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The *NOTCH* Pathway in Head and Neck Squamous Cell Carcinoma

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Abstract

Comprehensive genomic analyses have been performed for head and neck squamous cell carcinoma (HNSCC), revealing a significant rate of *NOTCH1* mutations and identifying *NOTCH1* as the second most frequently mutated gene after *TP53*. Most *NOTCH1* mutations are considered inactivating, indicating that *NOTCH1* is a tumor suppressor gene. On the other hand, cohorts from Asian populations with HNSCC have shown activating *NOTCH1* mutations. HNSCC with *NOTCH1* mutations have a worse prognosis than the *NOTCH1* wild-type tumors. Additional data on other *NOTCH* family members have shown that *NOTCH* promotes HNSCC progression. *NOTCH* family members, including *NOTCH* pathway genes, are upregulated in HNSCC compared with normal tissues, and inhibition of the *NOTCH* pathway decreases cell proliferation and invasion. *NOTCH* activity in HNSCC is therefore contextual, and *NOTCH* in HNSCC is considered to have a bimodal role as a tumor suppressor and an oncogene. In this review, recent understandings of *NOTCH* pathway genes, including *NOTCH* genes, in HNSCC are described. In addition, the implications of *NOTCH* pathway alteration for HNSCC-specific *NOTCH*-targeted cancer therapy are explored.

Keywords: TCGA, JAG, DLL, HES, HEY, anti-NOTCH therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide (Argiris et al. 2008), and its prognosis remains poor. HNSCC is considered to arise with the accumulation of genetic and epigenetic alterations. Previously, several mutations that lead to HNSCC development were reported, including *TP53, RB1, CDH1, CDKN2A, PTEN, EGFR*, and *PI3CA* mutations (Okami et al. 1998; Papadimitrakopoulou et al. 2001; Murugan et al. 2008; Poeta et al. 2009; Demokan et al. 2012). To elucidate the gene mutation profile of HNSCC, several comprehensive studies have been performed showing that the *NOTCH1* mutation rate is higher than previously considered and provides a new focus on its role in HNSCC (Table 1; Agrawal et al. 2011; Stransky et al. 2011).

In mammals, the *NOTCH* pathway has 4 receptors (*NOTCH1-4*) and 5 ligands (*JAG1* and *2* and *DLL1, 3*, and *4*). After a ligand binding to a *NOTCH* receptor, the γ-secretase complex releases the *NOTCH* intracellular domain (NICD), which moves to the nucleus, resulting in the transcriptional activation of *NOTCH* target genes, such as *HES* and *HEY* (Gordon et al. 2008). Each *NOTCH* receptor has different structures. Different from *NOTCH1* and *2, NOTCH3* and *4* have a shortened extracellular domain and lack the intracellular transcriptional activating domain. Only *NOTCH4* lacks the *NOTCH* cytokine response region (Fig. 1).

However, the complete diversity of *NOTCH* receptor functions and relationships with the downstream target genes in HNSCC is not well understood. Several clinical trials have examined the effect of *NOTCH* inhibitors on solid tumors. However, few studies have defined effects on each *NOTCH* receptor and its pathway genes. In this review, we introduce recent HNSCC studies addressing *NOTCH* pathway genes. Finally, we discuss the current understanding regarding anti-*NOTCH* therapy.

NOTCH1

In several cancers, including prostate (Zayzafoon et al. 2004), pancreas (Miyamoto et al. 2003), breast (Reedijk et al. 2005), and lung (Westhoff et al. 2009), *NOTCH1* is reported to have oncogenic functions and promote cancer growth. In HNSCC, several studies have shown that HNSCC has significantly higher *NOTCH1* expression than normal tissue (Table 2; Leethanakul et al. 2000; Hijioka et al. 2010; Zhang et al. 2011; Yoshida et al. 2013; Wirth et al. 2016). *NOTCH1* expression is correlated with both T stage and the clinical stage in oral squamous cell carcinoma (OSCC; Yoshida et al. 2013). Its expression is also significantly related to neck lymph node metastasis and the depth of invasion in tongue cancer patients (Joo et al. 2009; Zhang et al. 2011). Gu et al. (2010) found that *NOTCH1*

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Table 1. Comprehensive Analysis of *NOTCH* Mutation.

Authors	Year	*NOTCH1* Mutation Rate	*NOTCH2* Mutation Rate	*NOTCH3* Mutation Rate	*NOTCH4* Mutation Rate	No. of Tumors Analyzed
Agrawal et al.	2011	15%				120
Stransky et al.	2011	14%	5%	4%		74
Pickering et al.	2013	9%	5%			38
Lawrence et al.	2014	16.9%				384
Sun et al.	2014	13.5%				37
Gaykalova et al.	2014	8.1%				37
Song et al.	2014	43.1%				51
Izumchenko et al.	2015	54.0%				50
The Cancer Genome Atlas group	2015	19%				279
Vettore et al.	2015	5%	1.6%	5%		78
Ock et al.	2016	32.3%	39.4%	25.3%		71
Fukusumi et al.	2017	11%	2.5%	2.1%	1.3%	520

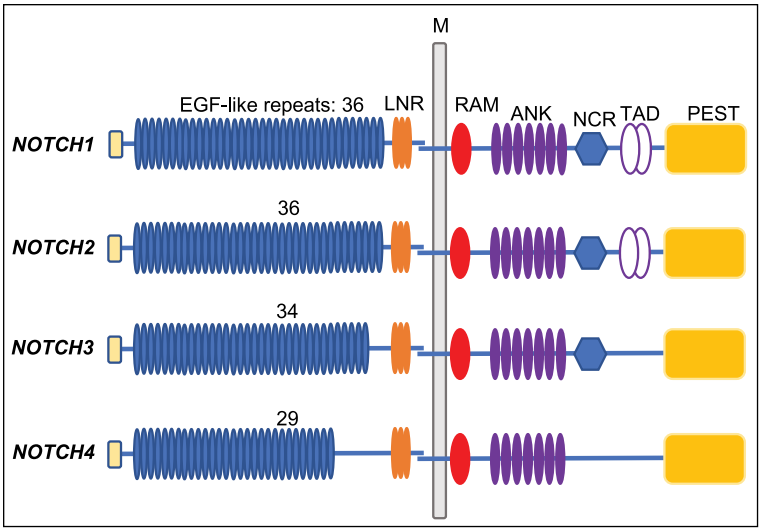

Figure 1. *NOTCH* receptor structures. Schema of *NOTCH* receptors. LNR, Lin-12 NOTCH repeats; RAM, RBP-Jk–associated molecule; ANK, ankyrin repeats; NCR, NOTCH cytokine response region; TAD, transcriptional activating domain; PEST, proline, glutamic acid, serine, and threonine degradation domain.

expression was significantly related to cisplatin resistance and that a gamma secretase inhibitor, which is a *NOTCH* inhibitor, showed a synergistic anticancer effect with cisplatin. Furthermore, *NOTCH1* is related to maintenance of a cancer stem cell (CSC) phenotype. Zhao et al. (2016) showed that *NOTCH1* inhibition reduces the HNSCC CSC fraction. We also examined the correlation between *NOTCH1* and its downstream genes using an updated the Cancer Genome Atlas (TCGA) data set excluding *NOTCH* mutant samples (HNSCC: 447, normal: 46 samples). Although the correlation coefficients are lower than 0.2, *NOTCH1* shows a weakly positive correlation with the *NOTCH* downstream gene activation in *HES1* and *HEY1*. *NOTCH1* expression also shows a significantly positive correlation with *BCL-2* expression (Table 3). In these contexts, *NOTCH1* is considered to be upregulated in HNSCC and is closely related to its progression.

