The prognostic value of inositol polyphosphate 5-phosphatase in cutaneous squamous cell carcinoma

Helen J. L. Cumsky, BS,[a,b] Collin M. Costello, MD,[a] Nan Zhang, MS,[c] Richard Butterfield, MA,[c] Matthew R. Buras, MS,[c] Jessica E. Schmidt, BS,[a] Kevin Drenner, BS,[a] Steven A. Nelson, MD,[a] Shari A. Ochoa, MS, MD,[a] Christian L. Baum, MS, MD,[d] Mark R. Pittelkow, MD,[a] David J. DiCaudo, MD,[a] Aleksandar Sekulic, MD, PhD,[a] and Aaron R. Mangold, MD[a]

Scottsdale, Arizona; Cleveland, Ohio; and Rochester, Minnesota

Background: Inositol polyphosphate 5-phosphatase (INPP5A) has been shown to play a role in development and progression of cutaneous squamous cell carcinoma (cSCC). The goal of the current study was to explore the prognostic value of INPP5A expression in cSCC.

Methods: A total of 189 cases of actinic keratosis and SCC in 174 patients were identified; clinical and outcome data were abstracted, histopathology was rereviewed, and immunohistochemical staining and interpretation was performed for INPP5A.

Results: The majority of tumors (89.4%) had an INPP5A score of 2 or 3. No patients had complete loss of INPP5A. Tumors with an INPP5A score of 1 were more likely to be intermediate- to high-risk tumors (Brigham and Women's Hospital stage $\geq$T2a 85.0% vs 23.7% [$P < .0001$]) characterized by a larger diameter (2.4 cm vs 1.3 cm [$P = .0004$]), moderate-to-poor differentiation (86.7% vs 17.6% [$P < .0001$]), and perineural invasion (37.5% vs 5.3%, [$P < .0001$]). An INPP5A score of 1 was associated with a worse 3-year survival (a rate of 42.3% [hazard ratio, 2.81, $P = .0006$]) and a local metastasis rate of 48.0% (hazard ratio, 4.71; $P < .0001$).

Conclusions: Low INPP5A scores are predictive of aggressive tumors and may be a useful adjunct to guide clinical management of cSCC. (J Am Acad Dermatol 2019;80:626-32.)

Key words: biomarker; cancer tumor marker; cutaneous squamous cell carcinoma; inositol polyphosphate-5-phosphatase; metastases; outcome; prognosis; prognostic marker; recurrence; squamous cell carcinoma; staging.

More than 2 million nonmelanoma skin cancers are diagnosed annually in the United States,[1] making it the most common cancer in humans and the fifth costliest.[2] Cutaneous squamous cell carcinoma (cSCC) of the skin is the second most common nonmelanoma skin cancer. In recent years, there has been an increased health and economic burden due to cSCC.[2-4] Other than surgery

for early disease, there is currently a lack of therapeutic options for advanced cSCC. Although cemiplimab (an anti-programmed cell death 1 inhibitor) has shown promising results in advanced cSCC, additional therapies are needed, specifically, in the transplant population.[5] Recent research into the genetic landscape of cSCC has demonstrated that several cancer-associated genes are involved in

development of the disease,[6] and as understanding of the genetics of cSCC grows, new and targeted therapies along with molecular diagnostic and prognostic tests can be developed.

cSCC can be staged according to American Joint Committee on Cancer (AJCC), International Union Against Cancer, and Brigham and Women's Hospital (BWH) staging systems. Although the new AJCC guidelines, as well as the recent BWH tumor staging, have improved disease staging, the available approaches remain inconsistent in their ability to accurately predict poor outcomes, including regional and distant metastasis.[7-11] The BWH tumor system staging risk factors include tumor size (≥2 cm), tumor differentiation (poorly differentiated), perineural invasion of 0.10 mm or more, and tumor invasion beyond the subcutaneous fat.[8,9] The BWH tumor staging system demonstrates better distinctiveness, homogeneity, and monotonicity than the AJCC staging system; however, there is a portion of BWH T2a tumors that metastasize, whereas the majority of T2b tumors never have metastatic disease. Therefore, it is important to examine new molecular markers with prognostic significance to guide clinical decision making.

