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Original Article

Elevated IL-7Rα is linked to recurrence and poorer survival of gastric adenocarcinoma

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Abstract: IL-7Rα expression has been suggested to be involved in the development of cancer and has been reported to be linked to poor overall survival in patients with lung cancer. However, no evidence has been reportedregarding the significance of IL-7Rα expression in gastric adenocarcinoma (GA) untilnow. In the present investigation, to understand the clinicopathological significance of IL-7Rα expression in GA, expression of IL-7Rα was evaluated using immunohistochemistry in 121 cases of paired GA and its corresponding normal controls. As further confirmation, detection of IL-7Rα expression was extended from the protein level to the mRNA level using qRT-PCR. Clinicopathological association was statistically analyzed between IL-7Rα expression and clinicopathological variables, including demographic, T classification, clinical stage, lymph nodes metastases, differentiation, recurrence or not, and overall prognosis. IL-7Rα expression was markedly up-regulated in GA tissues relative to paired normal controls at both the protein or mRNA level. Elevation of IL-7Rα was markedly associated with lymph nodes metastases (P=0.006), differentiation (P=0.005), recurrence (P=0.043), and poor overall prognosis (P=0.039). There were trends toward statistical significance forboth T classification (P=0.063) and clinical stage (P=0.065) despite no significant associations found. Together, our study is the first toanalyze the significance of IL-7Rα expression in GA, suggesting its potential predictive value for recurrence and overall prognosis.

Keywords: Gastric adenocarcinoma, IL-7Rα, metastases, differentiation, recurrence

Introduction

Gastric adenocarcinoma (GA), the third leading cause of cancer-related death in the world, ranks fifth among themost commonly diagnosed cancersin China [1]. Despite advances in early detection, diagnosis, and treatment of gastric cancer, the overall prognosis is still poor, with its 5-year survival rate being less than 25%, clinically mainly owing to the recurrence and metastasis after operation [2]. Nevertheless, underlying reasons for the recurrence and metastasis in gastric cancer remain unknown. Thus, a better understanding ofthe recurrence and metastasis of GA will undoubtedly be necessary for the improvement of diagnosis and therapy of GA.

Recently, extensive mechanistic studies have documented that the tumor microenvironment plays an important driving role in the progression of different types of cancer [3-5]. The tumor establishes a favorable environment [6] whereby cancer progression and metastasis are controlled and do not depend solely on cancer cell-autonomous defects [7]. Among the microenvironment factors, IL-7 has been reported to be able to promote the progression of human T-cell acute lymphoblastic leukemia [8], suggesting potential therapeutic application of IL-7 in hematological malignancy. Notably, IL-7, acting via IL-7 receptor (IL-7R), plays role.

The IL-7 receptor complex consists of the IL-7 receptor alpha (IL-7Rα) and the common γ chain (γc; CD132) [9]. IL-7Rα and IL-7 constitute the IL-7/IL-7Rα signaling axis that plays an important role in tumor progression [10, 11]. As mentioned previously, the IL-7/IL-7Rα axis has been extensively studied in hematopoietic neoplasms, however, the expression and clinico-

pathological significance of IL-7Rα remains unclear in GA despite rather limited, despite studies that have established the expression status of IL-7Rα in non-hematopoietic neoplasms, including breast cancer [12], lung cancer [13] and prostate cancer [10]. To understand the expression of IL-7Rα and its clinicopathological significance in GA, statistical analyses were carried out between clinicopathological characteristics available and IL-7Rα expression detected by immunohistochemistry in 121 cases of paired GA tissues and its matched normal controls. The results show that IL-7Rα is strikingly up-regulated in gastric cancer tissues relative to paired normal controland that up-regulation of IL-7Rα is significantly associated with lymph node metastases, differentiation, recurrence, and poor overall prognosis, thus indicating its potential predictor for recurrence and overall prognosis in GA.

