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ORIGINAL ARTICLE

Tumor heparanase expression in predicting response to induction chemotherapy in patients with locally advanced laryngeal cancer

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Summary

Purpose: *Induction chemotherapy is a feasible alternative to surgery for the treatment of locally advanced laryngeal cancer. Determining predictive factors associated with a better response to chemotherapy would help choose the patients most likely to benefit from larynx preservation.*

Methods: *Eighty-four patients diagnosed with locally advanced laryngeal cancer (stage III-IV) between April 1999 and May 2006 were retrospectively reviewed. Eighty-two of them received 2 cycles and 2 received only 1 cycle of cisplatin and 5-fluorouracil (5-FU) chemotherapy. Patients were then grouped, based on response to treatment, as either having complete response (CR), partial response (PR), stable (SD) or progressive disease (PD). Factors predicting response to treatment were evaluated. Paraffin blocks were immunohistochemically examined for heparanase activity to see for any link between heparanase expression and response to treatment.*

Results: *There were 73 males and 11 females with a mean age of 59 years. After induction chemotherapy (cisplatin and 5-FU), 33 patients achieved PR and 20 CR. SD and PD occurred in 9 and 21 patients, respectively. Patients with stage III disease had better overall (CR and PR) response rates when compared with those with stage IV disease. Moreover, development of bone marrow suppression and heparanase positivity were both associated with better overall response rates.*

Conclusion: *This study supports the hypothesis that heparanase positivity is associated with better responses to induction chemotherapy, regardless of TNM stage. Furthermore, a higher overall response rate was observed in patients who developed myelosuppression secondary to chemotherapy.*

Key words: chemotherapy, heparanase, laryngeal cancer, prediction, response

Introduction

Squamous cell carcinoma of the larynx is associated with a high morbidity and mortality, mainly because most patients are usually diagnosed with advanced local disease. Locally advanced squamous cell carcinomas of the head and neck are usually managed by surgery and radiation or by a combination of chemotherapy, radiotherapy, and selected surgery [1,2]. Combination chemotherapy with cisplatin and 5-fluorouracil is the most commonly used induction regimen. This regimen has been reported to result in combined CR and PR rates of up to 80%, with CR rates ranging from 20-30% [3-5]. In our previous report, response to induction chemotherapy was 71%, and CR rate was 17.8% [6]. Induction chemotherapy with cisplatin and 5-fluorouracil and radiotherapy are effective, organ-preserving therapies alternative to surgery in patients with carcinoma of the larynx and hypopharynx [7]. The precise clinical and molecular parameters that may help predict the response to treatment in patients chosen for organ-preserving chemotherapy have yet to be established [8-10]. Such knowledge would go a long way in making the decision to choose the most suitable patient for organ-preserving treatment a lot easier.

Several contradictory reports on tumor vascularity and its effect on response to chemotherapy may be encountered in the literature. It is widely believed that tu-

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mor blood supply determines response to treatment, and that susceptibility to chemotherapy increases in parallel to the extent of tumor vascularity. Furthermore, it has been suggested that other factors such as tumor size, age, sex, and stage also influence the response to treatment.

Heparanase is an endoglycosidase that specifically cleaves heparan sulphate (HS) side chains of heparan sulphate proteoglycans (HSPG). Traditionally, heparanase activity has been implicated in cellular invasion associated with angiogenesis, inflammation and cancer metastasis [11,12]. To the best of our knowledge, heparanase activity in laryngeal cancer patients has yet to be studied on a large scale.

The aim of this study was to investigate the role of several clinical and pathological factors, as well as the extent of tumor heparanase expression in predicting response to induction chemotherapy.

Methods

Patients with newly diagnosed resectable, locally advanced laryngeal cancer, who were followed up between April 1999 and May 2006 at the Department of Medical Oncology, Faculty of Medicine, Hacettepe University, were included in this study. Staging procedures consisted of history, physical examination, panendoscopy and biopsy, computed tomography (CT) of the primary tumor site and the neck, chest x-ray and routine laboratory studies. Patients were staged according to the American Joint Committee on Cancer Staging. Before being enrolled onto the study, each patient was reviewed at a joint conference with a representative from a surgical department, a radiation oncologist and a medical oncologist.

