Loss of Inositol Polyphosphate-5-Phosphatase in Development and Progression of Head and Neck Squamous Cell Carcinoma

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Abstract

Objective: Evaluate the expression patterns of inositol polyphosphate-5-phosphatase (INPP5A), a negative regulator of inositol signaling, in the development and progression of head and neck mucosal squamous cell carcinoma (SCCA).

Methods: A total of 40 case subjects of oropharyngeal tumor tissues were evaluated. Each tumor tissue included a section of invasive SCCA along with adjacent normal mucosa for comparison. Additionally, 39 matched lymph node metastases were analyzed in order to identify genetic changes associated with SCCA development. Immunohistochemistry (IHC) was performed to evaluate loss of INPP5A protein levels in primary SCCA tumors when compared to normal mucosa, as well as further loss with metastasis. Formalin fixed, paraffin embedded tissues were sectioned and incubated with anti-INPP5A mouse monoclonal antibody, and subsequently INPP5A protein expression levels were quantified.

Results: Deletions of a region on chromosome 10q harboring the INPP5A gene in cutaneous SCCA have previously been reported. INPP5A protein levels have been found to be frequently reduced in primary cutaneous SCCA tissues and demonstrate an early event in the progression of SCCA with further reduction in metastatic disease. Subsequent validation by IHC on a set of 40 mucosal head and neck SCCA tissues demonstrated reduced INPP5A protein levels in 55% of primary SCCA tumors. Importantly, further reduction of INPP5A levels were seen in 61.5% of matched metastatic lymph node tumors.

Conclusions: Previous studies have shown that INPP5A loss is seen with cutaneous SCCA proliferation. This study provides a comparison of INPP5A protein levels in progressional mucosal oropharyngeal SCCA with that seen in cutaneous SCCA. The observed frequency and pattern of loss indicate that INPP5A, a negative regulator of inositol signaling, may play a role in development and progression of mucosal head and neck SCCA tumors.

Introduction

Head and neck cancer is the 5th most common cancer diagnosed worldwide and the 8th most common cause of cancer death. SCCA is the most common malignancy of the oropharynx, comprising more than 90% of all malignant tumors of that origin. Current clinical prognostic algorithms are suboptimal and therapeutic options for aggressive disease inadequate. Incomplete understanding of the molecular mechanisms leading to the development and progression of SCCA have hindered development of accurate prognostic markers and targeted therapies as well as more effective early chemoprevention strategies.

Development of genomic technologies in recent years has provided unparalleled opportunities for rapid and detailed study of cancer on the molecular level. Use of IHC has significantly impacted understanding of cancer and facilitated better disease classification and development of novel diagnostic and therapeutic approaches. INPP5A belongs to a large family of inositol polyol polyphosphate 5-phosphatases. This 40 kDa membrane-associated type I inositol polyphosphate has preferential substrate affinity for inositol 1,4,5-trisphosphate (Ins(1,4,5)P3) and inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P4). Functioning mostly as a signal-terminating enzyme with implication for several cellular processes, including proliferation. Loss of INPP5A may be linked to cancer development and progression. Recent studies from our institution have noted that the pattern of loss of INPP5A may play a role in development and progression of cutaneous SCCA tumors. We herein demonstrate that marked decrease of INPP5A protein levels is observed in mucosal head and neck SCCA. This event occurs early in the development of SCCA, and progressive reduction of INPP5A levels is seen in a subset of SCCA patients as the tumor progresses from primary to metastatic stage.

Methods

Tissue samples analyzed in this study were formalin fixed paraffin embedded (FFPE) archived specimens obtained under the Institutional Review Board approved protocols at Mayo Clinic Hospital. FFPE tissue blocks were sectioned on glass slides at 5 μm thickness and baked for 60 minutes at 60 °C. Slides were subsequently subjected to heat induced epitope retrieval using a proprietary citrate based retrieval solution for 20 minutes. The tissue sections were incubated for 30 minutes with anti-INPP5A mouse monoclonal antibody, clone 3D8 (Novus Biologicals, Littleton, CO). The sections were visualized with the Bond Polymer Refine Detection kit (Leica Microsystems Inc., Bannockburn, IL) using diaminobenzidine chromogen as substrate.

Results and Discussion

INPP5A Protein Level is Frequently Reduced in Primary SCCA Tissues

A total of 40 case subjects of oropharyngeal tumor tissues were evaluated. Each tumor tissue included a section of invasive SCCA along with adjacent normal mucosa for comparison. Additionally, 39 matched lymph node metastases were analyzed in order to identify genetic changes associated with SCCA development.

Stained slides were evaluated using a standard scoring system based on the intensity of staining (0-3) with score of 0 representing no staining and score of 3 as intense staining. If a relative difference in signal was observed between tissues being compared, it was recorded as a change in INPP5A protein level.

Detection of INPP5A by IHC demonstrated mainly diffuse cytoplasmic signal. A comparison of INPP5A staining intensity between SCCA tissues and matched normal epidermis identified three general staining patterns. The most prevalent pattern of expression, observed in 22/40 (55%) of examined tissues, manifested as a relative reduction of INPP5A in SCCA tissues when compared to matched normal mucosa. Only 18/40 (45%) of examined tissues showed no difference in INPP5A staining between the SCCA and matched normal mucosa. Importantly, no single case was observed where INPP5A staining was more intense in SCCA tumor than in matched normal mucosa, further highlighting the specificity of the observed pattern. Furthermore, the more well-differentiated tumors seemed to retain their staining compared to the more poorly-differentiated tumors. Taken together, a high frequency of INPP5A loss at the protein level notes a mechanism of INPP5A suppression and deregulation.

Loss of INPP5A in Association With Progression to Metastatic Disease

To assess a potential role of INPP5A loss in the process of tumor maintenance and progressions, we queried whether reduction of INPP5A level is associated with the subsequent biological step in SCCA progression, development of metastatic disease. To this end, we evaluated INPP5A protein levels in a cohort of 39 matched lymph node with SCCA tumors that subsequently metastasized from the primary tumor tissue.

IHC analysis of these paired tissues detected further reduction of INPP5A levels in the transition from primary to metastatic SCCA in 24/39 (61.5%) of examined tissue pairs. Though the remaining 15/39 (38.5%) of studied pairs show no further loss of INPP5A levels in transition from primary to metastatic disease, it is important to note that no single case was identified where INPP5A staining was stronger in the metastatic tissue than in the primary SCCA tumor, further highlighting the specificity of the observed INPP5A loss in SCCA progression. These data suggest that reduction of INPP5A levels, although an early event in development of SCCA, may also play a role in progression of SCCA from primary to metastatic disease in a significant subset of aggressive primary SCCA tumors.

Figure 1: Decreased staining in invasive tumor (left) compared to normal mucosa (right).

In summary, we identify loss of INPP5A as an early event in development of mucosal SCCA. Protein levels are reduced in the majority of SCCA tumors. More frequent reduction of INPP5A levels in aggressive primary SCCA tumors, as well as further reduction in metastatic disease, point to a potential role of INPP5A in the development and progression of mucosal SCCA.

Our findings support the previously reported observations that implicate INPP5A as a novel tumor suppressor in other human cancers. Understanding the precise mechanism(s) of INPP5A loss in SCCA and exploring the connection between INPP5A and uncontrolled cellular proliferation in cutaneous and mucosal cancer may provide novel insights into relevant mechanisms of epithelial carcinogenesis and facilitate development of clinically applicable prognostic markers, therapeutic strategies as well as novel chemopreventive approaches.

Conclusions


References


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