Cancer Cell Research 12 (2016) 290-293

Available at http:// www.cancercellresearch.org ISSN 2161-2609

Research of Expression about HIF-1α in Non-Hodgkin's Lymphoma in Children and Its Influence to Prognosis and Chemotherapy Sensitivity

Xilin Xiong[1,2,*], Chaotao Zeng[1,3], Ling Liu[1,4]

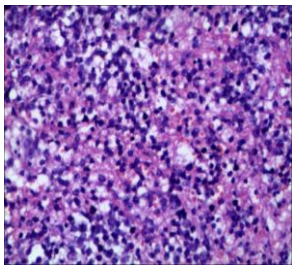
[1]Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China

[2]Pediatric Hematology/Oncology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China

[3]Department of Emergency, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China

[4]Department of Pediatric Hematology/Oncology, Affiliated hospital of Guangdong medical college, Zhanjiang, 524000, China

Abstract: **To explore the expression of (hypoxia-inducible factor-1α (HIF-1α) in Non-hodgkin's lymphoma in children and its influence in prognosis and sensitivity to chemotherapy. We conducted immunohistochemistry to determine the expression of HIF-1α in 125 cases of Non-hodgkin's lymphoma, and then investigated the correlation between the expression level of HIF-1α and the effect of preoperative chemotherapy, or 5-year survival rate. HIF-1α was highly expressed in Non-hodgkin's lymphoma, and was unrelated to Gender, ECOG (Eastern Cooperative Oncology Group) and IPI (international prognostic index)score of tumor (P＞0.05), but related to TNM clinical stage, B symptoms, LDH level (P＜0.05) . The efficiency of chemotherapy in HIF-1α negative patients (90%) was significantly higher (P＜0.05) than that of HIF-1α positive patients (68.75%). HIF-1α was highly expressed in Non-hodgkin's lymphoma, and was closely related to lymph node metastasis, prognosis, and sensitivity to chemotherapy. So, HIF-1α could be one of the indicators that evaluate the prognosis and effectiveness of chemotherapy in child with Non-hodgkin's lymphoma.**



Keywords: Non-hodgkin's lymphoma; HIF-1α; Neoadjuvant chemotherapy/NACT; Prognosis

Received 20 September 2016, Revised 23 October 2016, Accepted 25 October 2016

*Corresponding Author: Xilin Xiong, kikibear@163.com

1. Introduction

The burden of Non-hodgkin's lymphoma continues to increase according to the Global Cancer Statistics [1]. And unfortunately, Non-hodgkin's lymphoma is the most frequently diagnosed cancer and the leading cause of cancer death. Systemic chemotherapy appears to be a kind of important complementary treatments for Non-hodgkin's lymphoma. While effectiveness of chemotherapy significantly decreases or even resistance appears, it is crucial to look for definite predictors like the widely accepted factors PDCD5, HER2, TOP Ⅱα, etc [2] . HIF-1α belongs to the family of plasminogen-related growth factors (PRGFs). As is evidenced by previous studies, research found that it can be expressed in a variety of malignant tumor, and it is closely related to the process with the tumor blood, proliferation of tumor cells and apoptosis [3-6]. Serum level of HIF-1α in Non-hodgkin's lymphoma showed significant increase when compared with controls [7]. And it was reported that patients with more advanced TNM staging were shown to have higher serum soluble HIF-1α [8]. But few studies have demonstrated HIF-1α expression in Non-hodgkin's lymphoma, and there is little knowledge about the possibility that HIF-1α could be one of the predictors that contribute to predict the effect of chemotherapy [9,10]. Here, this study was designed to confirm the expression profile of HIF-1α in Non-hodgkin's lymphoma tissues from 125 cases, and to elucidate the possible relationship between the expression level ofHIF-1α and the effect of chemotherapy, and between it and the prognosis of child.

2. Materials and methods

2.1. Clinical data

The 125 samples were collected from child who diagnosed as Non-hodgkin's lymphoma before chemotherapy in our hospital, and all the patients' pathological findings after surgery were completed. Of the 125 Children, 62 received 1 week preoperational

chemotherapy among whom 41 cases adopted the CEF regime (intravenous injection of CTX at 800 mg/m^2 on day 1 and 8, intravenous drip of EPI at 60 mg/m^2 on day 2 and 3, intravenous drip of 5-FU at 500 mg/m^2 during day 4 and 6), and 21 adopted the CTF regime (intravenous injection of CTX at 800 mg/m^2 on day 1 and 8, intravenous drip of THF at 30 mg/m^2 on day 2 and 3, intravenous drip of 5-FU at 500 mg/m^2 during day 4 and 6). The average age was 10.5±1.2 years. 52 of them had postoperative lymph node metastasis. Their TNM clinical stages were as follows: 87 of them were stage Ⅰ and Ⅱ, 38 were Ⅲ.