In 2011, Agrawal et al. and Stransky et al. examined the whole exons of 120 and 74 HNSCC tumors, respectively (Table 1). Along with well-known mutations, both research groups reported novel mutations in *NOTCH1*. Mutations of *NOTCH1* were found in 10% to 15% of HNSCC tumors, making *NOTCH1* is the second most frequently mutated gene after *TP53*. They also revealed that *NOTCH1* mutations were inactivating and concluded that *NOTCH1* acted as a tumor suppressor in HNSCC (Agrawal et al. 2011; Stransky et al. 2011). In the other squamous cell carcinoma (SCC) studies for *NOTCH* mutations, 81% of cutaneous SCC samples were reported to have at least 1 *NOTCH1* or *NOTCH2* mutation. In addition, 12.5% of lung SCC samples have *NOTCH1* or *NOTCH2* mutation that is inactivating (Wang et al. 2011). Gao et al. (2014) showed mutation rates of *NOTCH1* (13%), *NOTCH2* (4%), and *NOTCH3* (6%) using exome sequencing of 113 pairs of tumor and normal DNA samples collected from esophageal SCC. Genomic comprehensive analysis for esophageal SCC in 144 patients revealed that 19% and 8% of tumors have *NOTCH1* and *NOTCH3* mutations, respectively (Sawada et al. 2016). These *NOTCH* mutation rates in lung and esophageal SCC are similar to those in HNSCC (Table 1).

The TCGA project was constructed to identify the genes and signal pathways that can be used as potential targets in cancer treatment (de Castro and Negrao 2014). In HNSCC, comprehensive analysis of somatic genome alterations was performed using this data set showing several gene mutation rates such as those for *TP53* (72%), *CDKN2A* (22%), and *PI3KCA* (21%). Furthermore, *NOTCH1* mutations were identified in approximately 20% (Cancer Genome Atlas 2015). After this study, the sample number was increased to 500. Fukusumi et al. (2017) showed that the *NOTCH1* mutation rate was 11% using this recent TCGA data set. This mutation rate is the highest among *NOTCH* receptors (Table 1; Fukusumi et al. 2017). Pickering et al. (2013) and Gaykalova et al. (2014) used their

Table 2. HNSCC Studies of Each *NOTCH* Receptor.

NOTCH Subtype	Authors	Year	Material	Functional Consequence
NOTCH1	Leethanakul et al.	2000	HNSCC (*n* = 5)	Elevated expression in tumors
	Joo et al.	2009	OSCC (*n* = 51)	Protein expression was correlated with lymph node metastasis, tumor invasion
	Gu et al.	2010	HNSCC (*n* = 25)	Cisplatin resistance
	Hijioka et al.	2010	OSCC (*n* = 4) cell line	Elevated expression in tumors
	Zhang et al.	2011	OSCC (*n* = 74) cell line	Elevated expression in tumors, correlated with lymph node metastasis
	Yoshida et al.	2013	OSCC (*n* = 12) cell line	Elevated expression in tumors, T stage, clinical stage Cell proliferation, invasion
	Sun et al.	2014	HNSCC (*n* = 44)	Elevated expression in tumors
	Retting et al.	2015	HNSCC (*n* = 79)	Nonperipheral NICD1 staining is associated with poor differentiation and extracapsular spread
	Wirth et al.	2016	HNSCC (n = 100)	Elevated expression in tumors
	Zhao et al.	2016	Cell line	CSC, tumorigenicity, elevated expression in tumors
NOTCH2	Leethanakul et al.	2000	HNSCC (*n* = 5)	Elevated expression in tumors
	Zou et al.	2016	Cell line	Cell growth, antiapoptosis
NOTCH3	Zhang et al.	2011	OSCC (*n* = 74) cell line	Elevated expression in tumors
	Man et al.	2012	Cell line	Cell proliferation, chemoresistance, sphere formation ability, tumorigenicity
	Zhang et al.	2013b	OSCC (*n* = 74) cell line	Elevated expression in tumors, clinical stage
	Sun et al.	2014	HNSCC (*n* = 44)	Elevated expression in tumors
	Kayamori et al.	2016	OSCC (*n* = 93) cell line	Cancer-associated fibrobrasts with *NOTCH3* expression promote angiogenesis and have worse OS
NOTCH4	Ha et al.	2003	HNSCC (*n* = 7)	Elevated expression in tumors
	Snijders et al.	2005	OSCC (*n* = 89)	Elevated expression in tumors
	Lunde et al.	2014	OSCC (*n* = 24)	elevated expression in tumors
	Mk et al.	2016	OSCC (*n* = 60) cell line	T stage, clinical stage, perineural invasion cell proliferation, migration
	Fukusumi et al.	2017	TCGA (*n* = 520) cell line	Cell proliferation, chemoresistance, sphere formation ability, cell cycle, antiapoptosis, EMT, CSC

CSC, cancer stem cells; EMT, epithelial-mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; OS, overall survival; OSCC, oral squamous cell carcinoma; TCGA, the Cancer Genome Atlas.

HNSCC samples and showed that 9% and 8.1% of patients, respectively, have a *NOTCH1* mutation. Lawrence et al. (2014) collected and analyzed data from 4,742 samples across 21 tumor types, and a *NOTCH1* mutation was detected in 16.9% of HNSCC. It should be noted that these studies are mostly from Caucasian patients. Interestingly, Asian studies have shown different results. Song et al. (2014) assessed the *NOTCH1* mutation rate (43%) in 51 OSCC tumors obtained from Chinese patients, and 60% of mutations were activating ones. The *NOTCH1* mutation group showed significantly worse overall survival (OS) and disease-free survival (DFS) than the *NOTCH1* wild-type group (Song et al. 2014; Mao 2015). Izumchenko et al. (2015) also examined the Chinese OSCC cohort showing *NOTCH1* mutations in 54% of patients, with 40% of these *NOTCH1* mutations showing gain of function. Ock et al. (2016) performed deep sequencing for 71 HNSCC samples in Korean patients. This study showed a relatively high rate of not only *NOTCH1* but also *NOTCH2, 3* mutations (*NOTCH1*: 32.3%, *NOTCH2*: 39.4%, and *NOTCH3*: 25.3%). The *NOTCH1* mutation domain and type were similar to Chinese ones, indicating this mutation was activating (Ock et al. 2016). Vettore et al. (2015) also examined HNSCC in a Singapore cohort and revealed that *NOTCH* pathway genes' mutation in OSCC is associated with a significantly worse DFS. However, this study showed a lower *NOTCH* mutation rate (*NOTCH1*: 5%, *NOTCH2*: 1.6%, and *NOTCH3*: 5%) compared with other Asian studies (Vettore et al. 2015).

