CD117 Expression in Squamous Cell Carcinoma of the Oesophagus

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Abstract. *Background/Aim:* Identification of changes in specific genes may help new attempts in finding targeted therapy for oesophageal cancer which still has a very poor prognosis. The aim of this study was to investigate CD117 expression in squamous cell carcinoma of the oesophagus (SCCO). *Materials and Methods:* A preliminary study was performed for CD117 immunoreactivity using a monoclonal antibody against CD117 on 27 SCCO specimens from patients who underwent surgical resection. Specimens of oesophageal mucosa obtained from 10 healthy individuals were studied as a control group. *Results:* Most patients had TNM American Joint Committee on Cancer stage IIb or III SCCO and mean overall survival was 21 (range=2-72) months. Cytoplasmic membrane CD117 immunoreactivity was demonstrated in only four (15%) out of 27 tumours and in none of the controls. *Conclusion:* Although immunohistochemical expression of CD117 was higher than previously demonstrated, the lack of expression does not warrant further use in targeted therapy of SCCO.

Squamous cell carcinoma of the oesophagus (SCCO) is a highly aggressive neoplasm. Statistically, oesophageal carcinoma is the sixth leading cause of cancer-related death worldwide (1). The incidence varies geographically, with highest rates found in developing countries, such as Southern and Eastern Africa and Eastern Asia (2, 3). In southern Brazil, in the state of Rio Grande do Sul, SCCO may reach an estimated incidence of 16.21 and 4.21 new cases/100,000 men or women in 2018 (4).

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At diagnosis, the majority of patients have locally or regionally advanced disease or disseminated cancer, which confers a poor prognosis (5). The underlying reasons for this disappointingly low survival rate are multiple: ineffective screening tools, cancer detection at an advanced stage, high risk for recurrent disease after oesophagectomy, definitive chemoradiotherapy, mutation of tumour-suppressor genes leading to reduced response to therapy and limited survival achieved with isolated palliative chemotherapy for patients with metastatic or unresectable disease (6). Surgical treatment has traditionally been the choice for patients with resectable tumours (7, 8). The combined modality approaches of chemotherapy, radiotherapy, and chemoradiotherapy with surgery have not led to a significant improvement in overall survival rate (9-12).

Several risk factors for development of oesophageal carcinoma have been identified, such as smoking and alcohol consumption, but the molecular mechanisms related to oesophageal carcinogenesis remain under investigation. Changes in specific genes that play important roles in several cellular functions such as adhesion, signal transduction, differentiation or DNA development and repair, as well as apoptosis, have been identified (8, 13-15).

CD117 (c-KIT) is a transmembrane tyrosine kinase that acts as a receptor for stem cell factor or KIT ligand. It belongs to the type-III family of receptor kinases and it can be detected in several normal cell types including hematopoietic cells, germ cells, interstitial cells of Cajal, ductal breast epithelium, mast cells and melanocytes (16-18). This molecule is also consistently expressed in a number of tumour types, such as gastrointestinal stromal tumours, seminoma/dysgerminoma, acute myelogenous leukaemia, neuroblastoma, Ewing's sarcoma, mast cell disorders, and small-cell or large-cell neuroendocrine carcinoma of the lungs (19).

The development of targeted therapy with protein kinase inhibitors has demonstrated clinical safety and efficacy for several cancer types. Thus, overexpressing of CD117 would

Table I. Analysis of CD117 expression.

Score	Expression	Staining pattern
0	Negative	No staining, or membranous staining in fewer than 10% of tumour cells.
1+	Negative	Faint barely perceptible membranous staining detected in more than 10% of tumour cells. The cells are only stained in part of the membrane.
2+	Positive	Weak to moderate complete membranous staining in more than 10% of the tumour cells.
3+	Positive	Strong complete membranous staining in more than 10% of tumour cells.

enable the use of inhibitors such as imatinib mesylate and sorafenib as treatment option for oesophageal carcinoma (20-23).

CD117 has not yet been assessed in oesophageal cancer in a Brazilian population, although it has a high prevalence among southern Brazilian states. Therefore, the aim of the present study was to evaluate the immunohistochemical expression of CD117 in SCCO, in an attempt to determine whether this kinase may be expressed in our hospital cases and to see whether it may allow any blocking therapy in the future.

