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ORIGINAL ARTICLE

Insulin-like growth factor-1 receptor is associated with better prognosis in classical Hodgkin's lymphoma: Correlation with MET expression

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SUMMARY

The purpose of this study was to examine the prognostic significance of insulin-like growth factor-1 receptor (IGF-1R) expression alone and in relation to the expression of the MET- receptor and the MET-homologous receptor RON, in classical Hodgkin's lymphoma (cHL). Tumour samples from patients with cHL (n = 202; median age 37.5 years) were analysed retrospectively for IGF-R1, MET or RON expression by immunohistochemistry using tissue microarrays. The median follow-up time was 3.7 years (range, 0.1–20 years). Twenty-nine patients (14.3%) expressed IGF-1R protein in Hodgkin/Reed–Sternberg (HRS) cells, which was associated with a better overall survival (OS) (P = 0.036). IGF-1R expression was closely associated with MET receptor expression and low level of lactate dehydrogenase. In patients with cHL receiving doxorubicin, bleomycin, vinblastine and dacarbazine, those expressing IGF-1R showed a trend towards better OS and event-free survival than IGF-1R-negative patients (P = 0.129 and P = 0.115 respectively), but statistical significance was not reached. This study suggests that IGF-1R expression could be associated with better clinical outcome in cHL but is significantly associated with the expression of MET receptor.

Keywords
Hodgkin's lymphoma, IGF-1R, MET, prognosis

Classical Hodgkin's lymphoma (cHL) is characterized by the disruption of the normal lymph node architecture by the presence of Hodgkin/Reed–Sternberg (HRS) cells, which are usually in a minority within a background of reactive bystander cells that are mainly composed of T and B lymphocytes and other cell types (Pileri *et al.* 2002). Although there have been important advances in treatment, approximately 20% of patients do not respond, or relapse after receiving the optimal initial therapeutic strategy, and may require adapted first-line treatment (DeVita & Costa 2010).

The International Prognostic Score (IPS) has been the gold standard for predicting prognosis of the patient with cHL (Hasenclever & Diehl 1998; Engert *et al.* 2010). However, the prognostic value of the IPS is limited to advanced stage cHL and does not fully reflect the biological spectrum of cHL. Several biologic factors have been suggested as predictors of prognosis in patients with cHL, including those identified by gene expression profiling (Steidl *et al.* 2010, 2012) or immunohistochemistry-based detection (Koh *et al.* 2013a,b; Mestre *et al.* 2012; Koh *et al.* 2013a,b; Xu *et al.* 2012; Yoon *et al.* 2012). However, whether they have prognostic value for patients with cHL remains to be determined.

Insulin produced by the pancreas and insulin-like growth factors (IGFs) produced mainly by the liver regulate cellular growth and metabolism. There are two IGFs, namely IGF-1 and IGF-2, which each bind insulin-like growth factor-1 receptor (IGF-1R) and insulin-like growth factor-2 receptor (Garofalo 2002; De Meyts 2004). The IGF-1/IGF-1R signalling pathway, which is a subfamily of receptor tyrosine

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kinases, has shown in previous studies to have an association with tumour cell proliferation, transformation, survival and resistance to chemotherapy (Liu *et al.* 2002; Baserga 2005; Yakar *et al.* 2005). This association has been noted to influence the incidence and prognosis of prostate, breast and colorectal cancers (Hellawell *et al.* 2002; Palmqvist *et al.* 2003; Law *et al.* 2008; Cox *et al.* 2009). The IGF-1/IGF-1R signalling pathway is closely associated with proliferation and survival in the haematological malignancies of multiple myeloma (Stromberg *et al.* 2006) and mantle cell lymphoma (Vishwamitra *et al.* 2011). Furthermore, studies of IGF-1R-targeted therapy in IGF-1R-expressing pulmonary non-small cell carcinoma are currently underway (Cappuzzo *et al.* 2010; Dziadziuszko *et al.* 2010; Ekman *et al.* 2011; Kim *et al.* 2012).