To reconcile the apparent discrepancy between *NOTCH* inactivating mutations and *NOTCH* pathway upregulation and activation in HNSCC, investigators have performed comprehensive analyses integrating mutation and network activation and expression data. Sun et al. (2014) found that 10.8% of *NOTCH1* mutations were identified in HNSCC tumors and performed a comprehensive analysis of *NOTCH* signaling in their cohort. They also compared the activation of *NOTCH* by the downstream genes *HES1*/*HEY1* between HNSCC tumors with and without *NOTCH1* mutations and found significantly lower *HES1*/*HEY1* expression in HNSCC tumors with *NOTCH1* mutation than in those with *NOTCH1* wild type. Furthermore, these *NOTCH1* mutant tumors have similar *HES1/HEY1* expression to normal tissues, consistent with the loss-of-function of *NOTCH1* mutations described above. On the other hand, they found that 30.3% of *NOTCH1* wild-type tumors exhibited *HES1*/*HEY1* overexpression, indicating *NOTCH* pathway activation. In the TCGA cohort, they observed that decreased expression of *HES1* showed borderline significance in the *NOTCH1* mutant versus wild-type HNSCC tumors, whereas increased expression of *HEY1* showed a statistically significant difference (Sun et al. 2014). Rettig et al. (2015) stained NICD in HNSCC tumors. NICD

Table 3. Correlation between *NOTCH* Receptors and Their Downstream Genes.[a]

	HEY1	*HES1*	*CCND1*	*MYC*	*BCL-2*	*p21*
NOTCH1	$P < 0.0001$	$P < 0.0001$	$P = 0.38$	$P = 0.042$	**$P < 0.0001$**	$P = 0.12$
	$r = 0.19$	$r = 0.19$	$r = -0.042$	$r = -0.096$	**$r = 0.35$**	$r = -0.073$
NOTCH2	$P = 0.010$	$P = 0.052$	$P = 0.65$	$P = 0.82$	$P = 0.0012$	$P = 0.32$
	$r = -0.12$	$r = -0.092$	$r = -0.021$	$r = -0.011$	$r = 0.15$	$r = 0.047$
NOTCH3	**$P < 0.0001$**	$P = 0.032$	$P = 0.94$	$P = 0.42$	$P = 0.011$	$P = 0.80$
	$r = 0.20$	$r = 0.10$	$r = 0.0036$	$r = 0.038$	$r = 0.12$	$r = -0.012$
NOTCH4	**$P < 0.0001$**	$P = 0.37$	$P = 0.057$	$P < 0.0001$	**$P < 0.0001$**	$P < 0.0001$
	$r = 0.39$	$r = -0.042$	$r = 0.090$	$r = -0.26$	**$r = 0.44$**	$r = -0.20$

[a]The correlations are examined using the Cancer Genome Atlas head and neck squamous cell carcinoma data set excluding *NOTCH* mutant samples ($n = 447$). r indicates Pearson's correlation coefficient. The groups with a significantly positive correlation ($r > 0.2$) are written in bold characters.

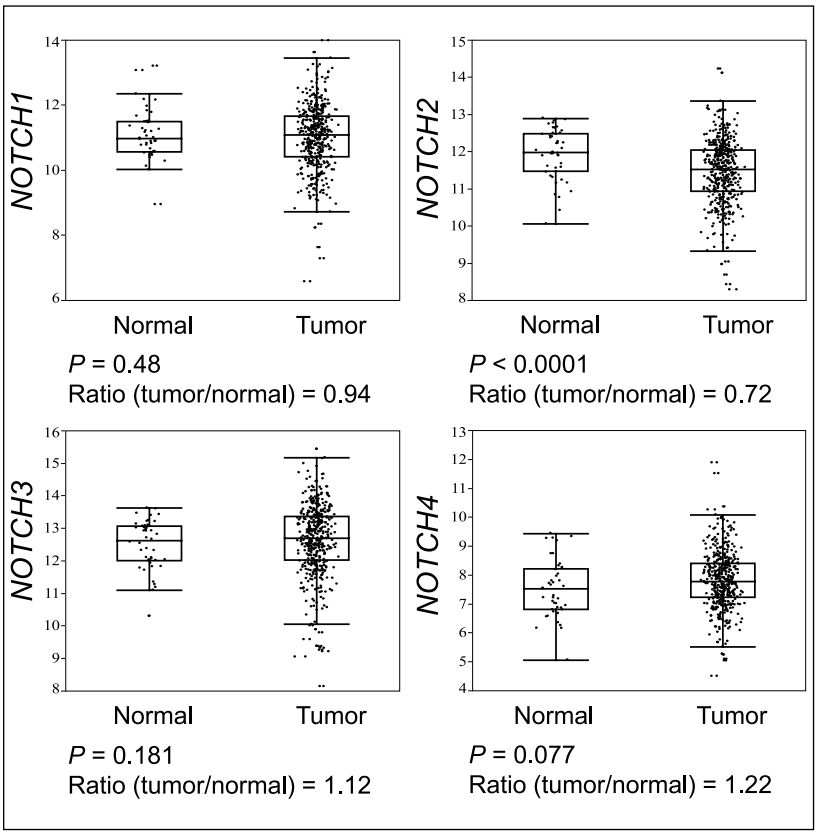

Figure 2. The Cancer Genome Atlas (TCGA) data set analysis of *NOTCH* receptors between head and neck squamous cell carcinoma (HNSCC) and normal samples. The mRNA expression of *NOTCH1-4* is compared between HNSCC ($n = 447$) and normal samples ($n = 46$) using the TCGA data set. Seventy-three HNSCC samples with *NOTCH* mutation are excluded. The ratio is calculated by dividing the mRNA expression of the tumor samples by that of the normal samples. Whiskers indicate the minimum and maximum values. The *P* value is calculated using Student's *t* test.

expression was significantly associated with the *NOTCH1* mutation status. *NOTCH1*-mutated tumors most commonly exhibited negative staining. There were no significant differences in recurrence, invasion, or clinical stages between *NOTCH1* wild-type and mutant patients (Rettig et al. 2015). These results indicate that inactivating *NOTCH* mutations do not necessarily correlate with a poorer clinical prognosis.

In summary, *NOTCH1* likely plays a bimodal role in HNSCC, with inactivating mutations indicating a tumor suppressor role and activating mutations and upregulation consistent with an oncogenic role.

NOTCH2

NOTCH2 is known to play an important role in hepatocellular and esophageal carcinoma. *NOTCH2* affects proliferation, cell cycle, chemoresistance, sphere formation ability, and tumorigenicity in hepatocellular carcinoma cells (Wu et al. 2016). *NOTCH2* is also an independent prognostic factor for OS and progression-free survival in esophageal SCC (Wang et al. 2016).

In HNSCC, higher *NOTCH2* expression was detected compared with normal tissues (Leethanakul et al. 2000; Zou et al. 2016). The *NOTCH2* expression was increased in HNSCC with lymph node metastasis compared with that without metastasis. *NOTCH2* also affects cell growth and apoptosis, and knockdown of *NOTCH2* inhibited the migration and invasion abilities and decreased the expression levels of its downstream genes such as *c-MYC* and *BCL-2* (Zou et al. 2016). In contrast, TCGA analysis showed a significantly decreased *NOTCH2* mRNA expression in HNSCC samples compared with that in normal samples (Fig. 2), and no significantly positive correlation was found between the expression of *NOTCH2* and *NOTCH* downstream genes (Table 3).

NOTCH3

NOTCH3 alteration is also reported to correlate with several cancers. A large meta-analysis was performed on 3,663 non–small-cell lung carcinomas showing that *NOTCH3* expression, as well as *NOTCH1* expression, was significantly correlated

with a worse OS (Yuan et al. 2015). In glioma, *NOTCH3* expression was also associated with a significantly worse prognosis (Alqudah et al. 2013). *NOTCH3* also plays a critical role in the development of prostate cancer as well as the prostate gland (Villaronga et al. 2008).

