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Expression of Helios in gastric tumor cells predicts better survival in gastric cancer patients

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Abstract

Purpose Helios belongs to Ikaros family, which plays an important role in the cell-fate decision and control cell proliferation; abnormal expressions in leukemia are associated with poor prognosis. In this study, we investigated the Helios expression between Helicobacter pylori infection and prognosis in gastric cancer patients.

Methods A total of 67 gastric cancer patients who received partial or full gastrectomies were enrolled. Helios expression by immunohistochemistry and mRNA was investigated with the clinical stage, Helicobacter pylori infection, CD4 expression, FoxP3 expression and prognosis.

Results From the immunohistochemistry stain, we found that the Helios was expressed in both cancer cell and tumor-infiltrated lymphocytes. The high expression of Helios in gastric tumor cells had a better median overall survival rate in gastric cancer patients (50.7 ± 3.2 vs. 34.1 ± 4.9 months; $P = 0.015$), Helicobacter pylori-infected patients (51.1 ± 3.5 vs. 30.4 ± 5.1 months;

$P = 0.007$) and advanced gastric cancer patients (42.1 ± 5.5 vs. 23.2 ± 4.8 months; $P = 0.043$). From multivariate analysis, the Helios expression in gastric tumor cells was an independent factor to predict better survival in all gastric cancers (HR = 2.78; 95 % confidence interval [CI], 1.09–7.09; $P = 0.032$) and advanced gastric cancer patients (HR = 2.85; 95 % confidence interval [CI], 1.00–8.13; $P = 0.03$).

Conclusions Higher expression of Helios in gastric tumor cells predicts better survival in gastric cancer patients, especially for Helicobacter pylori-infected and advanced-stage gastric cancer patients.

Keywords Gastric cancer · Helicobacter pylori · Helios · Prognosis

Introduction

Gastric cancer is the second leading cause of global cancer-related deaths, and it occurs with geographic variation with highest incidence in East Asian countries estimated in 2012 (Ferlay et al. 2012). Tumor heterogeneity is often observed in cancer cells due to the accumulation of gene mutations. In the recent report, the significantly mutated genes include TP53, KRAS, PIK3CA, ERBB3, PTEN, HLA-B, β-catenin and TGF-β pathways in gastric cancer (Network 2014). Except for the geographic differences in gastric cancer, recent clinical trial using Avastin also showed total different results between Asian and non-Asian patients (Ohtsu et al. 2011). Except for the genetic factors, the tumor microenvironments are also detrimental factors to influence the tumor development and therapeutic effects (Abe et. al. 2015). Immune cells, which infiltrated in the tumor, are one of the most important factors in tumor

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microenvironments (Kashimura et al. 2012). It especially has abundant immune cells in the gastrointestinal tract under normal conditions. The components of an adaptive immune system play important roles in anticancer immunity. Regulatory T cells (Treg) are essential cells with an immune suppressive function to maintain tolerance to the host's own tissues. The transcription factor forkhead box P3 (FoxP3) is a key nuclear molecule for Tregs development and function and is the most specific Treg marker to date (Hori et al. 2003). The higher numbers of suppressive Treg cells in tumor microenvironments will inhibit the anti-tumor responses (Kindlund et al. 2016); thus, it can be a potential prognostic marker (deLeeuw et al. 2012). However, there were studies which reported that an increased frequency of Foxp3+ Tregs was associated with improved prognosis (Kim et al. 2014). The diversity and the potential of plasticity produce a challenge to explore whether they are beneficial to the host by down-regulating excessive immune activation to avoid autoimmune responses or are disadvantageous for anti-tumor immune responses, resulting in promotion of tumor growth (deLeeuw et al. 2012; Shang et al. 2015).