Receptor crosstalk is apparently a common occurrence in cancer cells. MET, the hepatocyte growth factor (HGF) receptor, can promote tumour cell proliferation, survival, invasion and metastasis through the KRAS/BRAF/MAPK and the PTEN/PI3K/Akt pathways (Danilkovitch *et al.* 2000). IGF-1R can also activate KRAS/BRAF/MAPK and PTEN/PI3K/Akt pathways (LeRoith *et al.* 1995; Baserga 1999). A previous study demonstrated that IGF-1-induced delayed MET activation occurs in a prostate cancer cell line, suggesting that IGF-1R-mediated MET activation may contribute to tumorigenesis. (Varkaris *et al.* 2013). Furthermore, RON, a receptor tyrosine kinase with homology to MET, is involved in tumour progression and metastasis (Chen *et al.* 2000; Wang *et al.* 2003) and is overexpressed in human epithelial malignancies (Wang *et al.* 2000; Bardella *et al.* 2004). The expressions of both MET and RON have been reported to be prognostic biomarkers in cHL (Koh *et al.* 2013).

Recently, IGF-1R expression by immunohistochemistry was demonstrated to be a prognostic factor, but this study included only 80 patients with cHL (Liang *et al.* 2014). Here, we examine the prognostic significance of IGF-1R expression in 202 patients with cHL and also investigate its correlation with MET and RON expressions. Additionally, we performed a subgroup analysis according to chemotherapeutic regimen.

Materials and methods

Patients

A retrospective analysis was carried out in 202 consecutive patients diagnosed with cHL at the Asan Medical Center between 1989 and 2012, and at the Ulsan University Hospital between 2000 and 2013. All patients had pathologically confirmed cHL, had received no previous treatment, had no previous history of malignancy, had been treated with combination chemotherapy with or without radiotherapy, and had laboratory data and follow-up information available.

Responses were assessed using Cheson's criteria (Cheson *et al.* 2007). Routine follow-up imaging analyses were performed every 3 months for the first 2 years, every 6 months for the next 3 years, and then annually (or whenever clinically indicated) thereafter. Limited stage was defined as Ann Arbor stage I–II without B symptoms or bulky disease.

All tissues were reviewed by three pathologists and were classified, according to the World Health Organization criteria, into one of the four subgroups of cHL [nodular sclerosing (NS), lymphocyte-rich, mixed cellularity, lymphocyte-depleted] or as not otherwise specified (Swerdlow *et al.* 2008).

Immunohistochemistry

Tissue microarrays were constructed with three 1-mm-diameter tumour cores from selected areas of formalin-fixed, paraffin-embedded tumour samples. The tissue microarray section was stained using an automatic immunohistochemistry staining device (BenchMark XT; Ventana Medical Systems, Tucson, AZ, USA). Briefly, 5-μm-thick sections were transferred onto poly-L-lysine-coated adhesive slides and dried at 62°C for 30 min. After standard heat-induced epitope retrieval for 30 min in ethylenediaminetetraacetic acid (pH 8.0), the samples were incubated with an antibody against cleaved IGF-1R (dilution 1:1000, clone G11; Ventana Medical Systems). The sections were then incubated with biotinylated anti-mouse immunoglobulin, peroxidase-conjugated streptavidin (LSAB kit; DAKO, Glostrup, Denmark) and 3,3′-diaminobenzidine. Slides were counterstained with Harris haematoxylin.

In the three tissue cores from each patient sample, at least ten CD30-positive HRS cells in at least one core were analysed for expression of IGF-1R. A sample was considered IGF-1R positive if the marker was expressed in HRS cells in the cytoplasm and/or the membrane, according to the criteria previously described (Liang *et al.* 2014).

MET and RON immunohistochemistry data obtained from a previously reported study were used (Wha Koh *et al.* 2013).

In situ hybridization analysis of Epstein–Barr virus (EBV)-encoded RNA-1 and RNA-2 (EBER) was performed and scored as previously described (Huh *et al.* 1999).

