Fascin Over Expression is Associated with Dysplastic Changes in Sinonasal Inverted Papillomas: A Study of 47 Cases

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Abstract Sinonasal inverted papilloma (IP) is a primary benign lesion with a tendency for local recurrence. Malignant transformation may develop in up to 15% of cases. Fascin (Fascin 1) is an actin cross-link binding protein required for the formation of actin-based cell-surface protrusions and cell motility. Fascin up-regulation in lung, gastric, breast and hepatobiliary carcinomas correlates with aggressiveness and decreased survival. Here we evaluate immunohistochemical expression of fascin in 47 sinonasal IPs from 34 patients. Fascin over-expression is significantly more common in sinonasal IP with high-grade dysplasia than in those with no dysplastic or low-grade dysplastic epithelium ($P = 0.0001$). No significant change in fascin expression is seen with recurrence. Over expression of fascin in high-grade dysplastic epithelium in IP may be associated with tumor progression and malignant transformation.

Keywords Fascin · Sinonasal inverted papilloma · Dysplasia · Malignant transformation · Immunohistochemistry

Introduction

Sinonasal inverted papilloma (IP) is a benign tumor with a tendency for local recurrence and malignant transformation.

Sinonasal IP comprises 0.5–4% of all nasal cavity and paranasal tumors, and accounts for approximately 10% of all squamous cell carcinoma (SCC) cases [1, 2]. About 40% of patients with SCC-ex- IP will die of the disease within 3 years [3], yet its etiology is still not fully understood. Generally, human papilloma virus (HPV) is demonstrated in approximately one third of IP [4–6]. The incidence of HPV detection increases in IP with the degree of dysplasia; it has been speculated that this may relate to viral integration in dysplastic and transformed IP. Other important etiologic promoters may include cigarette smoke and occupational exposures [7, 8].

Various changes in protein expression have been described in dysplastic and transformed IP including increased matrix metalloproteinase (MMP)-2 and 9 [9], aberrant p53 up-regulated p21, p27, p16, and MIB 1 labeling index [4]. Further, expression of the gene p63, which bears a strong homology to the gene p53, correlates with the proliferation index in IP [10]. It has been described that increased proliferative activity (Ki67) and loss of the expression of basal cell keratin 14 (CK 14) may predict recurrence [11]. Increased epidermal growth factor receptor (EGFR) and transforming growth factor- α (TGF- α) are thought to be early changes in IP transformation [12].

Recently, the association of fascin (fascin 1) has been queried in the context of IP transformation [13]. Fascin was first identified in sea-urchin egg extracts [14] and was identified in human HeLa cells in 1985 [15]. It is one of the actin cross-linking binding proteins required for the formation of actin-based cell-surface protrusions and increased cell motility in various transformed epithelial cells [16, 17]. In normal human tissue, expression of fascin has been seen throughout development in the nervous system, lymphoid tissue, basal layer cells of stratified squamous epithelia, mesenchymal and vascular endothelial

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cells [18]. However, dramatic increases in fascin expression have been noted in certain cancers and lymphocytic disorders [19]. Up-regulation of fascin in many carcinomas correlates with the clinical aggressiveness of tumors and poor patient survival [20].

A recent study suggests that an increase in the expression of fascin is associated with malignant transformation of sinonasal IP [13]. Our goal was to validate these findings by examining fascin expression on 47 sinonasal IP by immunohistochemistry.

Materials and Methods

Forty-seven excisional biopsy specimens of the nasal cavity and paranasal sinuses were obtained from 34 patients over a 7 year period (2000–2007) from two major medical centers. Patients included 26 males and 8 females, ranging in age from 22 to 81 years (Mean: 50.6 years). Twenty six patients were diagnosed with IP for the first time. Eight patients developed recurrent disease, from 1 to 5 recurrences (Table 1). All the tissue was formalin fixed and paraffin embedded. Twelve cases of papillomas represented mixed oncocytic Schneiderian papillomas with inverted papillomas (Table 2). The extent of dysplasia was classified after histological review. The dysplasia was graded according to the standard from the low-grade (mild dysplasia) to the high-grade (moderate dysplasia, severe dysplasia and carcinoma in situ. Of 47 specimens, 12 (25.5%) specimens are no-minimal dysplasia, 17 (36%)

specimens are low-grade mild dysplasia, 12 (25.5%) specimens are high-grade moderate dysplasia, and 6 (13%) specimens are high-grade carcinoma in situ.