Recently, we discovered that the loss of inositol polyphosphate-5-phosphatase (INPP5A) may play a role in the development and progression of cSCC.[12] INPP5A is a membrane-associated type I inositol phosphatase that has been shown to play a role in development and progression of actinic keratosis (AK) to cSCC and localized oropharyngeal (opSCC) to metastatic disease.[12,13] Loss of INPP5A has been previously linked to cancer development and progression.[14] The goal of the current study was to explore the utility of INPP5A expression in predicting clinical outcomes in cSCC, focusing on tumor characteristics and outcome. We hypothesized that loss of INPP5A expression in cSCC correlates with aggressive primary tumor characteristics and poor outcomes.

METHODS
Subjects and tissue selection
This study was approved by the Mayo Clinic Institutional Review Board. A total of 189 cases of AK, squamous cell carcinoma in situ (SCCIS), and cSCC were identified from our retrospective cSCC database of patients of Mayo Clinic (Jacksonville, Florida; Rochester, Minnesota; and Scottsdale, Arizona). Tissue samples were all selected on the basis of clinical outcomes (locally recurrent, locally metastatic [including nodal and in-transit metastasis], and distant metastasis), histopathologic features (AK, SCCIS, low-risk cSCC, and high-risk cSCC), and tissue availability. Our goal was to examine AK (n = 40) and cSCC arising in AK (n = 40), primary SCC tissue, cSCC without high-risk features (n = 27), cSCC with high-risk features (n = 27), and metastatic and recurrent cSCC (n = 55). Tissue samples were selected consecutively between January 1, 1999, and December 31, 2016; 77.2% were initial biopsy specimens and 22.8% were excisional specimens.

All cases underwent histopathologic rereview (D.J.D. and S.A.N.) and had clinical follow-up data. We assessed INPP5A protein levels by immunohistochemistry (IHC) in AK, SCCIS, cSCC without high-risk features, recurrent cSCC after treatment, and metastatic cSCC. Additionally, age- and stage-matched, nonmetastatic controls were selected for each metastatic case. These controls had the same BWH staging and a minimum of 2 years of clinical follow-up with no documented metastasis. Clinical characteristics including age, sex, immune status, and tumor stage (BWH and AJCC seventh edition) were collected.

IHC and scoring
All samples were stained according to a standardized approach with use of INPP5A primary antibody (clone 3D8, Novus Biologicals, Littleton, CO) at a 1:100 dilution (Supplement 1; available at http://www.jaad.org). INPP5A expression levels were scored on a 4-point scale (0-3) (Fig 1) by consensus diagnosis by a board-certified dermatopathologist (D.J.D. or S.A.N.) and a dermatologist (A.R.M.). Reviewers were blinded to outcome. A score of 3 indicated normal INPP5A expression; a score of 2 indicated partially diminished expression; a score of 1 indicated significantly diminished expression, and a score of 0 indicated complete loss of INPP5A expression. All expression levels were scored by the lowest expression level within the tumor and relative to normal epidermis within

CAPSULE SUMMARY

- Inositol polyphosphate-5-phosphatase has been shown to play a role in cancer development and progression.

- We found that low inositol polyphosphate-5-phosphatase expression is associated with aggressive tumors and poor outcomes. Inositol polyphosphate-5-phosphatase is a novel tumor marker that may help in the management of cutaneous squamous cell carcinoma.

Abbreviations used:	
AJCC:	American Joint Committee on Cancer
AK:	actinic keratosis
BWH:	Brigham and Women's Hospital
cSCC:	cutaneous squamous cell carcinoma
HR:	hazard ratio
IHC:	immunohistochemistry
INPP5A:	inositol polyphosphate-5-phosphatase
opSCC:	oropharyngeal squamous cell carcinoma
SCCIS:	squamous cell carcinoma in situ

the specimen. For cases lacking an internal control, a batch control of normal-appearing epidermis specimens was treated under the same conditions. All clinical and outcome data were obtained through the retrospective cSCC database.

Statistical analysis

Demographics characteristics by patient level and clinical characteristics by patient level and tumor level were summarized and compared between INPP5A groups by using the 2-sample *t* test, Wilcoxon rank sum test, chi-square test, or Fisher exact test when applicable. The overall survival was estimated by using the Kaplan-Meier method and compared between INPP5A groups using log-rank test. Cox regression was used to estimate the hazard ratio for the INPP5A-high (a score of 2 or 3) versus INPP5A-low group (a score of 1). Cumulative incidences of cSCC disease-specific death, local recurrence, local metastasis, and distant metastasis were estimated by using the Fine and Gray method in the presence of a competing risk event (ie, death before any events of interest) and compared by using the Gray k-sample test. A proportional hazard model for the subdistribution of events of interest was used to estimate the hazard ratio for the INPP5A-high versus INPP5A-low group.

