Exp Oncol 2009
31, 2, 102–105

Experimental ONCOLOGY

EXPRESSION OF CATHEPSIN L IN NASOPHARYNGEAL CARCINOMA AND ITS CLINICAL SIGNIFICANCE

*X. Xu[1, §, *], G. Yuan[2, §], W. Liu[1], Y. Zhang[1], W. Chen[2]*

[1]*Cancer Center, Renmin Hospital of Wuhan University, Wuhan 430060, China*

[2]*Department of Oncology, the Third Affiliated Hospital of Wenhou Medical College, Rui'an 325200, Zhejiang Province, China*

Aim: **To study the expression of cathepsin L in nasopharyngeal carcinoma (NPC), and analyze its relationship with clinicopathologic factors.** ***Methods:*** **The expression of cathepsin L was determined immunohistochemically in NPC, adjacent normal nasopharyngeal tissues and metastatic cervical lymph nodes. The correlation between its protein expression and clinicopathologic parameters as well as with long term follow-up data in NPC was analyzed.** ***Results:*** **The protein of cathepsin L was overexpressed in 47% primary tumor tissues, and in 89% metastatic cervical lymph node samples. Overexpression of cathepsin L was found to correlate with lymph node metastasis ($P = 0.04$) and distant metastasis ($P = 0.01$), and marginally with clinical stage and T classification, but not with patient age, gender and histological classification of tumor. Patients with overexpression of cathepsin L in tumor tissue had worse 5-year survival than those without such expression pattern ($P = 0.033$). Multivariate survival analysis showed that cathepsin L protein expression level had a marginal significant correlation with prognosis of NPC.** ***Conclusion:*** **Cathepsin L is a potential biomarker for prognosis of NPC and contributes to NPC metastasis.**

Key Words: **nasopharyngeal carcinoma, cathepsin L, immunohistochemistry, prognosis.**

Nasopharyngeal carcinoma (NPC) is highly prevalent in southern China, with incidence rates of 15–50 per 100,000 [1]. Although as a subtype of head and neck carcinoma, NPC is unique in epidemiology, clinical characteristics, etiology and histopathology. Compared with other head and neck squamous cell carcinomas, NPC tends to present at a more advanced stage of disease and exhibits higher metastatic potential [2, 3]. Despite advances in diagnosis and treatment, the survival rate for patients with NPC remains unchanged in the past years. To guide treatment and improve prognosis, identification of new biomarkers is required.

Destruction of surrounding tissues, such as the extracellular matrix and basement membrane, is an essential component of tumor invasion and metastasis. Many proteolytic enzymes are known to be involved in this process, including cysteine, aspartyl and serine proteases [4]. Cathepsins belong to lysosomal hydrolases that degrade proteins in lysosomes at an acidic pH, and are composed of several classes, such as cathepsin A, B, C, D, H, L. Of the cathepsin subtypes, cathepsin L, also known as MEP (major excreted protein), was reported to be more potent than the other cathepsin classes in degrading extracellular matrix components [5]. Chauhan *et al.* [6] demonstrated that cancers in general express higher levels of cathepsin L than normal tissues, including kidney, testicular, lung, breast, ovary, colon, adrenal, bladder, prostate, thyroid tumors. Cathepsin L has previously been found to correlate with metastatic potential in a number of model tumor systems, both *in vivo* and *in vitro* [7], and has been associated with outcome in a number of human cancers [8]. However, information on the relationship between cathepsin L and NPC is limited.

Received: May 3, 2009.

[§]Equal contribution.

*Correspondence: Fax: 86-27-88042292
E-mail: doctorxu120@yahoo.com.cn

Abbreviations used: NPC – nasopharyngeal carcinoma.

In this study, we examined the expression of cathepsin L in primary NPC, adjacent normal nasopharyngeal tissues and metastatic cervical lymph nodes by immunohistochemistry, and analyzed the relationship between its expression and clinicopathologic factors and follow-up data of NPC patients.