Helios belongs to the family of Ikaros proteins, which contain Ikaros, Aiolos, Helios, Eos and Pegasus, which is a kind of zinc finger transcription factor. These proteins play important roles in the cell-fate decision in hematopoiesis and especially in lymphocyte development (John and Ward 2011; Georgopoulos 2002). In addition, Ikaros families performed as tumor suppressors because many studies reported that abnormal expressions of these proteins in leukemia are associated with poor prognosis (Mullighan et al. 2009). Helios is expressed in T cells, especially in regulatory T cells (Baine et al. 2013). Forced expression of Helios enhanced the suppressive function in foxp3+ CD4+ T cells (Takatori et al. 2015). Except for the hematopoietic cells, Helios is expressed by a small population of nestin-positive neural progenitor cells (Martin-Ibanez et al. 2012). In this study, we examined the Helios expression in gastric cancer, including gastric tumor cells and infiltrated lymphocytes, to investigate the associations of Helios expression between Helicobacter pylori infection and prognosis in gastric cancer patients.

Materials and methods

Tissue sample

From 2010 August to 2014 September, a total of 67 patients (consisting of 47 males and 20 females with a median age of 69 years in the age range of 47–87 years as depicted in Table 1) were pathologically diagnosed with gastric cancer samples collected from the Chang Gung Memorial

Table 1 Characteristics of total gastric cancer patients based on Helios presentation

	All ($N = 67$)	High ($N = 39$)	Low ($N = 28$)	P
Age	68.49 ± 10.02	67.9 ± 9.3	69.3 ± 11.0	0.581
Gender				0.846
Female	20	12	8	
Male	47	27	20	
TNM stage				0.289
I + II	29	19	10	
Advanced [III + IV]	38	20	18	
LN metastasis				0.294
No	24	16	8	
Yes	43	23	20	
Distal metastasis				0.368
No	58	35	23	
Yes	9	4	5	
HP infection				0.469
No	23	12	11	
Yes	44	27	17	
CD4				0.777
Low	42	25	17	
High	25	14	11	
FoxP3				0.982
Low	36	21	15	
High	31	18	13	

Hospital (CGMH), in Chia-Yi, Taiwan. The pathologic examination and further investigation were carried out under the informed consent of the patients. The Institutional Review Board (IRB) of the Chang Gung Memorial Hospital approved all human subject assessments (IRB No. 104-4187C).

Immunohistochemistry (IHC)

Formalin-fixed and paraffin-embedded tissue specimens were cut into 4-µm-thick sections. The samples were heated at 65 °C for 30 min and then deparaffinized in xylene and rehydrated using a 100–75 % series of ethanol. The slides were then washed three times for 3 min in phosphate-buffered saline (PBS). For antigen retrieval, the samples were microwaved twice in 10 mM citrate (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked with 3 % hydrogen peroxide for 10 min. Nonspecific binding was blocked using 1X phosphate-buffered saline with Tween 20 (PBST) containing 5 % bovine serum albumin (BSA) for 30 min at 4 °C. The samples were then reacted with rabbit polyclonal antibody anti-Helios

(1:100, Sigma-Aldrich® Life Science, St. Louis, MO) at 4 °C overnight and were subsequently washed three times for 3 min with PBS, and then, the samples were detected with a Super Sensitive™ Polymer-HRP IHC Detection System (BioGenex, Fremont, CA) according to the manufacturer's protocol, and the slides were counterstained with hematoxylin before mounting. Formalin-fixed and paraffin-embedded tissue samples were also examined with double-staining immunohistochemistry using rabbit polyclonal antibody anti-Helios and mouse monoclonal anti-FOXP3 (1:100, ab20034, Abcam, Cambridge, UK). The detection system utilized the Mouse/Rabbit Double Stain Kit (with AEC/HRP Green, TADS03A, BIOTnA). After the reaction of double-staining immunohistochemistry, the Helios signal presented a brown color and the FOXP3 signal presented a green color in the tissue samples.

The intensity, percentage and subcellular localization of the immunohistochemical staining of each case were recorded. The intensity of staining was recorded as 0, 1, 2, and 3 referring to negative, weak, moderate and strong staining, respectively. The percentages of positive cells were recorded from 0 to 100 %. The results of staining were scored using a quick (Q) score, which was obtained by multiplying the percentage of positive cells [P] by the intensity [I] ($Q = P \times I$; maximum = 300) (Charafe-Jauffret et al. 2004). One pathologist evaluated the results of immunohistochemical staining without knowledge of the clinicopathologic data.