RESULTS

Our study population consisted of 174 patients with 189 primary tumors. Its demographics are shown in Table I. The average age of the cohort was 74.9 years (standard deviation, 10.1 years). The population was 68.4% male and 31.6% female. Most patients were white (98.8%), with the rest of the study population being Asian (0.6%) and American Indian/Alaska Native (0.6%). Of those patients with documented Fitzpatrick skin types, 15.4% had type I skin, 69.2% had type II, 13.8% had type III, and 1.5% had type IV. A significant portion of the study cohort was immunosuppressed before occurrence of the cSCC of interest (19.2%). Low INPP5A expression was more common in those who were immunosuppressed (47.4% vs 15.5% [*P* = .0009]).

Tumor characteristics are shown in Table II. The majority of tumors (169 of 189 [89.4%]) had a high level of INPP5A expression (a score of 2 or 3), whereas a minority (20 of 189 [10.6%]) had low INPP5A expression (a score of 1). No patients had complete loss of INPP5A. Initial analyses indicated that tumors with INPP5A scores of 2 or 3 clustered together and were distinct from tumors with an INPP5A score of 1. Subsequent analyses compared these 2 groups. Tumors with low INPP5A expression versus high INPP5A expression were more likely to be intermediate- to high-risk tumors (BWH stage ≥T2a 85.0% vs 23.7% [*P* < .0001]), have a larger diameter (2.4 cm vs 1.3 cm [*P* = .0004]), be more invasive (*P* < .0001), have moderate-to-poor differentiation (86.7% vs 17.6% [*P* < .0001]), and have perineural invasion (37.5% vs 5.3% [*P* < .0001]).

Differences in outcomes were noted between the INPP5A-low and INPP5A-high groups. The overall survival and local metastasis rates for patients who had tumors with low INPP5A expression were significantly worse than those of patients who had tumors with high INPP5A expression (3-year survival rates of 42.3% vs 73.7% [hazard ratio (HR), 2.81; *P* = .0006] and local metastatic rates of 48.0% vs 13.3% [HR, 4.71; *P* < .0001], respectively). There was a trend towards higher rates of disease-specific death with low INPP5A expression (3-year cumulative incidence of 11.0% vs 4.7% [HR, 3.25; *P* = .061]). The rates of local recurrence and distant metastasis were nonsignificant (a local recurrence rate of 29.2% vs 15.5% [HR, 1.86; *P* = .20] and a distant metastasis rate of 6.4% vs 3.3% [HR, 1.77; *P* = .60]).

DISCUSSION

This study identified low INPP5A expression as a predictive tumor marker of aggressive clinical and histopathologic features for cSCC. Additionally, we found that low INPP5A expression predicts poor overall survival and a high rate of local metastasis. The loss of INPP5A has been previously linked to cancer development and progression.[14] Inositol signaling pathways are involved in intracellular calcium release, membrane trafficking, chemotaxis, ion channel activity, and many other nuclear functions.[15] Inositol signaling is highly conserved throughout the animal kingdom and seems to play a key role in gene regulation. The best-known inositol intracellular signaling messengers in this class are inositol triphosphate and diacylglycerol.