In HNSCC, Stransky et al. (2011) showed that the *NOTCH3* mutation rate was 4%, and these mutations were inactive (Table 1). Sun et al. (2014) examined *NOTCH*-related gene expression arrays using their cohort. The mRNA expression of *NOTCH3* was significantly higher in primary HNSCC tumors than in normal mucosa. Similarly, significantly increased expression of *NOTCH*3 was found in HNSCC tumors using the TCGA HNSCC data set (Sun et al. 2014). We examined the updated TCGA data set and show a significantly positive correlation between *NOTCH3* and *HEY1* expression (Table 3). However, moderate but not significantly increased *NOTCH3* expression in HNSCC was noted using this TCGA data set (Fig. 2). Using HNSCC cells, *NOTCH3* inhibition decreases cell proliferation, chemoresistance, sphere formation ability, xenograft tumor volume, and the expression of *NOTCH* downstream genes such as *HEY1, BCL-2, c-MYC*, and *CCND1* (Man et al. 2012). Tongue cancers had significantly higher *NOTCH3* expression than normal tongue tissue (Zhang et al. 2011; Zhang et al. 2013b), and *NOTCH3* expression showed a significant correlation with its clinical stage (Zhang et al. 2013b). Kayamori et al. (2016) noted that *NOTCH3* did not affect OSCC cell proliferation. However, they focused on cancer-associated fibroblasts (CAFs) in OSCC and indicated that *NOTCH3* in CAFs promoted angiogenesis, and immunohistochemical study of 93 OSCC cases indicated that *NOTCH3* expression in CAFs was significantly correlated with tumor size. Furthermore, OSCC with *NOTCH3* (+) CAFs showed a significantly worse OS than that with *NOTCH3* (–) CAFs (Kayamori et al. 2016). These data are consistent with a possible bimodal oncogenic and tumor suppressor role for *NOTCH3*, similar to that of *NOTCH1*.

NOTCH4

Ha et al. (2003) used their 26-patient cohort and showed that only *NOTCH4* expression in HNSCC was significantly increased compared with that in normal mucosa among *NOTCH* receptors. Using comparative genomic hybridization (CGH) analysis, *DLL1* and *NOTCH4* were upregulated in OSCC compared with that in normal tissue, whereas *NOTCH1, 2*, and *3* and *HES1* were expressed at lower levels (Snijders et al. 2005). The chromosomal region of *NOTCH4* was shown to amplify in OSCC using CGH analysis (Table 2; Lunde et al. 2014). Similar to these results, our TCGA analysis also showed that *NOTCH4* expression shows the highest ratio among *NOTCH* receptors in HNSCC compared with that in normal tissues, although the difference was not statistically significant (Fig. 2). The mutation rate of *NOTCH4* was the lowest among *NOTCH* receptors (Table 1).

Breast cancer cells were shown to induce epithelial-mesenchymal transition (EMT) via *NOTCH4* (Lombardo et al. 2014). *NOTCH4* is an EMT trigger and promotes the metastasis of melanoma cells (Lin et al. 2016). In these backgrounds, Fukusumi et al. (2107) examined *NOTCH4* function and demonstrated that *NOTCH4* was significantly related to HNSCC cell proliferation, chemotherapy resistance, cell cycle, apoptosis inhibition, and EMT using the TCGA data set and in vitro experiments. Clinically, *NOTCH4* expression is also significantly related to T stage, clinical stage, and perineural invasion (Mk et al. 2016).

As shown in Table 3, *NOTCH4* expression is significantly related to its downstream genes such as *HEY1* and *BCL2.* Fukusumi et al. (2014) indicated that *NOTCH4* promotes EMT through *HEY1*, as described below. *NOTCH4* expression was also reported to be increased in breast CSC (Simões Bruno et al. 2015). In HNSCC, *CD10* (Fukusumi et al. 2014), *CD44* (Prince et al. 2007), and *ALDH1* (Chen et al. 2009) are defined as CSC markers. Thus, the expression levels of these markers were compared using the TCGA data set. Significant differences in *ALDH1* were noted between the *NOTCH4/HEY1* high and low groups. Si-*NOTCH4* and si-*HEY1* cells also showed significantly increased *ALDH1* expression. From these results, the authors suggested that *ALDH1* could regulate the *NOTCH4-HEY1* pathway (Fukusumi et al. 2017).

NOTCH *Pathway Genes*

Similar to *NOTCH* receptors, *NOTCH* ligands also relate to HNSCC progression. *JAG1* and *2* expressions in HNSCC are significantly higher than that in normal mucosa (Zhang et al. 2011; Sun et al. 2014). *JAG1* expression is related to poor prognosis (Lin et al. 2010) and lymph node metastasis (Zhang et al. 2011). *JAG1* regulates the differentiation, proliferation, and angiogenesis in HNSCC (Zeng et al. 2005; Zhang et al. 2013c). Recently, several studies have shown that *DLL4* can regulate tumor angiogenesis (Noguera-Troise et al. 2006; Ridgway et al. 2006). In HNSCC, *DLL4* expression has a significantly positive correlation with expression of vascular endothelial growth factor. Moreover, *DLL4* expression is independently associated with poor prognosis and significantly elevated in distant metastases compared with primary HNSCC tumors (Zhang et al. 2013a).

After ligand binding to a *NOTCH* receptor, NICD activates *NOTCH* downstream genes. Rettig et al. (2015) performed immunohistochemical staining for NICD in the tonsils and HNSCC samples. All tonsil specimens expressed NICD. In the tumor samples, 81% stained positive. Among tumor samples, most of the *NOTCH1* wild-type samples had positive NICD staining (89%). Half of the mutated *NOTCH1* samples had negative staining. The authors also found that negative NICD staining was significantly associated with poor differentiation. Furthermore, NICD positive staining was significantly negatively associated with lymph node metastasis (Rettig et al. 2015). However, Gokulan and Halagowder (2014) showed that the normal oral epithelium predominantly exhibited negative staining for NICD, the expression of NICD was gradually increased from dysplasia to carcinoma, and NICD staining was

higher in stage III to IV cases than in stage I to II cases. Furthermore, a significant correlation was found between NICD expression and lymph node metastasis of OSCC. In this study, NICD expression in *NOTCH* wild-type HNSCC is consistent with a more aggressive phenotype characterized by and EMT phenotype (Gokulan and Halagowder 2014).

HES, HEY, CCND1, MYC, BCL-2, and *p21* are among a large number of *NOTCH* target genes. Among these genes, the roles of *HES* and *HEY* in HNSCC are not well understood. The most prominent targets of the *NOTCH* pathway are the *HES* and *HEY* families (Kalaitzidis and Armstrong 2011; Sethi et al. 2011). Thus, several recent studies have focused on these functions in HNSCC. To elucidate the HNSCC-specific correlation of *NOTCH* pathway genes, we examined the correlation between each *NOTCH* receptor and its associated *NOTCH* downstream genes using the updated TCGA data set. Several significantly positive correlations were found, such as *NOTCH1-BCL2, NOTCH3-HEY1, NOTCH4-HEY1*, and *NOTCH4-BCL2* (Table 3). Among them, *NOTCH3, 4* and *HEY1* have been shown to have a mutual relationship described below (Man et al. 2012; Sun et al. 2014; Fukusumi et al. 2017).