Materials and methods

Clinical samples

The present study was approved by the Medical Ethics Committee of the Affiliated Qingdao Hiser Hospital of Qingdao University, and written informed consent was obtained from each participant involved. Formalin-fixed and paraffin-embedded (FFPE) tissue blocks of 121 cases of GA and paired normal controls were retrieved from the archives of the Department of Pathology, the Affiliated Qingdao Hiser Hospital of Qingdao University. Clinicopathological characteristics available were retrieved from the Hospital Information System (HIS) of the Affiliated Qingdao Hiser Hospital of Qingdao University, including demographic information (age and gender), T classification, clinical stage, lymph nodes metastases, differentiation, recurrence or not, and overall prognosis. All the patients involved with recurrent GA were hospitalized between January 2011 and January 2017 at the Department of Oncology, the Affiliated Qingdao Hiser Hospital of Qingdao University. No chemo-or-radio therapy was prescribed or performed on these patients involved before pathological biopsy examination. Another independent cohort composed of 50 paired cases of GA and normal control was obtained from the Department of Internal Medicine, People's Hospital of Zhangqiu. Tissue from non-cancer that was at least 5 cm from the primary cancer site was defined as the paired normal control, and both the cancer and paired normal control tissues were immediately processed with FFPE method after being resected from patients. Patients who developed recurrent postoperative disease and underwent gastroectomy were recorded in the Department of Oncology of our hospital.

Immunohistochemistry (IHC)

IHC analysis was carried out following previously described methods [14]. Briefly, PPFE block sections were incubated at 60°C for 12 hours, followed by deparaffinization and dehydration in descending concentrations of ethanol and finally in ddH_2O. The activity of endogenous peroxide was blocked in 3% H_2O_2 for 10 minutes. The antigen was retrieved in 0.01 M citrate buffer heated in a microwave oven at 98°C for 10 minutes and then cooled at room temperature for 30 minutes. After washing in phosphate-buffered saline, tissue sections were incubated with normal serum to block nonspecific staining for 30 minutes. Rabbit polyclonal antibody to human IL-7Rα (1:100 dilution; Catalogue number: ab95024, Abcam, MA, USA) was incubated with the section overnight at 4°C in a humidified chamber. After washing with phosphate-buffered saline for 5 times with each 5 minutes, the tissue sections wereincubated with horseradish-conjugated mouse monoclonal anti-rabbit IgG heavy chainsecondary antibody (1:8000 dilution as suggested, Catalogue number: ab99702, Abcam, MA, USA), followed by washing with phosphate-buffered saline 5 times/5 minutes each. Staining was done with adiaminobenzidine Kit (Catalogue number: ZLI-9018, Beijing Zhongshan Golden Bridge Biotechnology Co, Ltd). IHC staining was assessed in a series of randomly selected 4 high-power fields, which were believed to be representative of the average in sections at ×200 magnification. The IL-7Rα antibody was replaced with isotype specific pre-immune rabbit serum IgG at the same dilution as the primary antibody for negative controlstaining as previously strongly suggested [15]. To ensure the specificity of the primary antibody (Supplementary Figure 1), recombinant IL-7Rα (Catalogue number: H00-003575-P01, Abnova) was used as a blocking peptide according to previously recommended [16].