Our findings strengthen the previously reported observations that implicate INPP5A as a novel tumor suppressor in human cancers. The loss of the chromosomal region 10q26, which encodes INPP5A, is associated with brain tumors, and
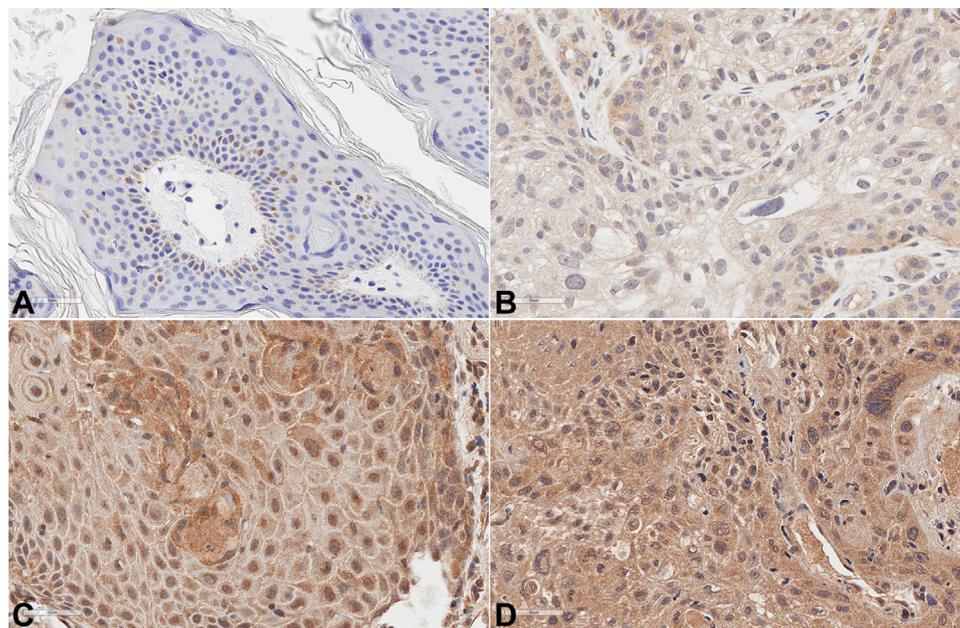
Fig 1. Representative sections of inositol polyphosphate-5-phosphatase (INPP5A) immuno-histochemical stains. **A,** Normal-appearing epidermis (INPP5A score of 0). **B,** Squamous cell carcinoma (INPP5A score of 1). **C,** Squamous cell carcinoma (INPP5A score of 2). **D,** Squamous cell carcinoma (INPP5A score of 3). (**a,** Immunoperoxidase stain incubated without INPP5A primary antibody and **b-d,** INPP5A immunoperoxidase stain; original magnifications: **a-d,** ×400.)

decreased INPP5A activity is associated with human leukemias.[16-20] Prior studies in cSCC found that the *INPP5A* gene was lost in 24% of tumors and had decreased protein expression in 72% of tumors.[12] Additionally, primary cSCC with metastasis showed a loss in 92% of cases.[12] A similar pattern is seen in opSCC, highlighting the importance of INPP5A loss in cSCC and opSCC.[12]

The precise mechanism(s) of INPP5A loss in cSCC and opSCC is unknown, and exploring the connection between INPP5A and uncontrolled cellular proliferation in cutaneous and mucosal cancers will provide novel insights into the molecular genetics of SCC and future therapy development. Inositol hexaphosphate (phytic acid), a downstream product of INPP5A, was found to reduce tumor formation at the initiation phase of carcinogenesis in murine models of cutaneous carcinogenesis.[21] In vitro studies of other cancers such as breast cancer have shown the efficacy of inositol hexaphosphate, both as monotherapy and in combination with chemotherapy in resistant cell lines.[22]

Additional IHC markers have been studied in cSCC. CD133, a cancer stem cell marker, was found to be an independent predictor of poor overall survival (HR, 1.9).[23] The transcriptional coactivator p300 was found to be associated with high-risk

disease and an independent prognostic factor of poor overall survival (HR, 2.4).[24] Low INPP5A expression may be the most promising tumor marker to date, with an HR of 2.71 for overall survival and an HR of 4.71 for local/regional metastasis. Future studies are needed to validate these findings and determine the impact on disease-specific death, local recurrence, and distant metastasis.

Whole exome sequencing and transcriptome analysis of head and neck and advanced cSCC found mutation and variation of expression in the following genes: tumor cell p53 gene (*TP53*), cyclin dependent kinase inhibitor 2A gene (*CDKN2A*), phosphatase and tensin homolog gene (*PTEN*), phosphatidylino-sitol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (*PIK3CA*), Harvey rat sarcoma viral oncogene homolog gene (*HRAS*), notch 1 gene (*NOTCH1*), notch 2 gene (*NOTCH2*), ajuba LIM protein gene (*AJUBA*), caspase 8 gene (*CASP8*), FAT atypical cadherin 1 gene (*FAT1*), lysine methyltransferase 2C gene (*KMT2C*), par-3 family cell polarity regulator gene (*PARD3*), RAS p21 protein activator 1 gene (*RASA1*), interferon regulatory factor 6 gene (*IRF6*), and tumor cell p53 gene (*TP63*).[6,25,26] Markers such as KMT2C were associated with more aggressive tumor behavior. An additional study was performed on lymph node metastasis of cSCC; it identified similar genomic

