁴ This coordinated network is very important in normal homeostasis and development. MDM2-null mice have early embryonic lethality that can be rescued with p53 deletion.¹⁵ Some cancers bypass p53 function by overexpressing MDM2 or MDMX.¹⁶ Responses of p53 on a cell are stress and tissue specific. Below, we explore some of the evidence supporting the role of p53 in stem cell homeostasis.

III. CSC HYPOTHESIS

The CSC hypothesis states that a small subset of cancer cells within a tumor have tumor-initiating, self-renewing, and multilineage differentiation potential.¹⁷ This hypothesis postulates that (1) only a small population of cancer cells within a tumor are capable of initiating and propagating tumors in immunodeficient mice; (2) tumors resulting from CSCs contain a mixture of tumorigenic and nontumorigenic cells that recapitulate original tumor heterogeneity; and (3) tumors generated by CSCs can be serially transplanted, demonstrating self-renewal capabilities. These CSCs can be isolated based on the expression of cell-surface markers and/or aldehyde dehydrogenase (ALDH) activity.¹⁸

The first studies supporting the CSC hypothesis showed that tumor tissues have a distinct hierarchy of cells, similar to that observed in adult tissues.¹⁹ Within a tumor, cells can be distinguished by the expression of different cell lineage markers. This hierarchical organization was first evident in leukemic cells and later proven to be true in breast cancer and other solid malignancies.^{20–23} Only a small population of cells has tumorigenic potential and stem cell properties of self-renewal and differentiation, but the rest of the tumor tissue is comprised of transit-amplifying and differentiated cells that do not contribute to tumor initiation. Furthermore, CSCs are in a translational phase between epithelial-and mesenchymal-cell stages that might contribute to the plasticity observed in cancer cells.^{24,25} Environmental cues can cause CSCs to acquire or lose these stem cell characteristics.^{26,27}

The frequency and prevalence of CSCs can vary by tumor type and among patients who have the same type of cancer.^{28–30} Studies have shown that CSCs can be used as predictive and prognostic markers for patient survival.^{28–30} We have not yet identified a CSC marker that is uniquely expressed in these cells. As such, they can only be used to enrich for CSC population rather than isolate a pure population of cells.³¹ This complicates the study of CSCs and presents challenges to the use of these markers for targeted therapeutic approaches. Significant effort is now in place to address these challenges and develop biomarkers that can be used to verify the efficacy of CSC targeted therapies.

IV. p53 IN STEM CELLS

There are several mechanisms by which p53 is thought to regulate both normal stem cells and CSCs.³² In this review, we focus on only two of these. The first involves the effects of p53 on stem cell self-renewal and the second on stem cell differentiation.

A. p53 in Self-Renewal

Stem cells are defined by their ability to self-renew and produce progenitor cells that can ultimately generate multiple cell lineages (i.e., multipotency). Both of these tasks are centered on a single mitotic division event. Whereas, symmetric stem cell division yields two identical daughter cells, asymmetric division produces one stem cell and one proliferative progenitor cell (Fig. 1). When a stem cell undergoes symmetric division, it can yield two identical daughter stem cells or two proliferative progenitor cells. Both of these processes are essential during development and tissue homeostasis to maintain a stem cell pool and produce specialized cells. It has been shown that p53 plays an important role in the balance between self-renewal and differentiation in embryonic and adult stem cells, and this balance is important in cancer development and progression.^{5,6}

In mammary stem cells, p53 has been found to regulate polarity of cellular divisions.³³ The absence of p53 promotes self-renewal of these cells, allowing for expansion of the stem cell pool and resulting in unlimited and symmetric self-renewing divisions. Asymmetric divisions can be measured by the distribution of Numb.³⁴ Numb can directly interact with p53 and MDM2 and is thought to regulate p53 signaling by preventing MDM2-mediated ubiquitination.³⁵ Interestingly, p53 knockout mice contain a higher percentage of cells that are capable of forming mammospheres in culture and repopulating the mammary gland *in vivo*, further supporting a role for p53 in maintaining the stem cell pool.³⁶

Several groups have shown that deregulation of pathways that control self-renewal of normal stem cells can lead to their transformation into cancer cells.^{17,37,38} Indeed, p53 regulates the self-renewal of myeloid progenitor cells, transforming them into leukemia-initiating cells that resemble CSCs.³⁷ Conversely, p53 loss promotes CSC-pool expansion.^{39,40} Meanwhile, mutant p53 induces CSC marker expression in colorectal cancer.⁴¹ Such findings suggest a strong role for p53 in regulating self-renewal of CSCs.

B. p53 in Differentiation

A correlation between grade of differentiation and presence of p53 mutations has been observed in several malignancies.^{42,43} Cancers with p53 mutations or functional p53 inactivation by downstream regulators (e.g., overexpression of *Mdm2*) correlate with poor-grade and undifferentiated tumors.⁴⁴ In many cancers, histological grade remains one of the best prognostic factors for patient survival.^{45,46} This has certainly been the case for most squamous cell carcinomas and salivary gland cancers (SGCs).^{47,48}

Endogenous p53 was shown to induce differentiation of mouse embryonic stem cells by suppressing *Nanog* expression after DNA damage.⁴⁹ *Nanog* is an important gene for embryonic stem cell self-renewal.⁵⁰ Not only does *Nanog* have a role in embryonic stem cells, it also regulates dedifferentiation of primary p53-deficient mouse astrocytes into CSC-like cells.⁵¹ Tumor-suppressor p53 can regulate differentiation of several cell types,⁵² and its loss can also induce reprogramming of pluripotent stem cells, further supporting a role for p53 in differentiation.

It has been shown that p53 also transcriptionally activates miRNAs responsible for down-regulating stem cell transcription factors⁵³ down-regulates canonical Wnt pathway components by several mechanisms.^{54,55} Canonical Wnt signaling was first thought to be essential for maintaining stem cells undifferentiated during development.^{56,57} But, the roles of p53 and Wnt in differentiation are cell and context dependent. For example, p53 can induce transcription of Wnt ligands while Wnt signaling is balanced between differentiation and self-renewal pathways.^{56,58–60} The role of p53 in regulating cell fate is a balance between stem cell differentiation and self-renewal (Fig. 2). This balance is “hijacked” in cancer to expand the CSC pool. Deregulation of these differentiation and self-renewal pathways can be responsible for the formation of cancer stem-like cells.

V. SENSITIZING CSCs TO CHEMOTHERAPY

Two main approaches can be used to target CSCs. The first, and most studied, is to find agents that can specifically kill these cells.⁶¹ CSCs are resistant to standard chemotherapy agents and radiotherapy used in the clinic.^{62–66} For example, breast CSCs (i.e., CD44^{high} and CD24^{low} cells) appear to be intrinsically resistant to conventional chemotherapy and radiation therapy.^{63–64} Their unique biology allows them to bypass many of the mechanisms used to target cancer cells. For this reason, a CSC-specific treatment is needed to kill these cells. Several clinical trials targeting CSC-specific pathways are currently underway globally.⁵³

The second approach is to find agents that can differentiate CSCs to sensitize them to chemotherapy and radiation therapy. CSCs exploit physiological stem cell pathways to maintain a more undifferentiated state in such a way that they self-renew and give rise to different cells comprising a tumor.⁷ In theory, differentiating CSCs will make them lose their potential to self-renew, therefore becoming short lived. This approach is gaining increasing interest because the idea of sensitizing otherwise resistant cells to standard chemotherapy regimens is an attractive approach for the clinic. Nevertheless, more about the potential to differentiate CSCs must be understood because CSCs in many different tumor types (different from adult stem cells) have not been fully characterized and may present undistinguished differentiation fates.

