American Journal of Otolaryngology–Head and Neck Medicine and Surgery xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

American Journal of Otolaryngology–Head and Neck Medicine and Surgery

journal homepage: www.elsevier.com/locate/amjoto

Cortactin and phosphorylated cortactin tyr^{466} expression in temporal bone carcinoma

Gino Marioni [a,*], Elisabetta Zanoletti [a], Antonio Mazzoni [a], Andrea Gianatti [b], Elisa Valentini [c], Laura Girasoli [a], Martina Guariento [a], Luciano Giacomelli [c], Alessandro Martini [a], Stella Blandamura [c]

[a] Department of Neurosciences DNS, Otolaryngology Section, Padova University, Padova, Italy
[b] Anatomic Pathology Unit, Ospedali Riuniti Bergamo, Bergamo, Italy
[c] Department of Medicine DIMED, University of Padova, Italy

ARTICLE INFO

Article history:
Received 17 November 2016
Available online xxxx

Keywords:
Temporal bone squamous cell carcinoma
Cortactin
Phosphorylated
Prognosis
Target therapy

ABSTRACT

Purpose: Cortactin is a multidomain protein engaged in several cellular mechanisms involving actin assembly and cytoskeletal arrangement. Cortactin overexpression in several malignancies has been associated with increased cell migration, invasion, and metastatic potential. Cortactin needs to be activated by tyrosine or serine/threonine phosphorylation. The role of cortactin and phosphorylated cortactin (residue tyr^{466}) was investigated in temporal bone squamous cell carcinoma (TBSCC).
Materials and methods: Immunohistochemical expression of cortactin and phosphorylated cortactin (residue tyr^{466}) was assessed in 27 consecutively-operated TBSCCs.
Results: Several clinicopathological variables correlated with recurrence (pT stage, *dura mater* involvement), and disease-free survival (DFS) (cT stage, pT stage, pN status, *dura mater* involvement). Twenty-three of 24 immunohistochemically evaluable TBSCCs were cortactin-positive. Median cortactin expression was 75.0%. Cortactin reaction in the cytoplasm was more intense in carcinoma cells than in normal adjacent tissue. Recurrence and DFS rates did not correlate with cortactin and phosphorylated cortactin (residue tyr^{466}) expression in TBSCC specimens.
Conclusions: Cortactin upregulation in TBSCC supports the conviction that inhibiting cortactin functions could have selective effects on this malignancy. Multi-institutional studies should further investigate the role of cortactin and phosphorylated cortactin in TBSCC, and their potential clinical application in integrated treatment modalities.

1. Introduction

Given the poor prognosis for patients with advanced temporal bone squamous cell carcinoma (TBSCC) [1], there is an undeniable need for novel, more effective diagnostic/therapeutic strategies to improve the outcomes of treatment. TBSCC primary therapies definitely need to be patient-tailored more rationally [1], also because managing TBSCC recurrences is particularly difficult, and any treatment carries a significant morbidity and mortality [2]. Extending surgery to ensure safe tissue margins is the premise for the adequate treatment of TBSCC.

Molecular changes occurring before any morphological changes become apparent are responsible for the disease's biological behavior, prognosis, and response to primary therapy [3]. In clinical practice, the ideal molecular marker should have: (i) prognostic value; (ii) a significant capacity to predict the efficacy of specific treatments; and (iii) the features needed for it to become the target of integrated therapeutic approaches [4].