2.2. Immunohistochemistry

Immunohistochemistry was carried out using the two-step EnVision procedure. Briefly, each tissue section was deparaffinised, hydrated and then incubated with fresh 3% hydrogen peroxide (H_2O_2) in methanol for 15 min. After rinsing with phosphate-buffered saline (PBS), antigen retrieval was carried out by microwave treatment in 0.01M sodium citrate buffer (pH 6.0) at 100℃ for 15min. Next, tissue sections were incubated with primary antibodies diluted in PBS containing 0.2% Triton X-100 for 30min at room temperature. After rinsing with PBS, slides were incubated with the ChemMate™ EnVision™+/HRP for 30 min at room temperature. The reaction is visualized by the ChemMate™ DAB+ Chromogen. Negative controls were prepared by substituting PBS for primary antibody.

Table 1. The expression of HIF-1α and the relationship with Patient characteristics.

Pathologic features		HIF-1α		*X2/T*	P
		-	+		
Gender	Boy	28	35	0.0529	＞0.05
	Girl	32	30		
ECOG	0-1	27	36	1.3459	＞0.05
	2-4	33	29		
IPI score	0-2	37	50	3.4322	＞0.05
	3-5	23	15		
LDH level	Normal	50	44	5.4597	＜0.05
	elevated	10	21		
TNM clinical stage	Ⅰ-Ⅱ	51	36	12.9332	＜0.01
	Ⅲ-Ⅳ	9	29		
B symptoms	Yes	14	38	15.8475	＜0.01
	No	46	27		
ER	+	33	43	1.6286	＞0.05
	-	27	22		
PR	+	35	36	0.1106	＞0.05
	-	25	29		

2.3. Assessment of chemotherapy effectiveness

The assessment of chemotherapy effectiveness is according to RECIST Guidelines established by NIH, and confirmed at 4 wks: CR/ complete response, complete disappearance of all target lesions; PR/partial response, at least a 30% decrease in the sum of the longest diameter of target lesions; SD/stable disease, neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease; PD/progressive disease, at least a 20% increase in the sum of the longest diameter of target lesions. Here, we defined “effective” as “CR+PR”, and “ineffective” as “SD+PD”.

2.4. Statistical analysis

All data were analyzed with SPSS 19.0 statistical software. Student’s paired t-test and chi-square test were performed to analyze statistical significance in continuous variables and categorical variables respectively. Survival was analyzed by a log-rank test. Differences were considered statistically significant at P＜0.05 with a 95% confidence interval.

3. Results

3.1.Expression of HIF-1α in Child with Non-hodgkin's lymphoma

In order to analyze the relationship between the expression of HIF-1α and the clinical parameters in our study, we determined the positive rate of HIF-1α in Non-hodgkin's lymphoma tissues. The Non-hodgkin's lymphoma tissues were diagnosed by 2 pathologists, and 92 of the 125 patients were diagnosed. The positive rate of HIF-1α in human breast cancer tissues was 52%, and was related to TNM clinical stage, B symptoms, LDH level (P＜ 0.05), but unrelated to Gender, ECOG and IPI score of

tumor (P＞0.05). (Table 1, Figure 1).

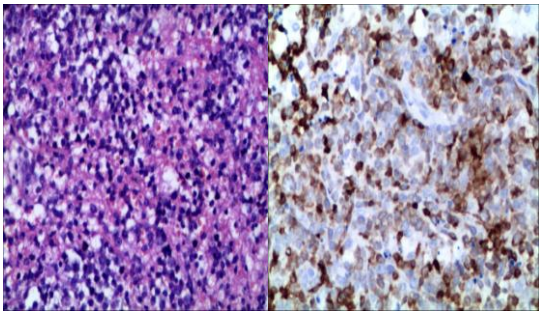


Figure 1. HIF-1α expression in the NHL, A: HE×200 non-hodgkin's lymphoma, B: HIF-1α×200.