One prominent challenge in studying CSCs is that the percentage and markers used to identify these cells in tumors varies by cancer and cancer subtype.⁶⁷ Compelling evidence for the importance of CSCs in tumorigenesis and correlations with patient outcomes has been observed in different tumors.^{68–70} Apart from surgery, chemotherapy and ionizing radiation are the most common therapies used to treat cancer, but a good number of cancers do not respond to therapy and some develop resistance over time.⁷¹ Furthermore, most chemotherapy agents are DNA damaging. CSCs can be resistant to conventional chemotherapy through several mechanisms.⁶² It is thought that these cells are intrinsically less susceptible to chemotherapy because they are slow cycling. Because chemotherapy agents are DNA damaging, they are most effective on rapidly proliferating cancer cells. Apart from this, CSCs have ABC transporters that can effectively efflux chemotherapy drugs and are resistant to DNA damaged-induced death, among other potential mechanisms.^{72,73}

Intravenously injected chemotherapy drugs are capable of penetrating just a few layers of cells in tumor tissues.^{74,75} This implies that deeper cell layers in the tumor receive lower doses of these agents. Given the intrinsic resistance of CSCs to chemotherapeutic drugs, subcytotoxic doses received by cells could explain the increase in fraction of CSCs observed when treated with chemotherapy.^{76–79} This increase could also be due to the intrinsic nature of these cells to be more resistant to chemotherapeutic agents, thus surviving while the rest of the tumor cells succumb to treatment. A combination of both mechanisms could contribute to this phenomenon. Residual CSCs that survive treatment are thought to be responsible for tumor recurrence and metastasis due to increased cell invasiveness, survival, and tumorigenic potential.⁸

Chemotherapy is capable of inducing trans-differentiation of cancer cells into CSCs, creating a population of chemotherapy-resistant cells that rely on similar mechanisms as those for generation and maintenance of induced pluripotent stem cells (iPSCs).^{80,81} A study by Auffinger and colleagues showed that temozolomide induced an increase in the glioma CSC pool, and the increase was a result of a phenotypic shift of the non-CSC pool to a CSC-like state.⁸¹ These authors showed that the newly transdifferentiated cells are more tumorigenic, invasive, and chemoresistant than the original tumor source.

Clinical studies monitoring the prevalence of CSCs before and after chemotherapy treatment have shown that CSCs are resistant to therapy, confirming results observed in *in vitro* and in preclinical studies.^{82,83} As an example, breast cancer patients undergoing neoadjuvant

chemotherapy who underwent biopsies before and after treatment showed that cells with CSC markers persisted even as tumor mass regressed.⁶³ CSCs resistant to radiotherapy have also been observed in patients who have received high doses of irradiation.⁸⁴

Studies attempting to exploit similarities between CSCs and iPSCs to sensitize cancers to conventional therapies have been attempted.⁸⁵ Using *Nanog* as a reporter of CSC formation, Saydamino and colleagues identified the compound GDM-1515 to be a regulator of histone demethylases that is capable of sensitizing CSCs to cisplatin-induced apoptosis.⁸⁵ This compound was able to inhibit epithelial-mesenchymal transition (EMT) and induction of CSCs by cisplatin. On the other hand, an unbiased screen for small molecules cytotoxic to CSCs revealed salinomycin, a natural compound that can reduce expression of CSC genes and mammary tumor growth and increase epithelial differentiation of mammary gland tumors.⁶² Further studies have proven the effectiveness of GDM-1515 for targeting CSCs in other cancers.^{86–89} However, its severe toxicity in normal cells impedes its clinical use.^{90,91} Meanwhile, other researchers have shown that targeting pathways that regulate CSC self-renewal can lead to CSC sensitization to chemotherapy.⁹² *Bmi-1* is a component of polycomb-repressor complex I and a known regulator of self-renewal. Targeting *Bmi-1* results in sensitization of cisplatin-based chemotherapies in CSCs.⁹²

IV. p53 IN HEAD AND NECK CANCER

Head and neck cancers, arising in mucosal surfaces of the oral cavity, nasopharynx, oropharynx, hypo-pharynx, larynx, paranasal sinuses, nasal cavity, and salivary glands, constitute ~4% of all cancers worldwide. Among those, more than 90% are head and neck squamous cell carcinomas (HNSCCs). HNSCC is the sixth most common cancer worldwide, occurring in more than 550,000 individuals and resulting in greater than 380,000 deaths each year.⁹³ Despite advancement in treatment modalities, there has been limited improvement in patient survival during the last three decades.

HNSCC can be stratified based on human papilloma virus (HPV) infection status; HPV-positive HNSCC carries a better survival rate than HPV-negative cancers.^{94–98} Accumulating epidemiological, molecular, and clinical evidence supports HPV-positive cancer to be a distinct subtype of head and neck cancers.^{99–101} Although the overall incidence of head and neck cancers has declined slightly in industrialized countries, there has been an increase in the incidence of HPV-positive cancers of the oropharynx.^{102–105}

Mutations in *TP53* are the most frequent genomic alterations in HNSCC and have been correlated with poor patient survival.^{95,106–108} The Cancer Genome Atlas (TCGA) analysis of 279 HNSCC patients showed *TP53* mutations in 70.4% of tumors.⁹⁵ *TP53* mutations are thought to be an early event in HNSCC carcinogenesis, because they have been found in premalignant lesions, and their incidence is associated with cancer progression.¹⁰⁹ Although *TP53* is frequently mutated in HPV-negative tumors, it is typically wild type in HPV-positive tumors.^{95,110,111} It has been shown that the HPV16 E6 protein in HPV-positive tumors can bind and target the p53 protein for proteasome degradation, resulting in functional inactivation of p53 signaling.¹¹² These results indicate that p53 signaling is key in both HPV-negative and -positive HNSCC.

Although *TP53* is commonly mutated in HNSCC, it is rarely mutated in SGCs when compared to other neoplasms.¹¹³ SGCs are rare malignant tumors that account for ~6% of all head and neck cancers. The most recent classification of the World Health Organization showed that 22 types of malignant salivary gland tumors exist.¹¹⁴ Their histological and clinical diversity results in diagnostic and management challenges for clinicians.¹¹⁵ Primary therapy commonly involves radical surgery and/or radiation therapy, with conventional chemotherapy as a palliative aim for recurrent or metastatic disease. The limited clinical trial data on systemic therapeutic approaches for SGCs is a problem that has largely been a result of challenges in recruiting enough patients (due to the rarity of these tumors) or lack of patient stratification by tumor type. SGCs can originate in any major or minor salivary gland. SGC subtype and gland location are prognostic factors of patient survival. Although little is known about the pathogenesis of SGCs, several recurrent chromosomal translocations have been identified in some of the most common SGC subtypes. These common translocations are reviewed elsewhere.¹¹⁶

Mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) are the two most common malignant SGCs.¹¹⁴ They are characterized by recurrent chromosomal translocations that are thought to play an important part in tumorigenesis.¹¹⁶ Despite these findings, the role of these translocations in tumor progression is not fully understood. Limited genomic studies of SGCs have hindered our understanding of the pathobiology of these cancers and identification of molecular targets for targeted therapies.

MEC is the most common malignant salivary gland tumor, accounting for 30%–35% of all salivary tumors.^{117,118} MEC, a heterogeneous cancer that consists of mucin-producing, epidermoid, and intermediate cells, is commonly classified into low-, intermediate-, and high-grade disease. High-grade MEC has a prominent epithelial/solid component and low-grade MEC a prominent cystic/mucous component. High-grade MEC has the worst patient prognosis and frequent regional and distant metastasis.⁴⁸ More than 80% of MEC cases contain a t(11;19)(q21–22;p13) translocation that results in fusion between *MAML2* and *CRCT1* or *CRCT3* genes.¹¹⁹ With this fusion, the Notch-binding domain of *MAML2* is replaced by the cyclic adenosine monophosphate (cAMP) response element-binding (CREB) domain of *CRCT1/3*.¹²⁰ The molecular and pathological consequences of this fusion are still in the process of being elucidated, but it is thought the CREB dysregulation, mediated by this fusion, participates in tumorigenesis.¹²¹ It has been suggested that *CRCT1-MAML* fusion is an early event in MEC pathogenesis that can serve as a biomarker of MEC. A subgroup of fusion-negative high-grade tumors are suspected of being a different class of carcinomas and only fusion-positive tumors should be classified as MEC.¹²² Reports correlating mutational status with positive patient outcomes must be reevaluated, because of increasing evidence confirming fusion-negative tumors to be different classification of SGCs.^{123,124}

Varying reports of p53 mutational status in MEC samples^{125,126} have been limited by small sample sizes and unbiased detection of p53 mutations. Whole-exome sequencing of a cohort of 18 patients found p53 to be mutated in 30% of all MEC cases,¹¹⁹ and p53 mutations were only found in intermediate- and high-grade tumors. Comprehensive genomic profiling of 48 MEC patient samples further confirmed p53 to be a common genetic alteration in MEC that

was found in ~40% of the cases analyzed and prevalent in higher disease grade samples.¹²⁷ Similar results were observed in a previous study using immunohistochemistry, in which aberrant expression of p53 was detected in higher histological graded tumors.¹²⁶ The p53 mutational status and its implications in MEC pathobiology must be further evaluated to determine frequency of these genetic alterations and to understand their implications in disease progression.

