

EGFR polysomy in squamous cell carcinoma of the thyroid. Report of two cases and review of the literature

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ABSTRACT

Aims and background. Primary squamous cell carcinoma of the thyroid gland (PSCCT) is an uncommon malignancy characterized by a poor prognosis. A radical surgical approach combined with radiotherapy or chemotherapy is the generally accepted treatment for this tumor. The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor modulating the cell proliferation and biological progression of many human epithelial tumors. The EGFR overexpression in PSCCT suggests an additional therapeutic option for the treatment of this tumor.

Methods and study design. The clinicopathological features and immunohistochemical profiles of two cases of primary squamous cell carcinoma of the thyroid in a 66-year-old and an 83-year-old woman are presented. EGFR status was valued in both cases.

Results. Overexpression of EGFR protein was detected in 50% and 75% of the tumor cell membranes. EGRF gene polysomy was detected in both tumors.

Conclusions. Pharmaceuticals targeting EGFR may help to provide the rationale for an additional, novel therapeutic option for this rare tumor, especially when other therapeutic options have been exhausted. Free full text available at www.tumorionline.it

Introduction

Primary squamous cell carcinoma of the thyroid gland (PSCCT) is an uncommon malignancy characterized by a poor prognosis¹. Its pathogenesis is uncertain and heterogeneous immunohistochemical profiles have been described². A radical surgical approach combined with radiotherapy or chemotherapy is the generally accepted treatment for this tumor². Long *et al.* recently described EGFR protein overexpression in PSCCT, suggesting an additional therapeutic option³. Further to this report, we investigated the epidermal growth factor receptor (EGFR) status in 2 cases of PSCCT using immunohistochemical analysis (IHC) and fluorescence *in situ* hybridization (FISH).

Case reports

Patient selection

Among 1024 cases of thyroid cancer diagnosed at the Pathology Department of the University of Modena and Reggio Emilia during the period 1991-2008, 2 cases of PSCCT were identified (Table 1). In accordance with the WHO definition⁴, "only tumors composed entirely of squamous cells and showing so-called intercellular bridges and/or forming keratin" were included in this study. Hematoxylin and eosin-stained

Key words: squamous cell carcinoma, thyroid, EGFR, polysomy.

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slides representative of all tumors were available and were reviewed by two pathologists. The mode of presentation, clinical course and follow-up information were obtained by review of the patients' charts.

Case history

Case 1

A 66-year-old woman complained of progressive enlargement on the right side of the neck, hoarseness, and difficulty swallowing. Physical examination revealed a semi-mobile painless and hard mass located in the right lobe of the thyroid. Palpable lymph nodes were present bilaterally. Ultrasound showed a solid nodule of approximately 4 cm in diameter, lobulated in shape and protruding through the thyroid. Radiological examinations including X-ray and CT of the neck and chest, endoscopic and otorhinolaryngological examinations were meticulously carried out to exclude the possibility of secondary thyroid involvement from other primary cancers in the aerodigestive tract. Plasma levels of thyroid hormone and calcitonin were in the normal range. No family history of thyroid cancer or previous neck irradiation was documented. Clinical malignancy was suspected and a total thyroidectomy was carried out. Grossly, the mass measured 40 × 32 cm and had ill-defined margins. Histologically the neoplasm was composed of infiltrating nests of irregular squamous cells with marked nuclear pleomorphism and focal keratin formation (Figure 1A). Entrapped remnant thyroid follicles were observed within the tumor while scant regular thyroid parenchyma was present at the periphery of the lesions. There was no evidence of associated papillary or follicular thyroid carcinoma. The patient underwent adjuvant chemotherapy after thyroidectomy. Dissection of the regional lymph nodes revealed the presence of multiple metastases. The patient developed liver and lung metastases within 8 months and died 1 year after presentation

Case 2

A 83-year-old woman with a 2-month history of swallowing difficulty and dysphagia presented to us. Her past medical history was significant only for goiter diagnosed 2 years before. No family history of thyroid cancer or previous neck irradiation was documented. Plasma levels of thyroid hormone and calcitonin were in the normal range. Radiological examinations including x-

