ONCOLOGY

Markers of chemoradiation resistance in patients with locally advanced head and neck squamous cell carcinoma, treated by intra-arterial carboplatin and concurrent radiation

Marcatori di chemo- e radio-resistenza in pazienti con carcinoma squamocellulare localmente avanzato del distretto cervico-cefalico, trattati con somministrazione intra-arteriosa di carboplatino e concomitante radioterapia

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SUMMARY

The onset of chemo- and/or radio-resistance in tumour cells is one of the main causes of failure of integrated treatment protocols combining intra-arterial administration of platinum derivatives and radiotherapy, and is associated with recurrent disease and/or distant metastases. In the present study, the expression of a series of markers of chemo- and/or radio-resistance was investigated in 21 patients with advanced squamous cell carcinoma of the head and neck treated with combined intra-arterial carboplatin and radiotherapy. The results were correlated with local response to treatment, recurrence and overall and disease-free survival. In non-responders or in patients presenting recurrence, caspase 8 was significantly (p 0.05) under-expressed while p-Gp (p 0.035) and MDR-3 (p 0.049) were significantly over-expressed. Tumours with unfavourable outcome more frequently over-expressed two or more anti-apoptotic factors (p-53, BCL-2, BCL-x) (p 0.01). Patients with shorter overall survival, significantly over-expressed p53 (p 0.04), LRP (p 0.038) and a larger number of trans-membrane transport proteins compared with those who survived more than one year (p 0.013). Finally, patients with the shortest disease-free survival presented over-expression of p53 (p 0.027) and BCL-x (p 0.023). Further studies are necessary to confirm the possibility, in a future perspective, of using a panel of markers of chemo- and radio-resistance to identify those patients potentially sensitive to the treatment and to avoid patients at high risk of resistance from being submitted to ineffective and toxic treatment protocols.

KEY WORDS: Head and neck tumours • Squamous cell carcinoma • Intraarterial chemotherapy • Radiotherapy • Tumoral markers

RIASSUNTO

L'insorgenza di meccanismi di chemio- e radio-resistenza nelle cellule tumorali rappresenta una delle più importanti cause di fallimento dei protocolli di trattamento integrato che prevedono la somministrazione per via intra-arteriosa di derivati del platino sincrona a radioterapia con conseguente recidiva di malattia e/o metastasi a distanza. Nella presente ricerca è stata studiata l'espressione di una serie di marcatori di chemio- e radio-resistenza in 21 pazienti con carcinoma squamocellulare del distretto cervico-cefalico localmente avanzato trattati con infusione intra-arteriosa di carboplatino sincrona a radioterapia. I risultati sono stati correlati con la risposta locale, comparsa di recidiva, sopravvivenza totale e sopravvivenza libera da malattia. Nei pazienti non responsivi alla terapia o in quelli con comparsa di recidiva Caspase 8 era significativamente (p 0,05) sottoespressa, mentre Gp (p 0,035) e MDR-3 (p 0,049) erano significativamente sovraespresse. Tumori con esito infausto sovraesprimevano più frequentemente due o più fattori antiapoptotici (p-53, BCL-2, BCL-x) (p 0,01). I pazienti con sopravvivenza minore sovraesprimevano in modo significativo p53 (p 0,04), LRP (p 0,038) e più proteine di trasporto transmembrana rispetto ai pazienti con sopravvivenza superiore all'anno (p 0,013). Infine, i pazienti con più breve sopravvivenza libera da malattia presentavano sovrespressione di p53 (p 0,027) e BCL-x (p 0,023). Sono necessari ulteriori studi per verificare la possibilità di utilizzare, in una prospettiva futura, un pannello di marcatori di chemio- e radio-resistenza per identificare i pazienti potenzialmente sensibili al trattamento ed evitare che pazienti ad alto rischio di resistenza siano sottoposti a cure inutili e dannose.