Eligibility criteria

Eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2, no history of prior chemotherapy or radiotherapy, histopathologic diagnosis of squamous cell carcinoma located in the larynx or hypopharynx, nonmetastatic clinical stages III and IV, resectable lesion, age less than 75 years, leukocyte count > 4,000/mm^3, platelet count > 100,000/mm^3, serum creatinine level <1.2 mg/dL, and serum bilirubin <2 mg/dL. Toxicity was recorded by utilizing the RTOG toxicity criteria.

Induction chemotherapy

Eighty-two patients received 2 cycles and 2 only 1 cycle of induction chemotherapy, consisting of cisplatin 20 mg/m^2/day on days 1-5, and 5-FU 600 mg/m^2/day by continuous infusion on days 1-5, repeated at 3-week intervals.

Local treatment

After the 2 cycles of induction chemotherapy, all patients were evaluated by direct laryngoscopic and physical examination besides CT for response evaluation. Patients with PR and CR were assigned to radiotherapy with Cobalt 60 or Linear accelerator.

Patients with SD or PD were planned to undergo surgery and postoperative radiotherapy.

Histopathological evaluation

After confirmation of the pathological diagnosis of squamous cell carcinoma of the larynx, the grade of differentiation was established, defined as undifferentiated, poorly differentiated, moderately differentiated and well differentiated. Heparanase activity was measured from the paraffin blocks using immunohistochemistry. Slices 5 μm in thickness were obtained from the paraffin blocks, which were fixed with formalin. Heparanase activity was measured using Polyclonal Rabbit Anti-Human heparanase 1 (HPA1) antibody kits (Cell Sciences Inc.), which showed values ranging from negative to 3+.

Treatment evaluation

Response to treatment was evaluated after induction chemotherapy. Response evaluation was done by direct laryngoscopic and physical examination besides CT. Response criteria were based on bidimensional tumor measurements and defined as CR, PR, SD, and PD. CR was defined as complete disappearance of clinical and radiologic evidence of disease, while PR was defined as any response with a reduction of 50% in the sum of the products of the crossed dimensions of all measurable lesions. Patients with tumor reduction of <50% were considered to have SD. PD was defined as an increase >25% in the sum of the products of the crossed dimensions of all measurable lesions or as the appearance of new areas of locally recurrent or metastatic tumor.

Statistical considerations

The study was designed to help determine clinical and pathological predictors of response to induction chemotherapy. Overall survival (OS) was assessed from the first day of treatment until death or until last patient contact. Disease free survival (DFS) was calculated from the date of CR. Statistical evaluation of the data was done with a two-tailed Student's t-test when simple comparison between two groups was required; chi-square test was used to establish the statistical significance of distributions. Nonparametric Spearman's correlation coefficient method was used to assess the statistical significance of the correlation between clinicopathologic tumor characteristics and heparanase expression. Patient survival curves were calculated using the Kaplan-Meier method and analysis was done by the log-rank test. Differences were significant at $p < 0.05$. All statistical tests were done using the Statistical Package for Social Sciences, v.13.0 for Windows.

Results

Patient characteristics

A total of 84 patients with locally advanced stage of laryngeal cancer who presented between April 1999 and May 2006 were included in this study. The patient baseline characteristics are summarized in Table 1.

There were 73 (86.9%) males with a mean age of 59 years (range 33-75). According to the TNM classification, 43 (51.2%) cases had stage III, 40 (47.6%) stage IVa, and 1 stage IVb. When evaluated for pretreatment PS, 28, 23 and 8 patients had a ECOG PS of 0, 1 and 2, respectively, and 80 (95.2%) patients had a his-

Table 1. Patient characteristics

Characteristics	*Patients N (%)*
Age (years)	
Mean	59
Range	33-75
Sex	
Male	73 (86.9)
Female	11 (13.1)
ECOG performance status	
0	28 (33.3)
1	23 (27.4)
2	8 (9.5)
Stage	
III	43 (51.2)
IVa	40 (47.6)
IVb	1 (1.2)

tory of smoking with a mean cigarette consumption of 45.5±2.8 pack/year. A survey of alcohol consumption revealed that 36.9% of the subjects consumed alcohol at least once a week, while 56% denied alcohol use. All patients included had received neoadjuvant chemotherapy: 82 (97.6%) patients received 2 cycles, while the remaining 2 (2.4%) patients received only 1 cycle.

