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Overexpression of CSF-1R in nasopharyngeal carcinoma

LI HUANG[1,2)], XIMING XU[1)], YANRONG HAO[2)], JIAXIN CHEN[2)], LEI LI[2)], JUNPING CHENG[2)], ZHAOHONG CHEN[2)], WUQING LIANG[2)], JIAO YANG[2)], WEN AO[2)], XIN HAO[2)], WEIWEI GAO[2)]

[1)]*Cancer Center, Renmin Hospital of Wuhan University, China*
[2)]*Cancer Center, People's Hospital of Guangxi Autonomous Region, China*

Abstract

Background: Tumor-associated macrophages play a significant role in tumor progression. CSF-1/CSF-1R is one of the most primary regulators of macrophage physiology in immune system. The expression of CSF-1/CSF-1R in nasopharyngeal carcinoma is unclear. *Objectives*: The aim of this study was to compare the expression of CSF-1R in nasopharyngeal carcinoma to nasopharyngitis for assessing the role CSF-1/CSF-1R in nasopharyngeal carcinoma. *Materials and Methods*: Diagnostic tissues from 56 nasopharyngeal carcinoma patients and 32 nasopharyngitis patients were evaluated retrospectively by immunohistochemical analysis for the expression of CSF-1R. *Results*: Significant differences of CSF-1R expression exists between nasopharyngeal carcinoma patients and nasopharyngitis patients (p<0.001). However, there is no relevance between CSF-1R and worse survival.

Keywords: CSF-1/CSF-1R, nasopharyngeal carcinoma, nasopharyngitis, metastasis, survival.

Introduction

Nasopharyngeal carcinoma (NPC) is a kind of common epithelial cancer in Southeast Asia. In China, the annual incidence rate of NPC is approximately 25-fold higher than that in the Western countries. Epidemiological studies show that NPC is multiple genes inherited tumors [1]. As NPC is highly sensitive to radiation, the first-line treatment for early stage NPC is radiotherapy. For advanced nasopharyngeal carcinoma, combined radiotherapy with chemoradiotherapy is the standard treatment. According to the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, the local relapse-free survival (LRFS) of N0 stage patients is at a rate of 88.6% [2]. Although the survival rate of NPC has been greatly improved, local recurrence and distant metastases after radiotherapy are the major causes for the failure of NPC treatment [3]. Although TNM stage is the most reliable indicator to predict the prognosis of nasopharyngeal carcinoma, it seems to reveal significantly differences for the prognosis of nasopharyngeal carcinoma patients in same stage and pathological type who received the same treatment. Study shows that different NPC patients put up different sensitivity to radiotherapy, and the survival is also different. Furthermore, it shows much difference in gene expression [4].

Last year, 14 112 human genes of 12 radiotherapy-resisted nasopharyngeal carcinoma patients and eight radiotherapy-sensitive nasopharyngeal carcinoma patients have been tested by DNA chips technology and 111 genes expression in radiotherapy-resisted patients have been found much different from radiotherapy-sensitive patients, especially colony-stimulating factor 1 receptor (CSF-1R). The expression of CSF-1R in radiotherapy-resisted patients is 4.1 times higher than radiation sensitive cases [5]. Therefore, we suspect that the CSF is an important factor to regulate and control tumor progression.

CSF-1R, encoded by the c-fms proto-oncogene, is one of the most primary regulators in immune system, which will further influence the development of tumor [6]. CSF-1 binds to CSF-1R and comes into being phosphorylation and kinase domain activation, meanwhile, CSF-1R forms into homodimer [7]. Then, tyrosine residues of the effect protein will be phosphorylated, which leads to macrophages' activation. The activated mononuclear scavenger system could promote the development of tumor through many pathways, like promoting tumor growth, angiogenesis, extracellular matrix breakdown, invasion, and metastasis. Besides, studies have shown that CSF-1/CSF-1R also affects tumor cells directly [8]. Various malignant tumors have been confirmed CSF-1R over-expression [9–11]. However, it is a pity that there is still no report about whether CSF is related to the progress of NPC.