Materials and Methods

Patients. In this preliminary study, 27 cases of SCCO were retrospectively studied randomly selected among those patients who underwent surgical resection at the University Attached Hospital, Faculty of Medicine, Rio Grande do Sul Federal University, Porto Alegre, RS, southern Brazil between 2006 and 2010. Clinical data were noted from patient records and biopsy-proven SCCO was obtained by endoscopic examination prior to any treatment. As a control group, 10 tissue specimens of oesophageal mucosa from healthy volunteers were studied. The study was performed by the Southern Surgical Oncology Group of the Post-Graduate Program of Surgery, Faculty of Medicine after approval by the Institutional Review Board of the Hospital de Clínicas de Porto Alegre University Attached Hospital (HCPA-UFRGS).

CD117 immunohistochemistry. Tissue sections (4-mm-thick) were deparaffinised and rehydrated in water. Sections were rinsed with phosphate-buffered saline and incubated with a monoclonal antibody anti-CD117 (DakoCytomation, San Diego, CA, USA) diluted at 1:50. To determine the antibody reactivity, the avidin-biotin peroxidase complex (ABC method; LSAB Kit; DakoCytomation) was used (Figure 1). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 15 min. Following incubation, all samples were rinsed with phosphate-buffered saline and were rinsed and counterstained with Harris haematoxylin. The procedure was carried out at the Pathology Department of the University Hospital. As positive controls for immunohistochemical reaction, interstitial cells of Cajal known to express CD117 were used (Figure 2). All immunostaining reactions were evaluated and double-checked by the same pathologist unaware of patient identity, tumour condition or clinical outcome.

The slides were analysed following a score of 0 to 3+, where 0 meant completely negative; 1+ weakly positive; 2+ moderately

Table II. Clinical and pathological features of the patient population.

Feature	n	Percent
Gender		
Male:female	21:6	78:22
Tumour site		
Middle third	10	37
Distal third	17	63
Histological type		
Well-differentiated	3	11
Moderately differentiated	22	82
Poorly differentiated	2	7
7th Edition AJCC Staging		
I	1	4
IIB	5	18
III	21	78
CD117 expression		
Positive	4	15
Negative	23	85

positive; 3+ strongly positive (Table I). Only staining scored 2+ and 3+ at membranous sites (no cytoplasmic staining considered) were regarded as positive.

Qualitative data were described as mean, median, minimum and maximum values, and standard deviation. Percentages and frequencies were used for categorical variables. The association between CD117 overexpression and clinical and pathological features was carried out using the chi-square test. Deaths unrelated to oesophageal cancer were not considered for overall survival analysis calculated from treatment onset. The statistical significance level (α) was set at 0.05.

Results

Twenty-one (78%) patients were male. The median age was 58 years and ranged from 36 to 77 years.

Tumour grade was considered according to the World Health Organisation classification (24) as follows: Three (11%) patients presented well-differentiated tumour, 22 (82%) moderately differentiated and two (7%) poorly differentiated (Table II). The immunohistochemical reaction produced by the anti-CD117 antibody was considered positive in only four cases (15%) out of 27 tumours and in none of the controls.

Figure 1. A representative case of an oesophageal tumour positive for CD117 expression (immunohistochemical, 400× magnification).

Figure 2. A Cajal cell expressing CD117, used as a positive control (immunohistochemical, 400× magnification).

The depth of invasion, according to the seventh edition of the American Joint Committee on Cancer classification (25), varied; the majority of the cases were of stage III (n=21; 78%). The median (SD) overall survival rate was 21 (8) months, ranging from 2 to 72 months. Survival was significantly lower for those with advanced pT stage ($p \leq 0.001$) and lymph node ($p \leq 0.001$) involvement. However, association with CD117 fail to be demonstrated due to the small number of positive cases of the subgroups in this sample.