For the Helios expression, the optimal cutoff points of the Q scores were determined using X-Tile 3.6.1 software, as previously described (Camp et al. 2004). The program calculated the Chi-square values at all possible divisions based on the log-rank test for Kaplan–Meier estimates. The cutoff points of the Q scores with the highest Chi-square values were 160 and 60 for Helios in tumor cells and stromal leukocytes, respectively.

For CD4+ lymphocytes in the tumor stroma, 50 % was used as a cutoff value. For FOXP3+ lymphocytes in the tumor stroma, cases with more than 1 % of the positive cells were classified as positive. The others were classified as negative.

Extraction of RNA and quantitative RT-PCR

RNA extraction was performed using the TRizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. For removing the potential contaminating DNA from the complementary DNA, 1 μg of total RNA was treated with DNase I (Amplification Grade, Invitrogen) prior to reverse transcription. First-strand cDNA synthesis was carried out using MMLV High-Performance Reverse Transcriptase (Epicentre, Chicago, IL). The real-time PCRs were performed on an ABI Step-One real-time PCR system (Applied Biosystems, Foster City, CA). The primer of *ikzf2* was GCGAGGTGGCTGACAACAG and CGTTCACCAG TGTGACTCCTTTT, and GAPDH was the internal control. The relative gene expression was determined by comparing the threshold cycle of the test gene against the Ct value of GAPDH in a given sample (i.e., through the comparative Ct method).

Statistical analysis

The Mann–Whitney test was used to assess the associations between the RNA expression and IHC score of Helios. The Kaplan–Meier method was used to construct the overall survival curves, and a log-rank test was used to assess the significance of differences in survival. All statistical analyses were performed using SPSS software version 18.0 (SPSS, Inc., Chicago, IL) or GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA). $P < 0.05$ was considered statistically significant.

Results

Helios was expressed in both cancer cells and tumor-infiltrated lymphocytes

To detect the infiltrated lymphocytes in gastric cancer expressed with Helios, we performed an immunohistochemistry stain by the anti-Helios antibody in gastric cancer samples (Fig. 1). The Helios was expressed in the infiltrated lymphocytes in the gastric cancer samples (Fig. 1a, brown, some of them indicated by arrow). To investigate whether the Helios expression lymphocytes were regulatory T cells, we performed double staining by using anti-Foxp3 (green) and anti-Helios (brown, arrow) in gastric cancer samples. There are many cells with positive staining including both Foxp3 and Helios in the infiltrated lymphocytes (Fig. 1b, arrow head). Strikingly, we found that not only were the tumor-infiltrated lymphocytes expressed with Helios, the gastric tumor cells (Fig. 1c) and adjacent normal cells (Fig. 1d) were also positive staining with Helios. The relative RNA expression was higher in adjacent normal tissues over gastric cancer tissues (Fig. 1e, $P < 0.05$). Also, the higher protein expression of Helios was found in adjacent normal tissues according to the immunohistochemical staining score (Fig. 1f, $P < 0.001$).

Helios expression in gastric tumor cells was associated with survival in gastric cancer and especially in Helicobacter pylori-infected patients