MATERIALS AND METHODS

Patients. Eighty-five patients with nasopharyngeal carcinoma (NPC) were included in this study. Their diagnosis was confirmed from 1999 to 2002 via nasopharyngeal biopsies at the Department of Pathology of Renmin Hospital of Wuhan University. All the patients presented no distant metastasis at their diagnosis. The patients received irradiation only or combined irradiation and chemotherapy. Clinical data on patient age, gender, stage of disease, histopathological classification (WHO) were collected. The median follow-up time was 61 months (range, 11 to 70 months). The 1992 NPC staging system of China was used for tumor staging [9], and the histopathological grade was defined according to WHO criteria. Our study was approved ethically by the institutional review board of Wuhan University.

Immunohistochemical staining. The expressions of cathepsin L in NPC, adjacent normal nasopharyngeal tissues and metastatic cervical lymph nodes, were detected by immunohistochemistry. The tissue sections were deparaffinized in xylene twice for 10 min each, progressively hydrated in graded ethanol, and then immersed in 3% hydrogen peroxide to quench endogenous peroxidase. Nonspecific binding sites were blocked by incubating the sections in 10% normal rabbit serum for 10–15 min. The sections were then incubated with mouse anti-human cathepsin L monoclonal antibody (dilution 1 : 1000; Jing Mei Biotechnology Co.) at 4 °C overnight, followed by incubation with biotinylated goat-anti-mouse secondary antibody (Boster Biotechnology Co.) conjugated to horseradish peroxidase (HRP) at room temperature for 10–15 min.

The staining was developed with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide, and the sections were counterstained with hematoxylin and mounted. For positive controls, a section of human liver tissue was used in each assay. Negative controls for all immunostainings were derived by substituting the primary antibody with normal rabbit serum.

Evaluation of immunostaining. The immunostained sections were observed under high-power magnification (× 200) and were evaluated independently by two pathologists without knowledge of the clinicopathologic details. Scoring of the immunohistochemical results was performed according to the methods described by Sinicrope et al. with minor modifications [10]. Briefly, the positive tumor cells were quantified, expressed as the percentage of the total number of tumor cells, and assigned to one of 5 categories: 0, ⩽ 5%; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, ⩾ 75%. The immunointensity was graded as: 1+, weak; 2+, moderate; and 3+, intense. Immunoreactivity scores for each case were produced by multiplication of the values for the two parameters. Cases with scores ⩾ 3 were considered as overexpression of cathepsin L.

Statistical analysis. Data were analyzed using SPSS 12.0 software. The chi-square test was used to analyze the relation between cathepsin L expression and clinicopathologic characteristics. Survival curves were plotted by the Kaplan — Meier method and compared by the log-rank test in relation to cathepsin L expression. Multivariate analysis was performed by Cox's proportional hazards regression model. $P \leqslant 0.05$ was considered statistically significant.

RESULTS

Patient or tumor characteristics. Patients consisted of 61 men and 24 women, and their age ranged in age from 24 to 66 years at the moment of diagnosis (mean, 47 years). Patient and tumor characteristics are presented in Table 1.

Table 1. Correlation between clinical pathological features and cathepsin L expression in nasopharyngeal carcinoma

Characteristics	Number of patients	Number (%) of cathepsin L overexpression	*P* value
Normal tissues	20	0	
NPC	85	40 (47%)	
Metastatic lymph nodes	18	16 (89%)	
Age (years)			
⩽ 45	40	18 (45.0%)	0.72
> 45	45	22 (48.9%)	
Gender			
Male	61	30 (49.2%)	0.532
Female	24	10 (41.7%)	
Stage			
I + II	27	9 (33.3%)	0.084
III + IV	58	31 (53.4%)	
T classification			
T1 + T2	36	13 (36.1%)	0.083
T3 + T4	49	27 (55.1%)	
Lymph node metastasis			
Negative	19	5 (26.3%)	0.04
Positive	66	35 (53.0%)	
Distant metastasis			
Negative	65	18 (27.7%)	0.01
Positive	20	12 (60.0%)	
Histological classification			
Type II	21	10 (47.6%)	0.953
Type III	64	30 (46.9%)	

Expression of cathepsin L. The expression of cathepsin L protein was observed in the cytolplasm of tumor cells and extracellular matrix adjacent to lesional cells. The immunostaining was shown as fine to coarse granular staining. The protein was overexpressed in 47% of primary tumor tissues, and in 89% metastatic cervical lymph node samples (Fig. 1, *a*, *b*). However, there was little immunoreactivity in the adjacent normal tissues (Fig. 1, *c*).

