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Enhanced Podoplanin Expression in Chronic Maxillary Sinusitis

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Abstract. *Podoplanin expression has been reported in oral squamous epithelium, myoepithelia of the salivary glands, and odontogenic lesions, and has been linked with inflammatory and neoplastic conditions. We hypothesized that inflamed respiratory mucosa of the maxillary sinus also express podoplanin, especially in cases with odontogenic sinusitis. We retrospectively investigated podoplanin expression in biopsies from maxillary sinus with inflammatory changes. Cases with chronic rhinosinusitis with polyp formation (n=5), chronic rhinosinusitis without polyps (n=5), chronic rhinosinusitis with eosinophilia (n=5), and odontogenic chronic rhinosinusitis (n=5) were investigated immunohistochemically using an established antibody for podoplanin (D2-40). Respiratory epithelium in chronic maxillary sinusitis with polyp formation did not exhibit enhanced podoplanin expression. However, D2-40 positivity was detected in the basal cells in all cases with chronic sinusitis associated with inflammatory infiltrations as well as in the parabasal epithelial layer in chronic sinusitis without polyp formation. We observed podoplanin expression in non-neoplastic maxillary sinus epithelium exhibiting inflammatory changes. We suggest that podoplanin is involved in the pathogenesis of chronic rhinosinusitis, particularly in the intraepithelial migration of inflammatory infiltrates.*

Podoplanin expression has been observed in oral mucosa and was associated with both neoplastic (1-10) and inflammatory (5, 11) conditions. Besides oral squamous epithelium, myoepithelial and acinar (12) cells of non-neoplastic salivary glands exhibited a positive reaction for D2-40 antibody to podoplanin. Furthermore, epithelia from tooth germ tissues (13, 14), odontogenic tumours (14-17) and oral cysts (18, 19) also displayed a positive reaction for podoplanin. Notably, inflamed gingiva (11) and radicular cysts (14) were also reported to be immunohistochemically positive for D2-40. Based on the anatomic proximity of oral structures to the maxillary sinus, we wished to investigate podoplanin expression in sinonasal biopsies obtained from the maxillary sinus. We hypothesized that chronic inflammation of the sinonasal respiratory epithelium is associated with podoplanin expression, particularly in cases with odontogenic rhinosinusitis.

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Materials and Methods

Study participants. We retrospectively searched the databases of the Institute of Pathology, University Medical Centre Hamburg-Eppendorf for cases of chronic rhinosinusitis diagnosed from January 2012 to July 2012. All 20 selected patients were adults operated on by the two senior Authors of the present study (RK, RF). Informed consent was obtained from the patients in accordance with German legal requirements. Paraffin-embedded specimens were available for all cases. All archival slides were stained with hematoxylin-eosin.

Histopathological criteria. Four non-neoplastic mucosal lesions of the maxillary sinus mucosa were chosen for further investigation. Histopathological diagnoses were defined in accordance with the EP3OS 2007 (20) criteria for rhinosinusitis. Chronic rhinosinusitis with nasal polyps was defined by moderate proliferation of the respiratory epithelium with increased number of goblet cells and subepithelial plasma cell and lymphocyte infiltration but with conspicuous edema or fibroblastic proliferation of the submucosal stroma, leading to the formation of mucosal polyps. Chronic rhinosinusitis without nasal polyps was characterized by moderate proliferation of the respiratory epithelium with an increased number of goblet cells and subepithelial plasma cells, and lymphocyte infiltration. Neither excessive edema nor fibroblastic proliferation of the submucosal stroma was observed. Chronic rhinosinusitis with eosinophilic leukocytes was defined as goblet cell hyperplasia, basement membrane thickening, subepithelial edema, focal hyperplasia of mucous glands and conspicuous subepithelial infiltration of eosinophilic granulocytes. No fungal organisms were detected microscopically. Odontogenic chronic rhinosinusitis was characterized by respiratory mucosa exhibiting severe plasma cell and lymphocyte infiltration of the subepithelial tissue associated

with epithelial proliferation. Every case was also associated with the presence of a severe inflamed tooth and/or periodontal tissue. Eosinophilic leukocytes were scarce and thickening of the basal membrane was not present.

Immunohistochemistry. From the archival paraffin-embedded tissues, freshly-cut sections were prepared for immunohistochemical analysis. Sections were deparaffinized and heated in the manufacturer's recommended unmasking solution at 95˚C for 20 min. The endogenous peroxidases were quenched with 0.3% H_2O_2 in PBS. Sections were incubated overnight at 4˚C using anti-podoplanin (clone D2-40; SIGNET, Dedham, MA, USA). Following this incubation, sections were incubated with Envision® system (DAKO Cytomation, Glostrup, Denmark) for 30 min at room temperature. Submucosal lymph vessels served as a positive control for the immunohistochemical reaction. Staining each sample without adding anti-human primary antibody was performed as a negative control. Finally, samples were incubated with diaminobenzidine peroxidase substrate to give a brown stain and counterstained with haematoxylin and mounted with coverslips. The intensity of the staining was graded as absent, weak, mild, moderate and strong.