Sun et al. (2014) found that both *HES1* and *HEY1* mRNA expressions in HNSCC were significantly higher than in normal mucosa. In addition, 14% and 25% of HNSCC tumors showed overexpression of *HES1* and *HEY1* compared with that in normal mucosa. In total, 31.8% HNSCC tumors showed overexpression of *HES1* and/or *HEY1*. In their microarray, *HES1* and *HEY1* were also overexpressed in tumor samples; either *HES1* or *HEY1* was overexpressed in 26.8% of HNSCC samples, a finding similar to that in the previous expression array data (31.8%; Sun et al. 2014). Wirth et al. (2016) also showed elevated expression of *HES1* and *HEY1* in HNSCC compared with normal tissues.

HES1 expression is upregulated in OSCC lesions compared with that in dysplastic lesions. *HES1* promoted sphere formation ability, indicating that *HES1* activates the CSC phenotype (Lee et al. 2012). Another study showed that the expression of *HES1* was higher in stage III to IV cases than in stage I to II OSCC cases. A higher expression of *HES1* was also found in lymph node metastasis–positive cases than in negative cases. *HES1*-positive OSCC showed significantly worse DFS than negative cases (Gokulan and Halagowder 2014).

TCGA mRNA sequence analysis showed that *HEY1* expression exhibited a significant positive correlation with all *NOTCH* receptors but that *HES1* did not show a similar association with *NOTCH* receptor expression. Among these receptors, *NOTCH4* exhibited the most significant correlation to *HEY1* expression. *HEY1* expression in HNSCC was significantly increased, approximately twice as high as that in normal samples, and in vitro experiments revealed the same results (Fukusumi et al. 2017). In general, *HEY1* is known to regulate EMT. *HEY1* expression in HNSCC was also related to an EMT phenotype as determined by gene expression in both the TCGA data set analysis and in vitro experiments (Fischer et al. 2007; Luna-Zurita et al. 2010; Fukusumi et al. 2017). Man et al. (2012) confirmed that *HEY1* expression of HNSCC cells was significantly higher than that of normal epithelial cells. In the studies noted above, there are consistent data demonstrating that the *NOTCH4-HEY1* pathway of HNSCC can be specifically up-regulated and promote EMT.

Anti-NOTCH *Therapy*

The *NOTCH* pathway is an attractive cancer therapeutic target, and its inhibition has been shown to decrease cell proliferation and invasion (Yao et al. 2007). Many types of *NOTCH* inhibitors exist, including monoclonal antibodies, RNAi, receptor decoys, and glycosylation/protease inhibitors (Ran et al. 2017). Among them, γ-secretase inhibitors (GSIs) are the most used inhibitors for several cancer studies and clinical trials (Strosberg et al. 2012; De Jesus-Acosta et al. 2014). DAPT used as a GSI enhanced the radiation-induced apoptosis of HNSCC cells (Yu et al. 2011). Furthermore, GSI can inhibit sphere formation ability and decrease the CSC fractions (Upadhyay et al. 2016). The combined therapy of DAPT and conventional drugs improved its anticancer effect synergistically (Zhao et al. 2016). These results are consistent with *NOTCH* being related to CSC. Thus, anti-*NOTCH* therapy can be efficient for HNSCC CSC that is considered chemoresistant and radioresistant, albeit in experimental systems.

However, GSIs cannot inhibit specific, individual *NOTCH* receptors. Ran et al. (2017) performed *NOTCH* substance activity assays using various GSIs (BMS-906024, PF-3084014, RO4929097, semagacestat, MK-0752, and DAPT) and showed that each GSI had different effects against each *NOTCH* receptor. Only BMS-906024 inhibited all *NOTCH* substrates nearly equivalently (Ran et al. 2017). *NOTCH* signaling is necessary for tissue homeostasis. Thus, nonspecific inhibition by GSIs can induce severe side effects such as gastrointestinal toxicity, diarrhea, hepatotoxicity, and nephrotoxicity (Searfoss et al. 2003; van Es et al. 2005; Garber 2007; Wu et al. 2010).

To avoid this nonspecific inhibition, anti-*NOTCH1, 2*, and *3* antibodies have been developed, although a functional anti-*NOTCH4* antibody has not been developed yet, as *NOTCH4* lacks an extracellular component for ligand binding that is a potential target for an inactivating antibody (Fig. 1). The anti-*NOTCH1* antibody significantly decreased the growth of mouse xenograft colon cancer cells without weight loss and severe side effects for normal goblet cells (Wu et al. 2010). Huntzicker et al. (2015) showed that the anti-*NOTCH2* antibody reduced mouse liver tumors, but the anti-*NOTCH3* antibody did not decrease the tumor burden. Anti-*DLL4* antibody and nanoparticles have been examined in terms of a potential anticancer effect for HNSCC cells. They indicate anti-*DLL4* therapy enhances radiation response and decreases angiogenesis (Liu et al. 2011, 2015).

These studies indicate the importance of specific *NOTCH* target cancer therapy, and further analysis of the HNSCC-specific *NOTCH* pathway and establishment of *NOTCH* subtype-specific therapies may offer the opportunity for therapeutic effect while minimizing side effects.

Discussion

Most studies in this review reveal *NOTCH* pathway is upregulated in HNSCC, and *NOTCH* expression shows significant correlations with clinical stage (Joo et al. 2009; Zhang et al. 2011). *NOTCH* is also related to EMT (Zhao et al. 2016; Fukusumi et al. 2017), and EMT has been related to the therapeutic resistance, invasion, and metastasis of cancers (Bao et al. 2006; Li et al. 2008). Thus, the *NOTCH* pathway can play an important role in HNSCC development, and anti-*NOTCH* therapy can be attractive.

However, as described above, *NOTCH1* is considered to play a bimodal role as a tumor suppressor and an oncogene unlike other highly mutated genes in HNSCC such as *TP53* and *PTEN*, which are well-established tumor suppressor genes. Of note, the *NOTCH1* mutations show divergence between Caucasian and Asian patient studies. There are no significant differences in recurrence, invasion, and clinical stages between *NOTCH1* wild-type and mutant patients (Agrawal et al. 2011; Stransky et al. 2011; Rettig et al. 2015). On the other hand, several Asian studies have indicated that *NOTCH1* mutation is activate type, and HNSCC with *NOTCH1* mutation has a worse prognosis than *NOTCH1* wild-type tumors (Song et al. 2014; Vettore et al. 2015). It will be important to examine whether the difference of *NOTCH1* mutation types are related to germ line genetic differences or exposures. The authors in Asian studies noted the higher alcohol concentration in Chinese liquor as a potential differential factor (Song et al. 2014; Izumchenko et al. 2015).

Human papilloma virus (HPV)–related HNSCC is considered to have different gene expression and pathways compared with HPV-negative HNSCC (Suárez et al. 2016). In HPV-positive cervical cancer, *NOTCH1* expression is significantly downregulated, and *NOTCH3* expression was significantly upregulated compared with normal cervix tissue (Tripathi et al. 2014). HPV E6 protein decreases *NOTCH1* expression (Kranjec et al. 2017). In HNSCC, *NOTCH1* is more mutated in HPV-negative samples than in HPV-positive samples (Rettig et al. 2015). Higher *NOTCH1* expression in HPV-positive HNSCC is shown compared with HPV-negative HNSCC (Kaka et al. 2017). However, there is no comprehensive analysis for each *NOTCH* pathway gene alterations comparing HPV-positive and -negative HNSCC and no definitive study defining whether HPV E6/7 affects the *NOTCH* pathway in HNSCC.