PAROLE CHIAVE: *Tumori testa e collo • Carcinoma squamoso • Chemioterapia intra-arteriosa • Radioterapia • Markers tumorali*

Introduction

Surgery has traditionally played an important role in the treatment of patients with locally advanced head and neck squamous cell carcinoma (SCCHN), but radical tumour ablation can not be achieved in all patients [1]. Combined treatment protocols with simultaneous intra-arterial (IA) cisplatin administration and radiotherapy (RADPLAT) [2] have been reported to improve overall survival in advanced head-and-neck cancer with less toxicity compared to systemic chemotherapy and radiation [3]. The onset of mechanisms of chemo- and/or radio-resistance in the tumour cells is one of the major causes of failure of these protocols, and is associated with recurrent disease and/or distant metastases. Major mechanisms involved in chemo-resistance are: increased leakage of the drug from neoplastic cells mediated by trans-membrane transport proteins, such as p-glycoprotein and multi-drug resistance proteins [4-9], disregulation of apoptosis with prevalence of anti-apoptotic factors [10-12], increased activity of DNA repair proteins, such as hMLH1 [13-18], and of detoxifying agents, such as glutathione S-transferases and metallothionein [19 20]. The same factors play a role in tumour cell resistance to radiation [21]; moreover, replicative index, tissue vascular density and expression of the epidermal growth factor receptor appear to be associated with tumour response to radiotherapy [22].

Aim of the present study was to investigate the degree of expression of a series of markers of chemo- and/or radio-resistance in a group of patients with advanced SCCHN treated with combined IA carboplatin and radiotherapy. The results were correlated with the response to therapy, in terms of local response and overall and disease-free survival.

Materials and methods

Patients

Between April 2003 and December 2004, 28 patients with primary locally advanced SCC of the oral cavity, oropharynx and hypopharynx were enrolled in the treatment protocol defined by the Interdisciplinary Group for Head and Neck Cancer of the IRCCS Policlinico "S. Matteo" Foundation (Table I). This was a phase II study designed to evaluate the tolerability and efficacy of combined hyper-fractionated radiation therapy (HFX-RT) and high-dose IA carboplatin administration in patients with locally advanced SCCHN.

Table I. Clinical characteristics of patients.

Patient	Sex	Age (yrs)	Site	TNM	Stage	Grade
1	M	64	Oropharynx	T4N2BM0	IVA	G2
2	M	49	Oropharynx	T2N1M0	III	G2
3	M	45	Oropharynx	T3N0M0	III	G2
4	M	49	Oropharynx	T4N1M0	IVA	G2
5	M	40	Oropharynx	T4N0M0	IVA	G2
6	M	62	Oropharynx	T2N3M0	IVB	G3
7	F	57	Oropharynx	T4N0M0	IVA	G2
8	F	47	Oropharynx	T3N2cM0	IVA	G2
9	M	61	Oropharynx	T2N0M0	II	G2
10	M	69	Oropharynx	T3N1M0	III	G2
11	M	54	Oral cavity	T4N2aM0	IVA	G2
12	M	64	Oropharynx	T4N2cM0	IVA	G2
13	M	65	Oral cavity	T2N0M0	II	G3
14	M	70	Oropharynx	T2N0M0	II	G2
15	M	53	Oropharynx	T3N2cM0	IVA	G3
16	M	72	Oropharynx	T2N1M0	III	G3
17	F	67	Oral cavity	T3N0M0	III	G3
18	M	59	Oropharynx	T4N2aM0	IVA	G2
19	M	48	Oropharynx	T4N0M0	IVA	G2
20	M	51	Oropharynx	T2N0M0	II	G2
21	M	50	Oropharynx	T4N2cM0	IVA	G2
22	M	67	Oropharynx	T2N0M0	II	G2
23	M	51	Oropharynx	T2N0M0	II	G2
24	M	57	Oropharynx	T3N1M0	III	G3
25	M	62	Oropharynx	T3N0M0	III	G3
26	M	66	Oral cavity	T3N0M0	III	G2
27	M	50	Oral cavity	T4N1M0	IVA	G2
28	M	62	Oropharynx	T2N0M0	II	G3

Table II. Antibodies, reaction protocols, immuno-reactivity patterns.