Therefore, in this study, we aimed immunoexpression analysis of differences in CSF-1R expression, in order to highlight some correlations of this marker with the aggressive variants of tumors. We hope to find the relationship between CSF-1R and the prognosis of NPC through contrasting the expression of CSF-1R in NPC patients to nasopharyngitis patients, and layer analysis data in NPC patients. We hope to find a new predictor or a therapeutic target for NPC patients in the future.

Materials and Methods

Patients

The study reviewed histological and immunohistochemical data from 56 patients diagnosed with NPC at the People's Hospital of Guangxi Zhuang Autonomous Region, China, between May 2007 and December 2013. All patients had been pathologically confirmed for NPC and had received no previous treatment before. All cases with a history of other cancers or with distant metastasis

were excluded. Referenced the American Joint Committee on Cancer (AJCC), patients with stages I–II were treated with intensity modulated radiation therapy (IMRT) alone and those with stages III–IVB received concurrent chemoradiotherapy (CRT). After one month of IMRT treatment, computed tomography (CT) or magnetic resonance imaging (MRI) should be used to evaluate the IMRT response. When CRT treatment was over, the patients needed more necessary adjuvant chemotherapy, if curative effect evaluation could not be complete remission (CR) or partial response (PR). The clinical characteristics of the 56 NPC patients included in the study are summarized in Table 1.

Table 1 – ***The clinical characteristics of the 56 NPC patients***

Characteristics	Values	%
Gender		
Males	40	71
Females	16	29
Age [years]		
Median	47	
Range	28–76	
WHO pathology classification		
I (keratinizing)	0	0
II (non-keratinizing)	41	73
III (undifferentiated)	15	27
AJCC group		
Stage I	1	2
Stage II	8	14
Stage III	25	45
Stage IV	22	39
KPS		
<2	40	71
≥2	16	29
Therapy		
RT alone	9	16
CRT	47	84

NPC: Nasopharyngeal carcinoma; WHO: World Health Organization; AJCC: American Joint Committee on Cancer; KPS: Karnofsky performance status; RT: Radiation therapy; CRT: Concurrent chemoradiotherapy.

At the same time, histological and immunohistochemical data from 32 nasopharyngitis patients in same Hospital are reviewed. Every nasopharyngitis patients were confirmed without NPC by pathological examination. The median follow-up time was 24.6 months (range, 5–61 months). Routine follow-up evaluation were performed every three months for the first two years, every six months for the next three years, and then annually (or whenever clinically indicated) thereafter. The study was approved by the Appropriate Committees for Human Rights in Research in our Hospital, and written informed consent was obtained from each patient.

Histopathological analysis and immunohistochemistry

Paraffin-embedded tumor tissue obtained from the 56 patients with NPC and 32 patients with nasopharyngitis before treatment were analyzed by immunohistochemical methods. Tissues were constructed with three tissue cores 1 mm in diameter from selected areas of formalin-fixed, paraffin-embedded tissue samples. Five sections (4 μm) from each tissue samples on poly-L-Lysine-coated slides dried at 60^0C for one hour. After standard heat-induced epitope retrieval for 3–5 minutes in boiled citrate buffer (pH 6.0), the sections were incubated for 10 minutes at 26^0C with peroxidase blocking solution. And then, before incubated overnight at 4^0C with monoclonal antibodies to CSF-1R (1:75 dilution; ab61137; Abcam, England), each section had been incubated for 10 minutes at 26^0C with normal non-immune serum. After that, the sections were incubated with biotin-conjugated second antibody at 26^0C for 15 minutes and incubated with *Streptomyces* anti-Biotin peroxidase solution at 26^0C for 10 minutes. In order to color, the slides were covered with fresh configuration of DAB (3,3'-Diaminobenzidine) at 26^0C for eight minutes and counterstained with Mayer's Hematoxylin solution and mounted in a non-aqueous mounting medium.