Immunohistochemistry staining was performed according to previously published methods, with minor modification [21, 22]. Immunohistochemical staining was performed on 4 µm thick paraffin embedded tissue section using the Streptavidin peroxidase conjugated method with human fascin 1 monoclonal antibody, clone 55 k-2 (1:100 dilution; Neomarkers, Fremont, CA). Appropriate positive and negative controls were performed along with the study cases. Immunoreactivity was scored based on the percentage of positive cells staining (0 = <5–25%, +1 = 26–50%, +2 = 51–75%, +3 = >76%). The immunostain intensity was scored from 0 to +3 (0 = no stain, +1 = light staining intensity, +2 = moderate staining intensity, +3 = strong staining intensity). A weighted fascin score of each specimen was produced by adding the percentage score (0 to +3) to the intensity score (0 to +3). The total score ranged from 0 (in sum of lowest score 0 plus 0) up to 6 (in sum of highest score +3 plus +3).

Statistical analysis was performed by calculation of Chi-square, using a 2 × 2 Chi-square analysis [23].

Results

In normal squamous epithelium, fascin is only expressed in the cytoplasm of the basal layer cells (Fig. 1). Fascin positivity was seen in 26 of 47 specimens from 34 patients

Table 1 Clinical data of 34 patients with 47 samples of sinonasal inverted papilloma

Cases (n) ^a	Age range	Sex (n) ^a	Tumor locations	Recurrences (n) ^b
3	22–62	M(3)	Ethmoid sinus	1
8	36–71	M(6)/F(2)	Maxillary sinus	3
1	57	M	Sphenoid sinus	1
1	72	M	Frontal sinus	1
20	30–81	M(14)/F(6)	Nasal cavity	8
1	58	M	Unspecified site	
<i>Total</i>				
34	22–81 (mean: 50.6)	M(26)/F(8)		14

^a (n) Number of cases

^b Number of cases had history of recurrences, the highest recurrences was up to 5 times

Table 2 Fascin scores and Schneiderian papillomas by category

Fascin Scores	Score 0 (n) ^a	Score 1 (n) ^a	Score 2	Score 3 (n) ^a	Score 4	Score 5	Score 6	Total
No dysplasia	8 (5) ^a	2 (1) ^a	0	2 (2) ^a	0	0	0	12
Low-grade mild dysplasia	3 (1) ^a	3 (2) ^a	0	6 (1) ^a	1	2	2	17
High-grade dysplasia	2	3	0	0	0	4	3	12
High-grade and carcinoma-in-situ	0	0	0	0	0	0	6	6
								47

^a (n) Number of oncocytic Schneiderian papillomas mixed with inverted papillomas

Fig. 1 Sections of sinonasal inverted papilloma with minimal basal cell atypia (**a** HE stain) Immunostain **b** shows no expression of Fascin (score: 0). Insert shows a closer view

Fig. 2 Sections of sinonasal inverted papilloma with low-grade dysplasia (Mild dysplasia **a**). Immunostain for Fascin shows score 1(**b**)

(55%). The Fascin immunostaining positivity was determined by dark brown cytoplasmic stain in dysplastic cells, which is corresponding to the extent of dysplasia (Figs. 2, 3, 4). The distribution of final scores is summarized in Table 2. We collapsed the four categories into two: low-grade and no dysplasia versus high-grade dysplasia plus carcinoma in situ (Table 3). Cut-offs of either 3 and 4, or 4 and 5, were significant associated with high-grade dysplasia/carcinoma in situ ($P = 0.0005$ and 0.0001 , respectively, Fischer exact test).