Statistical analysis

Overall survival (OS) was defined as the time between the date of diagnosis and the date of death from any cause. The follow-up of patients still alive was censored at their last follow-up date. Event-free survival (EFS) was defined as the interval between the date of diagnosis and the date of disease progression, relapse or death from any cause. The follow-up of patients still alive without progression or relapse was censored at their last follow-up date. The OS and EFS were estimated using Kaplan–Meier curves, which were compared by log-rank testing. Multivariate prognostic analyses of OS and EFS were performed with the Cox proportional hazards regression model using the enter method. All statistical analyses were performed using the SPSS

statistical software program (version 18.0; SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Ethical Approval Statement

The present research was approved by the Internal Review Board of the Asan Medical Center.

Results

Patient characteristics

The clinical characteristics of the 202 patients included in the study are summarized in Table 1. The median follow-up time was 3.7 years (range, 0.1–20 years). The median age of the patients was 37.5 years (range, 6–84 years). Seventy-three patients experienced relapse, disease progression or death during follow-up, and 34 patients died. Median EFS was 8.83 years. Median OS was not reached. The estimated 5-year OS and EFS were 83.8% and 57.9% respectively.

Table 1 Demographic and clinical characteristics of patients

Characteristic at diagnosis	No. of patients (%)
Median age, (range) years	37.5 (6–84)
Male gender	121 (59.9)
Histologic subtype	
Nodular sclerosing	120 (59.4)
Mixed cellularity	49 (24.3)
Lymphocyte-rich	15 (7.4)
Lymphocyte-depleted	9 (4.5)
Not classifiable	9 (4.5)
Ann Arbor stage	
I	32 (15.8)
II	72 (35.6)
III	47 (23.3)
IV	51 (25.2)
Stage	
Limited	84 (41.6)
Advanced	118 (58.4)
B symptoms present	70 (34.7)
International Prognostic Score ≥4 (high-risk)	39 (19.3)
EBER-positivity	70 (39.3)
Primary treatment	
Chemotherapy	146 (72.3)
Chemoradiotherapy	53 (26.2)
Radiotherapy only	3 (1.5)
Chemotherapeutic regimen	
ABVD	146 (72.3)
ABVD/C-MOPP hybrid	18 (8.9)
BEACOPP	10 (5)
C-MOPP	14 (6.9)
Other regimen	11 (5.4)

ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone and procarbazine; EBER: Epstein–Barr virus-encoded RNA-1 and RNA-2 assessed by *in situ* hybridization.

IGF-1R protein expression

The correlations between IGF-1R expression and clinicopathologic factors are summarized in Table 2. IGF-1R was

Table 2 Correlation of IGF-1R protein expression with clinicopathologic variables

Characteristic	IGF-1R expression		*P*-value
	Negative ($n = 173$)	Positive ($n = 29$)	
Age (%)			
<45 years	100 (57.8)	21 (72.4)	0.156*
≥45 years	73 (42.2)	8 (27.6)	
Gender (%)			
Male	105 (60.7)	16 (55.2)	0.683*
Female	68 (39.3)	13 (44.8)	
Disease subtype (%)			
Nodular sclerosing	96 (55.5)	24 (82.8)	0.054†
Mixed cellularity	48 (27.7)	1 (3.4)	
Lymphocyte-rich	13 (7.5)	2 (6.9)	
Lymphocyte-depleted	8 (4.6)	1 (3.4)	
Not classifiable	8 (4.6)	1 (3.4)	
B symptoms (%)			
Absent	116 (67.1)	16 (55.2)	0.291*
Present	57 (39.2)	13 (44.8)	
Ann Arbor stage (%)			
Limited	74 (42.8)	10 (34.5)	0.425*
Advanced	99 (57.2)	19 (65.5)	
IPS (%)			
<4	138 (79.8)	25 (86.2)	0.464†
≥4	35 (20.2)	4 (13.8)	
LDH (U/L) (%)			
<250	46 (27.9)	15 (51.7)	0.016*
≥250	119 (72.1)	14 (48.3)	
EBER (%)			
Negative	91 (59.5)	17 (68.0)	0.510*
Positive	62 (40.5)	8 (32.0)	
Primary treatment (%)			
Chemotherapy	123 (71.1)	23 (79.3)	0.689†
Chemoradiotherapy	47 (27.2)	6 (20.7)	
Radiotherapy	3 (1.7)	0 (0)	
MET expression (%)			
Negative	66 (80.5)	5 (35.7	<0.001*
Positive	16 (19.5)	9 (64.3)	
RON expression (%)			
Negative	50 (61)	10 (71.4)	0.559*
Positive	32 (39)	8 (28.6	
Primary chemotherapeutic regimen (%)			
ABVD	122 (71.8)	24 (82.8)	0.167†
ABVD/C-MOPP hybrid	18 (10.6)	0 (0)	
BEACOPP	10 (5.9)	0 (0)	
C-MOPP	11 (6.5)	3 (10.3)	
Other regimen	9 (5.3)	2 (6.9)	

ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone and procarbazine; EBER: Epstein–Barr virus-encoded RNA-1 and RNA-2 assessed by *in situ* hybridization; IPS, International Prognostic Score; LDH, lactate dehydrogenase.
*Chi-squared test by two-sided Pearson's test.
†Chi-squared test by two-sided Fisher's test.

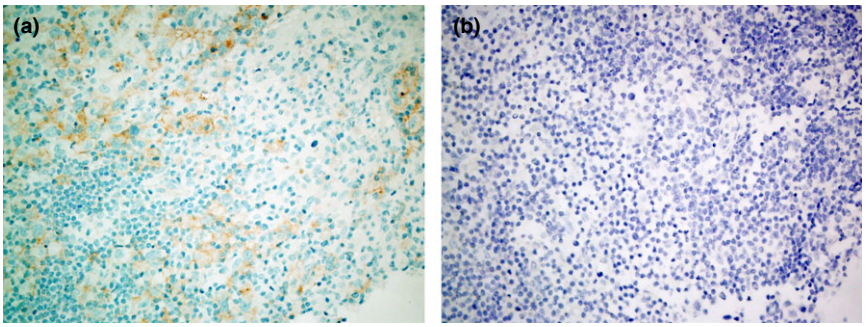

Figure 1 IGF-1R expression in classical Hodgkin's lymphoma tissues. Hodgkin/Reed–Sternberg cells were IGF-1R positive in the cytoplasm and/or the membrane (a) or negative in the cytoplasm and/or the membrane (b). Original magnification, 400×.