VII. HEAD AND NECK CSCs

A. CSCs in HNSCC

Increasing evidence supports a role for CSCs in the pathogenesis and progression of HNSCC. CSCs in HNSCC were first described in CD44^{high} cells by Prince and colleagues.¹²⁸ CD44, a type I transmembrane glycoprotein involved in cell-cell interactions, adhesion, and migration, can act as a receptor for hyaluronic acid and other extracellular matrix proteins. In these experiments, cells expressing high levels of CD44 had increased tumorigenic potential over cells that expressed low levels.¹²⁸ Moreover, tumors found to be generated from CD44^{high} cells could reproduce original tumor heterogeneity, as observed by the presence of both CD44^{high} and CD44^{low} cells. Gene expression analysis showed that CD44^{high} cells differentially expressed the *Bmi-1* gene, a self-renewal protein. Both self-renewal and differentiation potential were shown in this study of CD44^{high} cells, supporting a CSC hypothesis in HNSCC.

Similar experiments using ALDH showed that ALDH-positive cells had higher tumorigenic potential than ALDH-negative cells.^{129,130} ALDH is an enzyme involved in oxidation of aldehydes, cellular detoxification, retinoic acid metabolism, and protection from reactive oxygen species, among other important cellular pathways.¹³¹ Clay and colleagues found that as few as 500 ALDH^{high} cells were sufficient to form tumors in mice, and these tumors replicated original cell heterogeneity.¹²⁹ Notably, ALDH-positive cells isolated from patient samples were radioresistant and initiated tumors in mice.¹³² Krishnamurthy and colleagues went on to show that combined ALDH^{high} and CD44^{high} expression further enhanced the ability to identify CSCs and tumors generated with ALDH^{high} and CD44^{high} cells resembled original patient histology.¹³³ Moreover, using serial transplantations *in vivo*, the authors showed that these cells had increased *Bmi-1* expression and self-renewal abilities. Further studies using these markers continue to prove the existence and role of these CSCs in the pathogenesis of HNSCC.

CD44 expression has been correlated with worse disease grade and prognosis for pharyngeal and laryngeal cancers.^{134,135} Moreover, CD44 concentration in peripheral blood has the potential to serve as a prognostic and diagnostic tool for HNSCC patients.¹³⁵ CD44-positive cells in HNSCC were found to have an EMT phenotype, overexpress PD-L1, and were not as immunogenic as CD44-negative cells.¹³⁶ ALDH1 isoenzyme expression has also been correlated with decreased overall patient survival.¹³⁷ Clinical studies have shown that ALDH1A1 expression correlates with poor tumor differentiation and poor patient prognosis.¹³⁸ A recent study showed that NCT-501, an ALDH1A1 inhibitor, is able to sensitize cisplatin-resistant HNSCC cells to cisplatin and decrease CSC markers, self-renewal, and tumorigenic potential.¹³⁹ ALDH1A1 is immunogenic in HNSCC and an effective target for

CD8⁺ T-cell-mediated immune response.^{140,141} A recent transcriptome analysis of TCGA data for five different cancers including 520 HNSCC tumors versus 40 normal samples reviewed differential expression of 19 ALDH isoforms and found that differential expression of each isoform was similar among all cancers.¹⁴² Although some ALDH isoforms are up-regulated, others are down-regulated in these cancers. Finally, it was shown that differential expression of most isoforms correlated with cancer prognosis.

B. Salivary Gland CSCs

Recent studies have also identified ALDH⁺/CD44^{high} as markers for CSCs in salivary MEC and ACC.^{143,144} Similar to what was shown with HNSCC, serial transplantation and *in vitro* sphere assays demonstrated self-renewal and differentiation potential of these cells in both MEC and ACC. A recent study found that ALDH and CD44 markers enriched for CSCs in ACC and MEC patient-derived xenografts, validating the use of such markers in salivary gland CSC research.¹⁴⁵ Three patient-derived xenografts of a high-grade MEC were generated from successive relapse surgeries (~9 mo) in the same patient. Further genomic characterization of these tumors showed increased mutational burden and stem cell marker expression, with decreased expression of tumor suppressors such as TP53 during disease progression. Additionally, increased sphere-forming abilities and a larger fraction of ALDH⁺/CD44^{high} cells in later relapses were shown.

VIII. CONCLUSION

Given the increasing evidence supporting a significant role for head and neck CSCs in tumor progression, we postulate that targeting pathways involved in CSC maintenance can potentially sensitize resistant cancers to conventional therapies and result in better eradication of tumor cells. In this review, we highlight some of the current evidence showing the importance of p53 signaling in CSC maintenance. We know that p53 has a prominent role in self-renewal and differentiation of normal and tumorigenic stem cells.³² Aberrant p53 signaling or inactivation in either HPV-positive or -negative tumors correlates with tumor progression in head and neck cancers. Moreover, p53 status in head and neck cancers has been associated with poor patient prognosis.¹⁰⁹

MDM2 inhibitors can activate endogenous wildtype p53¹⁴⁶ and reduce tumor volume, rate of recurrence, and CSC population in SGC.^{147,148} They can also sensitize HNSCC to chemotherapy.¹⁴⁹ MDM2 inhibitors are in Phase I and II clinical trials for the treatment of several malignancies.^{150,151} Other methods for activating p53 include reactivation of wild-type p53 function in mutant p53 and use of viral vectors to deliver wild-type p53 in p53-deficient cells.¹⁵² A better understanding of the mechanisms by which p53 induces differentiation of CSCs in head and neck tumors could inform a new therapeutic paradigm for cancer. Cancer patients may benefit from p53-induced differentiation of CSCs that can sensitize these cells to conventional chemotherapy.

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ABBREVIATIONS:

ACC	adenoid cystic carcinoma
ALDH	aldehyde dehydrogenase
CSC	cancer stem cell
HNSCC	head and neck squamous cell carcinoma
HPV	human papilloma virus
iPSC	induced pluripotent stem cell
MEC	mucoepidermoid carcinoma
SGC	salivary gland carcinoma

REFERENCES

1. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*. 1992 3 19;356(6366):215–21. [PubMed: 1552940]
2. Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*. 1990 11 30;250(4985):1233–8. [PubMed: 1978757]
3. Armstrong JF, Kaufman MH, Harrison DJ, Clarke AR. High-frequency developmental abnormalities in p53-deficient mice. *Curr Biol*. 1995 8 1;5(8):931–6. [PubMed: 7583151]
4. Komarova EA, Chernov MV, Franks R, Wang K, Armin G, Zelnick CR, Chin DM, Bacus SS, Stark GR, Gudkov AV. Transgenic mice with p53-responsive lacZ: p53 Activity varies dramatically during normal development and determines radiation and drug sensitivity in vivo. *EMBO J*. 1997 3 17;16(6):1391–400. [PubMed: 9135154]
5. Verga Falzacappa MV, Ronchini C, Reavie LB, Pelicci PG. Regulation of self-renewal in normal and cancer stem cells. *FEBS J*. 2012 10;279(19):3559–72. [PubMed: 22846222]
6. Stiewe T. The p53 family in differentiation and tumorigenesis. *Nat Rev Cancer*. 2007 3;7(3):165–8. [PubMed: 17332760]
7. Clevers H. The cancer stem cell: Premises, promises and challenges. *Nat Med*. 2011 3;17(3):313–9. [PubMed: 21386835]
8. Chang JC. Cancer stem cells: Role in tumor growth, recurrence, metastasis, and treatment resistance. *Medicine (Baltimore)*. 2016 9;95(1 Suppl 1):S20–5. [PubMed: 27611935]
9. Lane DP. Cancer: p53, Guardian of the genome. *Nature*. 1992 7 2;358(6381):15–6. [PubMed: 1614522]
10. Soussi T, Wiman KG. Shaping genetic alterations in human cancer: The p53 mutation paradigm. *Cancer Cell*. 2007 10;12(4):303–12. [PubMed: 17936556]
11. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: Origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*. 2010 1;2(1):a001008. [PubMed: 20182602]
12. Xu Y. Regulation of p53 responses by post-translational modifications. *Cell Death Differ*. 2003 4;10(4):400–3. [PubMed: 12719715]
13. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by MDM2. *Nature*. 1997 5 15;387(6630): 299–303. [PubMed: 9153396]
14. Wang YV, Wade M, Wahl GM. Guarding the guardian: MDMX plays important roles in setting p53 basal activity and determining biological responses in vivo. *Cell Cycle (Georgetown, TX)*. 2009 11 1;8(21):3443–4.