Antibody	Source	Pretreatment	Dilution	Expression	Positive control
Drug transport					
p-Glycoprotein (p-Gp)	Novocastra, Newcastle, UK	MW 2 cycles 5' 650W Citrate buffer pH 6	1:20	Cytoplasm	Hepatocytes
Multi drug resistance 1 (MDR-1)	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:50	Cytoplasm	Small bowel epithelium
Multi drug resistance 3 (MDR-3)	Novocastra	MW 2 cycles 5' 650W EDTA in buffer pH 8	1:50	Cytoplasm	Small bowel epithelium
Multi drug resistance 5 (MDR-5)	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:200	Cytoplasm	
Lung resistance protein (LRP)	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:100	Cytoplasm	Small bowel epithelium
DNA repair					
MLH-1	BD Biosciences Pharmingen Worldwide	MW 4 cycles 5' 650W Buffer buffer pH 9.9	1:20	Cytoplasm	Small bowel epithelium
Pro-apoptosis					
Caspase 3	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:1200	Cytoplasm	Prostate glands
Caspase 8	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:60	Cytoplasm	Prostate glands
Fas (CD95)	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:80	Cytoplasm	Small bowel epithelium
Fas ligand (Fas-l)	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6	1:50	Cytoplasm	Prostate glands
Anti-apoptosis					
Bcl-x	Novocastra	//	1:20	Cytoplasm	Ovarian adenocarcinoma
Bcl-2	Dako Cytomation Carpinteria, CA	MW 3 cycles 5' 650W Citrate buffer pH 6	1:100	Cytoplasm	Lymphocytes
p53	Dako Cytomation	MW 3 cycles 5' 650W Citrate buffer pH 6	1:100	Nucleus	p53-mutated breast cancer
p16	Dako Cytomation	MW 3 cycles 5' 650W Citrate buffer pH 6	1:25	Nucleus	Squamous epithelium
Detoxification					
Metallothionein	Novocastra	MW 2 cycles 5' 650W Citrate buffer pH 6s	1:100	Cytoplasm	Hepatocytes
Glutathione S-Transferase pi (GST-pi)	Novocastra	//	1:100	Cytoplasm	Hepatocytes
Cell growth					
Epidermal growth factor receptor (EGFR)	Zymed, San Francisco CA	MW 2 cycles 5' 650W Citrate buffer pH 6	1:50	Cytoplasmic membrane	Respiratory epithelium
Proliferation marker					
Ki 67	Dako Cytomation	Trypsin 5' + MW 2 cycles 5' 650W Citrate buffer pH 6	1:50	Nucleus	Lymphocytes

MW: microwave oven

This study was approved by the Ethics Committee of the IRCCS Policlinico "S. Matteo" Foundation; all patients gave informed consent to the study. All patients underwent meticulous clinical evaluation [22] and the instrumental investigations considered more appropriate (fibroendoscopy, computed tomography (CT) scan, magnetic resonance imaging (MRI), arteriography, positron emission tomography (PET) including collection of biopsy tissue from the lesion for histological confirmation of the disease). Inclusion criteria of patients, in the study, have been reported elsewhere [23]. All patients received IA carboplatin (350 mg/m^2) administration in 130 ml saline solution (0.9%) rapidly

infused over a period of 15-20 min (infusion rate 4-6 mL/min) by way of percutaneous catheterization of the femoral artery and transfemoral-carotid artery angiography to selectively encompass the dominant blood supply of the targeted tumour. The treatment schedule for chemoinfusion was days 1, 15, 30, 45. The combined hyper-fractionated radiation therapy (HFX-RT) was started on day 1, patients received 1.8-2.0 Gy per fraction once daily, 5 days a week, for a total of 6-7 weeks; the total planned dose was 40.0-72.0 Gy according to the clinical stage and the primary target volume.

Treatment efficacy was evaluated according to the RECIST criteria [24], on the basis of clinical evaluation and fibroendoscopy performed immediately after the end of the treatment protocol and, thereafter, every 2 months. The first CT scan or MRI was performed after 5-6 months, since prior to this time, it is impossible to distinguish between scar tissue, due to the treatment, and persistence of the tumour [24]. In all those cases in which persistence of disease was suspected, multiple biopsies were performed for histological confirmation.

Complete response (CR) was defined as total disappearance of the macroscopically visible tumour; partial response (PR) was defined as the reduction of ≥ 50% of the tumour mass; no response (NR) was defined as a reduction of < 50% of the tumour.

Biopsy procedure and immuno-histochemical study
Biopsy samples were processed according to the routine protocol: formalin-fixed and paraffin-included. Diagnosis was made on haematoxylin and eosin-stained sections. Unstained sections, 4 micron-thick, were used for the immuno-histochemical analysis. At least one marker for each of the possible mechanisms of chemo-resistance, previously described, was included in the study. The selected antibodies with specific reaction protocols are outlined in Table II. Immunohistochemical reactions were revealed with peroxidase-conjugated streptavidin-biotin complex (LSAB + kit, Dako) using diaminobenzidine tetrahydrochlorohydrate as chromogen substrate (Dako liquid DAB substrate-chromogen system). Each reaction set included a negative control obtained with substitution of the primary antibody with dilution buffer and positive controls, as suggested by the provider.