Results

Chronic rhinosinusitis with nasal polyps exhibited minimal to moderate proliferation of the respiratory epithelium and loose fibrosis of the submucosal stromal tissue (Figure A). Besides proliferated fibroblasts, scattered lymphocytes were also seen in the submucosa. Although the epithelium was negative for reaction to D2-40 antibody, the submucosal lymph vessels exhibited a positive reaction (Figure B). Chronic maxillary sinusitis without polyp formation displayed conspicuous hyperplasia of goblet cells and both basal and parabasal epithelial cells. Furthermore, moderate to severe inflammatory infiltration with lymphocytes, plasma cells and neutrophils, as well as scattered eosinophilic granulocytes, was found within the subepithelial stroma (Figure C). Podoplanin was expressed in the enlarged basal epithelial cells and focally in parabasal epithelial cells (Figure D). Cases of chronic rhinosinusitis with eosinophilic leukocytes exhibited slight proliferation of goblet and basal cells. Moreover, mild to moderate thickening of the basal membrane was observed, in addition to moderate to severe edema of the deeper submucosal stroma. Inflammatory infiltration consisted of many eosinophilic leukocytes and only few plasma cells and scattered lymphocytes (Figure E). Strong membranous positive reaction to D2-40 was observed in non-enlarged basal epithelial cells (Figure F). Odontogenic maxillary sinusitis displayed mild epithelial proliferation associated with severe submucosal infiltration with plasma cells and lymphocytes, with a few neutrophilic granulocytes (Figure G). This soft tissue inflammatory infiltrate extented into the periradicular tissues associated with severely impacted teeth. Immunohistochemically, moderate to strong reaction to D2-40 was present in the basal layer epithelial cells with scattered negative basal cells (Figure H).

Discussion

Even though podoplanin expression was originally linked to neoplastic transformation of the oral squamous epithelium (1-7), it was also detected in inflammatory lesions of oral (5, 11) and gingival epithelium (11), as well as in radicular cysts (14). Therefore, we hypothesized there would be similar morphological changes of the maxillary sinus epithelium, particularly in those cases with propagation of the inflammatory process from inflamed tooth or periodontal tissues into the maxillary sinus. To ascertain whether the inflamed sinonasal mucosa expresses podoplanin, we retrospectively investigated a small cohort of archival morphologically well-defined lesions from the maxillary sinus, using an established antibody for podoplanin (D2-40). In the present study, we observed a podoplanin-positive reaction of the basal cells within the respiratory epithelium in all cases with chronic rhinosinusitis associated with inflammatory infiltration of the submucosa. Severe chronic rhinosinusitis without polyp formation exhibited enhanced podoplanin expression in both enlarged basal and parabasal epithelial layers. Interestingly, the cases of chronic sinusitis with polyp formation that were not associated with conspicuous inflammatory infiltration were podoplanin-negative. While podoplanin was detected in the inflammatory lesions of the maxillary sinus epithelium, every precaution should be taken in the consideration of the malignancy of the immunohistochemically-positive lesions. It can be suggested that coordination of podoplanin expression plays a substantial pathogenetic role in epithelial-mesenchymal adhesion and inflammatory cell migration. Although some authors (21-23) have recently reported on the association of podoplanin expression with directional cell migration of cancer cells, its role in non-neoplastic processes has not yet been explored in more detail on the molecular level. Our study, however, clearly demonstrated podoplanin expression in a non-neoplastic setting in the maxillary sinus epithelium. We recognise several limitations to our study. The present study was designed as a retrospective observative study and the materials used for the histopathological analysis were retrieved from the archive of our Institution. Therefore, we were not able to correlate our results with patient's quality of life (such as Medical Outcomes Study Short Form 36 or Sinonasal Outcome Tests). Furthermore, neither microbiological results for free-floating planktonic bacteria or fungi in chronic rhinosinusitis nor data on aspirin medication were available, but both factors play substantial roles in the pathogenesis of chronic rhinosinusitis. Data on the smoking status of the patients from our study were also unavailable. In conclusion, we detected podoplanin expression in chronic inflamed sinonasal mucosa of the maxillary sinus. We suggest that podoplanin is involved in the pathogenesis of chronic rhinosinusitis, particularly in the intraepithelial migration of inflammatory infiltrates.