Cortactin is a multidomain protein engaged in several cellular mechanisms based on actin assembly and cytoskeletal arrangement, such as endocytosis, cell migration, neuronal morphogenesis and tumor invasion. Cortactin is an actin-binding protein that promotes the weak activation of the actin-related protein (ARP) 2/3 complex in actin nucleation, and it stabilizes the formation of newly-branching network of F-actin [5]. Cortactin occurs in the cytoplasm and in actin-rich peripheral structures such as lamellipodia and podosomes. The cortactin locus CTTN is located in the 11q13 region, the amplification of which is one of the alterations most often seen in head and neck squamous cell carcinoma (HNSCC). Studies have shown that 11q13 amplification correlates with a poor prognosis in HNSCC patients, and cortactin has been accused of being the gene responsible for tumor progression because of its role in cell motility and carcinoma invasion. Cortactin overexpression promotes tumor aggressiveness, even without the 11q13 amplicon, suggesting a post-translational activation rather than a gene amplification in HNSCC [6,7]. Cortactin needs to be activated by tyrosine or serine/threonine phosphorylation. Tyrosine phosphorylation stems from

* Corresponding author at: Dept of Neurosciences DNS, Otolaryngology Section, University of Padova, Via Giustiniani 2, 35128 Padova, Italy.
E-mail address: gino.marioni@unipd.it (G. Marioni).

http://dx.doi.org/10.1016/j.amjoto.2017.01.012
0196-0709/

activation of the epidermal growth factor receptor (EGFR) and other receptor tyrosine kinases, and it occurs at residues tyr^{421}, tyr^{466} and tyr^{482}, in the proline-rich domain of the carboxy terminus. Phospho-cortactin interacts with the adaptor protein NCK1, which binds the neuronal Wiskott Aldrich Syndrome protein, resulting in ARP 2/3 activation. The role of phosphorylation in cortactin function is still not entirely clear, but it correlates positively with the ability to induce cell migration through matrix metalloproteinase recruitment and secretion in invadopodia [8,9].

Cancer has the characteristic ability to invade surrounding tissues and metastasize to regional and distant sites. The events attendant on local invasion and metastasis by epithelial tumors such as TBSCC include: (i) loss of adhesion to surrounding tumor cells and basement membrane; (ii) production of enzymes and mediators that facilitate the incursion of malignant cells into the subjacent connective tissue; (iii) attachment to extracellular membrane molecules; (iv) neovascularization; (v) passage into and out of the circulation *via* attachment to endothelial cell ligands; and (vi) a repeat of this cascade at a metastatic site [10]. The details of these mechanisms are not fully known in the case of TBSCC. The present study is the first to have investigated the immunohistochemical expression of cortactin and phosphorylated cortactin (residue tyr^{466}) in patients with primary TBSCC. The aim of the study was to conduct a preliminary assessment of the role of cortactin in this malignancy.

2. Methods

2.1. Patients (see Table 1)

The present investigation was approved by our Otolaryngology Section's in-house ethical committee. The study was carried out in accordance with the principles of the Helsinki Declaration.

The study was conducted on specimens from 27 patients with primary TBSCC operated by the same surgeon (A.Mz.) at a tertiary referral center. The patients included 15 women and 12 men with a mean age of 54.0 ± 12.7 years, who were part of a larger series investigated for other purposes in a previous study [11]. Preoperatively, all patients underwent micro-otoscopy with biopsy, temporal bone computerized tomography and/or contrast-enhanced magnetic resonance imaging, and neck ultrasonography (with or without fine-needle aspiration cytology). Positron emission tomography was performed in advanced cases to rule out distant metastasis.