In those cases of oncocytic Schneiderian papillomas mixed with IP, the Fascin positive staining was minimal and/or weak, scores ranging from 0 to 3, corresponding to their morphology, which exhibited no dysplasia or low grade dysplasia (Tables 2, 3). The majority of IP with low-grade dysplasia had scores less than 3, while IP with high-grade dysplasia /carcinoma in situ had scores between 4 and 6 (Table 2).

Table 3 Grouped Fascin scores and Schneiderian papillomas by collapsed category

Groups	Score ≤ 3 (n) ^a	Score = 4	Score ≥ 5
No dysplasia and LG dysplasia	24 (12) ^a	1	4
HG dysplasia and carcinoma in situ	5	0	13

^a (n) Number of oncocytic Schneiderian papillomas mixed with inverted papillomas

We studied 13 recurrences from 8 patients. One high-grade IP had 5 recurrences, and we examined 3 of these specimens. The original score was 5, and the recurrent samples had scores of 5 or 6. Other recurrent samples from low-grade groups including oncocytic Schneiderian papillomas had the same fascin scores, compared with the index lesions.

Discussion

We found significantly higher fascin expression scores in IP with high-grade dysplasia/carcinoma in situ. Wang and colleagues studied [13] 45 IP with dysplasia and 21 SCC-ex-IP. They scored cases according to fascin intensity and distribution, and found that increased and intense fascin expression was significantly associated with transformation.

Fascin over-expression is also seen in other neoplasms. For example, patients with intrahepatic biliary dysplasia have presented with fascin over-expression. It is usually expressed early in biliary carcinogenesis and might contribute to poor differentiation and to growth of intrahepatic cholangiocarcinoma [24]. Also, in thyroid neoplasms, fascin immunostaining was positive in 62% of thyroid carcinomas and 26% of thyroid adenomas. In thyroid papillary carcinoma, up-regulation of fascin was associated with both the Ki-67 labeling index and the occurrence of lymph node metastasis [25]. Fascin expression is tightly regulated during development of colon cancer metastases

[26]. High fascin immunostaining scores were significantly associated with advanced dysplasia in colorectal adenomas. Higher fascin scores were related to more advanced colorectal carcinomas [27, 28].

In renal cell carcinoma, higher extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN, also known as CD147) and fascin immunostain scores were associated with shorter survival times and correlated significantly with more advanced TNM stage [29]. Significant increase of matrix metalloproteinase (MMP)-2 and 9 were also observed in IP with moderate and severe dysplasia [9]. Fascin over-expression in high-grade dysplastic epithelium in sinonasal IP may also correlate to EMMPRIN.

Fascin over-expression is associated with increased invasiveness of carcinomas in the urinary bladder [21, 30], where benign proliferative lesions such as nephrogenic adenoma, IP, and exophytic papilloma were negative for fascin [21]. Its over-expression is seen more often in dysplastic epithelium in sinonasal IP, and may be organ specific. In our study, the fascin positive scores correspond

Fig. 3 Sections of sinonasal inverted papilloma with low to high-grade dysplasia (Mild to moderate dysplasia, Score: 1 to focally 3, see insert)

Fig. 4 Sections of sinonasal inverted papilloma with diffuse high-grade dysplasia (Severe dysplasia/Carcinoma in situ, a). Immunostain for Fascin shows diffusely positive staining with score 3 (b)

to the percentage of immunoreactivity in the dysplastic cells. Increasing the percentages of fascin immunoreactive cells correlates to the extent of dysplasia (Figs. 1, 2, 3, 4).

In summary, fascin over-expression is often seen in dysplastic epithelium in sinonasal IP, which corresponds to the extent of morphologically neoplastic change. Increased fascin in IP may be associated with tumor progression and malignant transformation. Fascin may play a role in early malignant transformation.

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