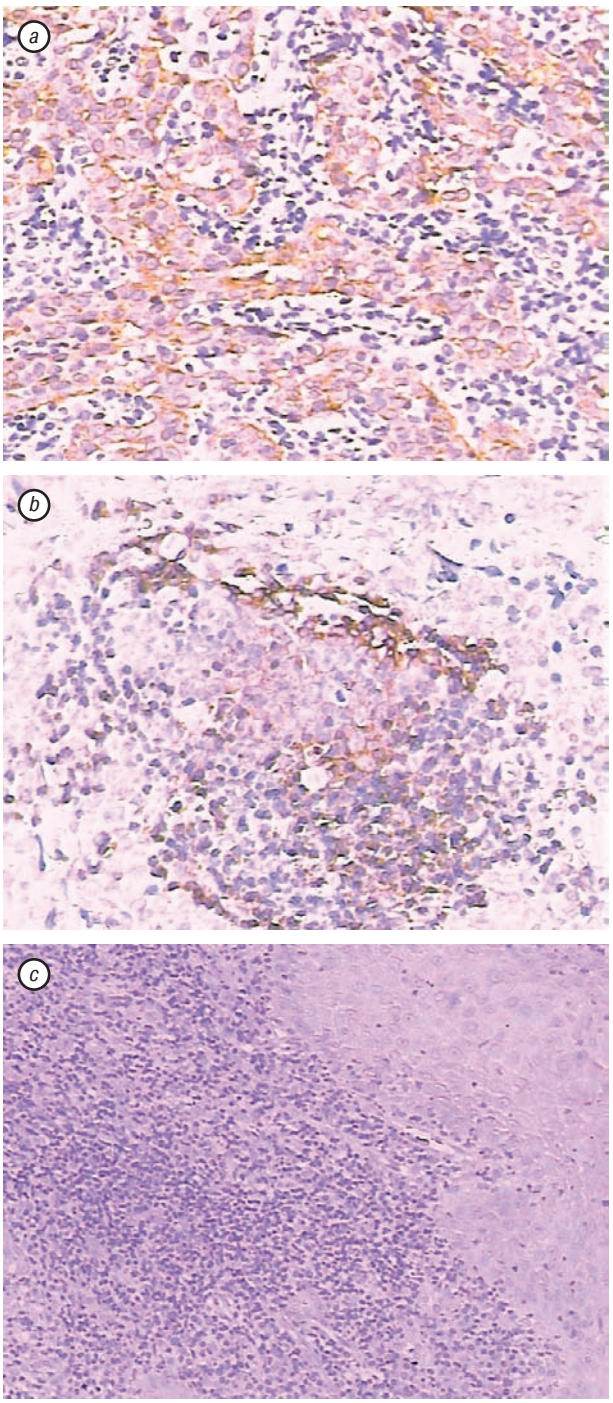

Fig. 1. Immunohistochemical analysis of cathepsin L expression. *a*, *b*, Overexpression of cathepsin L in NPC and metastatic lymph nodes; *c*, Negative expression in adjacent normal tissue. Original magnification x 100 or 200

Relation between cathepsin L expression and clinicopathologic factors, and follow-up data. The relationship between the expression of cathepsin L protein and various clinicopathologic features was

summarized in Table 1. There was no significant correlation between the expression of cathepsin L protein and age, gender of NPC patients, and histological classification of tumor. However, overexpression of cathepsin L was significantly associated with lymph node metastasis (P = 0.04), distant metastasis (P = 0.01), and marginally with clinical stage and T classification, as compared with tumors without cathepsin L overexpression.