Based on the revised Pittsburgh staging system [12,13], the primary TBSCCs were classified as cT1 in 5 cases, cT2 in 6, cT3 in 9, and cT4 in 7. En-bloc lateral temporal bone resection was performed in 19 cases (two of them partial), and en-bloc subtotal temporal bone resection in 8. The surgical techniques involved have been described in detail elsewhere [14]. The facial nerve was sacrificed in 9 of the 27 patients, *i.e.* in all the cases of subtotal temporal bone resection to enable *en-bloc* radical excision of the carcinoma, and in one case intraoperatively showing clinical signs of nerve involvement by the malignancy. *Dura mater* specimens were sent for frozen section during the surgical procedure to check for histological clearance: the carcinoma involved the *dura mater* in 4 of the 27 cases. Twenty-four patients underwent cervical lymph node dissection, and 25 had ipsilateral parotidectomy. On pathological T staging, 5 cases were pT1, 5 were pT2, 6 were pT3, and 11 were pT4. The pathological grade of the primary TBSCC was G1 in 17 of the 27 cases and G2 in 10. The surgical margins were negative on definitive histopathological examination in all cases. Regional lymph node status was classified as N_0 in 20 cases (3 cN_0 and 17 pN_0), and as N + in 7 (3 pN1, 3 pN2a, and 1 pN2b). Postoperative radiotherapy (RT) was performed in 15 cases (conventional external once-daily fractions of 2 Gy for a total dose ranging from 50 to 70 Gy, median 60 Gy). No distant metastases (M) were detected at diagnosis. The mean follow-up for the cohort was 82.9 ± 67.1 months (median 76 months). Survivors were followed up for at least 5 years.

2.2. Immunohistochemistry

Sections were obtained from each of the 27 tissue blocks for immunohistochemical examination, performed on 4 μm-thick formalin-fixed and paraffin-embedded sections from each tissue sample. Staining was done automatically (BondmaX, Menarini, Florence, Italy), as described elsewhere, using the Bond Polymer Refine Detection kit (Leica Microsystem, Wetzlar, Germany), with rabbit anti-cortactin antibody (monoclonal EP1922Y; Abcam, Cambridge, UK; working dilution

Table 1
Main clinical, pathological, and immunohistochemical characteristics (cortactin and phosphorylated cortactin [residue tyr^{466}]) of the patients with TBSCC.

Patient no.	Sex	cT	pT	G	N-status (0 *vs* +)	*Dura mater* involvement (0 *vs* 1)	Cortactin immunoreactivity (0 *vs* 1)	Cortactin %	Phosphorylated cortactin Y^{466} immunoreactivity (0 *vs* 1)	TBSCC recurrence (0 *vs* 1)
1	F	2	2	1	0	0	1	80%	1	0
2	M	2	2	2	0	0	1	90%	0	0
3	F	1	1	1	0	0	1	50%	0	0
4	F	4	4	2	0	0	1	60%	1	0
5	F	1	1	1	0	0	1	70%	1	0
6	M	2	2	1	0	0	1	90%	1	0
7	M	3	3	1	0	0	1	70%	1	0
8	F	4	4	1	0	0	1	90%	0	0
9	M	3	4	2	0	0	1	70%	0	0
10	M	1	1	2	0	0	1	95%	1	0
11	F	3	3	2	0	0	1	10%	0	0
12	F	3	3	1	+	0	1	80%	0	0
13	M	1	1	1	0	0	1	90%	0	0
14	F	2	2	1	+	0	Not evaluable	Not evaluable	Not evaluable	0
15	M	3	4	1	0	0	1	60%	Not evaluable	0
16	F	4	4	1	+	0	1	8%	0	0
17	M	2	2	1	0	0	0	0%	0	0
18	F	3	3	2	0	0	Not evaluable	Not evaluable	1	0
19	F	3	3	1	0	0	Not evaluable	Not evaluable	Not evaluable	0
20	M	4	4	1	0	1	1	30%	0	1
21	M	2	4	2	0	1	1	80%	1	1
22	M	4	4	1	0	0	1	80%	0	1
23	M	4	4	1	+	1	1	100%	0	1
24	F	1	1	1	0	0	1	10%	0	1
25	F	3	3	2	+	0	1	40%	0	1
26	F	4	4	2	+	1	1	80%	1	1
27	F	3	4	2	+	0	1	80%	0	1

Please cite this article as: Marioni G, et al, Cortactin and phosphorylated cortactin tyr466 expression in temporal bone carcinoma, American Journal of Otolaryngology–Head and Neck Medicine and Surgery (2017), http://dx.doi.org/10.1016/j.amjoto.2017.01.012