Table I. Demographics

INPP5A expression level	1 (n = 20)	2 and 3 (n = 154)	Total (N = 174)	*P* value
Age at biopsy of cSCC of interest, y				.3202*
Missing	0	0	0	
Mean (SD)	73.3 (10.6)	75.1 (10.0)	74.9 (10.1)	
Sex				.8693[†]
Female	6 (30.0%)	49 (31.8%)	55 (31.6%)	
Male	14 (70.0%)	105 (68.2%)	119 (68.4%)	
Race				.8754[†]
Missing	0	2	2	
White	20 (100.0%)	150 (98.7%)	170 (98.8%)	
Asian	0 (0.0%)	1 (0.7%)	1 (0.6%)	
American Indian/Alaskan Native	0 (0.0%)	1 (0.7%)	1 (0.6%)	
Fitzpatrick skin type				.7637[†]
Missing	15	94	109	
I	0 (0.0%)	10 (16.7%)	10 (15.4%)	
II	4 (80.0%)	41 (68.3%)	45 (69.2%)	
III	1 (20.0%)	8 (13.3%)	9 (13.8%)	
IV	0 (0.0%)	1 (1.7%)	1 (1.5%)	
Immunosuppressed before cSCC of interest				.0009[†]
Missing	1	6	7	
	9 (47.4%)	23 (15.5%)	32 (19.2%)	
Reason forimmunosuppression (≥1)				.2280[†]
Organ transplant	5 (55.6%)	12 (52.2%)	17 (53.1%)	
Chronic lymphocytic leukemia	2 (22.2%)	2 (8.7%)	4 (12.5%)	
Inflammatory disease and other	2 (22.1%)	9 (39.1%)	11 (34.4%)	
History of skin cancer				.1301[†]
	14 (70.0%)	129 (83.8%)	143 (82.2%)	
History of noncutaneous cancer				.1640[†]
	16 (80.0%)	104 (67.5%)	120 (69.0%)	

cSCC, Cutaneous squamous cell carcinoma; *SD*, standard deviation.
*Determined by chi-square test.
[†]Determined by Wilcoxon test.

aberrations as primary cSCC.[27] High NOTCH1 expression according to IHC was found to be a poor prognostic marker in laryngeal SCC.[28] Overexpression of p53 according to IHC was found to correlate with perineural invasion in cSCC of the head and neck.[29] These paradoxic findings are related to overexpression of the mutated gene, which was not degraded as quickly. However, a study examining Ki67 and p53 in cSCC did not find any correlation with expression and prognosis.[30] Additional genomic studies are needed to identify additional "high-risk" genes as potential biomarkers of disease behavior.

The current staging techniques, namely, the AJCC eighth edition and BWH systems, focus on clinical and histologic risk factors as a predictor of recurrence or metastasis. Both staging systems have defined intermediate-to-high risk as T2 or higher; however, the BWH has further refined this definition with a T2a and T2b designation on the basis of 1 versus 2 or 3 risk factors. The AJCC eighth edition has

made similar changes, with the refinement of T2, T3, and T4 tumors, although these changes are not as well validated as the BWH staging system.[11] The BWH staging system is ideal in that it stratifies low-, intermediate-, and high-risk tumors. T2a tumors (18.3% of all cSCCs) are identified as intermediate-risk tumors with a local recurrence rate of 5%, lymph node involvement in up to 7% of cases, and disease-specific death in 1% of cases.[31] Although T2a tumors are less likely to have poor outcomes, there is a subset of tumors within this group that are at high risk for local recurrence and nodal metastasis. For example, T2a tumors that invade the subcutaneous fat metastasize to the lymph nodes in up to 22% of cases. T2b tumors (4.7% of all cSCCs) are considered high-risk tumors, with a local recurrence rate of 21%, lymph node involvement in up to 30%, and disease-specific death in 10%. T2b has been defined as a group that may benefit from preoperative imaging, a sentinel lymph node biopsy, and postoperative radiation. Unfortunately, 163,000