There are several challenges that can be addressed for anti-*NOTCH* therapy in HNSCC. However, the *NOTCH* pathway is also important for oral normal tissue homeostasis as well as other organs (Harada et al. 1999). Thus, HNSCC-specific *NOTCH* pathway therapy would likely need to be tailored to specific *NOTCH* isoforms, to avoid systemic and gastrointestinal toxicities. Implicit in this concept is the need to characterize the contextual action of the *NOTCH* pathway in individual patients, such that *NOTCH*-targeted therapy is used exclusively for *NOTCH* pathway–activated tumors. Despite the challenges of *NOTCH* pathway–directed therapies, the high proportion of HNSCC with *NOTCH* pathway activation and the key role that *NOTCH* plays in development of this cancer indicate that *NOTCH*-based therapy has significant potential affect HNSCC outcomes.

Author Contributions

T. Fukusumi, contributed to design, data acquisition, analysis, and interpretation, drafted the manuscript; J.A. Califano, contributed to conception, critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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References

Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, et al. 2011. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 333(6046):1154–1157.

Alqudah MA, Agarwal S, Al-Keilani MS, Sibenaller ZA, Ryken TC, Assem M. 2013. NOTCH3 is a prognostic factor that promotes glioma cell proliferation, migration and invasion via activation of CCND1 and EGFR. PLoS One. 8(10):e77299.

Argiris A, Karamouzis MV, Raben D, Ferris RL. 2008. Head and neck cancer. Lancet. 371(9625):1695–1709.

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. 2006. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 444(7120):756–760.

Cancer Genome Atlas. 2015. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 517(7536):576–582.

Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, Chen DT, Tai LK, Yung MC, Chang SC, et al. 2009. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun. 385(3):307–313.

de Castro G Jr., Negrao MV. 2014. The Cancer Genome Atlas findings in head and neck cancer: a renewed hope. Curr Opin Oncol. 26(3):245–246.

De Jesus-Acosta A, Laheru D, Maitra A, Arcaroli J, Rudek MA, Dasari A, Blatchford PJ, Quackenbush K, Messersmith W. 2014. A phase II study of the gamma secretase inhibitor RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma. Invest New Drugs. 32(4):739–745.

Demokan S, Chuang A, Suoglu Y, Ulusan M, Yalniz Z, Califano JA, Dalay N. 2012. Promoter methylation and loss of p16(INK4a) gene expression in head and neck cancer. Head Neck. 34(10):1470–1475.

Fischer A, Steidl C, Wagner TU, Lang E, Jakob PM, Friedl P, Knobeloch KP, Gessler M. 2007. Combined loss of Hey1 and HeyL causes congenital heart defects because of impaired epithelial to mesenchymal transition. Circ Res. 100(6):856–863.

Fukusumi T, Guo T, Sakai A, Ando M, Ren S, Haft S, Liu C, Amornphimoltham P, Gutkind JS, Califano J. 2017. The NOTCH4-HEY1 pathway induces epithelial mesenchymal transition in head and neck squamous cell carcinoma [published online 2017 November 16]. Clin Cancer Res.

Fukusumi T, Ishii H, Konno M, Yasui T, Nakahara S, Takenaka Y, Yamamoto Y, Nishikawa S, Kano Y, Ogawa H, et al. 2014. CD10 as a novel marker of therapeutic resistance and cancer stem cells in head and neck squamous cell carcinoma. Br J Cancer. 111(3):506–514.

Gao YB, Chen ZL, Li JG, Hu XD, Shi XJ, Sun ZM, Zhang F, Zhao ZR, Li ZT, Liu ZY, et al. 2014. Genetic landscape of esophageal squamous cell carcinoma. Nat Genet. 46(10):1097–1102.

Garber K. 2007. Notch emerges as new cancer drug target. J Natl Cancer Inst. 99(17):1284–1285.

Gaykalova DA, Mambo E, Choudhary A, Houghton J, Buddavarapu K, Sanford T, Darden W, Adai A, Hadd A, Latham G, et al. 2014. Novel insight into mutational landscape of head and neck squamous cell carcinoma. PLoS One. 9(3):e93102.

Gokulan R, Halagowder D. 2014. Expression pattern of Notch intracellular domain (NICD) and Hes-1 in preneoplastic and neoplastic human oral squamous epithelium: their correlation with c-Myc, clinicopathological factors and prognosis in oral cancer. Med Oncol. 31(8):126.

Gordon WR, Arnett KL, Blacklow SC. 2008. The molecular logic of Notch signaling—a structural and biochemical perspective. J Cell Sci. 121(Pt 19):3109–3119.

Gu F, Ma Y, Zhang Z, Zhao J, Kobayashi H, Zhang L, Fu L. 2010. Expression of Stat3 and Notch1 is associated with cisplatin resistance in head and neck squamous cell carcinoma. Oncol Rep. 23(3):671–676.

Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, Pevsner J, Westra WH, Califano J. 2003. A transcriptional progression model for head and neck cancer. Clin Cancer Res. 9(8):3058–3064.

Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. 1999. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol. 147(1):105–120.

Hijioka H, Setoguchi T, Miyawaki A, Gao H, Ishida T, Komiya S, Nakamura N. 2010. Upregulation of Notch pathway molecules in oral squamous cell carcinoma. Int J Oncol. 36(4):817–822.

Huntzicker EG, Hotzel K, Choy L, Che L, Ross J, Pau G, Sharma N, Siebel CW, Chen X, French DM. 2015. Differential effects of targeting Notch receptors in a mouse model of liver cancer. Hepatology. 61(3):942–952.

Izumchenko E, Sun K, Jones S, Brait M, Agrawal N, Koch W, McCord CL, Riley DR, Angiuoli SV, Velculescu VE, et al. 2015. Notch1 mutations are drivers of oral tumorigenesis. Cancer Prev Res (Phila). 8(4):277–286.

Joo YH, Jung CK, Kim MS, Sun DI. 2009. Relationship between vascular endothelial growth factor and Notch1 expression and lymphatic metastasis in tongue cancer. Otolaryngol Head Neck Surg. 140(4):512–518.

Kaka AS, Nowacki NB, Kumar B, Zhao S, Old MO, Agrawal A, Ozer E, Carrau RL, Schuller DE, Kumar P, et al. 2017. Notch1 overexpression correlates to improved survival in cancer of the oropharynx. Otolaryngol Head Neck Surg. 156(4):652–659.

Kalaitzidis D, Armstrong SA. 2011. Cancer: the flipside of Notch. Nature. 473(7346):159–160.

Kayamori K, Katsube K, Sakamoto K, Ohyama Y, Hirai H, Yukimori A, Ohata Y, Akashi T, Saitoh M, Harada K, et al. 2016. NOTCH3 is induced in cancer-associated fibroblasts and promotes angiogenesis in oral squamous cell carcinoma. PLoS One. 11(4):e0154112.

Kranjec C, Holleywood C, Libert D, Griffin H, Mahmood R, Isaacson E, Doorbar J. 2017. Modulation of basal cell fate during productive and transforming HPV-16 infection is mediated by progressive E6-driven depletion of Notch. J Pathol. 242(4):448–462.

Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G. 2014. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. 505(7484):495–501.

Lee SH, Hong HS, Liu ZX, Kim RH, Kang MK, Park NH, Shin KH. 2012. TNFalpha enhances cancer stem cell-like phenotype via Notch-Hes1 activation in oral squamous cell carcinoma cells. Biochem Biophys Res Commun. 424(1):58–64.

