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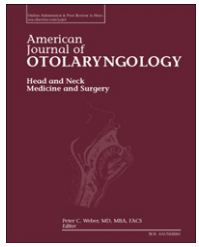

Postoperative radiotherapy for laryngeal carcinoma: the prognostic role of subcellular Maspin expression

Marco Lionello, MD[a,*], *Stella Blandamura, MD*[b], *Claudia Staffieri, MD, PhD*[c], *Giulia Tealdo*[a], *Luciano Giacomelli, BD*[b], *Rosario Marchese Ragona, MD*[a], *Cosimo de Filippis, MD*[a], *Alberto Staffieri, MD*[a], *Gino Marioni, MD*[a]

[a] *Department of Neurosciences, Otolaryngology Section, University of Padova, Padova, Italy*
[b] *Department of Medicine, DIMED, University of Padova, Padova, Italy*
[c] *Department of Neurosciences, Otolaryngology Section, Treviso Branch, University of Padova, Treviso, Italy*

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ABSTRACT

Purpose: Reported outcomes of postoperative radiotherapy (PORT) for laryngeal squamous cell carcinoma (LSCC) have varied and sometimes been disappointing. The aim of the present preliminary study was to investigate whether a given immunohistochemical pattern of Maspin expression in laryngeal carcinoma cells could be prognostically associated with response to PORT.
Materials and Methods: Thirty-two consecutive patients treated for LSCC with primary surgery and PORT. The subcellular (nuclear vs non-nuclear) pattern of Maspin expression was assessed immunohistochemically on LSCC surgical specimens and analyzed in relation to recurrence rate (RR) and disease-free survival (DFS).
Results: A non-nuclear Maspin expression was found in 23 of 32 cases (72%), and all recurrences (17 cases) occurred in this subgroup of patients. A non-nuclear Maspin expression was strongly associated with recurrence [p = 0.0002, hazard ratio (HR) 5.58] and a shorter DFS (p = 0.0004) after PORT for LSCC. Even in N0 patients, a non-nuclear Maspin expression was associated with a significantly higher RR (p = 0.04, HR 1.42) and a shorter DFS (p = 0.02). Among the common clinic-pathological parameters considered, only N stage showed a trend toward an association with prognosis in terms of DFS (p = 0.08).
Conclusion: Assessing subcellular patterns of Maspin expression in LSCC specimens could identify patients less likely to respond to PORT, who might benefit from combined chemo-radiotherapy to improve the efficacy of adjuvant protocols.

1. Introduction

Current indications for postoperative radiotherapy (PORT) in patients with laryngeal squamous cell carcinoma (LSCC) include several adverse tumor features, such as positive margins, pT3 or pT4 primary disease, selected N2 or N3 nodal disease, extracapsular nodal spread, and vascular, lymphatic or perineural invasion [1]. Although these

* *Corresponding author at:* Otolaryngology Section, Department of Neurosciences, University of Padova, via Giustiniani 2, 35128, Padova, Italy. Tel.: +39 049 8212010; fax: +39 049 8213113.
E-mail address: marcolionello@email.it (M. Lionello).

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guidelines are widely accepted, reported outcomes after PORT have varied quite considerably and sometimes been disappointing. There is a need for new parameters to help us identify patients at higher risk for recurrence after PORT, who might benefit from combining different adjuvant treatments (ie, chemotherapy and/or molecular target therapy) with conventional radiotherapy.

In recent years, improved diagnostic methods and advances in our understanding of tumor biology have led to the identification of subcellular pathways and specific molecules with key roles in tumor growth. Maspin is a member of the serine protease inhibitor/non-inhibitor (serpin) superfamily. Its gene is located on chromosome 18q21.3–q23, and was first described as a tumor suppressor in human breast cancer in 1994 [2]. Maspin reportedly inhibits tumor progression in several human malignancies [3] by regulating cell adhesion, motility, invasion, metastasis, and neoplastic angiogenesis and by inhibiting angiogenesis [4–6]. It was recently demonstrated that Maspin is located mainly in the nucleus of normal epithelial tissues [7], while a shift in its subcellular localization from the nucleus to the cytoplasm is considered an early marker of tumor progression [8]. There is evidence of Maspin sometimes having a pro-apoptotic effect [9], and it has been suggested that this may also apply to head and neck cancer [10], and to LSCC in particular [11]. To our knowledge, there is shortage of information on the possible role of Maspin in regulating tumor cell response to radiation-induced insult, and few clinical studies have dealt with the prognostic significance of Maspin in head and neck carcinoma patients treated with radiotherapy [12–16]. Our group has thoroughly investigated the prognostic role of different subcellular patterns of Maspin expression in LSCC [17,18,11,19–21], but the part played by Maspin in regulating response to radiation treatment in LSCC patients remains to be seen.