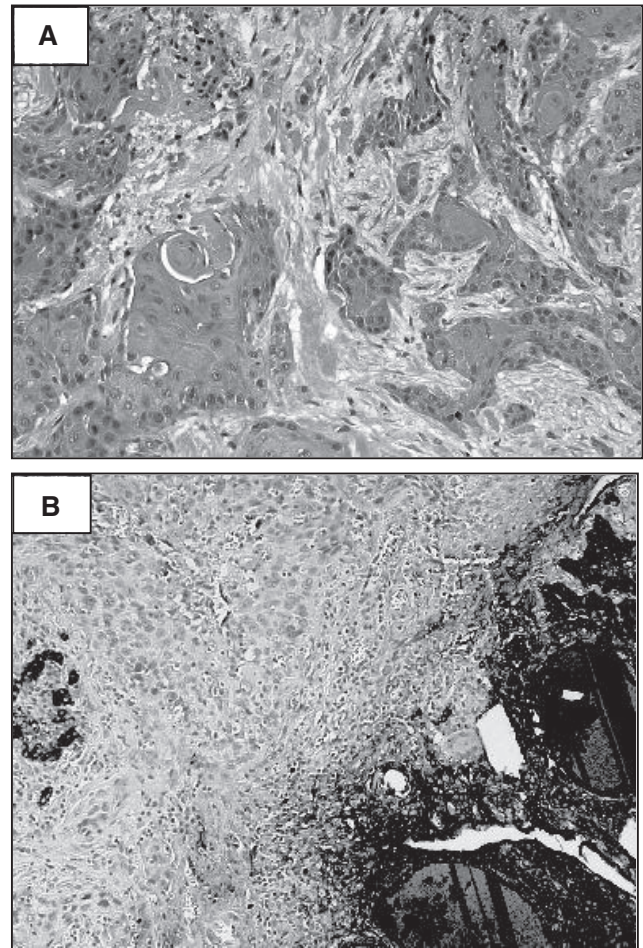


Figure 1 - Histological features of primary squamous cell carcinoma of the thyroid gland. The tumors were composed of infiltrating nests of irregular squamous cells with marked nuclear pleomorphism and focal keratin formation (A). Positive staining for thyroglobulin and TTF-1 was limited to normal follicles entrapped within the tumor and in the intrafollicular colloid (B).

ray and CT of the neck and chest, and endoscopic and otorhinolaryngological findings were unremarkable. The patient underwent radical thyroidectomy followed by chemotherapy. Grossly, the mass measured 42 × 36 mm and had ill-defined margins. Histologically, the neoplasm was composed of infiltrating nests of squamous cells with marked nuclear pleomorphism and focal keratin formation. Regional lymph node dissection

Table 1 - Clinical and pathological findings of the two cases of primary squamous cell carcinoma of the thyroid gland

	Sex	Age	Tumor site	Tumor size	Laboratory findings	Lymph node status	Cause of death	Symptoms	Concomitant thyroid disease
Case 1	♀	66	Right lobe	40 x 32 mm	Unremarkable	Metastatic	Metastatic disease	Hoarseness	Goiter
Case 2	♀	83	Right lobe	42 x 36 mm	Unremarkable	Metastatic	Cardiovascular disease	Hoarseness Dysphagia	Goiter

revealed the presence of metastatic lymph nodes. The patient died of cardiovascular disease about 4 months after surgery. Autopsy revealed no visceral metastases.

Pathological and immunohistochemical features

The panel of antibodies used for the immunohistochemical analyses is listed in Table 2. Appropriate negative and positive controls were run concurrently for all tests. Positive staining for thyroglobulin and TTF-1 was limited to normal follicles entrapped within the tumor and in the intrafollicular colloid (Figure 1B).

The aim of this study was to test the EGFR status in 2 cases of PSCCT. Expression of EGFR protein was determined by means of IHC using mouse antihuman EGFR monoclonal antibody (clone H11, Dako, Carpinteria, CA, USA). Assessment was based on the proportion of reactive cells within the tumors. IHC positivity was defined as more than 10% of tumor cells showing membranous staining of any intensity. Both our cases were uniformly and markedly immunoreactive for this EGFR antibody (Figure 2A).

EGFR fluorescence *in situ* hybridization

All samples provided enough diagnostic material for molecular determinations. FISH studies were performed on selected sections of paraffin-embedded tissue areas containing representative malignant cells, using the LSI EGFR SpectrumOrange/CEP7 Spectrum Green probe (Vysis, Abbott Laboratories, Abbott Park, IL, USA). Two observers analyzed 100 intact non-overlapping tumor cell nuclei for the observed number of red (EGFR gene) and green (chromosome - CEP7) signals. We determined the number of gene copies of the EGFR gene and classified them according to the FISH categories defined by Cappuzzo *et al.*⁵ EGFR amplification was considered present when the ratio of the EGFR gene to the chromosome (EGFR/CEP7) was ≥ 2 . Polysomy of the EGFR gene was defined as an increase in red EGFR signals in a large proportion of tumor cells. In particular, we defined low polysomy as the presence of >4 gene copies in 10-40% of scored cells and high

polysomy as the presence of >4 gene copies in $>40\%$ of scored cells. No EGFR gene amplification was found in our patients. Low polysomy was detected in case 1 (>4 gene copies in 38% of tumor cells) and high polysomy (>4 gene copies in 51.4% of tumor cells) in case 2. Details are summarized in Table 2 and shown in Figure 2B, 2C, and 2D.