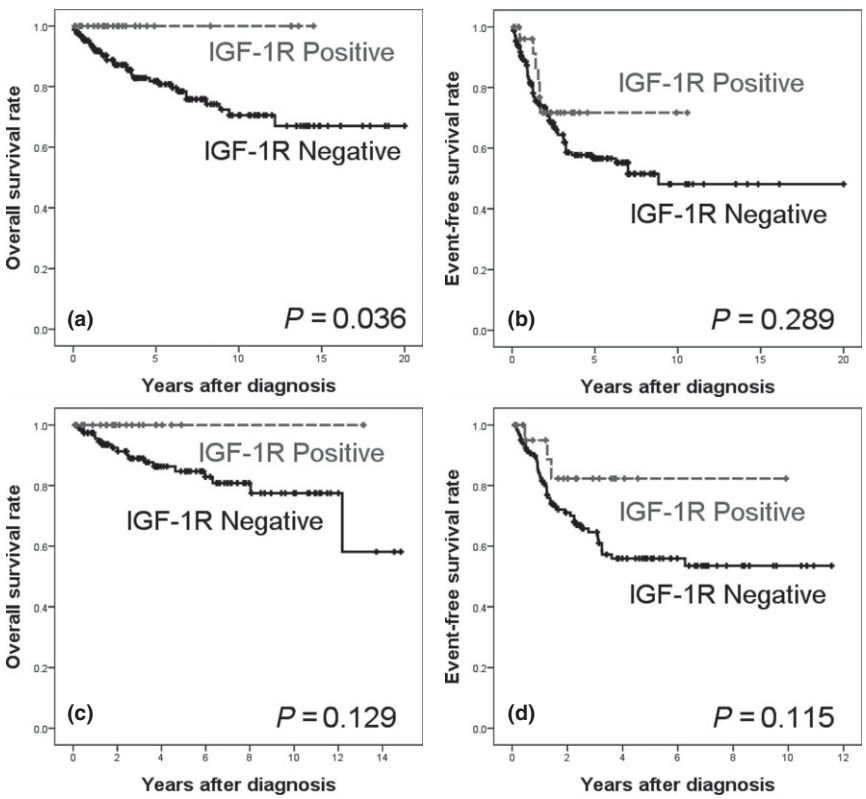

Figure 2 Comparison of survival rates according to IGF-1R expression. Overall survival was significantly worse in the IGF-1R-negative cases (a). IGF-1R expression was not associated with event-free survival (b). Among those treated with doxorubicin, bleomycin, vinblastine, and dacarbazine, IGF-1R-positive patients showed a trend towards better overall survival (c) or event-free survival (d) rates compared with IGF-1R-negative patients, but statistical significance was not reached.

expressed in both the cytoplasm and/or the membrane of HRS cells in 29 cHL tumour specimens (14.3%) (Figure 1). IGF-1R expression was associated with low level of lactate dehydrogenase (LDH) ($P = 0.016$) and MET protein expression ($P < 0.001$). The IGF-1R-positive group included more patients of NS subtype (82.8% *vs.* 55.5%, $P = 0.054$) and included less patients of MC subtype (3.4% *vs.* 27.7%), although statistical significance was not reached. No difference between the groups was identified with respect to age ($P = 0.156$), gender ($P = 0.683$), B symptoms ($P = 0.291$), Ann Arbor stage ($P = 0.425$), IPS ($P = 0.464$), EBER ($P = 0.510$), primary treatment method ($P = 0.689$), RON protein expression ($P = 0.559$) or primary chemotherapeutic regimen ($P = 0.167$). MET protein expression was not associated with RON protein expression ($P = 0.477$).

Prognostic significance of IGF-1R protein expression

The estimated 5-year OS rate of IGF-1R-positive patients was significantly higher than that of IGF-1R-negative patients (100% *vs.* 81.8%; $P = 0.036$) (Figure 2a), although the 5-year EFS rates were comparable (71.7% *vs.* 56.6%; $P = 0.289$) (Figure 2b). Among patients with cHL receiving doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD), those with an IGF-1R-positive tumour showed a trend towards better OS or EFS rates than those with an IGF-1R-negative tumour, ($P = 0.129$ and $P = 0.115$ respectively) (Figure 2c, d), but statistical significance was not reached. Multivariate analysis revealed that IGF-1R expression was not an independent prognostic marker for OS, along with high-risk IPS (≥4).

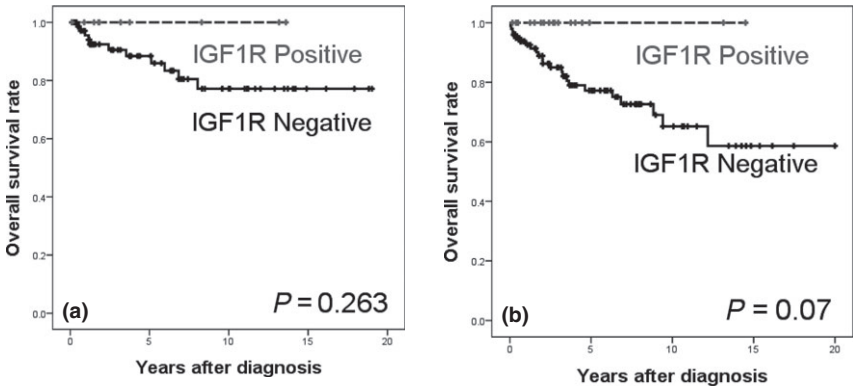

Figure 3 Comparison of survival rates according to Ann Arbor stage. IGF-1R-positive patients showed a trend towards a better overall survival rate compared with IGF-1R-negative patients in limited stage (a) and advanced stage (b), but the difference was not statistically significant.