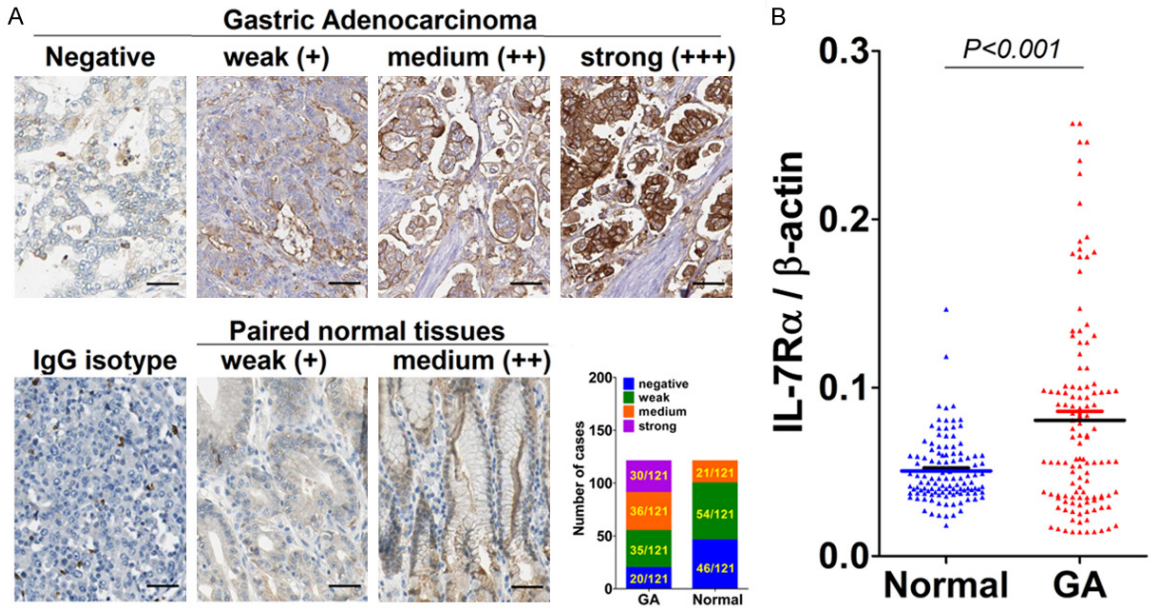

Figure 1. A: Expression of IL-7Rα in 121 cases of gastric adenocarcinoma (GA, upper panel) and paired normal control tissue (lower panel). IgG isotype means pre-immune isotype control of the primary antibody to IL-7Rα. Negative control, where primary antibody was replaced with isotype specific pre-immune IgG when performed IHC. Shown are representative figures among the candidates. The magnification fold was ×200, scale bar stands for 100 μm. B: Detection of IL-7Rα mRNA expression using qRT-PCR in 121 cases of gastric cancer (GC) and paired normal control tissue. Paired student's T-test was employed to analyze the difference between the two groups.

Evaluation of IHC staining

Evaluation of IHC staining was performed as previously described [17]. In brief, positive reactions were defined as those showing brown signals in the cell cytoplasm. Each separate tissue core was scored on the basis of the intensity and area of the positive staining. The intensity of positive staining was scored as follows: 0, negative (-); 1, weak staining (+); 2, moderate staining (++); and 3, strong staining (+++). The rate of positive cells was scored on a 0 to 4 scale: 0, 0-5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; and 4, >75%. If the positive staining was homogeneous, a final score was achieved by multiplication of the two scores above, giving a total range from 0 to 12. When the staining was heterogeneous, we scored it as follows: each component was scored independently and summed for the results. For example, a specimen containing 25% tumor cells with moderate intensity (1×2=2), 25% tumor cells with weak intensity (1×1=1), and 50% tumor cells without reactivity received a final score of 2+1+0=3. In our study, predominant expression of IL-7Rα was also present in adjacent normal gastric cells. The scores of adjacent normal gastric tissues were 0 to 9. For statistical analysis, we divided all the samples of GA into two groups according to positive intensity as follows: scores of 0 to 7 as low expression and scores of 8 to 12 as high expression.

Quantitative real time-PCR (qRT-PCR)

Total RNA of each PPFE slide was extracted using RNeasy mini kit (Catalogue number: 74106; 250 times; Qiagen, German) and was reverse transcribed into 1 μg cDNA with Revert Aid First Strand cDNA Synthesis Kit (Catalogue number: #K1622, Thermo Fisher Scientific, USA). Quantitative real time-PCR (qRT-PCR) was carried out using SYBR Green Premix PCR Master Mix (Roche, Mannheim, Germany) following the manufacturer's protocol. Relative mRNA expression of IL-7Rα was calculated using Ct method ($2^{-\Delta\Delta Ct}$) after being normalized to β-actin which was used as internal controls. All reactions were conducted independently in triplicate. Sequence of the all primers involved was listed below. IL-7Ra (NM_002185.4), forward primer: 5'-GAGCAATATATGTGTGAAGG-3'; reverse primer: 5'-ATAGACGACACTCAGGTCAAAAGG-3'. The product size using

Table 1. Clinicopathological significance of IL-7Rα expression in gastric cancer tissues

Clinicopathological variables	Total	IL-7Rα expression		χ^2	*P* value
		High	Low		
Gastric cancer	121	66	55	36.340	0.000
Matched normal control	121	21	100		
Gender					
Male	61	36	25	0.992	0.364
Female	60	30	30		
Age					
≤60	56	29	27	0.320	0.558
>60	65	37	28		
T classification					
T1-T2	48	21	27	3.740	0.063
T3-T4	73	45	28		
Clinical stage					
Stage I-II	52	23	29	3.913	0.065
Stage III-IV	69	43	26		
lymph node metastases					
N_o	58	24	34	7.789	0.006
N_{1-2}	63	42	21		
Differentiation degree					
Well-moderate	51	20	31	8.356	0.005
Low	70	46	24		
Recurrence					
No	53	23	30	4.728	0.043
Yes	68	43	25		

IL-7Ra primers was 106 bp, and the melting temperature was 60°C. β-actin (EF036500.1), forward primer: 5'-GGGAAATCGTGCGTGACATTAAG-3'; reverse primer: 5'-CATTGCCAATGGTGATGACC-3'. The product size using β-actin primers was 141 bp, and the melting temperature was 60°C.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS version 16.0 (SPSS, Inc, Chicago, IL, USA). Association was analyzed using the Chi-square test or Fisher's exact test (when expected numbers were less than 5) when appropriate between high and low expression of IL-7Rα in GA tissues based on clinicopathological variables, including age, gender, pathological stage, and TNM classification. Survival analysis of gastric cancer was conducted using the Log-Rank test for comparison between low and high expression of IL-7Rα IHC staining. Kaplan-Meier curve was plotted for the two groups based on overall survival. We accounted for clinical factors by univariate analysis and Cox proportional hazards models; *P* values of less than 0.05 were taken to be statistically significant.