Immunohistochemical reactions were evaluated by a pathologist blinded to the patients' features and follow-up. The presence of immuno-reactivity was assessed on neoplastic cells excluding normal epithelium, dysplastic areas, submucosal glands, connective stroma and inflammatory cells. For ki-67, p-53, p-16, Bcl-2, Bcl-x, LRP, MDR 1, 3 and 5, metallothionein, GST-pi, and p-Gp, the percentage of immuno-reactive cells was evaluated and scored as follows: 0: < 5% of cells; 1: 5-30%; 2: 31-50%; 3: 51-80%; 4: 81-100%. Degrees 0-2 were further aggregated as low expression (< 50% of neoplastic cells) and degrees 3-4 as high expression (> 50% of neoplastic cells). FAS, FAS-l, caspase 3 and caspase 8 were homogeneously expressed in all neoplastic cells, thus the degree of overall immuno-reactivity was semi-quantitatively evaluated as follows: 0: absence of expression 1: light expression; 2: moderate expression; 3: intense expression. For the statistical analysis, samples were also classified as positive or negative on the basis of the presence of any immuno-reactivity.

MLH-1 positive expression was characteristic of normal tissue; reduced expression was interpreted as pathological in neoplastic tissue, and classified as marked moderate or light. EGFR immuno-reactivity was scored as follows: 0: no stain or membrane reactivity in < 5% of cells; 1+: incomplete membrane reactivity in > 10% of cells; 2+: light or moderate complete membrane reactivity in > 10% of cells; 3+: strong complete membrane reactivity in > 10% of cells. Scores 0-1 were considered as negative, and scores 2-3 as positive.

Statistical analysis
The χ^2 test was used to analyze the association of each antigen expression with patient data (age, sex), tumour characteristics (degree of differentiation, stage, site) and with treatment outcome (complete response *vs.* no response or recurrence, disease-free survival and overall survival). Since recurrence and death from the disease usually occur within the first year after the beginning of the treatment, these two last parameters were divided into two categories: less than, or longer than one year; p values ≤ 0.05 were considered statistically significant.

Results

Immuno-histochemical analysis
Of the 28 patients recruited in the protocol, 21 (19 male, 2 female, mean age 57 years, SD 9.2, range 40-70) were considered suitable for the study. Five patients were excluded as biopsy samples were not available, having been obtained, processed and examined in other centres; 2 patients were excluded as IA therapy has been stopped after one or two infusions, on account of the appearance of severe haematological toxicity, even if these patients completed radiotherapy. Results of the immuno-histochemical investigation are outlined in Table III.

Treatment response and follow-up
Of the 21 patients, 20 (95.2%) showed complete response (CR) to the treatment while one patient (4.8%) did not respond (NR); analysis of follow-up (mean 13.5 months; range 4-26) showed that 17 patients (85%) were free from disease; and 3 (15%) had recurrent disease, 2 in the oro-pharyngeal area, and one both locally and at a distance, with bone metastases. The 2 patients with local recurrence were successfully treated with salvage surgery, while the patient with bone metastases died of the disease. The only NR patient died of the disease 9 months after the beginning of treatment; finally, one patient died from other causes, free from the disease, 7 months after the beginning of treatment. The mean disease-free survival (DFS) was 11.7 months (SD 6.8 months; range 0-26) and the mean overall survival (OS) was 13.2 months (SD 6.1 months; range 4-26).

Statistical analysis
None of the markers investigated showed a significant correlation with age and sex. As far as concerns the primary site of the disease, p-Gp was significantly over-expressed in the oro-pharyngeal tumours compared to tumours of the oral cavity (p 0.035). In poorly differentiated (G3) tumours, caspase 8, MDR-3 and GST-pi were significantly under-expressed (p 0.04, 0.02 and 0.035, respectively) compared to

Table III. Immuno-histochemical results.