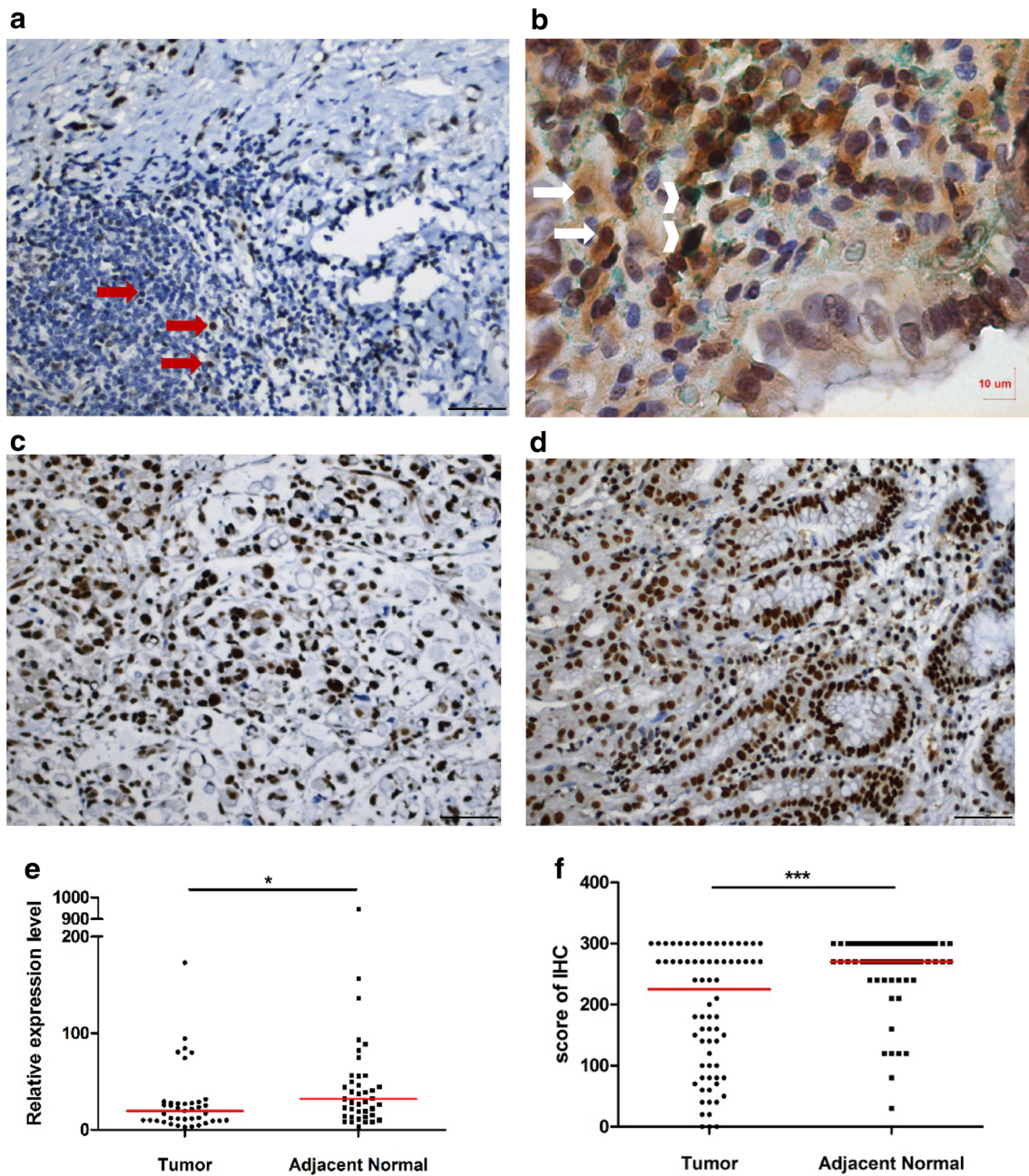Sixty-seven gastric tumor patients were divided into a high Helios expression group ($N = 39$) and a low Helios

Fig. 1 Immunohistochemical stains and the RNA expressions in gastric tumor. **a** Representative immunohistochemical staining of the infiltrated lymphocytes was stained with anti-Helios antibody (*arrow*). **b** Immunohistochemical staining of Helios (nuclear, *brown color*, *white arrow*) and Foxp3 (*green color, arrow head*) in infiltrated lymphocytes was indicated. The Helios expression (*brown*) was both detected in gastric tumor (**c**) and adjacent normal (**d**). **e** The Helios mRNA levels were determined by real-time PCR. **f** The IHC score of Helios expression in tumor and adjacent normal tissue from gastric tumor patients. *Scale bar* in **a**, **c**, **d** is 50 uM, and in **b** is 10 uM

expression group ($N = 28$) according to immunohistochemistry stain. There were no significant differences between these two groups in age, gender, cancer TNM stage, lymph nodule metastasis, distal metastasis, Helicobacter pylori (HP) infection, tumor-infiltrated CD4 T cell expression and tumor-infiltrated FoxP3+ T expression (Table 1). A Kaplan–Meier survival analysis revealed significantly

better median overall survival in the high Helios expression group than in the low Helios expression group (50.7 ± 3.2 vs. 34.1 ± 4.9 months; log-rank test, $P = 0.015$, Fig. 2a).