Results

IL-7Rα was markedly up-regulated in GA compared with paired normal control

To understand the expression of IL-7Rα in GA, IHC was carried out in 121 cases of GA and paired normal controls. First of all, to ensure the specificity of the primary antibody of IL-7Rα, antigen pre-adsorption pre-test was performed as recommended previously [16]. The positive immunoreactivity signals were hardly detectable after being pre-incubated with recombinant IL-7Rα that was commercially obtained from Abnova in comparison with control (Supplementary Figure 1), suggesting that the specificity of IL-7Rα antibody was adequately specific for further evaluation. On the basis of specific pre-evaluation, IHC was performed. Expression of IL-7Rα was mainly both membranous and cytoplasmic (**Figure 1**). IL-7Rα expression was shown to be highly heterogeneous in GA tissues, with its positive intensity varying from hardly detectable to weak, medium, and strong. Notably, positive staining of IL-7Rα was also be present in several cases of corresponding normal control, with the intensity being non-detection, low and at most medium level. Strong expression of IL-7Rα was absent in paired normal control tissues (**Figure 1A**). Taken as a whole, IL-7Rα was markedly over-expressed in GA tissues relative to paired normal controls (**Table 1**). To confirm, detection of IL-7Rα was extended from protein level to mRNA level using the qRT-PCR technique. Consistently, IL-7Rα was markedly elevated in GA tissues relative to paired normal controls as expected (**Figure 1B**). Therefore, both the protein level and mRNA level of IL-7Rα corroborated each other in our setting. As a further verification of IL-7Rα expression, another independent cohort consisting of 50 cases of GA tissues and matched normal controls collected from the Department

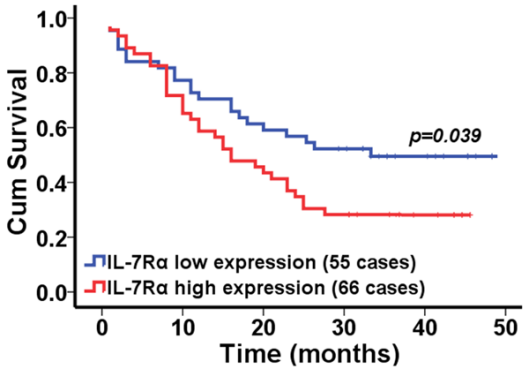

Figure 2. Prognostic analysis of IL-7Rα expression in 121 cases of gastric cancer tissues that were divided into two groups according to positive intensity as follows: scores of 0 to 7 as low expression and scores of 8 to 12 as high expression. Survival analysis was conducted using Log-Rank test.

of Internal Medicine, People's Hospital of Zhangqiu. As expected, IL-7Rα was remarkably up-regulated in gastric cancer tissues compared with corresponding normal controls (Supplementary Table 1). Our results thus demonstrated that IL-7Rα is significantly up-regulated in GA tissues relative to the paired normal control.