Three experienced pathologists used Volm double grading method to evaluate the results without clinical data of patients. Five visions were picked out to observe at high power microscope in each section. Average of five view scores would be the final score of this piece. Evaluation criteria: CSF-1R immunoexpression was valued by express area and expression intensity. Express area was described by the ratio of immunopositive cells in high-power microscope. According to the rate of positive cells, <25% was recorded as one point, 25~49% was two points, 50% or higher was three points. Then, according to the staining intensity grading, no coloring was 0 point, light yellow was one point, deep yellow or light brown was two points, tan was three points. Two scores together to judge the result: negative expression (-): 0 point, weakly positive expression (+): 1~2 points, moderate positive expression (++): 3~4 points, strong positive expression (+++): 5~6 points.

Statistical analysis

The rate of distant metastasis-free survival was the primary death from any cause endpoint of the study. Survival analysis was performed using the Kaplan–Meier method, and the curves were compared using the *log-rank* test. Multivariate prognostic analyses were performed using the Cox proportional hazards regression model using the Enter method. Categorical variables were compared using the χ^2 test. All statistical analyses were performed with SPSS *ver.* 17.0 statistical software program (SPSS, IBM, Chicago, IL, USA). $P<0.05$ is considered statistically significant.

Results

Immunostaining analysis

Positive expression of CSF-1R mainly locates in the cell nuclear membrane, a small amount in the cytoplasm. According to the expression level of CSF-1R, patients were divided into CSF-1R strong positive expression group [CSF-1R (+++), Figure 1A], and CSF-1R moderate positive expression group [CSF-1R (++), Figure 1B], CSF-1R weakly positive expression group [CSF-1R (+)] and CSF-1R negative expression group [CSF-1R (-), Figure 1C]. Forty-one (73.22%) NPC patients and three (9.38%) nasopharyngitis patients took on positive expression (Table 2).

There were significant differences between nasopha-

ryngeal carcinoma patients and nasopharyngitis patients about CSF-1R expression (p<0.001). And there was no difference between the expression level of CSF-1R and age, gender, pathological subtype, AJCC stage, KPS value or treatment of nasopharyngeal carcinoma patients (Table 3).