Leethanakul C, Patel V, Gillespie J, Pallente M, Ensley JF, Koontongkaew S, Liotta LA, Emmert-Buck M, Gutkind JS. 2000. Distinct pattern of expression of differentiation and growth-related genes in squamous cell carcinomas of the head and neck revealed by the use of laser capture microdissection and cDNA arrays. Oncogene. 19(28):3220–3224.

Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, Hilsenbeck SG, Pavlick A, Zhang X, Chamness GC, et al. 2008. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst. 100(9):672–679.

Lin JT, Chen MK, Yeh KT, Chang CS, Chang TH, Lin CY, Wu YC, Su BW, Lee KD, Chang PJ. 2010. Association of high levels of Jagged-1 and Notch-1 expression with poor prognosis in head and neck cancer. Ann Surg Oncol. 17(11):2976–2983.

Lin X, Sun B, Zhu D, Zhao X, Sun R, Zhang Y, Zhang D, Dong X, Gu Q, Li Y, et al. 2016. Notch4+ cancer stem-like cells promote the metastatic and invasive ability of melanoma. Cancer Sci. 107(8):1079–1091.

Liu SK, Bham SA, Fokas E, Beech J, Im J, Cho S, Harris AL, Muschel RJ. 2011. Delta-like ligand 4-notch blockade and tumor radiation response. J Natl Cancer Inst. 103(23):1778–1798.

Liu YR, Guan YY, Luan X, Lu Q, Wang C, Liu HJ, Gao YG, Yang SC, Dong X, Chen HZ, et al. 2015. Delta-like ligand 4-targeted nanomedicine for anti-angiogenic cancer therapy. Biomaterials. 42:161–171.

Lombardo Y, Faronato M, Filipovic A, Vircillo V, Magnani L, Coombes RC. 2014. Nicastrin and Notch4 drive endocrine therapy resistance and epithelial to mesenchymal transition in MCF7 breast cancer cells. Breast Cancer Res. 16(3):R62.

Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, del Monte G, Benguria A, Adams RH, Perez-Pomares JM, de la Pompa JL. 2010. Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve formation. J Clin Invest. 120(10):3493–3507.

Lunde ML, Roman E, Warnakulasuriya S, Mehrotra R, Laranne J, Vasstrand EN, Ibrahim SO. 2014. Profiling of chromosomal changes in potentially malignant and malignant oral mucosal lesions from South and South-East Asia using array-comparative genomic hybridization. Cancer Genomics Proteomics. 11(3):127–140.

Man CH, Wei-Man Lun S, Wai-Ying Hui J, To KF, Choy KW, Wing-Hung Chan A, Chow C, Tin-Yun Chung G, Tsao SW, Tak-Chun Yip T, et al. 2012. Inhibition of NOTCH3 signalling significantly enhances sensitivity to cisplatin in EBV-associated nasopharyngeal carcinoma. J Pathol. 226(3):471–481.

Mao L. 2015. NOTCH mutations: multiple faces in human malignancies. Cancer Prev Res (Phila). 8(4):259–261.

Miyamoto Y, Maitra A, Ghosh B, Zechner U, Argani P, Iacobuzio-Donahue CA, Sriuranpong V, Iso T, Meszoely IM, Wolfe MS, et al. 2003. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell. 3(6):565–576.

Mk H, Prince S, Mohan AM, Krishnan KV, Devi A. 2016. Association of Notch4 with metastasis in human oral squamous cell carcinoma. Life Sci. 156:38–46.

Murugan AK, Hong NT, Fukui Y, Munirajan AK, Tsuchida N. 2008. Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. Int J Oncol. 32(1):101–111.

Noguera-Troise I, Daly C, Papadopoulos NJ, Coetzee S, Boland P, Gale NW, Lin HC, Yancopoulos GD, Thurston G. 2006. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature. 444(7122):1032–1037.

Ock CY, Son B, Keam B, Lee SY, Moon J, Kwak H, Kim S, Kim TM, Jeon YK, Kwon SK, et al. 2016. Identification of genomic mutations associated with clinical outcomes of induction chemotherapy in patients with head and neck squamous cell carcinoma. J Cancer Res Clin Oncol. 142(4):873–883.

Okami K, Wu L, Riggins G, Cairns P, Goggins M, Evron E, Halachmi N, Ahrendt SA, Reed AL, Hilgers W, et al. 1998. Analysis of PTEN/MMAC1 alterations in aerodigestive tract tumors. Cancer Res. 58(3):509–511.

Papadimitrakopoulou VA, Izzo J, Mao L, Keck J, Hamilton D, Shin DM, El-Naggar A, den Hollander P, Liu D, Hittelman WN, et al. 2001. Cyclin D1 and p16 alterations in advanced premalignant lesions of the upper aerodigestive tract: role in response to chemoprevention and cancer development. Clin Cancer Res. 7(10):3127–3134.

Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, Zhao M, Ortega Alves MV, Chang K, Drummond J, et al. 2013. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov. 3(7):770–781.

Poeta ML, Manola J, Goldenberg D, Forastiere A, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, et al. 2009. The ligamp TP53 assay for detection of minimal residual disease in head and neck squamous cell carcinoma surgical margins. Clin Cancer Res. 15(24):7658–7665.

Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. 2007. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A. 104(3):973–978.

Ran Y, Hossain F, Pannuti A, Lessard CB, Ladd GZ, Jung JI, Minter LM, Osborne BA, Miele L, Golde TE. 2017. gamma-Secretase inhibitors in cancer clinical trials are pharmacologically and functionally distinct. EMBO Mol Med. 9(7):950–966.

Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, Lockwood G, Egan SE. 2005. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res. 65(18):8530–8537.

Rettig EM, Chung CH, Bishop JA, Howard JD, Sharma R, Li RJ, Douville C, Karchin R, Izumchenko E, Sidransky D, et al. 2015. Cleaved NOTCH1 expression pattern in head and neck squamous cell carcinoma is associated with NOTCH1 mutation, HPV status, and high-risk features. Cancer Prev Res (Phila). 8(4):287–295.

Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, Kowalski J, Watts RJ, Callahan C, Kasman I, et al. 2006. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature. 444(7122):1083–1087.

Sawada G, Niida A, Uchi R, Hirata H, Shimamura T, Suzuki Y, Shiraishi Y, Chiba K, Imoto S, Takahashi Y, et al. 2016. Genomic landscape of esophageal squamous cell carcinoma in a Japanese population. Gastroenterology. 150(5):1171–1182.

Searfoss GH, Jordan WH, Calligaro DO, Galbreath EJ, Schirtzinger LM, Berridge BR, Gao H, Higgins MA, May PC, Ryan TP. 2003. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. J Biol Chem. 278(46):46107–46116.

Sethi N, Dai X, Winter CG, Kang Y. 2011. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. Cancer Cell. 19(2):192–205.

Simões Bruno M, O'Brien Ciara S, Eyre R, Silva A, Yu L, Sarmiento-Castro A, Alférez Denis G, Spence K, Santiago-Gómez A, Chemi F, et al. 2015. Antiestrogen resistance in human breast tumors is driven by JAG1-NOTCH4-dependent cancer stem cell activity. Cell Rep. 12(12):1968–1977.

Snijders AM, Schmidt BL, Fridlyand J, Dekker N, Pinkel D, Jordan RC, Albertson DG. 2005. Rare amplicons implicate frequent deregulation of cell fate specification pathways in oral squamous cell carcinoma. Oncogene. 24(26):4232–4242.