The Helicobacter pylori infection is the most important risk factor for gastric malignancies. To explore the expression level of the Helios protein in different H. pylori infection conditions, we examined the survival curve between
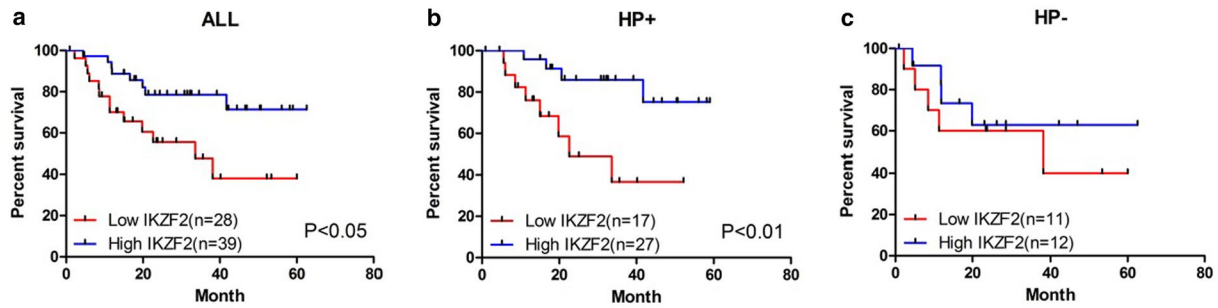
Fig. 2 Kaplan–Meier curves and log-rank test results for the overall survival of the patients with gastric cancer based on the expression of Helios in tumor tissues. **a** Kaplan–Meier curves of overall survival of gastric cancer patients obtained by the expression of Helios. The test resulted in a *P* value of <0.05. **b** Kaplan–Meier curves of overall survival of HP-positive gastric cancer patients obtained by the expression of Helios. The test resulted in a *P* value <0.01. **c** Kaplan–Meier curves of overall survival of HP-negative gastric cancer patients obtained by the expression of Helios. It did not show significant differences

Table 2 Association between hazard rate and risk factors based on the Cox model in gastric cancer patients

Risk factors	Hazard ratio [95 % CI]	*P*
Helios		0.032
Low	2.78 [1.09–7.09]	
High	1	
Gender		0.341
Male	2.32 [0.41–13.06]	
Female	1	
TMN stage		0.083
Advanced	5.29 [0.81–34.81]	
I + II	1	
LN metastasis		0.870
No	1.19 [0.15–9.35]	
Yes	1	
Distant metastasis		0.840
No	1.13 [0.35–3.69]	
Yes	1	
HP infection		0.258
No	2.58 [0.50–13.33]	
Yes	1	
CD4		0.499
Low	2.00 [0.69–5.81]	
High	1	
FoxP3		0.607
Low	1.65 [0.58–4.67]	
High	1	

Helios in gastric cancer did not show differences in the survival curve (44.0 ± 7.5 vs. 34.3 ± 8.0 months; log-rank test, *P* = 0.479, Fig. 2c). In a multivariate analysis, the hazard ratio for the overall survival rate between the high Helios and the low Helios was 2.78 (Table 2, 95 % confidence interval [CI], 1.09–7.09; *P* = 0.032). Other factors included tumor-infiltrated CD4 T cell levels, tumor-infiltrated FoxP3+ T cell levels, the cancer TNM stage, lymph node metastasis, distant metastasis and the HP infection, which did not show statistical significance in overall survival (Table 2).