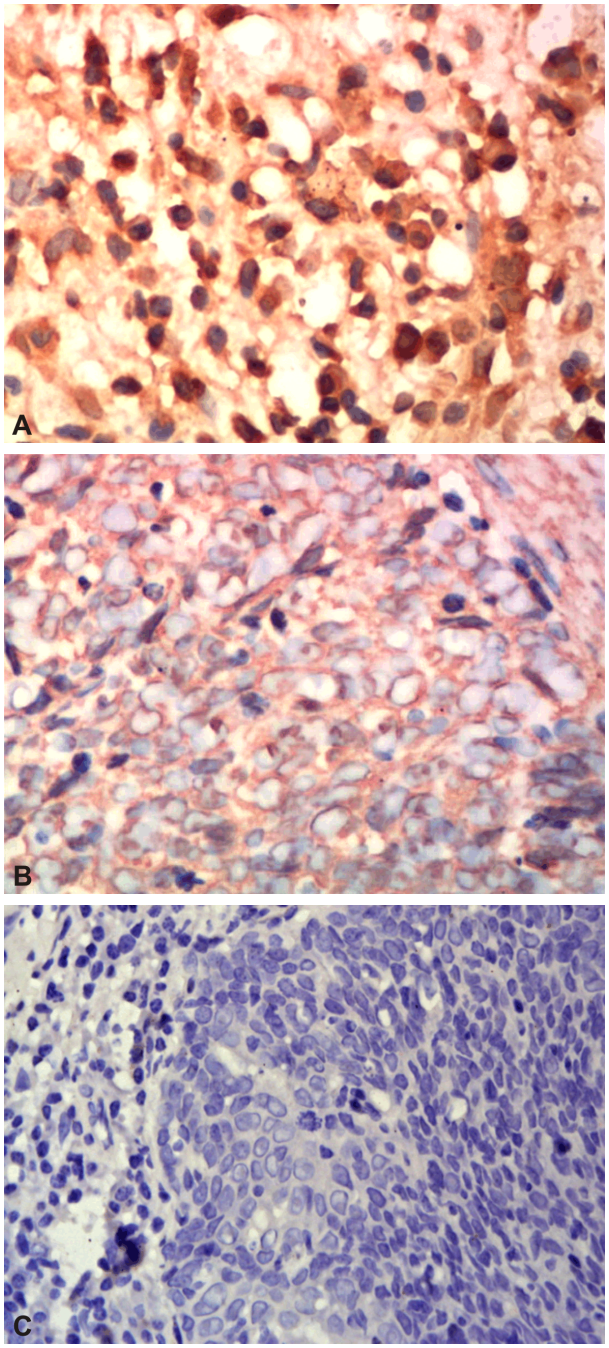

Figure 1 – *(A) Strong positive expression (+++) of CSF-1R in NPC cells (×400); (B) Moderate intensity of expression (++) of CSF-1R in NPC cells (×400); (C) Negative expression (-) of CSF-1R in NPC cells.*

Table 2 – *The distribution of CSF-1R expression intensity in NPC tissue and nasopharyngitis tissue*

	No. of cases	Expression intensity				*P*-value
		(-)	(+)	(++)	(+++)	
NPC	56	15	28	9	4	<0.001
Nasopharyngitis	32	29	3	0	0	

NPC: Nasopharyngeal carcinoma.

Table 3 – *Correlation between CSF-1R and clinical variables*

Characteristics	CSF-1R expression / No. of cases		*P*-value
	CSF-1R (+/++/+++) (≥30%)	*CSF-1R (-) (<30%)*	
Gender			0.600
Males	28	12	
Females	13	3	
Age [years]			0.854
<47	18	7	
≥47	23	8	
Disease subtype			0.730
Keratinizing	0	0	
Non-keratinizing	36	13	
Undifferentiated	5	2	
AJCC group			0.942
Stages I and II	6	3	
Stages III and IV	35	12	
KPS			0.616
<2	38	14	
≥2	3	1	
Therapy			0.454
RT alone	8	1	
CRT	33	14	

AJCC: American Joint Committee on Cancer; KPS: Karnofsky performance status; RT: Radiation therapy; CRT: Concurrent chemoradiotherapy.

Correlation between clinical outcome and CSF-1R expression

Twenty-four (42.86%), 29 (51.78%), and three (5.36%) of the 56 NPC patients achieved an initial complete response, partial response and stable disease to therapy, respectively. After a period of five to 61 months, 11 (19.6%) of the 56 presented progressive disease. These cases of distant metastases or deaths occurred in the parotid glands, lungs or livers. No nasopharyngitis patient developed into NPC.

Owing to the development of the technology of the NPC therapy and the too short follow-up time, only 11 patients revealed distant metastases or deaths, which leading to the drawback to value the survival rate, what was the indicator to illustrate the correlation between the status of prognosis with the expression of CSF-1R in NPC. It seemed no correlation existing between the expression of CSF-1R and distant metastasis (p>0.05; Figure 2). However, the result is obviously different if we stratified analysis from the aspects of the levels of CSF-1R expression. The risk of tumor recurrence, metastasis and death increased when the expression intensity is above of (++) or (+++) (Table 4).