Song X, Xia R, Li J, Long Z, Ren H, Chen W, Mao L. 2014. Common and complex Notch1 mutations in Chinese oral squamous cell carcinoma. Clin Cancer Res. 20(3):701–710.

Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, et al. 2011. The mutational landscape of head and neck squamous cell carcinoma. Science. 333(6046):1157–1160.

Strosberg JR, Yeatman T, Weber J, Coppola D, Schell MJ, Han G, Almhanna K, Kim R, Valone T, Jump H, et al. 2012. A phase II study of RO4929097 in metastatic colorectal cancer. Eur J Cancer. 48(7):997–1003.

Suárez E, González L, Pérez-Mitchell C, Ortiz AP, Ramírez-Sola M, Acosta J, Bernabe-Dones RD, González-Aquino C, Montes-Rodríguez I, Cadilla CL. 2016. Pathway analysis using gene-expression profiles of HPV-positive and HPV-negative oropharyngeal cancer patients in a Hispanic population: methodological procedures. P R Health Sci J. 35(1):3–8.

Sun W, Gaykalova DA, Ochs MF, Mambo E, Arnaoutakis D, Liu Y, Loyo M, Agrawal N, Howard J, Li R, et al. 2014. Activation of the NOTCH pathway in head and neck cancer. Cancer Res. 74(4):1091–1104.

Tripathi R, Rath G, Jawanjal P, Sharma S, Singhal P, Bhambhani S, Hussain S, Bharadwaj M. 2014. Clinical impact of de-regulated Notch-1 and Notch-3 in the development and progression of HPV-associated different histological subtypes of precancerous and cancerous lesions of human uterine cervix. PLoS One. 9(6):e98642.

Upadhyay P, Nair S, Kaur E, Aich J, Dani P, Sethunath V, Gardi N, Chandrani P, Godbole M, Sonawane K, et al. 2016. Notch pathway activation is essential for maintenance of stem-like cells in early tongue cancer. Oncotarget. 7(31):50437–50449.

van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, Begthel H, Cozijnsen M, Robine S, Winton DJ, Radtke F, et al. 2005. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature. 435(7044):959–963.

Vettore AL, Ramnarayanan K, Poore G, Lim K, Ong CK, Huang KK, Leong HS, Chong FT, Lim TK, Lim WK, et al. 2015. Mutational landscapes of tongue carcinoma reveal recurrent mutations in genes of therapeutic and prognostic relevance. Genome Med. 7:98.

Villaronga MA, Bevan CL, Belandia B. 2008. Notch signaling: a potential therapeutic target in prostate cancer. Curr Cancer Drug Targets. 8(7):566–580.

Wang C, Li Q, Liu F, Chen X, Liu B, Nesa EU, Guan S, Han L, Tan B, Wang N, et al. 2016. Notch2 as a promising prognostic biomarker for oesophageal squamous cell carcinoma. Sci Rep. 6:25722.

Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, Leigh IM, Collisson EA, Gordon PB, Jakkula L, et al. 2011. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci U S A. 108(43):17761–17766.

Westhoff B, Colaluca IN, D'Ario G, Donzelli M, Tosoni D, Volorio S, Pelosi G, Spaggiari L, Mazzarol G, Viale G, et al. 2009. Alterations of the Notch pathway in lung cancer. Proc Natl Acad Sci U S A. 106(52):22293–22298.

Wirth M, Doescher J, Jira D, Meier MA, Piontek G, Reiter R, Schlegel J, Pickhard A. 2016. HES1 mRNA expression is associated with survival in sinonasal squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 122(4):491–499.

Wu WR, Zhang R, Shi XD, Yi C, Xu LB, Liu C. 2016. Notch2 is a crucial regulator of self-renewal and tumorigenicity in human hepatocellular carcinoma cells. Oncol Rep. 36(1):181–188.

Wu Y, Cain-Hom C, Choy L, Hagenbeek TJ, de Leon GP, Chen Y, Finkle D, Venook R, Wu X, Ridgway J, et al. 2010. Therapeutic antibody targeting of individual Notch receptors. Nature. 464(7291):1052–1057.

Yao J, Duan L, Fan M, Wu X. 2007. Gamma-secretase inhibitors exerts antitumor activity via down-regulation of Notch and Nuclear factor kappa B in human tongue carcinoma cells. Oral Dis. 13(6):555–563.

Yoshida R, Nagata M, Nakayama H, Niimori-Kita K, Hassan W, Tanaka T, Shinohara M, Ito T. 2013. The pathological significance of Notch1 in oral squamous cell carcinoma. Lab Invest. 93(10):1068–1081.

Yu S, Zhang R, Liu F, Hu H, Yu S, Wang H. 2011. Down-regulation of Notch signaling by a gamma-secretase inhibitor enhances the radiosensitivity of nasopharyngeal carcinoma cells. Oncol Rep. 26(5):1323–1328.

Yuan X, Wu H, Xu H, Han N, Chu Q, Yu S, Chen Y, Wu K. 2015. Meta-analysis reveals the correlation of Notch signaling with non-small cell lung cancer progression and prognosis. Sci Rep. 5:10338.

Zayzafoon M, Abdulkadir SA, McDonald JM. 2004. Notch signaling and ERK activation are important for the osteomimetic properties of prostate cancer bone metastatic cell lines. J Biol Chem. 279(5):3662–3670.

Zeng Q, Li S, Chepeha DB, Giordano TJ, Li J, Zhang H, Polverini PJ, Nor J, Kitajewski J, Wang CY. 2005. Crosstalk between tumor and endothelial cells promotes tumor angiogenesis by MAPK activation of Notch signaling. Cancer Cell. 8(1):13–23.

Zhang JX, Cai MB, Wang XP, Duan LP, Shao Q, Tong ZT, Liao DZ, Li YY, Huang MY, Zeng YX, et al. 2013a. Elevated DLL4 expression is correlated with VEGF and predicts poor prognosis of nasopharyngeal carcinoma. Med Oncol. 30(1):390.

Zhang T, Liu H, Liang Y, Liang L, Liao G, Wu J, Huang H. 2013b. The expression and significance of the Notch signaling pathway molecules in tongue squamous cell carcinoma [in Chinese]. Hua Xi Kou Qiang Yi Xue Za Zhi. 31(3):303–309.

Zhang TH, Liu HC, Liang YJ, Liang LZ, Zheng GS, Huang HZ, Wu JN, Liao GQ. 2013c. Suppression of tongue squamous cell carcinoma growth by inhibition of Jagged1 in vitro and in vivo. J Oral Pathol Med. 42(4):322–331.

Zhang TH, Liu HC, Zhu LJ, Chu M, Liang YJ, Liang LZ, Liao GQ. 2011. Activation of Notch signaling in human tongue carcinoma. J Oral Pathol Med. 40(1):37–45.

Zhao ZL, Zhang L, Huang CF, Ma SR, Bu LL, Liu JF, Yu GT, Liu B, Gutkind JS, Kulkarni AB, et al. 2016. NOTCH1 inhibition enhances the efficacy of conventional chemotherapeutic agents by targeting head neck cancer stem cell. Sci Rep. 6:24704.

Zou Y, Fang F, Ding YJ, Dai MY, Yi X, Chen C, Tao ZZ, Chen SM. 2016. Notch 2 signaling contributes to cell growth, anti-apoptosis and metastasis in laryngeal squamous cell carcinoma. Mol Med Rep. 14(4):3517–3524.