Up-regulation of IL-7Rα is associated with recurrence and poor overall prognosis

Since the expression level of IL-7Rα in GA tissues was elevated, the relationship between high and low expression of IL-7Rα was explored on the basis of clinicopathological variables available, including age, gender, pathological stage, TNM classification, and recurrence. Up-regulation of IL-7Rα was strongly associated with lymph nodes metastases (P=0.006), differentiation (P=0.005), and recurrence (P=0.043). There were also trends toward statistical significance of both T classification (P=0.063) and clinical stage (P=0.065) despite no significant association (**Table 1**). To understand whether there was an association between of IL-7Rα expression and overall prognosis of patients with GA, Kaplan-Meier survival curves were plotted. A significant difference of overall prognosis between patients with high and low expression of IL-7Rα (P=0.039) was observed (**Figure 2**). These results suggest that IL-7Rα expression could be used as potential predictor for recurrence and prognosis of patients with GA with the exception of metastasis and differentiation. Unfortunately, confirmation of significant association with recurrence and prognosis failed during re-analysis in the additional in dependent cohort of GA consisting of 50 cases. As mentioned previously, this may be due to the unavailability of recurrent and prognostic information (Supplementary Table 1). To evaluate the effect of IL-7Rα expression and clinicopathological variables on prognosis of GA, both univariate and multivariate survival analyses were carried out. Univariate Cox regression analysis showed IL-7Rα expression (P=0.017), clinical stage (P=0.020), lymph node metastasis (P=0.017), differentiation degree (P=0.009) and recurrence (P=0.004) are prognostic factors for GA. By using multivariate analysis, we further analyzed the prognostic parameters of GA that were significant in univariate analysis. It was observed that IL-7Rα expression (P=0.036), lymph node metastasis (P=0.019), differentiation degree (P=0.025) and recurrence (P=0.011) are independent prognostic factors affecting the 5-year overall survival, indicating that IL-7Rα expression can be used as an independent prognostic predictor for patients with GA (**Table 2**). Moreover, lymph node metastasis, differentiation degree, and recurrence were all capable of being used as an independent prognostic factors in GA after multivariate analysis.

Discussion

This is the first report of IL-7Rα expression and its clinicopathological significance in GA. IL-7Rα was strongly elevated in GA tissues relative to paired normal controls. In comparison with patients with low expression of IL-7Rα, high expression of IL-7Rα was shown to be significantly increased, to be recurrent post operation, and tended to correlate with shorter survival. These results are suggestive of the recurrent and prognostic predictor value of IL-7Rα for patients diagnosed with gastric cancer.

Original reports regarding IL-7Rα in the setting of cancer came from malignant myeloid neoplasms [18], then it was extended to other hematopoietic malignancies, including, T-cell lymphoma [19], acute myeloid leukemia [20], acute lymphoblastic leukemia [21] and T-cell acute lymphoblastic leukemia [8, 22]. In non-

Table 2. Univariate and multivariate Cox-regression analysis of the prognostic parameters in patients with GA

P value		Univariate analysis	Multivariate analysis		95.0% *CI*
		Regression coefficient	*P* value	Relative risk	
IL-7Rα expression (negative vs. positive)	0.017	-0.576 (0.289)	0.036	1.878	1.313-3.687
Gender	0.074	0.672 (0.365)			
Age (years)	0.537	0.184 (0.291)			
Clinical stage	0.020	0.610 (0.313)	0.107	0.620	0.347-1.108
Lymph node metastasis	0.017	0.441 (0.150)	0.019	0.645	0.382-0.927
Differentiation degree	0.009	-0.572 (0.169)	0.025	1.450	1.293-1.945
Recurrence	0.004	0.483 (0.256)	0.011	0.605	0.426-0.911
T classification	0.061	0.169 (0.173)	0.460	1.147	0.723-1.820

Abbreviations: CI, confidence interval; GA, gastric adenocarcinoma; SE, standard error.

hematopoietic neoplasms, the first report with regard to IL-7Rα expression was from renal cell carcinoma [23]. Actually, the vast majority of earlier studies of IL-7Rα in cancers were focused on single nucleotide polymorphism (SNP) or mutation association research. In contrast, only a few of studies haveinvestigated expression IL-7Rα, not to mention the clinicopathological significance of IL-7Rα expression. The clinical role of IL-7Rα in cancers, especially in non-hematological malignancies therefore remains largely unknown. In a recent investigation [24] where Suzuki K and colleagues found that IL-7R expression was an independent predictors of recurrence in the setting of lung cancer, which prompted the conduction of our current study concerning IL-7Rα in GA. Taking into account these relevant previous studies of IL-7Rα in cancers [10, 12], we postulated that IL-7Rα could be elevated in GA compared with paired normal controls. In order to test this hypothesis, IHC was performed. As expected, IL-7Rα was significantly up-regulated in GA relative to paired normal controls, which was in concordance with earlier studies regarding IL-7Rα in other different types of cancers [10, 12]. Considering the potential technical limitations of IHC, further confirmation of IL-7Rα was extended from the protein level to the mRNA level using qRT-PCR. IL-7Rα was consistently shown to be remarkably up-regulated in GA tissues relative to paired normal controls, which corroborated with that at theprotein level. Clinicopathological analysis showed that IL-7Rα was significantly related to lymph nodes metastases, differentiation, and recurrence of GA; which was fundamentally in line with the data of Suzuki K et al. [25] in lung cancer, where IL-7R expression was significantly linked to higher stage, larger tumor size, lymphatic invasion, recurrence, and high grade morphology. Nevertheless, there was somewhat difference between our data and Suzuki K et al. in that they detected and mentioned the IL-7R throughout the study, but not IL7-Rα as we detected in our study. Regrettably, they also failed to provide the detailed information regarding the primary antibody of IL-7R they used, which make it not possible to judge whether the IL-7R they measured was actually IL-7Rα. Furthermore, IL-7Rα was markedly linked with inferior overall prognosis in GA, which was totally in agreement with Suzuki K et al. [25]. In order to verify the clinicopathological significance of IL-7Rα we established, detection of IL-7Rα expression that was then extended to another additional independent cohort consisting of 50 cases of GA and corresponding normal controls. As expected, IL-7Rα was elevated in GA compared with normal control. However, no significant relationship was observed mainly due to the unavailability of clinicopathological variables including recurrence and overall prognosis. Therefore, the underlying mechanism by which IL-7R plays role in GA, which remains to be further investigated.