Helios expression was an independent factor for survival in advanced gastric cancer patients

We also analyzed the effects of Helios expression in the early and advanced cancer TNM stages in overall survival. The Kaplan–Meier survival analysis revealed that a high Helios expression is a significantly preferred prognostic factor for median overall survival than for low Helios expression in advanced gastric cancer patients (42.1 ± 5.5 vs. 23.2 ± 4.8 months; log-rank test, *P* = 0.043, Fig. 3a) but not in early cancer stage patients (56.4 ± 4.0 vs. 49.4 ± 6.4 months; log-rank test, *P* = 0.420, Fig. 3b). In the subgroup study of advanced gastric cancer patients, there was no significant difference of patient characteristics in age, gender, distant metastasis, HP infection, tumor-infiltrated CD4 T cells and tumor-infiltrated FoxP3+ T cells (Table 3). In a multivariate analysis, the hazard ratio for overall survival among the high Helios subjects versus the low Helios subjects was 2.85 in advanced gastric cancer patients (Table 4, 95 % confidence interval [CI], 1.00–8.13; *P* = 0.05). Other factors included the tumor-infiltrated CD4 T cells, the tumor-infiltrated FoxP3+ T cells, distant metastasis and HP infection, which did not show statistical significance in overall survival.
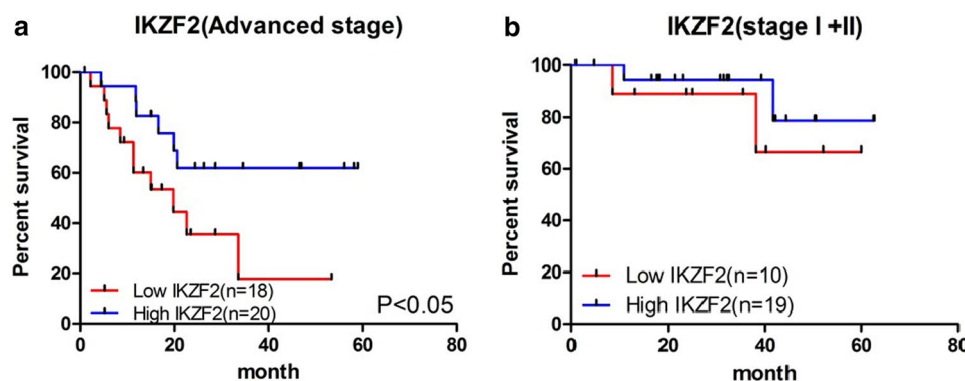
different HP-infected subgroups. In the subgroup of HP-infected gastric cancer patients, a high Helios expression group showed a significantly better survival rate than in the low Helios expression group (51.1 ± 3.5 vs. 30.4 ± 5.1 months; log-rank test, *P* = 0.007, Fig. 2b). However, in HP-negative patients, the expression level of

Fig. 3 Kaplan–Meier curves and log-rank test results for the overall survival of the patients with advanced gastric cancer based on the expression of Helios in tumor tissues. **a** Kaplan–Meier curves of overall survival of advanced-stage (T3+T4) gastric cancer patients obtained by the expression of Helios. The test resulted in a P value <0.05. **b** Kaplan–Meier curves of overall survival of lower-stage (T1+T2) gastric cancer patients obtained by the expression of Helios. It did not show significant differences

Table 3 Characteristics of advanced gastric cancer patients based on Helios presentation

	High ($N = 20$)	Low ($N = 18$)	P
Age	66.4 ± 11.6	69.2 ± 11.7	0.450
Gender			0.386
Female	7	4	
Male	13	14	
T stage			0.336
T1 + T2	1	0	
T3 + T4	19	18	
LN metastasis			0.336
No	1	0	
Yes	19	18	
Distal metastasis			0.573
No	16	13	
Yes	4	5	
HP infection			0.469
No	7	7	
Yes	13	11	
CD4			0.208
Low	14	9	
High	6	9	
FoxP3			0.782
Low	12	10	
High	8	8	

Discussion

In this study, we demonstrated that Helios expression in gastric tumor cells was an independent factor for survival in gastric cancer patients (Table 2; Fig. 2). We also found that the correlation with survival was especially evident in those Helicobacter pylori-infected patients (Fig. 2b) and

advanced gastric cancer patients (Fig. 3a). In our results, it showed that higher expression of Helios in gastric cancer cells yielded better survival.