15. Montes de Oca Luna R, Wagner DS, Lozano G. Rescue of early embryonic lethality in MDM2-deficient mice by deletion of p53. *Nature*. 1995 11 9;378(6553):203–6. [PubMed: 7477326]
16. Marine JC, Francoz S, Maetens M, Wahl G, Toledo F, Lozano G. Keeping p53 in check: Essential and synergistic functions of MDM2 and MDM4. *Cell Death Differ*. 2006 6;13(6):927–34. [PubMed: 16543935]
17. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001 11 1;414 (6859):105–11. [PubMed: 11689955]
18. Medema JP. Cancer stem cells: The challenges ahead. *Nat Cell Biol*. 2013 4;15(4):338–44. [PubMed: 23548926]
19. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: Cancer stem cells versus clonal evolution. *Cell*. 2009 9 4;138(5):822–9. [PubMed: 19737509]
20. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994 2 17;367(6464):645–8. [PubMed: 7509044]
21. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997 7;3(7):730–7. [PubMed: 9212098]
22. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA*. 2003 4 1;100(7):3983–8. [PubMed: 12629218]
23. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature*. 2004 11 18;432(7015):396–401. [PubMed: 15549107]
24. Ye X, Weinberg RA. Epithelial-mesenchymal plasticity: A central regulator of cancer progression. *Trends Cell Biol*. 2015 11;25(11):675–86. [PubMed: 26437589]
25. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: Acquisition of malignant and stem cell traits. *Nat Rev Cancer*. 2009 4;9(4):265–73. [PubMed: 19262571]
26. Lee G, Hall RR, 3rd, Ahmed AU. Cancer stem cells: Cellular plasticity, niche, and its clinical relevance. *J Stem Cell Res Ther*. 2016 10;6(10):363. [PubMed: 27891292]
27. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle (Georgetown, TX)*. 2009 10 15;8(20):3274–84.
28. Lu L, Wu M, Sun L, Li W, Fu W, Zhang X, Liu T. Clinicopathological and prognostic significance of cancer stem cell markers CD44 and CD133 in patients with gastric cancer: A comprehensive meta-analysis with 4729 patients involved. *Medicine (Baltimore)*. 2016 10;95(42):e5163. [PubMed: 27759647]
29. Tao Y, Li H, Huang R, Mo D, Zeng T, Fang M, Li M. Clinicopathological and prognostic significance of cancer stem cell markers in ovarian cancer patients: Evidence from 52 studies. *Cell Physiol Biochem*. 2018;46(4):1716–26. [PubMed: 29730663]
30. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG, Di Fiore PP. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. *Cell*. 2010 1 8;140(1):62–73. [PubMed: 20074520]
31. Kim WT, Ryu CJ. Cancer stem cell surface markers on normal stem cells. *BMB Rep*. 2017 6;50(6):285–98. [PubMed: 28270302]
32. Jain AK, Barton MC. p53: Emerging roles in stem cells, development and beyond. *Development*. 2018 4 13;145(8):158360.
33. Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, Giulini B, Briskin C, Minucci S, Di Fiore PP, Pelicci PG. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell*. 2009 9 18;138(6):1083–95. [PubMed: 19766563]
34. Knoblich JA, Jan LY, Jan YN. Asymmetric segregation of Numb and Prospero during cell division. *Nature*. 1995 10 19;377(6550):624–7. [PubMed: 7566172]
35. Colaluca IN, Tosoni D, Nuciforo P, Senic-Matuglia F, Galimberti V, Viale G, Pece S, Di Fiore PP. NUMB controls p53 tumour suppressor activity. *Nature*. 2008 1 3;451(7174):76–80. [PubMed: 18172499]

36. Tao L, Roberts AL, Dunphy KA, Bigelow C, Yan H, Jerry DJ. Repression of mammary stem/progenitor cells by p53 is mediated by Notch and separable from apoptotic activity. *Stem Cells*. 2011 1;29(1):119–27. [PubMed: 21280161]
37. Zhao Z, Zuber J, Diaz-Flores E, Lintault L, Kogan SC, Shannon K, Lowe SW. p53 Loss promotes acute myeloid leukemia by enabling aberrant self-renewal. *Genes Dev*. 2010 7 1;24(13):1389–402. [PubMed: 20595231]
38. Zhou Z, Flesken-Nikitin A, Nikitin AY. Prostate cancer associated with p53 and Rb deficiency arises from the stem/progenitor cell-enriched proximal region of prostatic ducts. *Cancer Res*. 2007 6 15;67(12):5683–90. [PubMed: 17553900]
39. Chiche A, Moumen M, Romagnoli M, Petit V, Lasla H, Jezequel P, de la Grange P, Jonkers J, Deugnier MA, Glukhova MA, Faraldo MM. p53 Deficiency induces cancer stem cell pool expansion in a mouse model of triple-negative breast tumors. *Oncogene*. 2017 4 27;36(17):2355–65. [PubMed: 27775073]
40. Liu K, Lee J, Kim JY, Wang L, Tian Y, Chan ST, Cho C, Machida K, Chen D, Ou JJ. Mitophagy controls the activities of tumor suppressor p53 to regulate hepatic cancer stem cells. *Mol Cell*. 2017 10 19;68(2):281–92 e5. [PubMed: 29033320]
41. Solomon H, Dinowitz N, Pateras IS, Cooks T, Shetzer Y, Molchadsky A, Charni M, Rabani S, Koifman G, Tarcic O, Porat Z, Kogan-Sakin I, Goldfinger N, Oren M, Harris CC, Gorgoulis VG, Rotter V. Mutant p53 gain of function underlies high expression levels of colorectal cancer stem cells markers. *Oncogene*. 2018 3;37(12):1669–84. [PubMed: 29343849]
42. Telugu RB, Chowhan AK, Rukmangadha N, Patnayak R, Phaneendra BV, Prasad BC, Reddy MK. Histopathological and immunohistochemical evaluation of meningiomas with reference to proliferative markers p53 and Ki-67. *J Clin Diagnostic Res*. 2016 1;10(1):EC15–9.
43. Akshatha C, Mysorekar V, Arundhati S, Arul P, Raj A, Shetty S. Correlation of p53 overexpression with the clinicopathological prognostic factors in colorectal adenocarcinoma. *J Clin Diagnostic Res*. 2016 12;10(12): EC05–08.
44. Robles AI, Harris CC. Clinical outcomes and correlates of TP53 mutations and cancer. *Cold Spring Harb Perspect Biol*. 2010 3;2(3):a001016. [PubMed: 20300207]
45. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan PH, Tse GM, Badve S, Ellis IO. Breast cancer prognostic classification in the molecular era: The role of histological grade. *Breast Cancer Res*. 2010;12(4):207. [PubMed: 20804570]
46. Arshad H, Ahmad Z, Hasan SH. Gliomas: Correlation of histologic grade, Ki67 and p53 expression with patient survival. *Asian Pac J Cancer Prev*. 2010;11(6):1637–40. [PubMed: 21338209]
47. Sawazaki-Calone I, Rangel A, Bueno AG, Moraes CF, Nagai HM, Kunz RP, Souza RL, Rutkauskis L, Salo T, Almangush A, Coletta RD. The prognostic value of histopathological grading systems in oral squamous cell carcinomas. *Oral Dis*. 2015 9;21(6):755–61. [PubMed: 25825335]
48. Nance MA, Seethala RR, Wang Y, Chiosea SI, Myers EN, Johnson JT, Lai SY. Treatment and survival outcomes based on histologic grading in patients with head and neck mucoepidermoid carcinoma. *Cancer*. 2008 10 15;113(8):2082–9. [PubMed: 18720358]
49. Lin T, Chao C, Saito S, Mazur SJ, Murphy ME, Appella E, Xu Y. p53 Induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol*. 2005 2;7(2):165–71. [PubMed: 15619621]
50. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*. 2003 5 30;113(5):631–42. [PubMed: 12787504]
51. Moon JH, Kwon S, Jun EK, Kim A, Whang KY, Kim H, Oh S, Yoon BS, You S. Nanog-induced dedifferentiation of p53-deficient mouse astrocytes into brain cancer stem-like cells. *Biochem Biophys Res Commun*. 2011 8 19;412(1):175–81. [PubMed: 21810410]
52. Matas D, Milyavsky M, Shats I, Nissim L, Goldfinger N, Rotter V. p53 Is a regulator of macrophage differentiation. *Cell Death Differ*. 2004 4;11(4):458–67. [PubMed: 14713961]
53. Lin CP, Choi YJ, Hicks GG, He L. The emerging functions of the p53-miRNA network in stem cell biology. *Cell Cycle (Georgetown, TX)*. 2012 6 1;11(11):2063–72.

