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SHORT COMMUNICATION

K-ras status in squamous cell anal carcinoma (SCC): it's time for target-oriented treatment?

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Abstract
Purpose Squamous cell anal carcinoma (SCC) is an uncommon disease comprising only 1–5% of all intestinal tumours. SCC is now considered the prototype for the successful application of conservative treatment as chemoradiation instead of aggressive surgery. The EGFR status and k-ras mutations in SCC of the anal canal has not been well investigated. The purpose of our evaluation was to give information about this issue.
Methods From June 1999 to December 2008, 32 patients affected by SCC were treated in our institution with chemotherapy containing fluoropyrimidine and platinum salt concomitant with pelvic radiotherapy. Immunohistochemistry for EGFR and k-ras mutation was retrospectively evaluated.
Results Twenty-six specimens were considered evaluable for biological objectives: K-ras mutation was performed in all cases, while EGFR in 12. In all cases of our series wild-type K-ras was observed.
Conclusions Such information is, in our knowledge, the first reported in literature on this setting. This observation previously reported in other tumours has supported the effective use of EGFR-inhibitors in recurrent or metastatic disease. This observation could support the role of EGFR-inhibitors in the treatment of SCC.

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Introduction

Squamous cell anal carcinoma (SCC) is an uncommon disease comprising only 1–5% of all intestinal tumours.

In most institutions only a small series of cases has been cured even if its noteworthy that its incidence has been increasing in western countries. This trend has been associated with persistent high-risk human papilloma virus genotype infection, previous lower genital tract dysplasia/carcinoma, high-frequency anoreceptive intercourse, heavy cigarette smoking, immunosuppression and/or immunodepression [1].

The standard option in locally advanced anal SCC is chemoradiotherapy [2].

Prognosis is related to clinical features, while biological correlation is still unknown.

The epidermal growth factor receptor (EGFR) status and most recently k-ras mutations have acquired a predictive role for treatment response and have justified the use of EGFR-inhibitors in colorectal, non-small cell lung and head and neck cancers' therapies [3, 4].

The ras gene family (H, K and N-ras) encodes the ras protein, a GTPase-activating protein that regulates several signal transduction pathways including cellular proliferation and differentiation. Ras genes are the most frequently mutated oncogenes in human cancer with typically studied alterations in codons 12, 13 and 61 [3, 4].

The EGFR status and k-ras mutations in anal SCC has not been well investigated.

The purpose of our study was to evaluate biological aspects of anal SCC by describing k-ras mutations and

EGFR status. The endpoint is to confirm wild-type k-ras status as reported in other previous literature data in anal and other squamous cell cancers and to justify the use of specific target treatment in anal cancer.

Materials and methods

From June 1999 to December 2008, 32 consecutive patients affected by SCC were treated in our institution with chemotherapy containing fluoropyrimidine and platinum salt concomitant with regional radiotherapy. It was possible to review histology in 26 patients for biological objective. Samples of anal tumour were embedded in paraffin and immunohistochemical analysis was performed for EGFR (3-μm-thick tissue section, using the EGFR pharmDX assay, Dako Cytomation, Carpinteria, CA, USA): EGFR positive was considered if at least 1% of malignant cells were stained for EGFR. Amplification of the k-ras gene exon 1 was done by laser capture micro-dissection. Then, DNA extraction, PCR and sequencing were evaluated for possible mutation in codons 12 and 13, two hotpots that cumulatively include more than 95% of mutations in this gene.

Results

Patients' characteristics and tumours are described in the Table 1.

Table 1 Patients characteristics

Variables	*N*
Median age (year)	59 (range 39–75)
Gender	
Males/females	5/27
Performance status (ECOG)	
0/1/2	24/7/1
Grading	
G1/G2/G3	0/8/12
Unknown	12
Clinical staging	
T1/T2	2/13
T3/T4	8/7
N0/N+	10/20
M1	2
EGFR	
Positive/negative/NA	7/5/20
K-ras status	
Wild-type/mutant/NA	26/0/6

NA not assessable

Among 30 patients with locally extended disease, 28 obtained a complete clinical remission (93%); among them 2 relapsed, one in lung and the other in liver and cervical nodes; both of them are still alive.