Helios belongs to the Ikaros family, which is characterized by a highly conserved C2H2 zinc finger DNA-binding domain near the N terminus and a second zinc finger protein–protein interaction domain in the C terminus (John and Ward 2011; Rebollo and Schmitt 2003). Ikaros is the founding member of this family. It is expressed in most hematopoietic cells, whereas Helios is expressed primarily in T-lineage cells and early multipotential precursor cells (Kelley et al. 1998; Hahm et al. 1998). Over-expression of short isoforms of either Ikaros or Helios was found in leukemia and is sufficient to perturb lymphocyte homeostasis (Fujii et al. 2003). The abnormal splicing or loss of Helios expression appears as a part of the advantage of cell growth and survival in adult T cell leukemia (Asanuma et al. 2013). In recent reports, it was found that there was frequent intragenic deletion of *ikzf2*, which is the gene of Helios in the adult T cell leukemia/lymphoma (Kataoka et al. 2015). There are papers that report that mutation of these proteins can contribute to leukemogenesis and lymphomagenesis (Rebollo and Schmitt 2003; Dovat et al. 2005). Thus, it has been shown that they have tumor-suppressor characteristics, although the research studies are focusing on the hematopoietic lineages. The gene regulation mechanisms of these transcription factors are complex. In recent reports, it indicated that Helios regulated IL-2 production in regulatory T cells by suppressing *IL2* gene transcription through histone deacetylation in *IL2* promoter (Baine et al. 2013). Furthermore, they can also interact with other transcription factors to modulate their functions. Helios interacts with Foxp3 to enhance the foxp3 inhibitory functions in vitro and in vivo (Takatori et al. 2015; Getnet et al. 2010). Except for the hematopoietic cells or T cell lineage,

Table 4 Association between hazard rate and risk factors based on the Cox model in advanced gastric cancer patients

Risk factors	Hazard ratio [95 % CI]	P
Helios		0.050
Low	2.85 [1.00–8.13]	
High	1	
Gender		0.551
Male	1.70 [0.30–9.73]	
Female	1	
CD4		0.311
Low	1.87 [0.56–6.29]	
High	1	
FoxP3		0.376
Low	1.71 [0.52–5.59]	
High	1	
Distant metastasis		0.949
No	1.04 [0.32–3.36]	
Yes	1	
HP infection		0.308
No	2.38 [0.45–12.50]	
Yes	1	

Helios is expressed by a small population of nestin-positive neural progenitor cells as well as by a larger population of immature neurons distributed throughout the mantle zone (Martin-Ibanez et al. 2012).

In our results, we found that high Helios expression is a significant better prognostic factor in gastric cancer patients, especially in H. pylori-infected and advanced gastric cancer patients. H. pylori infection is the major factor for gastric carcinogenesis. There are many factors involved after H. pylori infection including bacterial virulence, host genetic, immune responses and environmental factors. These factors interact with each other and elicit variable clinical outcomes over a time span of decades. It is still to be investigated whether the infection will perturb the Helios expression in gastric cancer patients.

In conclusion, our present study revealed a novel aspect of Helios expression in gastric cancer patients. The Helios expression was an independent factor for survival in gastric cancer patients, especially in advanced gastric cancer patients. A high Helios expression is a significantly better prognostic factor for median overall survival than low a Helios expression.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights statement The Institutional Review Board of the Chang Gung Memorial Hospital approved all human subject assessments (IRB No. 104-4187C).

Informed consent The pathologic examination and further investigation were carried out under the informed consent of the patients.

References

Abe A, Nagatsuma AK, Higuchi Y, Nakamura Y, Yanagihara K, Ochiai A (2015) Site-specific fibroblasts regulate sitespecific inflammatory niche formation in gastric cancer. Gastric Cancer. doi:10.1007/s10120-015-0584-y

Asanuma S, Yamagishi M, Kawanami K, Nakano K, Sato-Otsubo A, Muto S et al (2013) Adult T-cell leukemia cells are characterized by abnormalities of Helios expression that promote T cell growth. Cancer Sci 104:1097–1106

Baine IU, Basu S, Ames R, Sellers RS, Macian F (2013) Helios induces epigenetic silencing of IL2 gene expression in regulatory T cells. J Immunol 190:1008–1016