54. Kim NH, Kim HS, Kim NG, Lee I, Choi HS, Li XY, Kang SE, Cha SY, Ryu JK, Na JM, Park C, Kim K, Lee S, Gumbiner BM, Yook JI, Weiss SJ. p53 and microRNA-34 Are suppressors of canonical Wnt signaling. *Sci Signal*. 2011 11 1;4(197):ra71. [PubMed: 22045851]
55. Iwai A, Marusawa H, Matsuzawa S, Fukushima T, Hijikata M, Reed JC, Shimotohno K, Chiba T. Siah-1L, a Novel transcript variant belonging to the human Siah family of proteins, regulates P-catenin activity in a p53-dependent manner. *Oncogene*. 2004 9 30;23(45):7593–600. [PubMed: 15326481]
56. Atlasi Y, Noori R, Gaspar C, Franken P, Sacchetti A, Rafati H, Mahmoudi T, Decraene C, Calin GA, Merrill BJ, Fodde R. Wnt signaling regulates the lineage differentiation potential of mouse embryonic stem cells through Tcf3 down-regulation. *PLoS Gen*. 2013 5;9(5):e1003424.
57. Xu Z, Robitaille AM, Berndt JD, Davidson KC, Fischer KA, Mathieu J, Potter JC, Ruohola-Baker H, Moon RT. Wnt/ β -catenin signaling promotes self-renewal and inhibits the primed state transition in naive human embryonic stem cells. *Proc Natl Acad Sci USA*. 2016 10 18;113(42):E638290.
58. Lee KH, Li M, Michalowski AM, Zhang X, Liao H, Chen L, Xu Y, Wu X, Huang J. A genomewide study identifies the Wnt signaling pathway as a major target of p53 in murine embryonic stem cells. *Proc Natl Acad Sci USA*. 2010 1 5;107(1):69–74. [PubMed: 20018659]
59. Szemes M, Greenhough A, Melegh Z, Malik S, Yuksel A, Catchpoole D, Gallacher K, Kollareddy M, Park JH, Malik K. Wnt signalling drives context-dependent differentiation or proliferation in neuroblastoma. *Neoplasia (New York)*. 2018 4;20(4):335–50.
60. Davidson KC, Adams AM, Goodson JM, McDonald CE, Potter JC, Berndt JD, Biechele TL, Taylor RJ, Moon RT. Wnt/ β -catenin signaling promotes differentiation, not self-renewal, of human embryonic stem cells and is repressed by Oct4. *Proc Natl Acad Sci USA*. 2012 3 20;109(12):4485–90. [PubMed: 22392999]
61. Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: Therapeutic implications and challenges. *Acta Pharmacol Sinica*. 2013 6;34(6):732–40.
62. Zhao J. Cancer stem cells and chemoresistance: The smartest survives the raid. *Pharmacol Ther*. 2016 4;160: 145–58. [PubMed: 26899500]
63. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, Hilsenbeck SG, Pavlick A, Zhang X, Chamness GC, Wong H, Rosen J, Chang JC. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst*. 2008 5 7;100(9):672–9. [PubMed: 18445819]
64. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, Clarke MF. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 2009 4 9;458(7239):780–3. [PubMed: 19194462]
65. O'Hare T, Corbin AS, Druker BJ. Targeted CML therapy: Controlling drug resistance, seeking cure. *Curr Opin Genet Dev*. 2006 2;16(1):92–9. [PubMed: 16343892]
66. Oravec-Wilson KI, Philips ST, Yilmaz OH, Ames HM, Li L, Crawford BD, Gauvin AM, Lucas PC, Sitwala K, Downing JR, Morrison SJ, Ross TS. Persistence of leukemia-initiating cells in a conditional knockin model of an imatinib-responsive myeloproliferative disorder. *Cancer Cell*. 2009 8 4;16(2):137–48. [PubMed: 19647224]
67. Park SY, Lee HE, Li H, Shipitsin M, Gelman R, Polyak K. Heterogeneity for stem cell-related markers according to tumor subtype and histologic stage in breast cancer. *Clin Cancer Res*. 2010 2 1;16(3):876–87. [PubMed: 20103682]
68. Cheng B, Yang G, Jiang R, Cheng Y, Yang H, Pei L, Qiu X. Cancer stem cell markers predict a poor prognosis in renal cell carcinoma: A meta-analysis. *Oncotarget*. 2016 10 4;7(40):65862–75. [PubMed: 27588469]
69. Senel F, Kokenek Unal TD, Karaman H, Inanc M, Aytekin A. Prognostic value of cancer stem cell markers CD44 and ALDH1/2 in gastric cancer cases. *Asian Pac J Cancer Prev*. 2017 9 27;18(9): 2527–31. [PubMed: 28952294]
70. Yang B, Yan X, Liu L, Jiang C, Hou S. Overexpression of the cancer stem cell marker CD117 predicts poor prognosis in epithelial ovarian cancer patients: Evidence from meta-analysis. *Oncotargets Ther*. 2017;10:2951–61. [PubMed: 28652777]