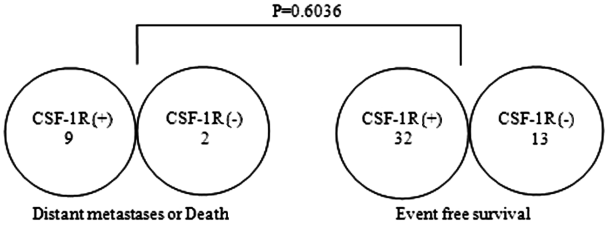

Figure 2 – *Relationship between the status of distant metastasis and the expression levels of CSF-1R (p>0.05).*

Table 4 – ***Relationship between the event and the expression level of CSF-1R***

Expression of CSF-1R	Distant metastases or Death		*P*-value
	Yes	*No*	
(-)	2	13	
(+)	2	26	0.6036
(++)	4	5	<0.0001
(+++)	3	1	0.0014

Especially when the expression intensity level of CSF-1R was graded with (++) and (+++), distant metastasis or deaths occurred in seven (63.64%) of the 11 cases. Figure 3 illustrate the Kaplan–Meier estimated survival curves for event free survival in patients classified according to expression intensity level of CSF-1R in the tumor (p=0.468, p<0.0001, p=0.002) when we used receiver operating characteristic (ROC) curve to define the critical time of prognosis. If the follow-up time was longer, the gap may be bigger.

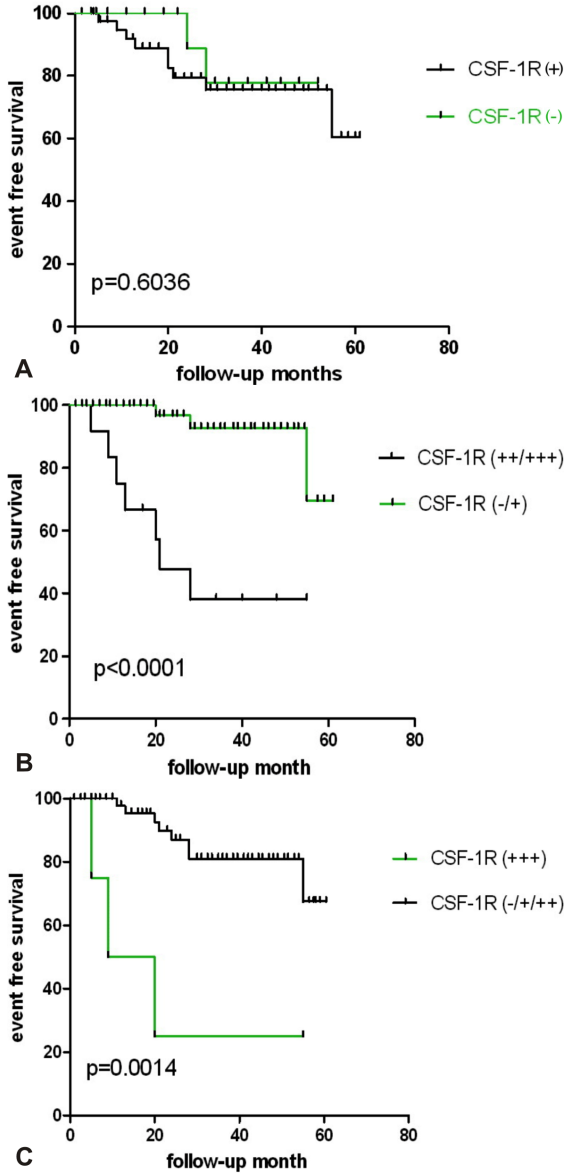

Figure 3 – ***Event-free survival (EFS) in NPC: (A) EFS of CSF-1R (+/++/+++) and CSF-1R (-) in NPC (p=0.468); (B) EFS of CSF-1R (++/+++) and CSF-1R (-/+) (p<0.0001); (C) EFS of CSF-1R (+++) and CSF-1R (-/+/++) (p=0.002).***

Then, we used Cox model in 56 patients with nasopharyngeal carcinoma analyzing metastasis-free survival in multivariate survival, the results showed that the expression levels of CSF-1R and the AJCC stage are prognostic factors of nasopharyngeal carcinoma (p<0.0001, p=0.034) (Table 5).

