Human Pathology (2008) **39**, 350–358

ELSEVIER

Human PATHOLOGY

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Original contribution

Nasal inverted papilloma expresses the muscle segment homeobox gene Msx2: possible prognostic implications

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Received 9 February 2007; revised 23 May 2007; accepted 19 June 2007

Keywords:
Inverted papilloma;
Msx2;
Recurrence;
Resorption

Summary Nasal inverted papilloma is a rare benign tumor of epithelial origin with aggressive evolution, bone destruction, recurrence, and malignant transformation. Msx2 is a homeobox gene implicated in organ development, bone metabolism, and tumorigenesis. Using reverse transcriptase–polymerase chain reaction and immunohistochemistry, Msx2 expression was examined in nasal inverted papilloma and in nontumorigenic tissue counterparts. For the first time, Msx2 was detected in all inverted papillomas but not in the nasal polyps or in the normal mucosa. The protein expression level was directly and significantly associated with tumor recurrence. Furthermore, Msx2 was associated with bone resorption markers receptor activator of nuclear factor-kappa B ligand and tartrate-resistant acid phosphatase, suggesting a role in osteolysis. In conclusion, Msx2 expression may represent a useful prognostic marker in inverted papilloma.

1. Introduction

Inverted papilloma (IP) of the nasal cavity and paranasal sinuses is a rather rare benign tumor, representing only 0.5% to 4% of all nasal neoplasms [1].

An IP most commonly arises from the lateral nasal wall, especially from the middle turbinate and middle meatus. The involvement of maxillary and ethmoid sinuses usually represents a secondary extension of the tumor from the lateral nasal wall. This tumor is rarely multicentric and bilateral [2].

The etiology of inverted papilloma remains uncertain. However, occupational exposure, allergy, chronic inflammation, and viral infection, notably with human papilloma viruses 6B, 11, 16, and 18, have been proposed [3]. The characteristic microscopic feature is the increase in thickness of the covering epithelium with extensive invasion into the underlying connective tissue stroma. The tumor is considered a true epithelial neoplasm with patchy squamous metaplasia and numerous microcysts containing macrophages. Inverted papilloma is associated with squamous cell carcinoma in 10% to 15% of the cases, though the proven malignant transformation rate in inverted papilloma varies between 2% and 6% [4]. These tumors show a high

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doi:10.1016/j.humpath.2007.06.017

recurrence rate of almost 60%. They are locally aggressive, causing intensive bone resorption. Consequently, the management of inverted papilloma may vary from conservative to more radical en bloc resection of the tumor combined with medial maxillectomy.

Homeobox genes represent a large family of developmental regulatory genes that have diverse activities during embryogenesis. The biologic functions of many homeobox genes have been elucidated through loss- and gain-of-function analyses, which have demonstrated their essential roles in controlling cellular proliferation and differentiation during development. However, the molecular mechanisms by which homeoproteins mediate these activities remain largely unknown. The vertebrate muscle segment homeobox (Msx) gene family contains 3 members, 2 of which (Msx1 and Msx2) have been well studied with respect to their expression patterns and biochemical properties (reviewed in reference [5]). These genes encode closely related homeoproteins that function as transcriptional repressors through G/CTAATTG motif recognition in target gene promoters and through interactions with components of the core transcription complex as well as other homeoproteins [6,7].

Msx2 has been implicated in the regulation of craniofacial bone metabolism and in organ development including tooth, hair, and submandibular salivary gland [8-10]. This gene is also implicated in epithelial mesenchymal interactions during development, and it was shown to regulate cell proliferation, differentiation, and survival. Several tumor cell lines were found to express Msx2, a fact that made some authors suggest an oncogenic role for this gene. Msx2 was reported in some tumors including osteosarcoma, fibrous dysplasia, myositis ossificans, and epithelial breast tumors [11,12]. Taken together, these data led us to investigate the potential involvement of Msx2 in the pathogenesis of inverted papilloma. The present study describes for the first time the expression of the transcription factor Msx2 in nasal inverted papilloma and attempts to correlate this expression to different clinicopathologic parameters of the patients.

2. Materials and methods

2.1. Patients

The present study is based on the analysis of unfixed frozen tissues from 12 inverted papilloma cases as well as 10 controls made of 7 polyposes and 3 clinically normal nasal mucosae (from patients operated on for plastic nasal surgery). All the tissues were obtained with the signed consent of the patients. The study was conducted in accordance with French bioethical rules. All the patients were admitted at the Department of Otolaryngology and Head and Neck Surgery, Bichat Hospital (Paris, France) between May 2003 and May 2007. The biopsy specimens were obtained from the patients during surgical dissection under general anesthesia. Fragments of approximately 5 mm^3 were cut from the lateral side of each tumor and immediately snap-frozen in liquid nitrogen. These fragments usually included both surface and deep fronts of the tumor. The remainder of the biopsy material was fixed in 10% formol saline solution and processed for routine histopathologic examination in the Department of Pathology, Bichat Hospital. The frozen tissue was cut into 2 equal parts; the first was used for immunohistochemistry and the second for RNA extraction. The diagnosis reported by the pathologist was confirmed on the frozen tissues by staining the first and last sections cut from each fragment used in the study. Serial sections (6 μm thick) were obtained from each tumor sample using a cryomicrotome (LEICA CM3050S; Leica Microsystemes SAS, Rueil-Malmaison, France); the sections were then air-dried and stored at −80 °C until use.

The patients included in the study ranged in age from 41 to 68 years (mean, 51.5 years), and the male-to-female ratio was 17:5 (Table 1). The patients with inverted papilloma (cases 1 to 12) were 1 woman and 11 men, with a mean age of 48.9 years. All patients experienced unilateral nasal obstruction. Extension to the neighboring sinuses occurred in 8 patients, and another patient had his nasal septum invaded (case 1 in Table 1). Sinus extension was found in the maxillary in 6 cases and in the ethmoid in 2 cases. Seven patients had history of nasal inverted papilloma during the preceding 24 months. The patients with polyposis (cases 13 to 19) were 3 women and 4 men, with a mean age of 54.5 years. Three patients presented with bilateral polyps of the nasal cavity. One of them had 2 previous interventions for inflammatory polyps (case 15). Cases 13, 14, and 18 had extensions to the maxillary and/or to the ethmoid sinuses and were suspected as inverted papilloma. The tissues from cases 20 to 22 presented histologic aspects of a normal respiratory mucosa, and the patients had no relevant medical history.

2.2. Immunolocalization

A standard indirect immunofluorescence method was used for the detection of Msx2 and the receptor activator of nuclear factor-kappa B ligand (RANKL). Tissue labeling was performed using TRANCE anti-RANKL, R&D Systems (Lille, France), and H70 anti-Msx2 amino terminal domain from Santa Cruz. Reaction sites (Le Perray en Yvelines, France), were detected with the appropriate secondary antibodies labeled with Alexa 594 or Alexa 488 from Molecular Probes (Cergy, France). All antibody incubations were made at 24°C for 1 hour in a moist chamber. No staining was obtained when nonimmune serum was used instead of the primary antibody. Mice skin served as a positive control for Msx2 expression in hair follicles.