The 5-year survival rate, assessed by the Kaplan — Meier method, was 75.6% in patients without overexpression of cathepsin L in tumor tissue; whereas it was only 52.5% in those with its overexpression. There was a negative correlation between 5-year survival rate and cathepsin L protein overexpression. That is, there was a significantly lower 5-year survival rate in patients with cathepsin L protein overexpression than in those without it (Fig. 2; log-rank test: P = 0.033).

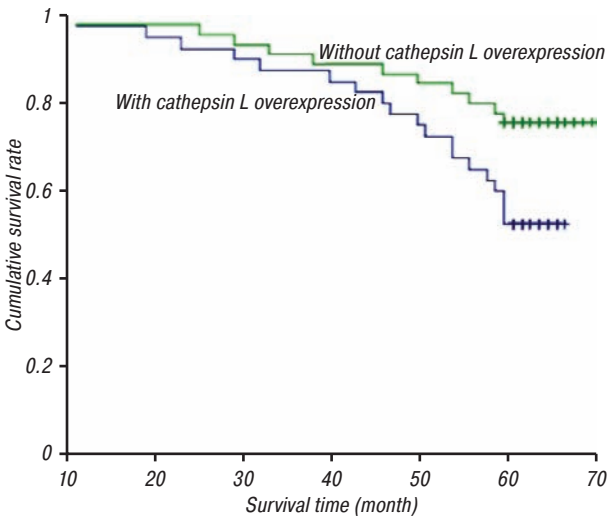

Fig. 2. Survival curves of patients with NPC, subdivided according to cathepsin L level

Besides cathepsin L protein expression level, T and N classification, and clinical stage were also significantly correlated with survival in Kaplan — Meier analysis and Log-rank test (for T classification, P = 0.02; for N classification, P = 0.012; for clinical stage, P < 0.001). We performed multivariate survival analysis, which included cathepsin L protein expression level, T and N classification, and clinical stage of the disease to determine whether cathepsin L protein expression level is an independent prognostic factor of outcome. The results showed that clinical stage was recognized as an independent prognostic factor, and cathepsin L protein expression level had a marginal significant correlation with prognosis of NPC (Table 2).

Table 2. Multivariate analysis of different prognostic variables in 85 patients with NPC by Cox's regression model

Factors	β value	Standard error value	*P* value
T classification	0.512	0.467	0.273
N classification	1.06	0.748	0.153
Clinical stage	2.393	1.038	0.021
Cathepsin L expression	–0.686	0.385	0.055

DISCUSSION

Proteases have been thought to play a role in tumor invasion and metastasis due to their destructive effects on extracellular matrix. As a lysosomal thiol protease, cathepsin L is more potent than many other cathepsin classes in degrading extracellular matrix as well as basement membrane components [5, 6]. It is synthesized and secreted in latent form. Cathepsin D and specific metalloproteases may play a role in its activation [11, 12]. Cathepsin L can also undergo autoactivation [13]. We assessed the protein level of cathepsin L semiquantitatively in NPC using immunohistochemical method with a specific monoclonal antibody that can discriminate between cathepsin L and other cathepsin classes but react with both active and latent forms. Therefore, the present data revealed not enzyme activity but the potential of the tumor to produce cathepsin L. In the present study, we showed that cathepsin L was more highly expressed in the primary NPC tissues than in normal nasopharyngeal tissues. Furthermore, the protein was overexpressed in the metastatic cervical lymph node samples. Cancers in general have been demonstrated to exrpess higher levels of cathepsin L than normal tissues, including kidney, testicular, lung, breast, ovary, colon, adrenal, bladder, prostate, thyroid tumors [6].

