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Original contribution

The notch pathway is activated in neoplastic progression in esophageal squamous cell carcinoma☆

Daniel J. Lubin MD[a,*], Rosemarie Mick MS[b], Stuti G. Shroff MD, PhD[a], Kristen Stashek MD[a], Emma E. Furth MD[a]

[a]*Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, PA, 19104*
[b]*Department of Biostatistics and Epidemiology, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA, 19104*

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Summary The Notch signaling pathway is integral to normal human development and homeostasis and has a deterministic function on cell differentiation. Recent studies suggest aberrant Notch signaling may contribute to neoplastic progression by an increase in stem cell survival, chemoresistance, and the promotion of epithelial-to-mesenchymal transition. The goals of our study were to determine, utilizing quantitative technologies, the expression of activated Notch 1 in esophageal squamous cell carcinoma (SCC) and to determine the relationship between Notch 1 expression and various clinicopathologic parameters. Immunohistochemical staining for Notch intracellular domain (NICD) was performed on 60 consecutive cases of esophageal squamous cell carcinoma, 42 cases of benign esophageal squamous epithelium, and 13 cases of eosinophilic esophagitis diagnosed in our department from 2007 through 2015, and exact nuclear staining and nuclear characteristics were graded using the Vectra imaging system. Clinicopathologic data (gender, age at diagnosis, smoking status, tumor grade, tumor stage, tumor location, and survival) were collected for each SCC case and these were correlated with NICD staining. Cases of esophageal SCC demonstrated significantly higher NICD staining compared to cases of benign and reactive esophageal epithelium (P = .003 and .005, respectively). Among cases of esophageal SCC, nuclear NICD staining was significantly correlated with both tumor grade and stage. Following classification and regression tree analysis, esophageal SCC patients with increased NICD expression were found to be more likely to die from their disease than those with lower levels of expression. Taken together, the findings suggest that increased Notch 1 may contribute to the development and aggressiveness of esophageal SCC.

1. Introduction

The Notch signaling pathway is involved in human development and homeostasis, playing a critical role in cell fate through cell-to-cell interaction and maintenance of the stem

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* Corresponding author at: Hospital of the University of Pennsylvania, 6 Founders, 3400 Spruce Street, Philadelphia, PA, 19104.
E-mail address: Daniel.Lubin@uphs.upenn.edu (D. Lubin).

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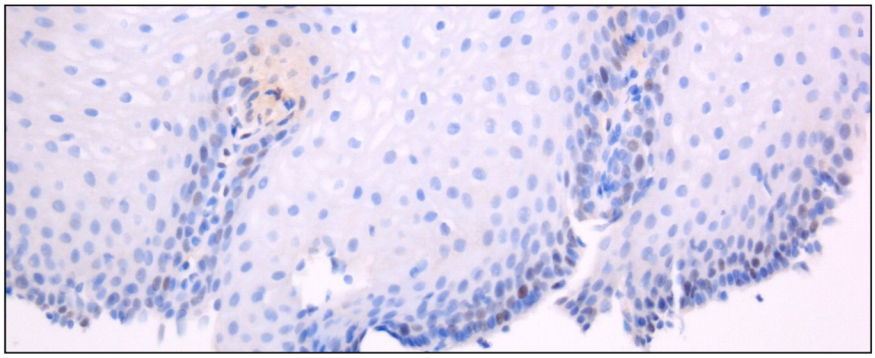

Fig. 1 NICD staining in a case of benign squamous epithelium of the esophagus. NICD, 20×.

cell niche. As a result of this process, referred to as "lateral specification," the fate of a primitive stem cell can dictate that of its immediate neighboring cells. The mammalian Notch pathway consists of four receptors (Notch 1–4) and five ligands, both Jagged-like (Jagged-1, −2) and Delta-like (DLL-1, −3, −4). The Notch receptors are single-pass transmembrane heterodimers composed of extracellular and intracellular domains bound non-covalently by calcium. When the Notch ligand binds to its corresponding Notch receptor, a conformational change allows for cleavage of the receptor within its extracellular domain by TNF-alpha-converting enzyme, which is immediately followed by intracellular cleavage by a γ-secretase complex. This releases the Notch intracellular domain (NICD), which subsequently translocates to the nucleus in order to modulate gene expression.

The Notch gene was discovered in 1917 by Thomas Hunt Morgan, who noted that a "notched" wing adult phenotype of *Drosophila melanogaster* followed an X-linked inheritance pattern [1]. Studying homozygous null *Notch* mutant *Drosophila* embryos in the 1930s, Poulson described a "neurogenic phenotype" which displayed prominent nervous system components at the expense of ectoderm, a phenotype incompatible with life [2]. Given its critical role in modulating stem cell fate and development, it follows that defective Notch signaling should contribute to human disease both in embryologic development and neoplastic progression. Aberrant Notch expression has been implicated in a number of inherited disorders, including bicuspid aortic valve (Notch 1 loss of function), Alagille Syndrome (Notch 2 loss of function), Hadju-Cheney Syndrome (Notch 2 gain of function), and CADASIL (Notch 3 loss of function) [3] and in human cancers.

The goals of our study were to determine, utilizing quantitative technologies, the nuclear expression (ie, the activated form) of Notch 1 in esophageal squamous cell carcinoma (SCC) and to determine the relationship between Notch 1 expression and various clinicopathologic parameters and patient survival.

2. Materials and methods

2.1. Case selection and analysis

Following approval by the Institutional Review Board for the University of Pennsylvania, we performed a database search which identified 60 cases of esophageal SCC, 42 cases of benign esophageal squamous epithelium, and 13 cases of eosinophilic esophagitis diagnosed in our department from 2007 through 2015. All specimens had been fixed in 10% non-buffered formalin and paraffin-embedded. For each case, an immunohistochemical stain for NICD [Cleaved Notch1 (Clone D3B8) (Val1774); Rabbit mAb #4147; 1:20 dilution; Cell Signaling Technology, Danvers, MA] was performed on a tissue section cut at 5 microns. For each run, staining was validated using cell lines HCC 1599-72D as a positive control and HCC 72-GSI as a negative control. The exact nuclear level of staining and nuclear characteristics (size and spindling) were graded using the Vectra imaging system and inform

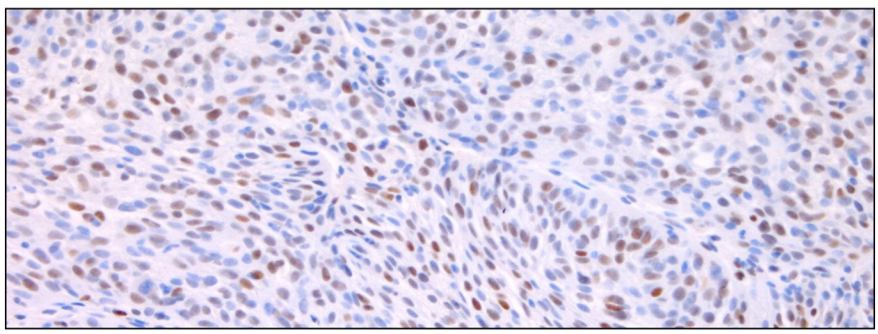

Fig. 2 NICD staining in a case of squamous cell carcinoma of the esophagus. NICD, 20×.