Prognostic significance of IL-7Rα expression has been analyzed in different types of cancers, including breast cancer [12], lung cancer [25, 26] and prostate cancer [10], with consistent results showing that higher expression of IL-7Rα is statistically associated with poorer overall prognosis. Despite this, it should be noted that in these previous relevant literature regarding IL-7Rα, these authors didn't specify

which subtype of IL-7R they detected and analyzed. The alpha subunit (CD127) and gamma subunit (CD132), compose the IL-7R complex. Our study first specified and analyzed the prognostic and clinicopathological significance of IL-7Rα expression in the context of GA, which mainly differs from the relevant previous reports. Furthermore, the difference in analysis of IL-7Rα we picked and IL-7R mentioned by earlier studies might to some extent account for the divergent results with respect to the clinicopathological significance of IL-7R expression. Our findings therefore confirm the prognostic potential of IL-7R in cancer. On the other hand, the varying cutoff values for determining the positive staining or high expression of IL-7Rα has been applied in different studies to study the prognostic impact, which could be another explanation for the inconsistent results compared with earlier studies. In one previous study of the IL-7R performed by Cleaver AL and colleagues [27] in pediatric T-cell acute lymphoblastic leukemia (T-ALL), low-expression of IL-7R was found to be independently predictive of relapse in T-ALL patients using microarray technique. This is in stark contrast with our observation that high expression of IL-7Rα is linked with recurrence of patients with GA.

There are several potential limitations that deserve to be noted. First, our conclusion warrants further verification in another independent cohort with larger sample size. Second, some technical issues, such as sample quality and experimental performance might potentially bias the final results. Third, detection of soluble IL-7Rα in peripheral blood could be desirably complementary for IHC analysis of IL-7Rα in GA.

In summary, our study for the first time and to the best of our knowledge, presents that IL-7Rα is remarkably elevated in GA tissues and this correlates with recurrence and shorter survival of patients with GA. Our observations establish useful evidence for potential application of IL-7Rα in the prediction of recurrence and prognosis of GA.

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Disclosure of conflict of interest

None.

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References

[1] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[2] Yang K, Chen XZ, Hu JK. Factors associated with recurrence and survival in N0 gastric cancer. Ann Surg 2017; 266: e10-e11.

[3] Casadei L, Calore F, Creighton CJ, Guescini M, Batte K, Iwenofu OH, Zewdu A, Braggio DA, Bill KL, Fadda P, Lovat F, Lopez G, Gasparini P, Chen JL, Kladney RD, Leone G, Lev D, Croce CM, Pollock RE. Exosome-derived miR-25-3p and miR-92a-3p stimulate liposarcoma progression. Cancer Res 2017; 77: 3846-3856.

[4] Henry CE, Emmanuel C, Lambie N, Loo C, Kan B, Kennedy CJ, de Fazio A, Hacker NF, Ford CE. Distinct patterns of stromal and tumor expression of ROR1 and ROR2 in histological subtypes of epithelial ovarian cancer. Transl Oncol 2017; 10: 346-56.

[5] Tsai YF, Tseng LM, Hsu CY, Yang MH, Chiu JH, Shyr YM. Brain-derived neurotrophic factor (BDNF) -TrKB signaling modulates cancer-endothelial cells interaction and affects the outcomes of triple negative breast cancer. PLoS One 2017; 12: e0178173.

[6] Kuol N, Stojanovska L, Nurgali K, Apostolopoulos V. The mechanisms tumor cells utilize to evade the host's immune system. Maturitas 2017; 105: 8-15.