Discussion

SCCO is still a disease of poor prognosis. Many therapies, such chemotherapy and radiotherapy, have been combined with surgery in an attempt to change this scenario, yet no clear improvement in survival has been reported. The outcome of patients with advanced disease remains poor, and 5-year survival rates rarely exceed 10% in Western countries (26). SCCO is highly prevalent in Southern Brazil and the overall mortality rate in this region for oesophageal cancer was 14.0 and 4.5 per 100,000 inhabitants for men and women respectively in 2014-2015 (4).

The multiple stages of oesophageal carcinogenesis have been histologically defined. It has been established that oesophageal carcinoma usually evolves through a series of progressively severe histopathological changes which involves dysplasia of the epithelium, carcinoma *in situ* and, finally, invasive tumour (27). Based on the concept that precancerous cells may have early alterations at a molecular level, identification of new potential biomarkers might play an important role in detection of high-risk lesions for malignant transformation and early diagnosis of SCCO (13, 28).

Immunohistochemical expression of gene by-products has become an easy, simple and very practical and widespread method for determining the involvement of a number of factors in paraffin-embedded samples from a wide range of

tissues, including tumours. It is therefore more routinely used to study the expression of new potential therapeutic targets and to determine which patients are the most likely to respond to these specific therapies (29).

The improvements achieved in molecular biology certainly favour appropriate methods for molecular screening, and may result in early diagnosis, allowing curative therapy. CD117, a trans-membrane tyrosine kinase receptor, is expressed in a large variety of human neoplasms. Little, however, is known about the prevalence and clinical implications of CD117 in SCCO, with few studies reported (30).

CD117 expression in malignant tumours plays an important role for cancer treatment since it is one of the targets of the tyrosine kinase inhibitors. The tyrosine kinase inhibitor imatinib was initially shown to be effective in the treatment of chronic myeloid leukaemia, in which it targets the kinase function of the breakpoint cluster region–Abelson proto-oncogene (BCR–ABL) fusion protein–Philadelphia chromosome. Subsequently, significant treatment responses were also reported in patients with advanced CD117-positive gastrointestinal stromal tumours. It has been suggested that the response rate to imatinib may be particularly high in CD117-expressing tumours that also harbour activating CD117 mutations (23).

Went *et al.* studied the prevalence of CD117 expression in more than 3,000 human tumours from 120 different tumour types by immunohistochemistry in a tissue microarray set. The epidemiologically most important CD117-positive tumours included gastrointestinal stromal tumours (100%), seminoma (84%), malignant melanoma (36%), large-cell (17%) and small-cell carcinoma of the lungs (7%). For SCCO, immunoreactivity was documented in only two out of 34 cases (5%) (31).

Zhang *et al.* studied the expression of CD117 in tumour tissue, surrounding-tumour tissue and normal tissue of 50 specimens of either squamous or adenocarcinoma of

oesophageal tumours. The strong expression rate was low in tumour, para-tumour, and normal tissues (4%, 4%, and 12%), respectively (21). Patients in that series similarly presented with tumours of the middle and lower oesophagus. No statistically significant differences were noted for CD117 expression and differentiation, depth of invasion, lymph node metastasis or stage and these findings are in agreement with those results from Zhang *et al.* (21).

Gilbault *et al.* showed expression of CD117 to be very rare in SCCO finding it in fewer than 3% of the cases (3/107). Like our cases, the use of external or internal positive controls (positive mastocytes in cancer stromal reaction for CD117) during immunohistochemical procedures ruled out the possibility of false-negative reactions (29), which corroborates those findings of lack of expression of CD117 for alternative associated target therapy.

In the present study, overexpression of CD117 was found in 15% of cases, which is almost 4-fold higher than the expression reported by Zhang *et al.* for both squamous carcinoma and adenocarcinoma. This difference may be explained by the fact that to date, this is the first study addressing patients with cancer from an area with a high incidence of SCCO, and that tumours in this series were of slightly more advanced stage (66% vs. 78%) as compared to the series of Zhang *et al.* (21).

Our results suggest that CD117 is overexpressed at a low rate in tumour tissue, corroborating previous findings. Therefore, failure to demonstrate overexpression of CD117 in SCCO may be due to a lack of control of the cell cycle in these tumours. However, this low expression was not significantly related to any prognostic feature and may not justify target therapy against CD117, such as c-KIT inhibitors.

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