NOTCH Mutations: Multiple Faces in Human Malignancies

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Abstract

NOTCH proteins have been implicated in multiple cellular functions, such as stem cell maintenance and cell fate determination. Initially identified as proto-oncogenes because they promote the development of certain types of leukemia, inactivating mutations of NOTCH were later reported. Together with the potential distinct functions of NOTCH family members, their ligands and associated niches, the precise roles of NOTCH in human cancers, particularly solid tumors, remain unsettled. In oral squamous cell carcinoma (OSCC), mutations of NOTCH1 are found in 10% to 15% tumors from Caucasian patients, mostly inactivating mutations. Recent studies of OSCC from Chinese patients, however, showed mutation rates of NOTCH1 about 50% with a considerable portion of potential activating mutations. These findings add another twist into the already complex picture of NOTCH alterations in human cancers, calling for further investigation to uncover what role exactly these molecules play in cancer initiation and progression to develop strategies targeting NOTCH signaling for cancer prevention and treatment. Cancer Prev Res; 8(4), 259–61. ©2015 AACR.

See related article by Izumchenko et al., p. 277

The NOTCH signaling pathway is evolutionally conserved and critical in stem cell differentiation and cell fate determination in development. Its roles in malignant initiation and progression, however, are complex and appear organ/cell-type dependent.

Four NOTCH receptors (NOTCH1–4) exist in mammals with a different number of EGF-like repeats and are activated upon binding to ligands (1). The activation requires two sequential protein cleavage steps by ADAM10/17 metalloproteases and presenilin–γ-secretase complex (γ-secretase) to release the intracellular portion of the NOTCH, also known as ICN, which is translocated into the nucleus and mediates activation of the NOTCH pathway (2–4). Therefore, NOTCH pathway activities can be impacted by not only NOTCH receptors themselves but also their ligands, the protein cleavage steps, and ICN nuclear translocation.

Genetic alterations of genes in the NOTCH pathway have been noted in a number of human malignancies both in hematopoietic and solid tumors (5). Most genetic alterations of NOTCH receptors in malignancies are observed in the NOTCH1 gene. The first reported NOTCH1 alteration was the identification of a chromosome translocation by fusing T-cell receptor-β to ICN1 in T-cell acute lymphoblastic leukemia (T-ALL; ref. 6) to create a constitutively active and oncogenic NOTCH1 in leukemia cells (7, 8). The subsequent discovery of activating NOTCH1 mutations in up to 50% of T-ALL cases and in other hematopoietic malignancies, such as chronic lymphocytic leukemia and lymphoma, further solidified the oncogenic role of NOTCH in leukemia (9–11).

These mutations concentrate on heterodimerization and the PEST domains, which may make NOTCH1 more sensitive to protease cleavage or reduce ICN degradation. In solid tumors, activating NOTCH1 mutations were documented in lung adenocarcinoma (12). However, the NOTCH signaling pathway is frequently activated in multiple types of solid tumors, such as melanoma, colorectal carcinoma, and cholangiocarcinoma, through other mechanisms (13–16).

In contrast with the oncogenic roles of NOTCH observed in malignancies, tumor-suppressive roles of the NOTCH pathway have been recently noted in multiple tumor types. In acute myeloid leukemia (AML), activation of the NOTCH signaling pathway can result in a disease remission (17). A similar tumor-suppressor role of the NOTCH pathway activation was also observed in B-cell ALL (18). Interestingly, several independent research groups in the United States recently reported that up to 15% of head and neck squamous cell carcinoma (HNSCC) carry NOTCH1 mutations, most of them likely to lead to NOTCH1 inactivation by whole-genome sequencing methods (19, 20). The finding is clinically important, because it is difficult clinically to restore functions of loss-of-function genes, and therefore, NOTCH1 was considered less likely a therapeutic target for patients with NNSCC. It should be noted that most of the tumors studied in the U.S. reports are from Caucasian patients.

More recently, we investigated a cohort of OSCC patients, the most common type of HNSCC, from Han Chinese for NOTCH1 mutation status. Using a conservative definition, we reported a NOTCH1 mutation rate of 43%, a rate comparable with the mutation rate of TP53 in the same patient cohort, although there was no association between mutations in NOTCH1 and TP53 (21). We noted that the NOTCH1 mutation rate observed in this Chinese OSCC cohort was substantially higher than the mutation frequencies observed in Caucasian OSCC populations (19, 20).

In addition to the higher rate of NOTCH1 mutations in the Chinese cohort, the mutation spectrum was also drastically different between our study and the U.S. studies (21). In contrast with the virtually universal inactivating mutations in the
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Caucasian cohorts, 60% of the mutations identified in the Chinese cohort are in domains likely to result in activating NOTCH signaling (21).

The relatively small sample size (51 patients) and the lack of validation in an independent cohort are some of the limitations of our earlier study (21). In this issue, Izumchenko and colleagues (22) studied an independent Han Chinese OSCC cohort (50 patients) and found that NOTCH1 mutations in 54% of the tumors with approximately 40% of the mutations are potentially gain-of-functions. The results are consistent with our findings in the Han Chinese, which provide further support for the considerable differences in NOTCH1 mutation rate and mutation spectrum between Caucasian and Chinese OSCC.