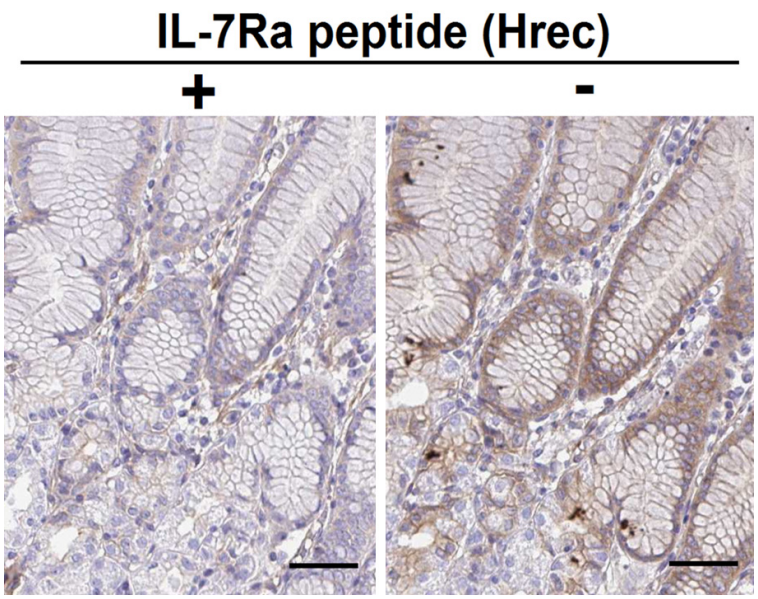
[7] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013; 19: 1423-37.

[8] Silva A, Laranjeira AB, Martins LR, Cardoso BA, Demengeot J, Yunes JA, Seddon B, Barata JT. IL-7 contributes to the progression of human T-cell acute lymphoblastic leukemias. Cancer Res 2011; 71: 4780-9.

[9] Lundstrom W, Highfill S, Walsh ST, Beq S, Morse E, Kockum I, Alfredsson L, Olsson T, Hillert J, Mackall CL. Soluble IL-7Ralpha potentiates IL-7 bioactivity and promotes autoimmunity. Proc Natl Acad Sci U S A 2013; 110: E1761-70.

[10] Qu H, Zou Z, Pan Z, Zhang T, Deng N, Chen G, Wang Z. IL-7/IL-7 receptor axis stimulates pros-