Marker	Positive N (%)	Negative N (%)	High expression N (%)	Low expression N (%)
Proliferation marker				
Ki 67	21 (100)	0 (0)	9 (42.8)	12 (57.2)
Anti-apoptosis				
p53	13 (62)	8 (38)	9 (42.8)	4 (19)
p16	13 (62)	8 (38)	9 (42.8)	4 (19)
Bcl-2	12 (57.2)	9 (42.8)	3 (14.3)	9 (42.8)
Bcl-x	18 (85.7)	3 (14.3)	6 (28.6)	12 (57.2)
Pro-apoptosis				
Fas (CD95)	17 (81)	4 (19)	9 (42.8)	8 (38)
Fas ligand (Fas-l)	21 (100)	0 (0)	16 (76)	5 (24)
Caspase 3	18 (85.7)	3 (14.3)	10 (47.6)	8 (38)
Caspase 8	17 (81)	4 (19)	12 (57.2)	5 (24)
DNA repair				
MLH-1	13 (62)	8 (38)[*]	3 (14.3)[*]	5 (24)[*]
Drug transport				
p-Glycoprotein (p-Gp)	11 (52.4)	10 (47.6)	1 (4.7)	10 (47.6)
Multi drug resistance 1 (MDR-1)	16 (76)	5 (24)	3 (14.3)	13 (62)
Multi drug resistance 3 (MDR-3)	7 (33.3)	14 (66.7)	0 (0)	7 (33.3)
Multi drug resistance 5 (MDR-5)	12 (57.2)	9 (42.8)	1 (4.7)	11 (52.4)
Lung resistance protein (LRP)	21 (100)	0 (0)	15 (71.4)	6 (28.6)
Detoxification				
Metallothionein	18 (85.7)	3 (14.3)	8 (38)	10 (47.6)
Glutathione S-Transferase pi (GST-pi)	19 (90.5)	2 (9.5)	13 (62)	6 (28.6)
Cell growth				
Epidermal growth factor receptor (EGFR)	6 (28.6)	15 (71.4)	2 (9.5)	4 (19)

[*] Positive expression is characteristic of normal tissue; expression was pathologically reduced in neoplastic tissue in 8 cases, in 5 of which the reduction was marked, and in 3 was light.
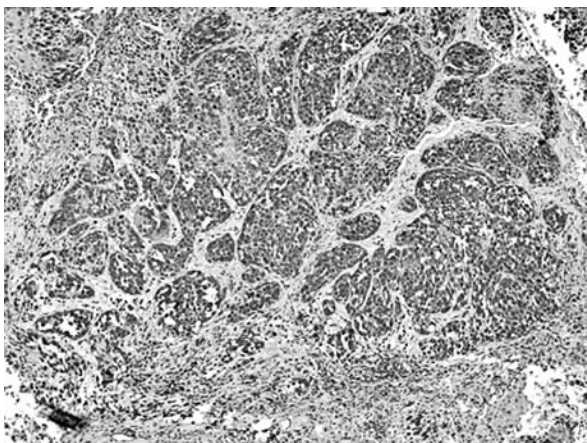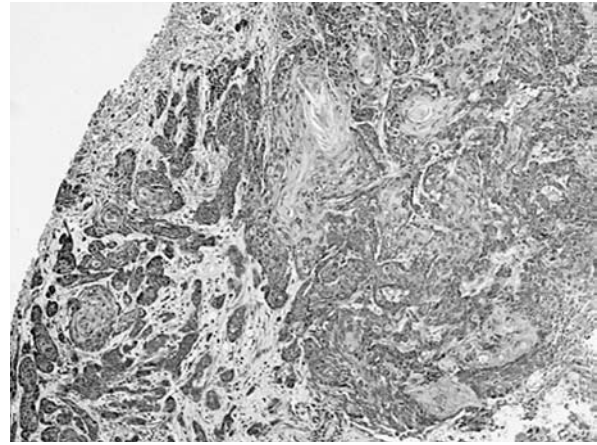

Fig 1. Squamous cell carcinoma showing marked immunoreactivity for the pro-apoptotic factor caspase 8.
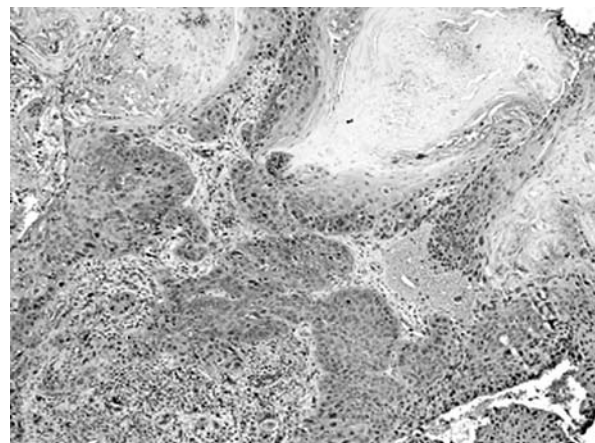