Discussion

To date, fewer than 200 cases of PSCCT have been described in the English medical literature¹. Its reported incidence ranges from 0.7% to 3.4% and it is more common in women, with a male:female ratio of approximately 1:2². Although the neoplasm tends to occur more often in elderly patients, sporadic young patients affected by PSCCT have been reported¹⁻³. Aggressive clinical behavior and an unfavorable prognosis characterize PSCCT. Most patients die with metastatic disease within a year of the diagnosis and only rare cases of a long disease history have been reported^{4,5}. The clinical manifestation of obstructive symptoms attests to the tendency of this tumor to invade adjacent aerodigestive structures such as the trachea and esophagus^{1-3,6}. The origin of PSCCT is unknown and different theories concerning its pathogenesis have been put forward, including development from embryonic remnants^{1,7} and malignant transformation of squamous metaplastic changes associated with chronic diseases such as goiter or Hashimoto thyroiditis⁸. The main differential diagnosis includes squamous cell carcinoma extending into or metastasizing to the thyroid gland from the lungs, thymus, or – most frequently – upper aerodigestive tract. Radiological examinations may be useful to rule out secondary involvement of the thyroid^{1-2,5,9}. Different cytokeratins can be tested to characterize the tumor^{1,5,9}. CD5 is practical for distinguishing CASTLE (thyroid gland carcinoma showing thymus-like differentiation), which is positive for this marker, from PSCCT, which is CD5-negative. Overexpression of the oncoprotein p53 and a high MIB-1 index ($\geq 20\%$) are reported to be asso-

Table 2 - Immunohistochemical profiles and EGFR gene status (immunohistochemical analysis and fluorescence *in situ* hybridization results) in two cases of PSCCT

	Immunohistochemical antibodies													FISH
	CK5-6	CK7	CK10	CK13	CK18	CK19	CK20	TTF-1	TG	p53	MIB-1	CD5	EGFR	
Case 1	+	+	-	-	+	±	-	-	-	+	24%	-	3+	Low polysomy* (38%)
Case 2	+	+	-	-	+	±	-	-	-	+	26%	-	3+	High polysomy** (51.4%)

*Low polysomy: >4 EGFR gene copies in 10-40% of tumor cells. **High polysomy: >4 EGFR gene copies in $>40\%$ of tumor cells. IHC, immunohistochemical analysis; FISH, fluorescence *in situ* hybridization.