tate cancer cell invasion and migration via AKT/NF-kappaB pathway. Int Immunopharmacol 2016; 40: 203-10.
[11] Andersson A, Srivastava MK, Harris-White M, Huang M, Zhu L, Elashoff D, Strieter RM, Dubinett SM, Sharma S. Role of CXCR3 ligands in IL-7/IL-7R alpha-Fc-mediated antitumor activity in lung cancer. Clin Cancer Res 2011; 17: 3660-72.
[12] Al-Rawi MA, Rmali K, Watkins G, Mansel RE, Jiang WG. Aberrant expression of interleukin-7 (IL-7) and its signalling complex in human breast cancer. Eur J Cancer 2004; 40: 494-502.
[13] Ming J, Zhang Q, Jiang Y, Qiu X, Bai X. [The expressions of IL-7 and IL-7R and the relationship between them with lymph node metastasis and prognosis in non-small cell lung cancer]. Zhongguo Fei Ai Za Zhi 2010; 13: 1101-6.
[14] Watson AS, Soilleux EJ. Detection of p62 on paraffin sections by immunohistochemistry. Cold Spring Harb Protoc 2015; 2015: 756-60.
[15] Hewitt SM, Baskin DG, Frevert CW, Stahl WL, Rosa-Molinar E. Controls for immunohistochemistry: the Histochemical Society's standards of practice for validation of immunohistochemical assays. J Histochem Cytochem 2014; 62: 693-7.
[16] Holmseth S, Zhou Y, Follin-Arbelet VV, Lehre KP, Bergles DE, Danbolt NC. Specificity controls for immunocytochemistry: the antigen preadsorption test can lead to inaccurate assessment of antibody specificity. J Histochem Cytochem 2012; 60: 174-87.
[17] Wu ZY, Chen T, Zhao Q, Huang JH, Chen JX, Zheng CP, Xu XE, Wu JY, Xu LY, Li EM. Altered expression of endogenous soluble vascular endothelial growth factor receptor-2 is involved in the progression of esophageal squamous cell carcinoma. J Histochem Cytochem 2013; 61: 340-7.
[18] Schumann RR, Nakarai T, Gruss HJ, Brach MA, von Arnim U, Kirschning C, Karawajew L, Ludwig WD, Renauld JC, Ritz J, Herrmann F. Transcript synthesis and surface expression of the interleukin-2 receptor (alpha-, beta-, and gamma-chain) by normal and malignant myeloid cells. Blood 1996; 87: 2419-27.
[19] Hahtola S, Tuomela S, Elo L, Hakkinen T, Karenko L, Nedoszytko B, Heikkilä H, Saarialho-Kere U, Roszkiewicz J, Aittokallio T, Lahesmaa R, Ranki A. Th1 response and cytotoxicity genes are down-regulated in cutaneous T-cell lymphoma. Clin Cancer Res 2006; 12: 4812-21.
[20] Dascalescu A, Zlei M, Grigore G, Danaila C, Jitaru D, Carasevici E. [Prognostic significance of leukemia-associated phenotype in correlation with other biologic markers in acute myeloid leukemia patients]. Rev Med Chir Soc Med Nat Iasi 2009; 113: 1176-81.
[21] Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, Cario G, Cazzaniga G, Kulozik AE, Stanulla M, Schrappe M, Biondi A, Basso G, Bercovich D, Muckenthaler MU, Izraeli S. Gain-of-function mutations in interleukin-7 receptor-alpha (IL-7R) in childhood acute lymphoblastic leukemias. J Exp Med 2011; 208: 901-8.
[22] Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, Tritapoe J, Hixon JA, Silveira AB, Cardoso BA, Sarmento LM, Correia N, Toribio ML, Kobarg J, Horstmann M, Pieters R, Brandalise SR, Ferrando AA, Meijerink JP, Durum SK, Yunes JA, Barata JT. Oncogenic IL-7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet 2011; 43: 932-9.
[23] Jeron A, Pfoertner S, Bruder D, Geffers R, Hammerer P, Hofmann R, Buer J, Schrader AJ. Frequency and gene expression profile of regulatory T cells in renal cell carcinoma. Tumour Biol 2009; 30: 160-70.
[24] Shimizu K, Okita R, Nakata M. Clinical significance of the tumor microenvironment in non-small cell lung cancer. Ann Transl Med 2013; 1: 20.
[25] Suzuki K, Kadota K, Sima CS, Nitadori J, Rusch VW, Travis WD, Sadelain M, Adusumilli PS. Clinical impact of immune microenvironment in stage I lung adenocarcinoma: tumor interleukin-12 receptor beta2 (IL-12Rbeta2), IL-7R, and stromal FoxP3/CD3 ratio are independent predictors of recurrence. J Clin Oncol 2013; 31: 490-8.
[26] Ming J, Zhang Q, Qiu X, Wang E. Interleukin 7/interleukin 7 receptor induce c-Fos/c-Jun-dependent vascular endothelial growth factor-D up-regulation: a mechanism of lymphangiogenesis in lung cancer. Eur J Cancer 2009; 45: 866-73.
[27] Cleaver AL, Beesley AH, Firth MJ, Sturges NC, O'Leary RA, Hunger SP, Baker DL, Kees UR. Gene-based outcome prediction in multiple cohorts of pediatric T-cell acute lymphoblastic leukemia: a Children's Oncology Group study. Mol Cancer 2010; 9: 105.



Supplementary Figure 1. Determination of the specificity of primary antibody using antigen pre-adsorption method. Whencarried out usingIHC, the primary antibody was incubated with sections in the presence (left, 10 μg/ml) and absence (right) of human recombinant (Hrec) IL-7Rα peptide that was commercially obtained from Abnova company. Scale bar represents 200 μm.

Supplementary Table 1. IL-7Rα expression in another independent cohort consisting of 50 cases of gastric cancer and paired normal control tissues

Clinicopathological variables	Total	IL-7Rα expression		χ^2	*P*
		Low	High		
Gastric cancer	50	18	32	19.869	0.000
Paired normal control	50	40	10		
Gender					
Male	28	10	18	0.002	1.000
Female	22	8	14		
Age					
<55	27	11	16	0.573	0.559
≥55	23	7	16		
T classification					
T_{1-2}	21	10	11	1.873	0.234
T_{3-4}	29	8	21		
N classification					
N_0	18	9	9	2.393	0.139
N_{1-3}	32	9	23		

Confirmation of IL-7Rα expression in additionally independent cohort consisting of 50 cases of gastric cancer and paired normal control tissues.