Fig. 2. Squamous cell carcinoma showing low immuno-reactivity for the pro-apoptotic factor caspase 8. The degree of expression was significantly reduced in non-responders and in patients with disease recurrence.

more differentiated neoplasms. Moreover, in these tumours, the loss of MLH-1 expression was significantly more frequent (p 0.02). p-Gp was also significantly over-expressed in cancers in the advanced stage (p 0.004). Tumour stage and grade did not show any correlation with treatment outcome. In non-responders and in patients presenting recurrence, caspase 8 (Figs. 1, 2) was significantly (p 0.05) under-expressed while p-Gp (p 0.035) and MDR-3 (p 0.049) were significantly over-expressed. Furthermore, evaluating, in a cumulative fashion, the expression of the three anti-apoptotic factors included in the study (p-53, BCL-2, BCL-x), the tumours with NR or recurrence more frequently over-expressed two or more of these factors (p 0.01). The patients with a shorter overall survival (< 1 year) significantly over-expressed p53 (Figs. 3, 4) (p 0.04), LRP (Figs. 5, 6) (p 0.038) and a larger number of trans-membrane transport proteins compared with those who survived > 1 year (p 0.013). Finally, patients with the shortest disease-free survival presented over-expression of p53 (p 0.027) and BCL-x (p 0.023).


Fig. 5. Squamous cell carcinoma showing marked immuno-reactivity for the trans-membrane drug transport protein LRP. Over-expression of LRP was significantly more frequent in patients with shorter overall survival.
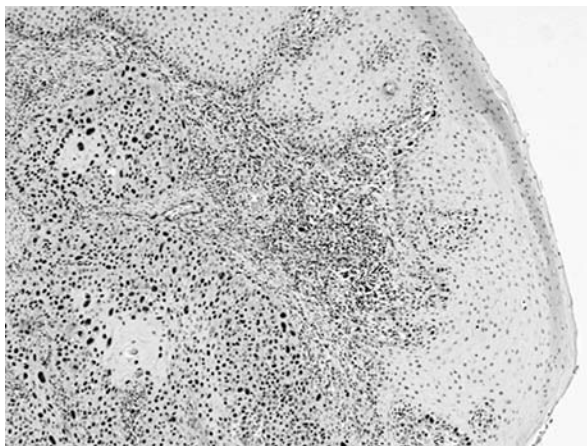
Fig. 3. Accumulation of abnormal, non-functional p53 is revealed in a large proportion of tumour cell nuclei by the immuno-histochemical reaction. Over-expression of p53 was significantly more frequent in patients with shorter overall and disease-free survival.

Fig. 6. Squamous cell carcinoma showing low immuno-reactivity for the trans-membrane drug transport protein LRP.
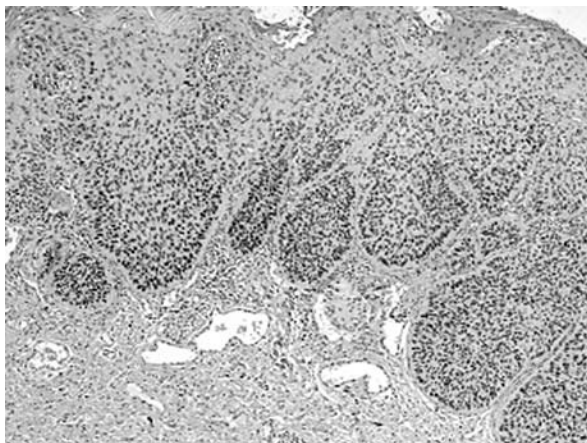
Fig. 4. Absence of immuno-reactivity for p53 in a squamous cell carcinoma with normal p53.

Discussion

The present study demonstrated over-expression, in SCCHNs, of a series of molecules known to play a role in resistance to chemo- and/or radiotherapy. The degree of expression of some of these molecules showed an association with tumour characteristics, and, more interestingly, with tumour outcome and patient survival after combined IA carboplatin and radiotherapy.