Camp RL, Dolled-Filhart M, Rimm DL (2004) X-tile: a new bioinformatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res 10:7252–7259

Cancer Genome Atlas Research Network (2014) Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513:202–209

Charafe-Jauffret E, Tarpin C, Bardou VJ, Bertucci F, Ginestier C, Braud AC et al (2004) Immunophenotypic analysis of inflammatory breast cancers: identification of an 'inflammatory signature'. J Pathol 202:265–273

deLeeuw RJ, Kost SE, Kakal JA, Nelson BH (2012) The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. Clin Cancer Res 18:3022–3029

Dovat S, Montecino-Rodriguez E, Schuman V, Teitell MA, Dorshkind K, Smale ST (2005) Transgenic expression of Helios in B lineage cells alters B cell properties and promotes lymphomagenesis. J Immunol 175:3508–3515

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M et al (2012) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN. Int J Cancer 136:E359–E386

Fujii K, Ishimaru F, Nakase K, Tabayashi T, Kozuka T, Naoki K et al (2003) Over-expression of short isoforms of Helios in patients with adult T-cell leukaemia/lymphoma. Br J Haematol 120:986–989

Georgopoulos K (2002) Haematopoietic cell-fate decisions, chromatin regulation and ikaros. Nat Rev Immunol 2:162–174

Getnet D, Grosso JF, Goldberg MV, Harris TJ, Yen HR, Bruno TC et al (2010) A role for the transcription factor Helios in

human CD4^{+}CD25^{+} regulatory T cells. Mol Immunol 47:1595–1600

Hahm K, Cobb BS, McCarty AS, Brown KE, Klug CA, Lee R et al (1998) Helios, a T cell-restricted Ikaros family member that quantitatively associates with Ikaros at centromeric heterochromatin. Genes Dev 12:782–796

Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061

John LB, Ward AC (2011) The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol Immunol 48:1272–1278

Kashimura S, Saze Z, Terashima M, Soeta N, Ohtani S, Osuka F et al (2012) CD83^{+} dendritic cells and Foxp3^{+} regulatory T cells in primary lesions and regional lymph nodes are inversely correlated with prognosis of gastric cancer. Gastric Cancer 15:144–153

Kataoka K, Nagata Y, Kitanaka A, Shiraishi Y, Shimamura T, Yasunaga J et al (2015) Integrated molecular analysis of adult T cell leukemia/lymphoma. Nat Genet 47:1304–1315

Kelley CM, Ikeda T, Koipally J, Avitahl N, Wu L, Georgopoulos K et al (1998) Helios, a novel dimerization partner of Ikaros expressed in the earliest hematopoietic progenitors. Curr Biol 8:508–515

Kim KJ, Lee KS, Cho HJ, Kim YH, Yang HK, Kim WH et al (2014) Prognostic implications of tumor-infiltrating FoxP3+ regulatory T cells and CD8+ cytotoxic T cells in microsatellite-unstable gastric cancers. Hum Pathol 45:285–293

Kindlund B, Sjoling A, Yakkala C, Adamsson J, Janzon A, Hansson LE et al (2016) CD4 regulatory T cells in gastric cancer mucosa are proliferating and express high levels of IL-10 but little TGF-beta. Gastric Cancer. doi:10.1007/s10120-015-0591-z

Martin-Ibanez R, Crespo E, Esgleas M, Urban N, Wang B, Waclaw R et al (2012) Helios transcription factor expression depends on Gsx2 and Dlx1&2 function in developing striatal matrix neurons. Stem Cells Dev 21:2239–2251

Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB et al (2009) Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 360:470–480

Ohtsu A, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR et al (2011) Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol 29:3968–3976

Rebollo A, Schmitt C (2003) Ikaros, Aiolos and Helios: transcription regulators and lymphoid malignancies. Immunol Cell Biol 81:171–175

Shang B, Liu Y, Jiang SJ, Liu Y (2015) Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep 5:15179

Takatori H, Kawashima H, Matsuki A, Meguro K, Tanaka S, Iwamoto T et al (2015) Helios enhances Treg cell function in cooperation with FoxP3. Arthritis Rheumatol 67:1491–1502