71. Efferth T, Konkimalla VB, Wang YF, Sauerbrey A, Meinhardt S, Zintl F, Mattern J, Volm M. Prediction of broad spectrum resistance of tumors towards anticancer drugs. *Clin Cancer Res.* 2008 4 15;14(8):2405–12. [PubMed: 18413831]
72. Begicevic RR, Falasca M. ABC Transporters in cancer stem cells: Beyond chemoresistance. *Int J Mol Sci.* 2017 11 8;18(11):E2362. [PubMed: 29117122]
73. Prieto-Vila M, Takahashi RU, Usuba W, Kohama I, Ochiya T. Drug resistance driven by cancer stem cells and their niche. *Int J Mol Sci.* 2017 12 1;18(12):E2574. [PubMed: 29194401]
74. Primeau AJ, Rendon A, Hedley D, Lilge L, Tannock IF. The distribution of the anticancer drug doxorubicin in relation to blood vessels in solid tumors. *Clin Cancer Res.* 2005 12 15;11(24 Pt 1): 8782–8. [PubMed: 16361566]
75. Patel KJ, Tredan O, Tannock IF. Distribution of the anticancer drugs doxorubicin, mitoxantrone and topotecan in tumors and normal tissues. *Cancer Chemother Pharmacol.* 2013 7;72(1):127–38. [PubMed: 23680920]
76. Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, van Buren G, 2nd, Samuel S, Kim MP, Lim SJ, Ellis LM Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. *Cancer Res.* 2009 3 1;69(5):1951–7. [PubMed: 19244128]
77. Nor C, Zhang Z, Warner KA, Bernardi L, Visioli F, Helman JI, Roesler R, Nor JE. Cisplatin induces Bmi-1 and enhances the stem cell fraction in head and neck cancer. *Neoplasia (New York).* 2014 2;16(2):137–46.
78. Abubaker K, Latifi A, Luwor R, Nazaretian S, Zhu H, Quinn MA, Thompson EW, Findlay JK, Ahmed N. Shortterm single treatment of chemotherapy results in the enrichment of ovarian cancer stem cell-like cells leading to an increased tumor burden. *Mol Cancer.* 2013 3 27;12:24. [PubMed: 23537295]
79. Lagadec C, Vlashi E, Della Donna L, Dekmezian C, Pajonk F. Radiation-induced reprogramming of breast cancer cells. *Stem Cells.* 2012 5;30(5):833–44. [PubMed: 22489015]
80. Hu X, Ghisolfi L, Keates AC, Zhang J, Xiang S, Lee DK, Li CJ. Induction of cancer cell stemness by chemotherapy. *Cell Cycle.* 2012 7 15;11(14):2691–8. [PubMed: 22732500]
81. Auffinger B, Tobias AL, Han Y, Lee G, Guo D, Dey M, Lesniak MS, Ahmed AU. Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. *Cell Death Differ.* 2014 7;21(7):1119–31. [PubMed: 24608791]
82. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, Rimm DL, Wong H, Rodriguez A, Herschkowitz JI, Fan C, Zhang X, He X, Pavlick A, Gutierrez MC, Renshaw L, Larionov AA, Faratian D, Hilsenbeck SG, Perou CM, Lewis MT, Rosen JM, Chang JC. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc Natl Acad Sci USA.* 2009 8 18;106(33):13820–5. [PubMed: 19666588]
83. Wilson BJ, Schatton T, Zhan Q, Gasser M, Ma J, Saab KR, Schanche R, Waaga-Gasser AM, Gold JS, Huang Q, Murphy GF, Frank MH, Frank NY. ABCB5 Identifies a therapy-refractory tumor cell population in colorectal cancer patients. *Cancer Res.* 2011 8 1;71(15):5307–16. [PubMed: 21652540]
84. Tamura K, Aoyagi M, Wakimoto H, Ando N, Nariai T, Yamamoto M, Ohno K. Accumulation of CD133-positive glioma cells after high-dose irradiation by gamma knife surgery plus external beam radiation. *J Neurosurg.* 2010 8;113(2):310–8. [PubMed: 20205512]
85. Saydaminova K, Strauss R, Xie M, Bartek J, Richter M, van Rensburg R, Drescher C, Ehrhardt A, Ding S, Lieber A. Sensitizing ovarian cancer cells to chemotherapy by interfering with pathways that are involved in the formation of cancer stem cells. *Cancer Biol Ther.* 2016 10 2;17(10):1079–88. [PubMed: 27574825]
86. Tang QL, Zhao ZQ, Li JC, Liang Y, Yin JQ, Zou CY, Xie XB, Zeng YX, Shen JN, Kang T, Wang J. Salinomycin inhibits osteosarcoma by targeting its tumor stem cells. *Cancer Lett.* 2011 12 1;311(1):113–21. [PubMed: 21835542]
87. Dewangan J, Srivastava S, Rath SK. Salinomycin: A new paradigm in cancer therapy. *Tumour Biol.* 2017 3;39(3):[1010428317695035](https://doi.org/10.10428317695035).
88. Wang Y Effects of salinomycin on cancer stem cell in human lung adenocarcinoma A549 cells. *Med Chem.* 2011 3;7(2):106–11. [PubMed: 21222617]

89. Zhi QM, Chen XH, Ji J, Zhang JN, Li JF, Cai Q, Liu BY, Gu QL, Zhu ZG, Yu YY, Salinomycin can effectively kill ALDH(high) stem-like cells on gastric cancer. *Biomed Pharmacother.* 2011 Oct; 65(7):509–15. [PubMed: 21996439]
90. Boehmerle W, Endres M. Salinomycin induces calpain and cytochrome c-mediated neuronal cell death. *Cell Death Dis.* 2011 6 2;2:e168. [PubMed: 21633391]
91. Ojo OO, Bhadauria S, Rath SK. Dose-dependent adverse effects of salinomycin on male reproductive organs and fertility in mice. *PLoS One.* 2013;8(7):e69086. [PubMed: 23840907]
92. Chen D, Wu M, Li Y, Chang I, Yuan Q, Ekimyan-Salvo M, Deng P, Yu B, Yu Y, Dong J, Szymanski JM, Ramadoss S, Li J, Wang CY Targeting BMI1⁺ cancer stem cells overcomes chemoresistance and inhibits metastases in squamous cell carcinoma. *Cell Stem Cell.* 2017 5 4;20(5):621–34 e6. [PubMed: 28285905]
93. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandona R, Dandona L, Fleming T, Forouzanfar MH, Hancock J, Hay RJ, Hunter-Merrill R, Huynh C, Hosgood HD, Johnson CO, Jonas JB, Khubchandani J, Kumar GA, Kutz M, Lan Q, Larson HJ, Liang X, Lim SS, Lopez AD, MacIntyre MF, Marczak L, Marquez N, Mokdad AH, Pinho C, Pourmalek F, Salomon JA, Sanabria JR, Sandar L, Sartorius B, Schwartz SM, Shackelford KA, Shibuya K, Stanaway J, Steiner C, Sun J, Takahashi K, Vollset SE, Vos T, Wagner JA, Wang H, Westerman R, Zeeb H, Zoeckler L, Abd-Allah F, Ahmed MB, Alabed S, Alam NK, Aldhahri SF, Alem G, Alemayohu MA, Ali R, Al-Raddadi R, Amare A, Amoako Y, Artaman A, Asayesh H, Atnafu N, Awasthi A, Saleem HB, Barac A, Bedi N, Bensenor I, Berhane A, Bernabe E, Betsu B, Binagwaho A, Boneya D, Campos-Nonato I, Castaneda-Orjuela C, Catala-Lopez F, Chiang P, Chibueze C, Chittheer A, Choi JY, Cowie B, Damtew S, das Neves J, Dey S, Dharmaratne S, Dhillon P, Ding E, Driscoll T, Ekwueme D, Endries AY, Farvid M, Farzadfar F, Fernandes J, Fischer F, TT GH, Gebru A, Gopalani S, Hailu A, Horino M, Horita N, Hussein A, Huybrechts I, Inoue M, Islami F, Jakovljevic M, James S, Javanbakht M, Jee SH, Kasaeian A, Kedir MS, Khader YS, Khang YH, Kim D, Leigh J, Linn S, Lunevicius R, El Razek HMA, Malekzadeh R, Malta DC, Marcenes W, Markos D, Melaku YA, Meles KG, Mendoza W, Mengiste DT, Meretoja TJ, Miller TR, Mohammad KA, Mohammadi A, Mohammed S, Moradi-Lakeh M, Nagel G, Nand D, Le Nguyen Q, Nolte S, Ogbo FA, Oladimeji KE, Oren E, Pa M, Park EK, Pereira DM, Plass D, Qorbani M, Radfar A, Rafay A, Rahman M, Rana SM, Soreide K, Satpathy M, Sawhney M, Sepanlou SG, Shaikh MA, She J, Shiue I, Shore HR, Shrimme MG, So S, Soneji S, Stathopoulou V, Stroumpoulis K, Sufiyan MB, Sykes BL, Tabares-Seisdedos R, Tadese F, Tedla BA, Tessema GA, Thakur JS, Tran BX, Ukwaja KN, Uzochukwu BSC, Vlassov VV, Weiderpass E, Wubshet Terefe M, Yebo HG, Yimam HH, Yonemoto N, Younis MZ, Yu C, Zaidi Z, Zaki MES, Zenebe ZM, Murray CJL, Naghavi M. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. *JAMA Oncol.* 2017 4 1;3(4):524–48. [PubMed: 27918777]
94. Dayyani F, Etzel CJ, Liu M, Ho CH, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol.* 2010 6 29;2:15. [PubMed: 20587061]
95. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature.* 2015 1 29;517(7536):576–82. [PubMed: 25631445]
96. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst.* 2008 2 20;100(4):261–9. [PubMed: 18270337]
97. Ko HC, Harari PM, Sacotte RM, Chen S, Wieland AM, Yu M, Baschnagel AM, Bruce JY, Kimple RJ, Wittek ME. Prognostic implications of human papillomavirus status for patients with non-opharyngeal head and neck squamous cell carcinomas. *J Cancer Res Clin Oncol.* 2017 11;143(11):2341–50. [PubMed: 28752235]
98. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst.* 2000 5 3;92(9):709–20. [PubMed: 10793107]

99. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst.* 2008 3 19;100(6):407–20. [PubMed: 18334711]
100. Seiwert TY, Zuo Z, Keck MK, Khattri A, Pedomallu CS, Stricker T, Brown C, Pugh TJ, Stojanov P, Cho J, Lawrence MS, Getz G, Bragelmann J, DeBoer R, Weichselbaum RR, Langerman A, Portugal L, Blair E, Stenson K, Lingen MW, Cohen EE, Vokes EE, White KP, Hammerman PS. Integrative and comparative genomic analysis of HPV- positive and HPV-negative head and neck squamous cell carcinomas. *Clin Cancer Res.* 2015 2 1;21(3):632–41. [PubMed: 25056374]
101. Stelow EB, Jo VY, Stoler MH, Mills SE. Human papillomavirus-associated squamous cell carcinoma of the upper aerodigestive tract. *Am J Surg Pathol.* 2010 7;34(7):e15–24. [PubMed: 20534998]
102. Mourad M, Jetmore T, Jategaonkar AA, Moubayed S, Moshier E, Urken ML. Epidemiological trends of head and neck cancer in the United States: A SEER population study. *J Oral Maxillofac Surg.* 2017 12;75(12):2562–72. [PubMed: 28618252]
103. Ramqvist T, Dalianis T. Oropharyngeal cancer epidemic and human papillomavirus. *Emerg Infect Dis.* 2010 11;16(11):1671–7. [PubMed: 21029523]
104. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol.* 2011 11 10;29(32):4294–301. [PubMed: 21969503]
105. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, Rosenberg PS, Bray F, Gillison ML. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol.* 2013 12 20;31(36):4550–9.
106. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, Sidransky D, Koch WM. TP53 Mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2007 12 20;357(25):2552–61. [PubMed: 18094376]
107. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, Leiserson MDM, Miller CA, Welch JS, Walter MJ, Wendl MC, Ley TJ, Wilson RK, Raphael BJ, Ding L. Mutational landscape and significance across 12 major cancer types. *Nature.* 2013 10 17;502(7471):333–9. [PubMed: 24132290]
108. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest.* 2012 6;122(6):1951–7. [PubMed: 22833868]
109. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Eby YJ, Ruppert JM, Sidransky D. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res.* 1993 10 1;53(19):4477–80. [PubMed: 8402617]
110. Wiest T, Schwarz E, Enders C, Flechtenmacher C, Bosch FX. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. *Oncogene.* 2002 2 28;21(10):1510–7. [PubMed: 11896579]
111. Braakhuis BJ, Snijders PJ, Keune WJ, Meijer CJ, Ruijter- Schippers HJ, Leemans CR, Brakenhoff RH. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst.* 2004 7 7;96(13):998–1006. [PubMed: 15240783]
112. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell.* 1990 12 21;63(6):1129–36. [PubMed: 2175676]
113. Gomes CC, Diniz MG, Orsine LA, Duarte AP, Fonseca-Silva T, Conn BI, De Marco L, Pereira CM, Gomez RS. Assessment of TP53 mutations in benign and malignant salivary gland neoplasms. *PLoS One.* 2012;7(7):e41261. [PubMed: 22829934]
114. Seethala RR, Stenman G. Update from the 4th edition of the World Health Organization classification of head and neck tumours: Tumors of the salivary gland. *Head Neck Pathol.* 2017 3;11(1):55–67. [PubMed: 28247227]
115. Jegadeesh N, Liu Y, Prabhu RS, Magliocca KR, Marcus DM, Higgins KA, Vainshtein JM, Trad Wadsworth J, Beitler JJ. Outcomes and prognostic factors in modern era management of major salivary gland cancer. *Oral Oncol.* 2015 8;51(8):770–7. [PubMed: 26033470]

116. Stenman G Fusion oncogenes in salivary gland tumors: Molecular and clinical consequences. *Head Neck Pathol.* 2013 7;7 (Suppl 1):S12–9. [PubMed: 23821214]
117. Wahlberg P, Anderson H, Biorklund A, Moller T, Perfekt R. Carcinoma of the parotid and submandibular glands—a study of survival in 2465 patients. *Oral Oncol.* 2002 10;38(7):706–13. [PubMed: 12167424]
118. Jones AV, Craig GT, Speight PM, Franklin CD. The range and demographics of salivary gland tumours diagnosed in a UK population. *Oral Oncol.* 2008 4;44(4):407–17. [PubMed: 17825603]
119. Kang H, Tan M, Bishop JA, Jones S, Sausen M, Ha PK, Agrawal N. Whole-exome sequencing of salivary gland mucoepidermoid carcinoma. *Clin Cancer Res.* 2017 1 1;23(1):283–8. [PubMed: 27340278]
120. Nordkvist A, Gustafsson H, Juberg-Ode M, Stenman G. Recurrent rearrangements of 11q14–22 in mucoepidermoid carcinoma. *Cancer Genet Cytogenet.* 1994 6;74(2):77–83. [PubMed: 8019965]
121. Chen J, Li JL, Chen Z, Griffin JD, Wu L. Gene expression profiling analysis of CRTC1-MAML2 fusion oncogene-induced transcriptional program in human mucoepidermoid carcinoma cells. *BMC Cancer.* 2015 10 26;15:803. [PubMed: 26503699]
122. Jee KJ, Persson M, Heikinheimo K, Passador-Santos F, Aro K, Knuutila S, Odell EW, Makitie A, Sundelin K, Stenman G, Leivo I. Genomic profiles and CRTC1-MAML2 fusion distinguish different subtypes of mucoepidermoid carcinoma. *Mod Pathol.* 2013 2;26(2):213–22. [PubMed: 23018873]
123. Behboudi A, Enlund F, Winnes M, Andren Y, Nordkvist A, Leivo I, Flaberg E, Szekely L, Makitie A, Grenman R, Mark J, Stenman G. Molecular classification of mucoepidermoid carcinomas-prognostic significance of the MECT1-MAML2 fusion oncogene. *Genes Chromo Cancer.* 2006 5;45(5):470–81.
124. Seethala RR, Dacic S, Cieply K, Kelly LM, Nikiforova MN. A reappraisal of the MECT1/MAML2 translocation in salivary mucoepidermoid carcinomas. *Am J Surg Pathol.* 2010 8;34(8):1106–21. [PubMed: 20588178]
125. Matizonkas-Antonio LF, de Mesquita RA, de Souza SC, Nunes FD. TP53 mutations in salivary gland neoplasms. *Brazil Dent J.* 2005;16(2):162–6.
126. Abd-Elhamid ES, Elmalahy MH. Image cytometric analysis of p53 and MDM-2 expression in primary and recurrent mucoepidermoid carcinoma of parotid gland: Immunohistochemical study. *Diagn Pathol.* 2010 11 22;5:72. [PubMed: 21092204]
127. Wang K, McDermott JD, Schrock AB, Elvin JA, Gay L, Karam SD, Raben D, Somerset H, Ali SM, Ross JS, Bowles DW. Comprehensive genomic profiling of salivary mucoepidermoid carcinomas reveals frequent BAP1, PIK3CA, and other actionable genomic alterations. *Ann Oncol.* 2017 4 1;28(4):748–53. [PubMed: 28327999]
128. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA.* 2007 1 16;104(3):973–8. [PubMed: 17210912]
129. Clay MR, Tabor M, Owen JH, Carey TE, Bradford CR, Wolf GT, Wicha MS, Prince ME. Single-marker identification of head and neck squamous cell carcinoma cancer stem cells with aldehyde dehydrogenase. *Head Neck.* 2010 9;32(9):1195–201. [PubMed: 20073073]
130. Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, Chen DT, Tai LK, Yung MC, Chang SC, Ku HH, Chiou SH, Lo WL. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. *Biochem Biophys Res Commun.* 2009 7 31;385(3):307–13. [PubMed: 19450560]
131. Clark DW, Palte K. Aldehyde dehydrogenases in cancer stem cells: Potential as therapeutic targets. *Ann Transl Med.* 2016 12;4(24):518. [PubMed: 28149880]
132. Chen YC, Chang CJ, Hsu HS, Chen YW, Tai LK, Tseng LM, Chiou GY, Chang SC, Kao SY, Chiou SH, Lo WL. Inhibition of tumorigenicity and enhancement of radiochemosensitivity in head and neck squamous cell cancer-derived ALDH1-positive cells by knockdown of Bmi-1. *Oral Oncol.* 2010 3;46(3):158–65. [PubMed: 20036608]