3.2. The correlation between HIF-1α level and patient sensitivity to chemotherapy

In order to demonstrate the correlation between HIF-1α level and patient sensitivity to chemotherapy, we compared the effective rate of chemotherapy in HIF-1α positive or negative patients. As is shown in Table 2, the effective rate of chemotherapy in HIF-1α negative patients was 90%, which was significantly higher (P＜0.05) than that in HIF-1α positive child (68.75%).

Table 2. The correlation between HIF-1α and chemotherapy sensitivity.

	CR	PR	SD	PD	Efficiency	P
HIF-1α+	3	19	8	2	68.75%	＜0.05
HIF-1α-	7	20	2	1	90%	

3.3. Correlation between HIF-1α level and prognosis of Non-hodgkin's lymphoma

Correlation analysis was performed to characterize the relationship between expression of HIF-1α and child survival time. As is shown in Fig. 2, the 5-year survival rate of HIF-1α positive patients was 78%, which was significantly lower (P＜0.05) than that of HIF-1α negative child (90%) (Figure 2.). The correlation analysis suggested that HIF-1α could be an important factor in patient survival with Non-hodgkin's lymphoma.

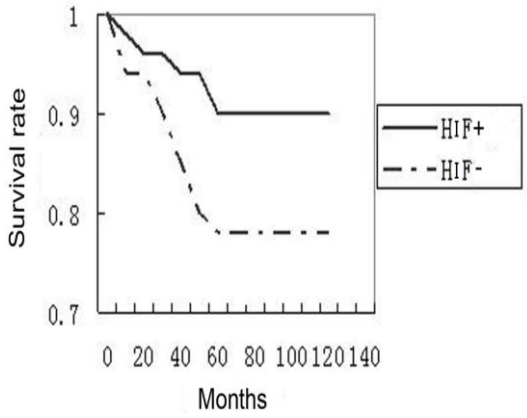


Figure 2. Correlation analysis of the relationship between expression of HIF-1α and 5-year survival rate. The 5-year survival rate of HIF-1α positive Child was 78%, which was significantly lower (P＜0.05) than that of HIF-1α nagtive child (90%).

4. Discussion

Non-hodgkin's lymphoma is clinically characterized as high morbidity, low chance of success through surgery alone, high recurrence and metastasis rate, poor prognosis, and resistant to chemotherapy. Thus, it is of great importance to explore the factors that can be used in predicting the metastasis, prognosis, and sensitivity to chemotherapy post-operationally. HIF-1α is a kind of multifunctional peptide factor that is secreted by epithelial cells. It promotes the process of proliferation, migration, invasion, and angiogenesis of tumors [11]. It has been widely reported that high expression of HIF-1α is closely related to the prognosis of patients. Wang and his co-workers [12] have also shown that HIF-1α was highly expressed in Non-hodgkin's lymphoma, and the expression level was closely related to the prognosis of patients. Bakshi and his colleagues [13] have demonstrated that HIF-1α was significantly correlated to the histological grade, tumor size, and while studying the relationship between the expression level of HIF-1α and the pathological parameters in Non-hodgkin's lymphoma. Fujiwara [14] reported that the Non-hodgkin's lymphoma specimens expressed a significantly higher level of HIF-1α, which may indicate that the HIF-1α regulatory system may have an important role in the progression of Non-hodgkin's lymphoma. Here, we detected the expression of HIF-1α in Non-hodgkin's lymphoma tissues of 125 patients by immunohistochemistry. The results indicated that HIF-1α was highly expressed in Non-hodgkin's lymphoma tissues of Child, and the positive rate was 52%. The expression of HIF-1α was unrelated to patient Gender, ECOG and IPI score of tumor, but related to TNM clinical stage and prognosis. Besides, all the 125 patients were followed up and their survival time and survival rate were compared and evaluated. The 5-year survival rate of HIF-1α positive patients was significantly lower than that of

HIF-1α negative patients. This result indicated that HIF-1α may be one of the essential predictors that contribute to predict the prognosis of Non-hodgkin's lymphoma, which was consistent with the report of HisamitsuIde et al. [15].