Response to neoadjuvant chemotherapy, heparanase activity and overall survival

Thirty-three (39.3%) patients achieved PR and 20 (23.8%) CR. SD and PD were encountered in 9 (10.7%) and 21 (25%) patients, respectively. Table 2 summarizes the different responses to treatment.

When treatment response was evaluated with respect to stage of disease, it was found that patients with stage III disease had higher PR or CR rates than those with stage IV disease (p=0.006), with more patients with the latter stage having SD or PR disease.

Each patient's biopsy samples (paraffin blocks) were reevaluated to ascertain tumor differentiation and in particular heparanase expression (Table 3). When the response to treatment was evaluated in terms of heparanase expression regardless of stage of disease, it was discovered that most of the patients with (+2) and (+3)

Table 2. Response rates to neoadjuvant chemotherapy

Response	*Patients N (%)*
Complete response	20 (23.8)
Partial response	33 (39.3)
Stable disease	9 (10.7)
Progressive disease	21 (25)
Undetermined	1 (1.2)

Table 3. Tumor heparanase expression and treatment response

	Hep^{-}/Hep^{+1}	Hep^{+2}/Hep^{+3}
CR/PR	20	33
SD/PD	23	7

CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, Hep^{-}: heparanase negative, Hep^{+1}: Heparanase 1+, Hep^{+2}: Heparanase 2+, Hep^{+3}: Heparanase 3+

heparanase expression had a PR or CR when compared with those with poor expression (+1) or no detectable heparanase at all (p=0.04).

The median follow-up period for the patients included in the study was 23.8 months (range 1-75), with a median OS of 38.2 (95% CI 19.5-56.8; Figure 1) and a median DFS of 35.7 (95% CI 10.7-61.2). When OS was evaluated with respect to heparanase expression, the difference between the patients negative for heparanase and those with varying levels of positivity was statistically insignificant (p=0.07; Figure 2).

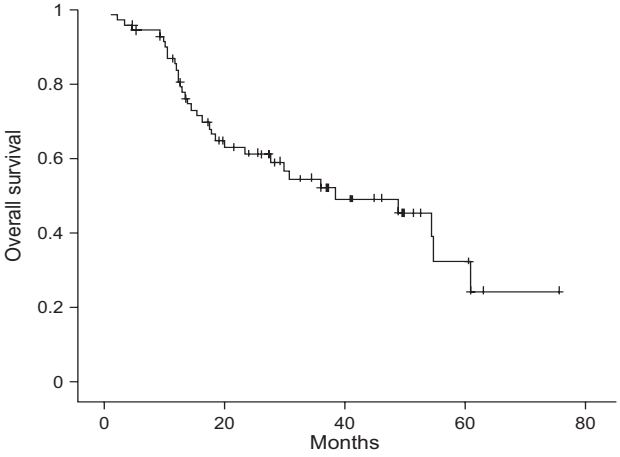

Figure 1. Patient overall survival.

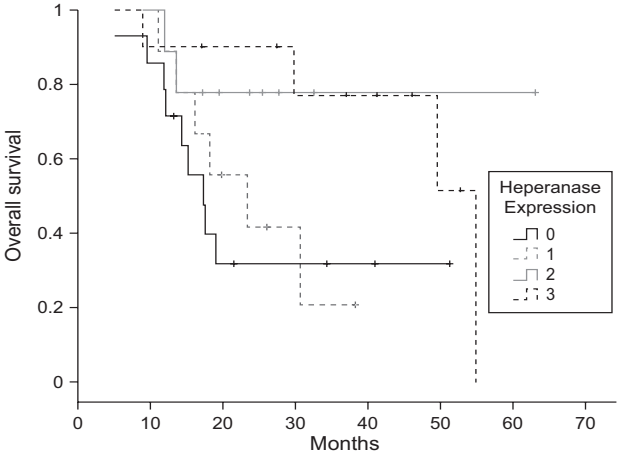

Figure 2. Patient overall survival according to heparanase expression (p=0.07).