133. Krishnamurthy S, Dong Z, Vodopyanov D, Imai A, Helman JI, Prince ME, Wicha MS, Nor JE. Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells. *Cancer Res.* 2010 12 1;70(23):9969–78. [PubMed: 21098716]
134. Chen J, Zhou J, Lu J, Xiong H, Shi X, Gong L. Significance of CD44 expression in head and neck cancer: A systemic review and meta-analysis. *BMC Cancer.* 2014 1 13;14:15. [PubMed: 24410905]
135. Kokko LL, Hurme S, Maula SM, Alanen K, Grenman R, Kinnunen I, Ventela S. Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous-cell carcinoma. *Oral Oncol.* 2011 6;47(6): 510–6. [PubMed: 21514878]
136. Lee Y, Shin JH, Longmire M, Wang H, Kohrt HE, Chang HY, Sunwoo JB. CD44+ Cells in head and neck squamous cell carcinoma suppress T-cell-mediated immunity by selective constitutive and inducible expression of PD-L1. *Clin Cancer Res.* 2016 7 15;22(14):3571–81. [PubMed: 26864211]
137. Dong Y, Ochsenreither S, Cai C, Kaufmann AM, Albers AE, Qian X. Aldehyde dehydrogenase 1 isoenzyme expression as a marker of cancer stem cells correlates to histopathological features in head and neck cancer: A meta-analysis. *PLoS One.* 2017;12(11):e0187615. [PubMed: 29112953]
138. Qian X, Wagner S, Ma C, Coordes A, Gekeler J, Klussmann JP, Hummel M, Kaufmann AM, Albers AE. Prognostic significance of ALDH1A1-positive cancer stem cells in patients with locally advanced, metastasized head and neck squamous cell carcinoma. *J Cancer Res Clin Oncol.* 2014 7;140(7):1151–8. [PubMed: 24770634]
139. Kulsum S, Sudheendra HV, Pandian R, Ravindra DR, Siddappa G, R N, Chevour P, Ramachandran B, Sagar M, Jayaprakash A, Mehta A, Kekatpure V, Hedne N, Kuriakose MA, Suresh A. Cancer stem cell mediated acquired chemoresistance in head and neck cancer can be abrogated by aldehyde dehydrogenase 1 A1 inhibition. *Mol Carcinog.* 2017 2;56(2):694–711. [PubMed: 27380877]
140. Visus C, Ito D, Amoscato A, Maciejewska-Franczak M, Abdelsalem A, Dhir R, Shin DM, Donnenberg VS, Whiteside TL, DeLeo AB. Identification of human aldehyde dehydrogenase 1 family member A1 as a novel CD8⁺ T-cell-defined tumor antigen in squamous cell carcinoma of the head and neck. *Cancer Res.* 2007 11 1;67(21):10538–45. [PubMed: 17974998]
141. Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, Szczepanski MJ, Brand RE, Ferrone CR, Whiteside TL, Ferrone S, DeLeo AB, Wang X. Targeting ALDH^{bright} human carcinoma-initiating cells with ALDH1A1-specific CD8⁺ T cells. *Clin Cancer Res.* 2011 10 1;17(19):6174–84. [PubMed: 21856769]
142. Chang PM, Chen CH, Yeh CC, Lu HJ, Liu TT, Chen MH, Liu CY, Wu ATH, Yang MH, Tai SK, Mochly-Rosen D, Huang CF. Transcriptome analysis and prognosis of ALDH isoforms in human cancer. *Sci Rep.* 2018 2 9;8(1): 2713. [PubMed: 29426835]
143. Sun S, Wang Z. ALDH High adenoid cystic carcinoma cells display cancer stem cell properties and are responsible for mediating metastasis. *Biochem Biophys Res Commun.* 2010 6 11;396(4): 843–8. [PubMed: 20450887]
144. Adams A, Warner K, Pearson AT, Zhang Z, Kim HS, Mochizuki D, Basura G, Helman J, Mantesso A, Castilho RM, Wicha MS, Nor JE. ALDH/CD44 Identifies uniquely tumorigenic cancer stem cells in salivary gland mucoepidermoid carcinomas. *Oncotarget.* 2015 9 29;6(29): 26633–50. [PubMed: 26449187]
145. Keysar SB, Eagles JR, Miller B, Jackson BC, Chowdhury FN, Reisinger J, Chimed TS, Le PN, Morton JJ, Somerset HL, Varella-Garcia M, Tan AC, Song JI, Bowles DW, Reyland ME, Jimeno A. salivary gland cancer patient-derived xenografts enable characterization of cancer stem cells and new gene events associated with tumor progression. *Clin Cancer Res.* 2018 6 15;24(12): 2935–43. [PubMed: 29555661]
146. Wang S, Sun W, Zhao Y, McEachern D, Meaux I, Barriere Stuckey JA, Meagher JL, Bai L, Liu L, Hoffman-Luca CG, Lu J, Shangary S, Yu S, Bernard D, Aguilar A, Dos-Santos O, Besret L, Guerif S, Pannier P, Gorge-Bernat D, Debussche L. SAR405838: An optimized inhibitor of MDM2-p53 interaction that induces complete and durable tumor regression. *Cancer Res.* 2014 10 15;74(20):5855–65. [PubMed: 25145672]
147. Warner KA, Nor F, Acasigua GA, Martins MD, Zhang Z, McLean SA, Spector ME, Chepeha DB, Helman J, Wick MJ, Moskaluk CA, Castilho RM, Pearson AT, Wang S, Nor JE. Targeting

- MDM2 for treatment of adenoid cystic carcinoma. *Clin Cancer Res.* 2016 7 15;22(14):3550–9. [PubMed: 26936915]
148. Nor F, Warner KA, Zhang Z, Acasigua GA, Pearson AT, Kerk SA, Helman JI, Sant'Ana Filho M, Wang S, Nor JE. Therapeutic inhibition of the MDM2-p53 interaction prevents recurrence of adenoid cystic carcinomas. *Clin Cancer Res.* 2017 2 15;23(4):1036–48. [PubMed: 27550999]
149. Roh JL, Kang SK, Minn I, Califano JA, Sidransky D, Koch WM. p53-Reactivating small molecules induce apoptosis and enhance chemotherapeutic cytotoxicity in head and neck squamous cell carcinoma. *Oral Oncol.* 2011 1;47(1):8–15. [PubMed: 21109480]
150. Zhao Y, Aguilar A, Bernard D, Wang S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 inhibitors) in clinical trials for cancer treatment. *J Med Chem.* 2015 2 12;58(3):1038–52. [PubMed: 25396320]
151. de Jonge M, de Weger VA, Dickson MA, Langenberg M, Le Cesne A, Wagner AJ, Hsu K, Zheng W, Mace S, Tuffal G, Thomas K, Schellens JH. A phase I study of SAR405838, a novel human double minute 2 (HDM2) antagonist, in patients with solid tumours. *Eur J Cancer.* 2017 5;76:144–51. [PubMed: 28324749]
152. Castellanos MR, Pan Q. Novel p53 therapies for head and neck cancer. *World J Otorhinolaryngol Head Neck Surg.* 2016 6;2(2):68–75. [PubMed: 29204550]

FIG. 1:

Stem cells can undergo both symmetric and asymmetric division events, resulting in expansion, exhaustion, or maintenance of the stem cell pool. When a stem cell undergoes asymmetric division, it can produce an identical daughter cell (self-renewal) and committed progenitor (differentiation). If a stem cell divides symmetrically, two daughter stem cells or two daughter progenitor cells can result. These modes of division are regulated by the stem cell pathways of self-renewal and differentiation.

FIG. 2:

Tumor suppressor p53 is a key regulator in stem cell fate, balancing self-renewal and differentiation. When p53 is activated in a stem cell, it shifts the balance to asymmetric division. CSCs frequently down-regulate p53 activity, leading to changes in cell polarity that increases symmetric stem cell division and expand the stem cell pool. Activating p53 in CSCs will differentiate these cells, lead to a decreased stem cell pool, and sensitize these otherwise resistant cells to conventional chemotherapies.