As it can improve the survival rate and surgical outcomes of Non-hodgkin's lymphoma, chemotherapy/ NACT has been used more and more widely in the clinical practice. Nevertheless, few studies investigated the effect of NACT on HIF-1α, and HIF-1αas a predictor for sensitivity to chemotherapy was still insufficiently characterized. In an effort to avoid the unnecessary side effects of chemotherapy that patients may undergo, and to improve the sensitivity to chemotherapy, defined valuable indicators in NACT therefore is in need of a better understanding. 62 Non-hodgkin's lymphoma children in this study were collected as preparation NACT group. Among them, the effective rate of chemotherapy in HIF-1α negative patients was about 90%, and was significantly higher than that in HIF-1α positive patients (68.75%), which meant HIF-1αis closely related to sensitivity to chemotherapy. Analogous results were reported by Delsol [16]. They identified that the HIF-1α was closely related to sensitivity to chemotherapy.

In conclusion, HIF-1α may be a new promising therapeutic target for Non-hodgkin's lymphoma, and may enable clinical practitioners to better predict patient sensitivity to chemotherapy and prognosis through detecting patient HIF-1αlevels. Although this presented study demonstrates the possibility and availability of HIF-1α as a useful predictor, understanding the mechanisms underlying the effect will require further studies.

References

[1] Gazzola A，Bertuzzi C，Agostinelli C，et al．Physiological PTEN ex-pression in peripheral T-cell lymphoma not otherwise specifi[J]. Haematologica, 2009, 94(7):1036 -103.

[2] Wang L, Liu P. Serum neuron-specific enolase is correlated with clinical outcome of patients with non-germinal center B cell-like subtype of diffuse large B-cell lymphoma treated with rituximab-based immunochemotherap[J]. Medical Oncology, 2011, 8(30):1533-1602.

[3] Wan J, Chai H, Yu Z, et al. HIF -1α effects on angiogenic poten-tial in human small cell lung carcinoma[J]. J. Exp. Clin. Cancer Res., 2011, 15(30):77-79.

[4] Dungwa JV, Hunt LP, Ramani P. Overexpression of carbonic anhydrase and HIF-1α in Wilms tumours[J]. BMC Cancer, 2011, 12(11):390.

[5] Chilov D, Camenisch G, Kvietikova I, et al. Induction and nucleartranslocation of hypoxia-inducible factor: het-erodimerization with ARNT is not necessary for nuclear accumulation of HIF-1α[J]. J. Cell. Sci., 1999, 112(8):1203 -1212.

[6] Semenza GL. HIF-1: mediator of physiological and pathophysio-logical responses to hypoxia[J]. J. Appl. Physiol, 2000, 88(4):1474-1480.

[7] Zundel W, Schindler C, Hass-Kogan D. Loss of PTEN facilitates HIF-1-mediated gene expression[J]. Genes. Dev., 2000, 14:391-396.

[8] Huang J, Kontos CD. PTEN modulates vascular endothelial growth factor-mediated signaling and angiogenic effects[J]. J. Biol. Chem., 2002, 277(13):10760-10766.

[9] Brito LG, Schiavon VF, Andrade JM, et al. Expression of Hypoxia-inducible factor 1α and vascular endothelial growth factor-C inlocally advanced breast cancer patients[J]. Clinics (Sao Paulo), 2011, 66(8):1313-1320.

[10] Zhu K, Hu R. Expression and significance of HIF-1α and PTEN in non-hodgkin lymphoma[J]. Modern Oncology, 2013, 21(09):2087-2090.

[11] Wu Y, Karas M, Dupont J, et al. Multiple signaling pathways areinvolved in the regulation of IGF-I receptor inhibition of PTEN-enhanced apoptosis[J]. Growth Horm. IGF. Res., 2004, 14:52-58.

[12] Wang L, Liu P, et al. Prognostic significance of neuron-specific enolase in patients with diffuse large B-cell lymphoma treated with rituximab-based immunochemotherapy[J]. Lymphoma, 2011, 52(9):1697-1703.

[13] Bakshi NA, Ross CW, Finn WG, et al. ALK-positive anaplastic large cell lymphoma with primary bone involvement in children[J]. Am. J. Clin. Pathol., 2006, 125(1):57-63.

[14] Fujiwara H, Arima N, et al. Clinical significance of serum neuron-specific enolase in patients withadult T-cell lymphoma[J]. Am. J. Hematology, 2002, 71(5):80-84.

[15] HisamitsuIde, YuichiTerado, et al. Novel Germ Line Mutation p53-P177R in Adult Adrenocortical Carcinoma Producing Neuron-specific Enolase as a Possible Marker[J]. J. Clin. Oncol., 2010, 40(8):815-818.

[16] Delsol G. The 2008 WHO lymphoma classification[J]. Ann. Pathol., 2008, 28(1):20-24.