Toxicity and side effects

After treatment, 33 (39.2%) and 18 (21.42%) patients developed grade I and II nausea and vomiting, respectively, with varying levels of renal toxicity (increase in serum creatinine > 30% of baseline value) occurring in 15 (17.9%) patients (13 patients: grade I and II, 2 patients grade 4; Table 4). Eighteen (21.4%) patients had some degree of mucositis.

In terms of bone marrow toxicity, grade III neutropenia developed in 16 (19%) patients, while grade IV neutropenia was established in 13 (15.5%) patients. On the other hand, 34 (40.5%), 9 (10.7%) and 15 (17.9%) patients developed grade I, II and III thrombocytopenia, respectively. While 38 (45.2%) patients did not develop anemia, 26 (31%) cases had grade I-II anemia, 15 of whom required blood transfusion.

When the relationship between response to treatment and the development of neutropenia or thrombocytopenia were evaluated, it was discovered that patients with some degree of bone marrow toxicity in the form of neutropenia or thrombocytopenia had more favorable response rates, while in the absence of bone marrow toxicity, the rate of SD and PD was higher (p = 0.001) (Tables 5 and 6). Similarly, when patients were assessed according to whether they developed anemia or not, anemic patients had higher rates of PR or CR (p=0.007).

Table 4. Toxicity of induction chemotherapy regimen

	N (%)			
	Grade 1	*Grade 2*	*Grade 3*	*Grade 4*
Nausea/vomiting	33 (39.2)	18 (21.42)	5 (5.95)	–
Renal*	8 (9.52)	5 (5.95)	–	2 (2.4)
Neutropenia	24 (28.6)	16 (19)	16 (19)	13 (15.5)
Thrombocytopenia	34 (40.5)	9 (10.7)	15 (17.9)	–

*Increase of serum creatinine >30% of baseline

Table 5. Frequency of neoadjuvant chemotherapy-associated neutropenia based on response

	Partial response N (%)	*Complete response N (%)*	*Stable disease N (%)*	*Progressive disease N (%)*
Grade 0	–	–	–	5 (29.4)
Grade 1	6 (20.7)	1 (5.2)	7 (87.5)	10 (58.8)
Grade 2	11 (38.0)	2 (10.5)	1 (12.5)	2 (11.8)
Grade 3-4	12 (41.3)	16 (84.3)	–	–

Table 6. Frequency of neoadjuvant chemotherapy-associated thrombocytopenia based on response

	Partial response N (%)	*Complete response N (%)*	*Stable disease N (%)*	*Progressive disease N (%)*
Grade 0	4 (14.2)	–	1 (12.5)	10 (58.8)
Grade 1	16 (57.1)	5 (26.3)	6 (75.0)	7 (41.2)
Grade 2	5 (17.8)	3 (15.8)	1 (12.5)	–
Grade 3-4	3 (10.7)	11 (57.9)	–	–