Table II. Tumor characteristics

INPP5A expression level	1 (n = 20)	2 and 3 (n = 169)	Total (N = 189*)	P value
BWH T stage				<.0001[†]
T0	1 (5.0%)	84 (49.7%)	85 (45.0%)	
T1	2 (10.0%)	45 (26.6%)	47 (24.9%)	
T2a	9 (45.0%)	29 (17.2%)	38 (20.1%)	
T2b	7 (35.0%)	9 (5.3%)	16 (8.5%)	
T3	1 (5.0%)	2 (1.2%)	3 (1.6%)	
Primary tumor diameter, cm				.0004[‡]
Missing	0	8	8	
Mean (SD)	2.4 (1.9)	1.3 (1.3)	1.4 (1.4)	
Primary tumor depth of invasion				<.0001[†]
Missing	15	76	91	
In situ	1 (20.0%)	84 (90.3%)	85 (86.7%)	
Dermis/subcutaneous fat	3 (60.0%)	7 (7.5%)	10 (10.2%)	
Cartilage/muscle/bone	1 (20.0%)	2 (2.2%)	3 (3.1%)	
Primary tumor differentiation				<.0001[†]
Missing	5	67	72	
Well	2 (13.3%)	84 (82.4%)	86 (73.5%)	
Moderate	4 (26.7%)	10 (9.8%)	14 (12.0%)	
Poor	9 (60.0%)	8 (7.8%)	17 (14.5%)	
Perinieural invasion				<.0001[†]
Missing	4	36	40	
	6 (37.5%)	7 (5.3%)	13 (8.7%)	
Location of primary tumor[§]				.2177[†]
Not a high-risk location	16 (80.0%)	151 (89.3%)	167 (88.4%)	
High-risk location	4 (20.0%)	18 (10.7%)	22 (11.6%)	

BWH, Brigham and Women's Hospital; *T stage*, tumor stage.
*The total of 189 primary tumors came from 174 patients; 161 patients had 1 primary tumor, 11 patients had 2 primary tumors, and 2 patients had 3 primary tumors.
[†]Determined by chi-square test.
[‡]Determined by Wilcoxon test.
[§]High-risk locations were defined as the ear and non—hair-bearing lip.

cSCCs are defined as being at intermediate and high risk of poor outcome.

Although determination of whether INPP5A could further risk-stratify these intermediate- and high-risk cSCCs was not a primary end point of our study and although the study was underpowered to make such a determination, we investigated this question by performing a subgroup analysis of T2 (T2a and T2b) and T2a tumors. A nonsignificant trend for worse outcomes was noted in T2 tumors with low INPP5A expression (a 3-year overall survival rate of 32.2% vs 53.3% [HR = 1.9, P = .099] and a local metastatic rate of 54.4% vs 40.4% [HR = 1.61, P = .16]). A similar analysis of T2a tumors found that low INPP5A expression was associated with a worse 3-year overall survival rate (31.7% vs 47.1% [HR = 1.55, P = .37]) and a higher local metastatic rate of 44.4% vs 40.1 % [HR = 1.34, P = .34]). Future studies are needed to determine whether INPP5A can further risk-stratify T2a and T2b tumors by outcome.

Limitations

This was a single-institution study with a predominately white population. Our study was underpowered to detect differences in disease-specific death, local recurrence, and distant metastasis. Despite this, we observed trends in disease-specific death. Larger studies validating these findings together with the integration of INPP5A expression into the current staging system is warranted.

CONCLUSIONS

Low INPP5A scores of initial tumor biopsy specimens are predictive of aggressive cSCC tumors; these tumors are more likely to exhibit high-risk features such as large clinical tumor size, poor differentiation, and perineural invasion and are associated with a poor overall survival and a high risk of local/regional metastasis. Our study demonstrates the potential for INPP5A expression level to be used as an adjunct tumor marker for clinical management of cSCC.

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SUPPLEMENT 1

Tissue sectioning and IHC staining was performed at the Pathology Research Core (Mayo Clinic, Rochester, MN) using the Leica Bond RX stainer for slides stained with INPP5A. Formalin fixed paraffin-embedded tissues were sectioned at 5 microns and IHC staining was performed online. Slides for INPP5A stain were retrieved for 20 minutes using Epitope Retrieval 1 (Citrate; Leica Biosystems; Buffalo Grove, IL, USA) and incubated in Protein Block (Dako; Agilent Technologies; Santa Clara) for 5 minutes. The INPP5A primary antibody (clone 3D8, Novus Biologicals; Centennial, CO) was diluted to 1:100 in Background Reducing Diluent (Dako; Agilent Technologics; Santa Clara, CA) and incubated for 15 minutes. Positive batch controls of normal epidermis were stained in the same manner. Negative controls were done in the absence of primary antibody.

The detection system used was a Polymer Refine Detection Kit (Leica Biosystems; Buffalo Grove, IL). Slides were counterstained online with hematoxylin for five minutes followed by several rinses in 1X Bond wash buffer and distilled water. Once the immunochemistry process was completed, slides were removed from the stainer and rinsed in tap water for five minutes. Slides were dehydrated in increasing concentrations of ethyl alcohol and cleared in 3 changes of xylene prior to permanent coverslipping in xylene-based medium.