Table 5 – ***Multivariate analysis of metastasis-free survival in patients with nasopharyngeal carcinoma***

	B	SE	Wald test	*P*	Exp(B)	95% CI for Exp(B)
Gender	1.559	1.178	1.750	0.186	4.752	0.472 to 47.827
Age	0.024	0.038	0.402	0.526	1.024	0.951 to 1.103
Disease subtype	-3.450	4.072	0.718	0.397	0.032	0.000 to 92.804
AJCC group	1.629	0.769	4.489	0.034	5.097	1.130 to 22.997
KPS	-1.036	1.236	0.703	0.402	0.355	0.031 to 4.002
Expression of CSF-1R	2.019	0.575	12.322	0.000	7.529	2.439 to 23.242

B: Beta coefficient; SE: Standard error; CI: Confidence interval; AJCC: American Joint Committee on Cancer; KPS: Karnofsky performance status.

Discussion

CSF-1 is expressed in non-hematopoietic cells, like macrophages, epithelial and fibroblasts cells and tumor cells [12]. The growth factor is an important hematopoietic growth factor responsible for activation proliferation and differentiation of macrophage [11]. Effects of CSF-1 on these target cells are mediated by the CSF-1 receptor (CSF-1R) [13]. Ligand binding to receptor tyrosine kinases (RTKs) translates into conformational alterations of the extracellular domain and subsequent receptor dimerization stabilizing the interactions between adjacent cytoplasmic domains of the receptors. These all result in phosphorylation of cytoplasmic tyrosine residues, which mediates development and mature cell function and activation together with other molecules [14]. Aligeti *et al.* demonstrate that in the pathology of endometriosis, they have found increased levels of CSF-1. When endogenous CSF-1/CSF-1R overexpresses in ovarian cancer cells, the ability of invasiveness, adhesion, and motility would increase [11]. Studies have substantiated that increased inflammation and poorer prognosis of breast cancer correlate with the protein expression of CSF-1/CSF-1R [15]. However, as so far, to our knowledge, the present study is the first to evaluate the expression of CSF-1R in NPC tissue and nasopharyngitis tissue. We found that CSF-1R expression in NPC is much higher than nasopharyngitis. The CSF-1R expressed on nuclear membrane of the tumor cells, with little expressed on nasopharyngitis cells. Therefore, it is necessary for us to further test and verify the expression of CSF-1R at the protein and gene levels. And this is also the key point of our next work.

According to the prognosis, 56 nasopharyngeal carcinoma patients have been divided into two subgroups, one group with distant metastases, and another group without metastasis. We collected and statistic analyses the expression of CSF-1R in two groups, founding the p-value was more than 0.05. There was little significant association between the expression of CSF-1R and the

event of distant metastasis in NPC. However, when the expression level of CSF-1R was graded with (++) and (+++), the consequence became different. And this result maybe more obvious if the follow-up time was longer. The drawbacks of the present study include its retrospective design, short follow-up time, and small sample size. Therefore, the next step, in order to find the link between CSF-1R and distant metastases of NPC, we will expand the sample size and continue to track the patient's prognosis. We hope the CSF-1R expression scores would be a predictive of survival.

Conclusions

Compared with nasopharyngitis tissue, CSF-1/CSF-1R is significantly higher expressed in nasopharyngeal carcinoma tissue. What is more, patients with moderate or strong intensity of expression of CSF-1/CSF-1R show a worse prognosis – are prone to nasopharyngeal carcinoma metastasis and recurrent.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Corresponding authors

Ximing Xu, Archiater, Master's Supervisor, Cancer Center, Renmin Hospital of Wuhan University, 430000 China; Phone/Fax 13707120651, 02788042292, e-mail: whuxxm@yahoo.com

Yanrong Hao, Associate Senior Doctor, Master's Supervisor, Cancer Center, People's Hospital of Guangxi Autonomous Region, 530000 China; Phone/Fax 13471018839, 07712186869, e-mail: yanronghao.2008@aliyun.com

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