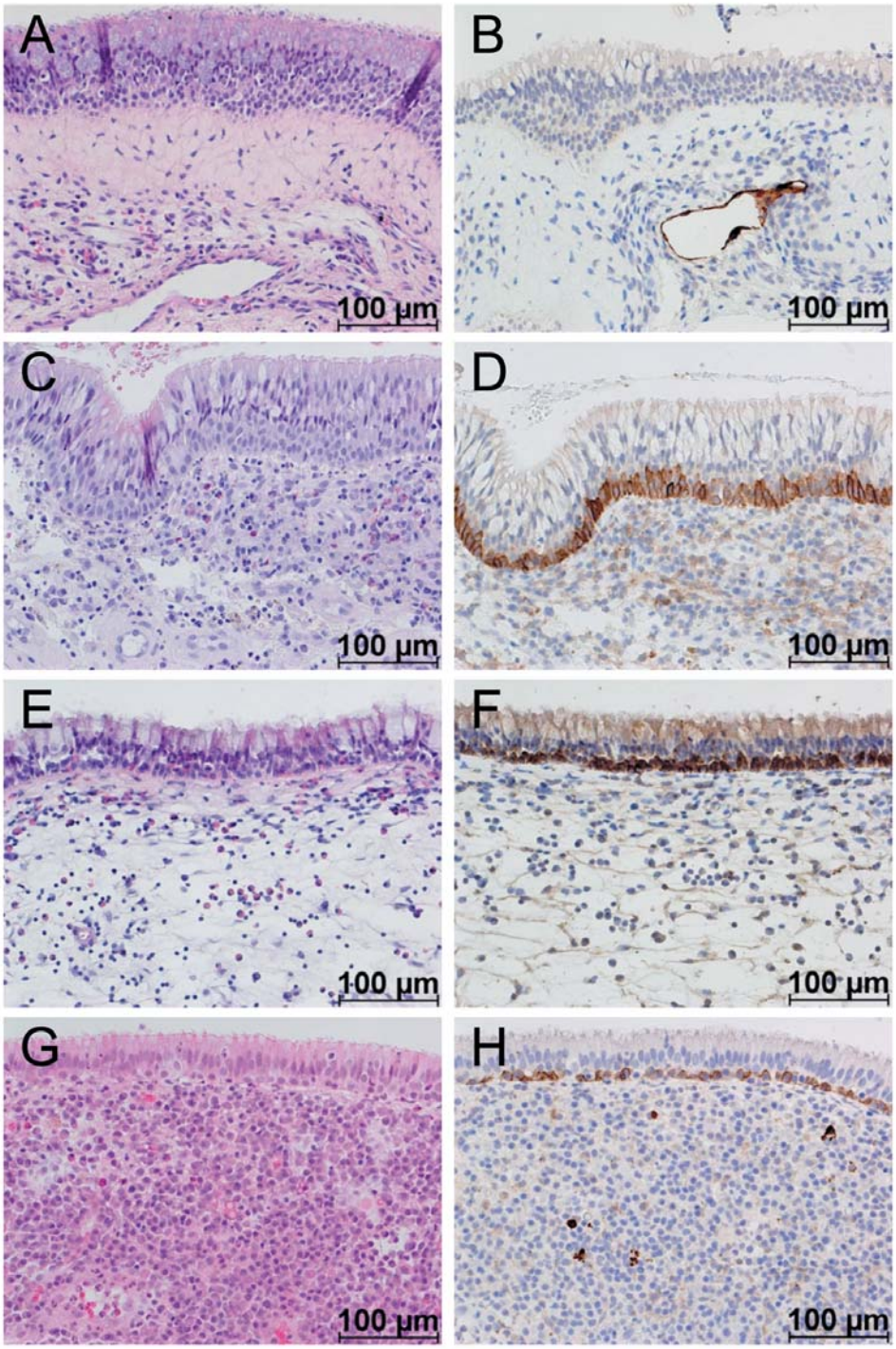

Figure 1. *Podoplanin expression in inflammatory lesions of the maxillary sinus epithelium (histopathology). A: Chronic rhinosinusitis with polyp formation displaying proliferated epithelium and fibrous stroma with mild inflammatory infiltration. B: Podoplanin expression was observed exclusively in lymph vessel endothelium. C: Chronic sinusitis without polyp formation was associated with more conspicuous inflammatory infiltration and strong podoplanin expression in the enlarged basal cells with focal extension also into the parabasal layer (D). E: Chronic maxillary sinusitis with eosinophilia showing mild epithelial proliferation, slight thickening of the basal membrane and moderate infiltration of eosinophilic leukocytes within edematous loosened subepithelial stroma. F: Strong podoplanin expression was seen in the basal cell layer in chronic maxillary sinusitis. G: Odontogenic maxillary chronic sinusitis displayed dense subepithelial infiltration by plasma cells, lymphocytes, neutrophils and a few eosinophile leukocytes. H: Podoplanin expression was seen in the basal cell layer in odontogenic maxillary chronic sinusitis (A,C,E,G: hematoxylin-eosin; B,D,F,H immunohistochemistry: D2-40; original magnification: ×200).*

Conflicts of Interest

We declare that we have no conflicts of interest (either financial or personal) in regard to this study. This work has not been published or presented (oral, print or online) elsewhere in whole nor in part.

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