K-ras mutation was performed in 26 cases, immunohistochemistry for EGFR was evaluable in only 12 patients due to poor availability of tissue samples.

In all cases of our series wild-type K-ras was observed.

In all K-ras wild type locally advanced cancer, a clinical complete remission after chemoradiotherapy was observed: one patient developed liver metastasis few months after the end of chemoradiotherapy.

The estimated 7-year failure-free survival in patients with locally advanced disease was 85.2% (68.3–100%) and the estimated 7-year OS was 86.2% (67.0–100%).

Discussion

SCC is now considered the prototype for the successful application of conservative treatment as chemoradiation instead of aggressive surgery. Anal lesion size of more than 4–5 cm and regional nodes involvement are features related to 30–40% treatment failure when fluorouracil combined to mitomycin or cisplatin were administered, with the overall survival rates still in the region of 50–60% at 5 years; more tailored approach is needed [2].

The EGFR and its mitogen-activated protein kinase signalling cascade represent an important pathway in cancer development. The availability of EGFR-inhibitors was greeted with interest in the therapy of a series of solid tumours, based on the nearly diffuse EGFR over-expression and its negative prognostic implication [3, 4]. Unfortunately, the impact of EGFR expression levels on EGFR-blockers sensitivity, is still an issue since preclinical data and clinical trial did not report correlation with response. Thus, the biology of responsiveness to these agents remains unclear and predictive molecular markers of response or resistance have yet to be fully delineated.

Recently, phase II and III trials, conducted for investigating the role of K-ras status on anti-EGFR target-therapy, revealed that colorectal cancer patients with wild-type K-ras had better clinical outcome in terms of prolonged median progression-free survival and overall response rates when compared to mutant K-ras [3, 4].

The evaluation of molecular biomarkers in anal carcinoma is limited because this is a rare tumour [5–8]. Recently in a setting of 38 primary anal SCC, EGFR expression was immunohistochemically examined and EGFR gene copy numbers were analysed by fluorescence in situ hybridization (FISH). EGFR was overexpressed in 21 (55%) of cases while FISH analysis showed that none evaluable cases had EGFR gene amplification: this data

confirmed the absence of any correlation between the two, as observed in a series of other solid tumours [5]. Of interest, EGFR expression rate reported in another study, differs significantly and was reported in all 21 (100%) biopsies on anal SCC [6]. These differences are not surprising because immunohistochemical findings are subject to inter-observer variability and to potential tissue storage time effect on protein degradation and loss of text sensitivity [8]. The poor reliability of EGFR by ICH does not justify the clinical use as predictive test.

First report in literature on K-ras and anal SCC was reported 20 years ago by Hiorn and coworkers that described K-ras mutation as an uncommon event in the genesis of this malignancy and apparently not-cooperating with HPV in cell-induced transformation, contrary to previous observations reported in experimental model in vitro [9].

It is noteworthy that in a recently published case report, an excellent response to the combination of an EGFR-inhibitor, cetuximab, plus irinotecan in a patient with refractory anal cancer was documented [10]. This regimen was proposed without specific biological evaluation but supported to the evidence of activity demonstrated in other squamous cell cancers.

Our results concerning K-ras status in SCC demonstrated the absence mutations in all population evaluated. These findings, also reported in head and neck squamous cell histology, could be explained by the involvement of ras in early stage of carcinogenesis and its activation by other mechanism different from mutation, such as epigenetic events [3].

The determination of K-ras status represents a more reliable condition if compared with immunohistochemistry-examined EGFR in predicting response to EGFR-inhibitors. Although of interest, this is a retrospective observation that warrants prospective evaluation in clinical trials focus on target therapies, with the aim to improve the cure of high-risk locally advanced tumour and to increase disease control rate of recurrent disease.

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