The aim of the present preliminary study was to investigate whether a given pattern of Maspin expression could be prognostically associated with response to PORT in a cohort of consecutive LSCC patients treated with primary surgery followed by radiotherapy.

2. Materials and methods

2.1. Patients

The study was approved by the internal committee of our Otolaryngology Section and was conducted on 32 consecutive LSCC patients (27 men and 5 women, mean age 67.3 ± 9.2 years). For all patients, the preoperative workup consisted in micro-laryngoscopy with laryngeal biopsy, upper aerodigestive tract endoscopy, esophagoscopy, neck ultrasonography (with or without fine needle aspiration cytology), contrast-enhanced computerized tomography and/or magnetic resonance imaging of the head and neck, chest x-rays, and liver ultrasonography. All patients underwent primary laryngeal surgery, performed by the same surgical team (at the Otolaryngology Section, Padova University), followed by radiation treatment according to currently-accepted indications for PORT [1]. Based on the 2009 TNM classification published by the Union Internationale Contre le Cancer (UICC) (7th edition) [22], the pathological stage of the primary laryngeal lesions (pT) was T1 in 1 case, T2 in 8, T3 in 14, and T4 in 9; for the regional lymph nodes (pN), it was N_0 in 13 cases, N2 in16, and N3 in 1; neck dissection was not performed in 2 patients. No distant metastases (M) were detected at diagnosis. As for pathological grade, 4 of the 32 cases were G1, 17 were G2, and 11 were G3. The mean follow-up was 53.6 ± 36.7 months.

2.2. Radiotherapy

All 32 patients received external beam radiation therapy postoperatively using the same 6 MV photon beam delivered from a linear accelerator, at the Radiotherapy and Nuclear Medicine Unit, Istituto Oncologico Veneto, Padova. Patients were first immobilized with a thermoplastic mask, and CT images were acquired in the treatment position to allow for 3D treatment planning. The method used to administer the radiotherapy depended on the target volume. In most cases, the dose was delivered using 2 parallel opposite fields up to 40 Gy, then the fields were shielded to cover the spine and matched with electron beams (8–10 MeV) on the spinal chains. In selected cases, the dose was delivered with multiple beams to cover the whole planned target volume. Conventional fractionation was used, i.e. 1.8–2 Gy/fraction daily for a total dose ranging from 50 to 70 Gy.

2.3. Immunohistochemistry

All tissues were fixed in 4% para-formaldehyde and embedded in paraffin. Sections 5 μm thick were cut from each of the 32 tissue blocks for immunohistochemistry, after staining with a fully-automated system (Bond-maX; Vision Bio Systems, UK). Tissue sections were dewaxed and rehydrated by successive incubations at 72 °C in Bond Dewax Solution (Vision BioSystems), ethanol, and distilled water. Antigens were retrieved by heating sections at 100 °C for 30 min in Bond Epitope Retrieval Solution 1 (Vision BioSystems, UK). Endogenous peroxidase was blocked with 3% hydrogen peroxide before 15 min of incubation with anti-human Maspin (mouse monoclonal antibody, clone EAW24, Novocastra Laboratories, Newcastle Upon Tyne, UK; diluted 1:100). Specimens were then washed with phosphate-buffered saline (pH 7.0) and incubated with the Bond Polymer Refine Detection system (Vision BioSystems, UK). The sections were dehydrated, cleared, mounted, and counterstained with Meyer's hematoxylin. Normal breast tissue was used as a positive MASPIN control. Primary antibodies were replaced with phosphate-buffered solutions for negative controls.

2.4. Subcellular Maspin expression

The pathologist (S.B.) interpreting the sections was blinded to the patients' clinical outcomes. Sections were scanned to select the less well-differentiated areas of carcinoma with no signs of necrosis or hemorrhage. Considering at least 600 carcinoma cells at 200× magnification in the areas with the strongest reactivity, the pathologist scored the subcellular MASPIN distribution as nuclear when more than 40% of the