Caspase 8, MDR-3 and GST-pi were under-expressed in poorly differentiated SC tumours. All these proteins are known to be involved in cell homeostasis, thus their loss could be explained as a consequence of disorganization of cell function in less differentiated tumour cells. Moreover, poorly differentiated tumours showed a significant loss of expression of the protein encoded by the mismatch repair gene MLH-1, which highlights a

progressive reduction of the capacity to repair damaged DNA strands, with the consequent accumulation of mutations and their transmission with mitotic division [13-18]. It has been shown [15] that MLH-1 inactivation, in head and neck tumours, depends on the hyper-methylation of the gene promoter and not on somatic mutations, as occurs in hereditary colon carcinoma [12]. Hyper-methylation occurs late in tumour progression and is not the trigger of neoplastic transformation, but rather the result of progressive loss of differentiation of tumour cells, thus explaining the reduced protein expression in poorly differentiated tumours.

In our series, tumour grading and clinical stage were not correlated with treatment outcome. The pro-apoptotic factor caspase 8 was associated with outcome, being significantly under-expressed in patients with no response or recurrence. Two other proteins, MDR-3 and p-Gp, involved in trans-membrane drug transport, were expressed with greater frequency and/or intensity in tumours with negative outcome. No data in the literature have, so far, documented the association of caspase 8 with chemo- or radio-sensitivity. However, since both types of resistance have been associated with a reduction in apoptotic activity, as confirmed in our study showing an association of the over-expression of several anti-apoptotic factors and negative outcome, the loss of expression of a key pro-apoptotic protein could play a role in the development of chemo- and radio-resistance.

The expression of p-Gp in SCCHN has been widely documented [4-7], and its possible association with response to treatment has been investigated. p-Gp is a trans-membrane pump that regulates the leaking of toxic substances, including drugs, from the cell. Our results are supported by recent *in vivo* studies demonstrating p-Gp over-expression associated with resistance to associated chemo-radiotherapy in SCCHN [5 6]. Likewise, MDR-3 expression has been associated with resistance to platinum derivates, at least in some *in vitro* studies on glial and lung tumours [9].

The observed over-expression of anti-apoptotic factors, such as BCL-2, BCL-x and mutated p-53, in tumours of patients with unfavourable outcome and/or reduced disease-free or overall survival is widely confirmed in the international literature [25-33]. Clearly, the prevalence of factors that inhibit programmed cell death is associated with a more aggressive tumour cell phenotype and with resistance to treatment protocols based on cell DNA damage and subsequent induction of the apoptotic process.

Finally, we observed a significant association between reduced survival and over-expression of membrane transport factors. Data in the literature do not confirm our results and demonstrate the absence of any significant association [31 34 35]. We also found no significant results when analysing single proteins, but found a significant negative association between the number of co-expressed transport factors and the length of survival. On the basis of all these data, it is necessary, in our opinion, to more deeply investigate the expression of LRP, MDRs and p-Gp in SCCHN in basal conditions and after chemotherapy, particularly in view of new therapeutic protocols based on the association of carboplatin with other chemotherapeutic agents.

Data obtained in this study did not confirm the extensively reported correlation between nuclear replicative index ki-67, and the response to radiotherapy [26 29 30 36-38]. The concomitant use of chemotherapy may account for the lack of correlation. On the other hand, GST-pi has frequently been associated with unsuccessful platinum treatment [30 33 34]. Again, these data were not confirmed in the present study.

Combined chemo-radiation appears to be highly effective in the treatment of locally advanced SCCHN, with a high percentage of complete responses and a low recurrence rate. The treatment is, however, burdened by a not negligible haematological toxicity. The present study represents a preliminary investigation aimed at defining the expression of a panel of markers of chemo- and radio-resistance, covering all known mechanisms, in SCCHN, and a first attempt to correlate this expression with the outcome of an experimental protocol of concurrent chemo-radiation. In a future perspective, a panel of markers of chemo- and radio-resistance could help to identify those patients potentially sensitive to the treatment and to avoid patients at high risk of resistance being submitted to ineffective and toxic treatment. Further studies are necessary, both on biological grounds, to assess the role of various markers in the mechanisms of chemo-radio-resistance, and on a larger clinical series to clarify their association with the clinical outcome of treatment.

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