A limitation noted in this study is the lack of matched constitutional DNA controls for more than 50% of the patients, which may complicate data interpretation because of the potential mis-calls of rare single-nucleotide polymorphisms (SNP) as somatic mutations. Nevertheless, the impact of this lack of matched controls is likely small, because the NOTCH1 mutations were observed in 50% of the 22 OSCC tumors with matched controls, which is comparable with 57% of the 28 tumors without matched controls in this study (22). The mutation frequency is also similar to our previously reported data where 90% of the OSCC patients had matched DNA controls and the NOTCH1 mutation rate was 43% (21). Statistically speaking, if any of the nonsynonymous mutations are SNPs, it is likely rare, because they are not registered in the database of the 1000 Genomes Project (www.1000genomes.org), where 523 samples were from Asian ancestry, including >300 Chinese origin. However, it cannot be ruled out that SNPs may be enriched in individuals susceptible to OSCC development, although the likelihood is small because these SNPs are not commonly observed in other OSCCs.

The most interesting observation by Izumchenko and colleagues (22) is the high rate of NOTCH1 mutation in oral leukoplakia lesions from a Chinese cohort. Based on the calling criteria by the investigators, 60% of the 45 lesions carried at least one NOTCH1 mutation (22), representing the most common genetic alterations in a single gene ever observed in such lesions. It should be noted, however, the data are derived from a relatively small patient cohort with no matched DNA controls and are not validated in independent cohorts. Therefore, the mutation frequency observed in the study should be viewed with caution. Further studies with independent cohorts from similar ethnic background will be needed to validate the finding.

Although malignant transformation rates from oral leukoplakia lesions are likely much lower than 60%, high frequent rates of genetic alterations have been found in such lesions. One example is the frequent deletions at chromosomes 3p and 9p (23–25). Though leukoplakia lesions carrying such deletions may not always develop into OSCC, they do possess a higher probability of malignant transformation. It is therefore possible that the leukoplakia lesions carrying NOTCH1 mutations have a higher risk for malignant transformation. The study reported by Izumchenko and colleagues (22), however, cannot address this issue because of the lack of analysis of patient outcomes, probably due to short follow-up period or incomplete follow-up data. In our study, however, we found that patients whose OSCC carried NOTCH1 mutations had poorer clinical outcomes (21), suggesting that these mutations play a role in OSCC progression.

The existence of an approximately equal portion of activating and inactivating NOTCH1 mutations in OSCC is of particular interest from both etiology and treatment standpoints. The major difference in terms of NOTCH1 mutations between Caucasian and Chinese OSCC is that no activating mutation was observed in any of the few hundred HNSCC tumors analyzed, whereas half of the mutations in the Chinese cohorts are activating mutations. Because the total number of Chinese patients analyzed is relatively small and comes from a relatively narrow geographic region, it is possible that the true frequency of inactivating NOTCH1 mutations in OSCC from ethnic Chinese OSCC is similar to the frequency in OSCC from Caucasian. What sets the two ethnic groups apart is that the activating mutations observed only in OSCC and oral leukoplakia are from ethnic Chinese. Although tobacco is the most common etiologic factor for OSCC in both Caucasian and Chinese, the inherited genetic background and other environmental factors, such as soil, contaminants in water and food styles, all contribute to the ways how oral mucosal is insulted and how the epithelial cells respond to the insults. Both Izumchenko and colleagues (22) and our study (21) cited the higher alcohol concentration in Chinese liquor as a potential differential factor. It will be important to conduct large studies to include all common environmental and inherited genetic factors to determine the potential association with the occurrence of activating NOTCH1 mutations.

It is important to note that, although no activating mutation was observed in OSCC from Caucasian, Sun and colleagues (26) recently showed that one third of HNSCC from Caucasians exhibited the activated NOTCH signaling pathway through over-expression of NOTCH ligands or receptors, which is consistent with frequent NOTCH signaling activation observed in other solid tumors (27–29). Therefore, activation of NOTCH signaling may be more common in OSCC than previously thought, but through different mechanisms dependent on inherited genetic background, etiologic factors, and environmental cofactors. In fact, we found that Presenilin 1 (PSEN1), a key component of γ-secretase, is frequently overexpressed in OSCC from ethnic Chinese (30), providing another possible mechanism to activate the NOTCH signaling pathway in OSCC.

All of this new information is critically important, because it provides a strong rationale to further investigate the complex manifestations of NOTCH signaling changes in oral tumorigenesis. We may have to take a comprehensive approach to understand how the NOTCH pathway is altered in a given tumor or premalignant lesion, which will include the common ligands, NOTCH receptors, molecules critical in NOTCH processing, and the downstream effectors. Tumors or premalignant lesions may be classified based on types of mutations or other alterations in the NOTCH pathway. More importantly, we need to understand how these alterations are triggered and their biologic consequences at different stages of oral tumorigenesis. Given the importance of the NOTCH pathway in cell differentiation and stem cell maintenance, it is likely that alterations of the NOTCH pathway promote oral malignant transformation and progression and, therefore, can serve as targets in preventing and treating OSCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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