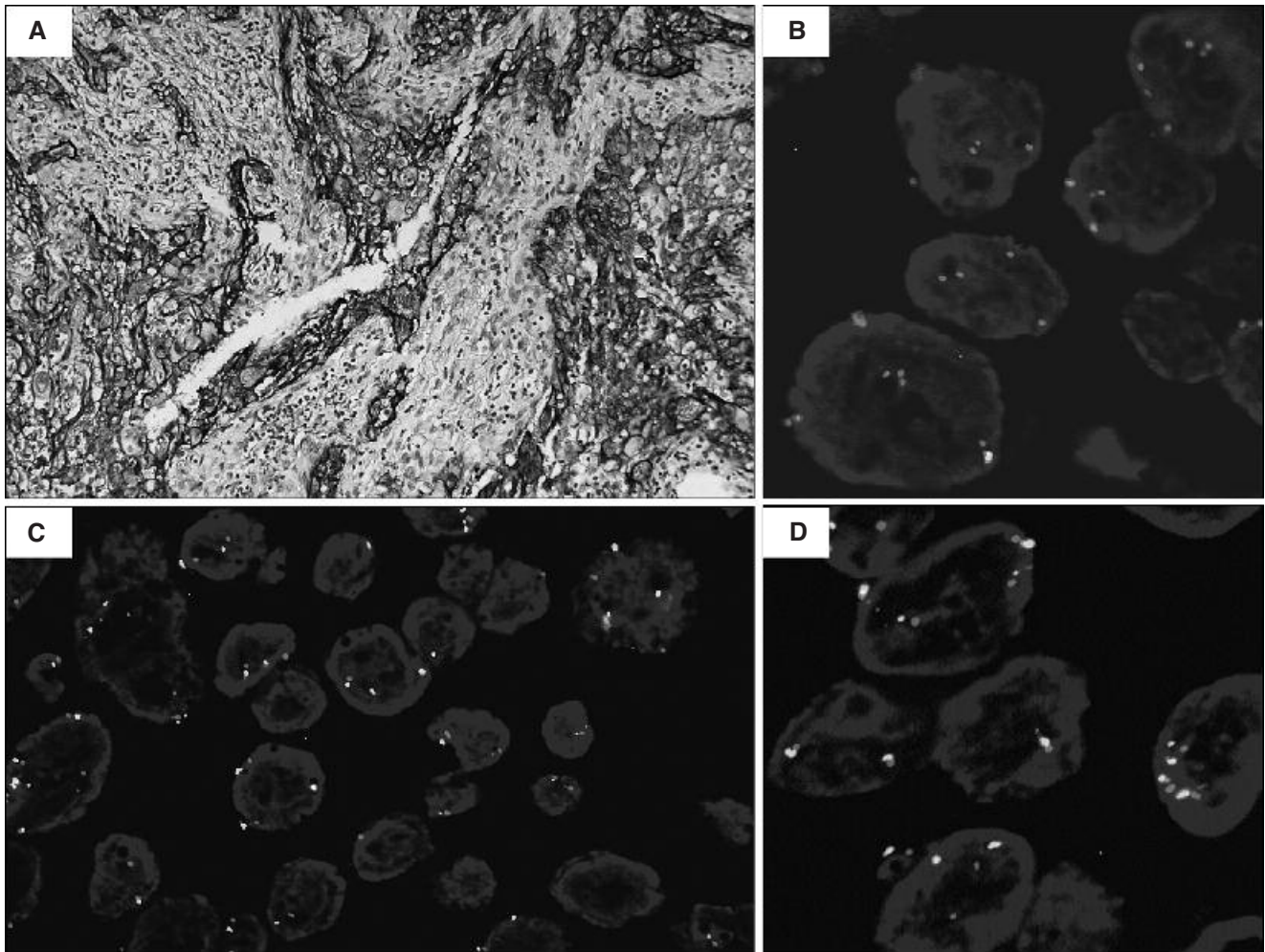


Figure 2 - Strong membrane staining for EGFR in tumor cells (A). EGFR gene polysomy was defined as an increase in red EGFR signals paralleled by the same increase in chromosomes 7 measured by the number of green CEP7 signals per nucleus (B, C, D).

ciated with the degree of differentiation and with a poor prognosis⁹.

Long *et al.*³ documented a case of PSCCT that was strongly immunoreactive for EGFR. EGFR is a transmembrane tyrosine kinase receptor modulating the cell proliferation and biological progression of many human epithelial tumors. Upregulation of EGFR with a significantly increased binding capacity for EGF compared to normal thyroid tissue was demonstrated in a case of SCC involving the thyroid¹⁰. Further to these reports, we tested the functional status of EGFR. Both our cases were uniformly and markedly immunoreactive for the EGFR antibody. Expression of EGFR protein was demonstrated in 50% and 75% of the tumor cell membranes. Two studies analyzing EGFR expression in benign and malignant thyroid tumors demonstrated protein overexpression in thyroid tumors and suggested its prognostic and diagnostic significance¹¹⁻¹³. Lee *et al.*¹² using FISH in a series

of anaplastic thyroid carcinomas reported high EGFR gene polysomy in a large proportion of tumor cells (≥ 4 copies in $\geq 40\%$ of cells). Four of these cases showed squamoid differentiation. In the present study, we found low EGFR polysomy (>4 copies in 38% of scored tumor cells) and high polysomy (>4 copies in 51.4% scored cells) in case 1 and case 2, respectively.

Although the main treatment for PSCCT is surgery followed by radiotherapy or chemotherapy, most reported cases were found to be unresponsive^{1,2}. This report documents EGFR protein overexpression and EGFR gene polysomy in a high rate of PSCCT cells. These preliminary results prompt consideration of EGFR as a therapeutic target for this rare tumor. It may help to provide the rationale for the use of new targeted therapies for refractory PSCCT when other options have been exhausted. Additional information will be needed to clarify these findings.

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