A large body of literature has accumulated to suggest that cathepsin L is involved in tumor invasion and metastasis. Alterations in the expression at protein or mRNA levels, as well as in the activity of the protease, have been found to correlate with malignancy of various murine and human tumors [14]. Overexpression of cathepsin L has been shown to be related to survival in clinical studies of malignant melanoma, breast, colorectal, gastric and brain cancers [15–19]. In the current study, we found that overexpression of cathepsin L in NPC was associated with lymph node metastasis and distant metastasis, and marginally with clinical stage and T classification. Expression of cathepsin L was also closely related to long-term outcome of patients with NPC. Patients with overexpression of cathepsin L in NPC tissue had a poorer 5-year survival rate than those without it. Multivariate survival analysis showed that cathepsin L protein expression level had a marginal significant correlation with prognosis of NPC. So, cathepsin L contributes to NPC metastasis and may be used as a potential biomarker for prognosis of NPC.

REFERENCES

1. **Yu MC, Yuan JM.** Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol 2002; **12**: 421–9.

2. **Vokes EE, Liebowitz DN, Weichselbaum RR.** Nasopharyngeal carcinoma. Lancet 1997; **350**: 1087–91.

3. **Ahmad A, Stefani S.** Distant metastases of nasopharyngeal carcinoma: a study of 256 male patients. J Surg Oncol 1986; **33**: 194–7.

4. **Duffy MJ.** The role of proteolytic enzymes in cancer invasion and metastasis. Clin Exp Metastasis 1992; **10**: 145–55.

5. **Mason RW, Johnson DA, Barrett AJ, Chapman HA.** Elastinolytic activity of human cathepsin L. Biochem J 1986; **233**: 925–7.

6. **Chauhan SS, Goldstein LJ, Gottesman MM.** Expression of cathepsin L in human tumors. Cancer Res 1991; **51**: 1478–81.

7. **Ishidoh K, Kominami E.** Gene regulation and extracellular functions of procathepsin L. Biol Chem 1998; **379**: 131–5.

8. **Duffy MJ.** Proteases as prognostic markers in cancer. Clin Cancer Res 1996; **2**: 613–8.

9. **Min H, Hong M, Ma J, *et al.*** A new staging system for nasopharyngeal carcinoma in China. Int J Radiat Oncol Biol Phys 1994; **30**: 1037–42.

10. **Sinicrope FA, Ruan SB, Cleary KR, *et al.*** bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 1995; **55**: 237–41.

11. **Nishimura Y, Furuno K, Kato K.** Biosynthesis and processing of lysosomal cathepsin L in primary cultures of rat hepatocytes. Arch Biochem Biophys 1988; **263**: 107–16.

12. **Hara K, Kominami E, Katunuma N.** Effect of proteinase inhibitors on intracellular processing of cathepsin B, H and L in rat macrophages. FEBS Lett 1988; **231**: 229–31.

13. **Gal S, Gottesman MM.** The major excreted protein of transformed fibroblasts is an activable acid-protease. J Biol Chem 1986; **261**: 1760–5.

14. **Lah TT, Kos J.** Cysteine proteinases in cancer progression and their clinical relevance for prognosis. Biol Chem 1998; **379**: 125–30.

15. **Stabuc B, Mrevlje Z, Markovic J, Stabuc-Silih M.** Expression and prognostic significance of Cathepsin L in early cutaneous malignant melanoma. Neoplasma 2006; **53**: 259–62.

16. **Harbeck N, Alt U, Berger U, *et al.*** Long-term follow-up confirms prognostic impact of PAI-1 and cathepsin D and L in primary breast cancer. Int J Biol Markers 2000; **15**: 79–83.

17. **Troy AM, Sheahan K, Mulcahy HE, *et al.*** Expression of cathepsin B and L antigen and activity is associated with early colorectal cancer progression. Eur J Cancer 2004; **40**: 1610–6.

18. **Dohchin A, Suzuki JI, Seki H, *et al.*** Immunostained cathepsins B and L correlate with depth of invasion and different metastatic pathways in early stage gastric carcinoma. Cancer 2000; **89**: 482–7.

19. **Strojnik T, Kavalar R, Trinkaus M, *et al.*** Cathepsin L in glioma progression: comparison with cathepsin B. Cancer Detect Prev 2005; **29**: 448–55.