Discussion

PR was achieved in 39.9% of the patients included in this study, while CR was observed in 23.8%, with 10.7% having stable disease. Our results are consistent with other reports concerning response rates observed in patients with squamous cell head-neck tumors who received cisplatin and 5-fluorouracil combination chemotherapy [4,5]. When the response to chemotherapy was evaluated according to stage of disease, patients with stage III disease were found to have a higher PR or CR rate when compared to those with stage IV disease [13]. Neutropenia developed in 82.1% of our patients, while 69.1% had some degree of thrombocytopenia. Similar results with cisplatin and 5-fluorouracil chemotherapy for advanced-stage local head-neck cancer may be encountered in the literature, with neutropenia reported at 44% [14], and in another study bone marrow suppression was reported in 62% of patients [15]. Our results seem consistent with the current relevant literature. Moreover, our study demonstrates that the development of neutropenia, thrombocytopenia or anemia is associated with better responses to chemotherapy (p=0.001). The link between both the therapeutic and side effects of chemotherapy has been a topic of discussion for many years. In one study, the prognostic value of post-treatment bone marrow suppression in determining response to treatment was investigated. It was observed that responders to treatment had a higher rate of bone marrow suppression [16]. A more recent study with a similar theme on non-small cell lung cancer patients demonstrated a more favorable outcome in patients who developed neutropenia in terms of response to treatment and OS, when compared with those in whom neutropenia did not occur [17]. Some authors have hypothesized that the degree of bone marrow suppression after treatment is an indirect indicator of the biological activity of the chemotherapeutic agents [18]. Both tumor cells and healthy cells are bound by the same pharmacokinetic laws when exposed to any drug (distribution, metabolism, etc). The development of post-treatment bone marrow suppression is a sign of effective drug distribution, ensuring that tumor cells are sufficiently exposed to a chemotherapeutic agent, while a similar study in breast cancer patients also es-

tablished a link between failure to develop neutropenia and a higher relapse rate [19].

In this study, a possible correlation between tumoral heparanase expression and response to treatment was investigated. Those with substantial heparanase expression had higher PR and CR rates when compared with those with poor expression or undetectable levels of heparanase (p=0.04). Several laboratory and clinical studies have demonstrated a link between heparanase activity and cellular invasion, angiogenesis and inflammation. Heparanase expression has been reported to increase the frequency of tumor invasion and metastasis [18]. Positive correlation between the degree of heparanase positivity and the vascular density of tumor tissue has been established [20,21]. An increase in intratumoral vascularity is paralleled by a similar increase in heparanase expression [22,23]. Although it is widely accepted that tumoral blood supply is one of the most important factors influencing the response to chemotherapy, there still exists much controversy in the literature regarding this issue. In 2003, a study by Gadduci et al. in cancer patients demonstrated that the extent of intratumoral micro-vascularization potentiated the clinical response, with a better blood supply associated with a higher CR rate [24]. In another study by Zotterstrom et al. on 48 head-neck cancer patients, response to chemotherapy was found to increase in parallel with the degree of tumor vascularity, and better CR rates were observed in tumors with a better blood supply. However, no benefit with regard to OS or DFS was reported [8]. In a separate set of studies, a more or less similar link was established, this time between tumor vascularity and response to radiotherapy [25,26]. In our study, heparanase positivity was associated with a better response rate when compared to heparanase negative patients. Our data is consistent with the above mentioned studies in that increased intratumoral vascularity augments the response to chemotherapy. However, in the present study we failed to ascertain a direct link between heparanase activity and survival. Only a handful of reports investigating the association between heparanase positivity in head-neck cancer tissue and response to treatment, as well as survival, may be encountered in the literature. In a recent study on 46 patients with nasopharyngeal cancer by Bar-Sela et al., heparanase expression and survival were found to be inversely correlated [18]. In yet another study, it was established that the presence of heparanase in head-neck tumors was associated with increased invasiveness [27].

The importance of neovascularization for tumor growth has been emphasized through the years by several authors and the exact mechanism as well as to what extent cytotoxic agents target tumor vascularization remains somewhat of a mystery that requires further investigation. Although many reports have established a direct link between the degree of vascularization and response to treatment, several authors have also reported the opposite. It has been hypothesized that the better the blood supply, the higher the concentration of chemotherapy is achieved within the tumor tissue, thus potentiating the effect of chemotherapy. On the other hand, some have suggested that a better blood supply improves oxygenation, thus preventing spontaneous cell death. On a similar note, it has been argued that better vascularization would result in faster clonal proliferation between chemotherapy cycles, and therefore contributing to hyperkinetic resistance [28]. Further studies are required to shed light on this matter.

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