As the IPS is a significant prognostic factor only in advanced stage disease (Hasenclever & Diehl 1998), we performed a subgroup analysis according to the disease stage (limited and advanced), to determine whether IGF-1R expression was more widely applicable than the IPS. IGF-1R-positive patients showed a trend towards a better OS rate than IGF-1R-negative patients in both limited stage ($P = 0.263$) and advanced stage ($P = 0.07$) (Figure 3a, b), but the difference was not statistically significant.

Our previous study revealed that MET protein was associated with a better survival outcome in patients with cHL (Wha Koh *et al.* 2013). Therefore, we combined the dichotomized IGF-1R and MET, and stratified patients into four groups (IGF-1R+/MET+, IGF-1R+/MET−, IGF-1R−/MET+ and IGF-1R−/MET−). IGF-1R−/MET− patients had inferior OS and EFS rates than those with the other expression patterns although the results did not reach statistical significance ($P = 0.228$, Figure S1a and $P = 0.301$, Figure S1b respectively).

Discussion

Receptor tyrosine kinases are associated with the regulation of cell proliferation, survival, growth and differentiation. Activating mutations of specific receptor tyrosine kinases may be a common occurrence in numerous tumours. HRS cells aberrantly express multiple different receptor tyrosine kinases (Renne *et al.* 2005). Receptor tyrosine kinase expression is observed frequently in the NS subtype of cHL, but is also detected at various levels in the other subtypes (Renne *et al.* 2005). Consistent with these findings, IGF-1R is also predominantly expressed in the NS subtype (Liang *et al.* 2014). In the present study, the IGF-1R-positive patients were more likely to have the NS subtype and less likely to have the MC subtype, although statistical significance was not reached.

Crosstalk between IGF1-R and MET has only been reported in a study of prostate cancer cells (Varkaris *et al.* 2013). Although cooperation between IGF-1R and MET has not been previously demonstrated in cHL or other malignancies, several lines of evidence suggest that it is likely. First, activation of the IGF-1/IGF-1R pathway in breast carcinoma (Dunn *et al.* 2001; Nielsen *et al.* 2004) and the HGF/MET pathway in various malignancies (Monvoisin *et al.* 1999; Ried *et al.* 1999; Tacchini *et al.* 2003) result in activation of the urokinase plasminogen activator/urokinase plasminogen activator receptor system, a common downstream mediator of invasion. Second, IGF-1 signalling induces hypoxia-inducible factor-1-alpha in pancreatic carcinoma cells (Stoeltzing *et al.* 2003), and hypoxia activates transcription of the MET proto-oncogene, resulting in higher levels of MET in human lung, hepatocellular and other carcinomas (Pennacchietti *et al.* 2003). Third, growth factor receptor-binding protein 2-associated binder-1 has been reported to mediate the interaction with the MET receptor (Weidner *et al.* 1996) and has also been shown to function as a signalling intermediate for IGF-1 (Winnay *et al.* 2000). Fourth, IGF-1 acts as a comitogen with HGF in a rat hepatoma cell line (Price *et al.* 2002), and fifth, IGF-1R and MET have been shown to cooperate to induce migration and invasion of human pancreatic carcinoma cells (Bauer *et al.* 2006). Furthermore, MET is required for both HGF- and IGF-1-mediated migration and invasion (Bauer *et al.* 2006).

Previous studies reported an expression of IGF-1R in 55% of the patients with cHL (Liang *et al.* 2014) and an expression of MET in 52%(Xu *et al.* 2012) respectively. However, the previous results showed no correlation between IGF-1R and MET expression (Liang *et al.* 2014). Our data showed a lower level of IGF-1R (14.3%) or MET expression (26.4%) than previous results. A correlation between IGF-1R and MET was also noted in the present study. Furthermore, IGF-1R-/MET- patients had worse OS or EFS than those with the other expression patterns, although the statistical significance was not reached. Possible reasons for interstudy discrepancy include disparate study populations and technical differences, such as using different antibody clones, the use of tissue microarray *vs.* whole sections or disparate assessments of outcome. Liang *et al.* (2014) used anti-IGF-1R antibody clones 3C8B1 and 3G5C1 on whole sections, while in the current study, we used anti-IGF-1R antibody clone G11 on tissue microarray sections.

Studies assessing the prognostic significance of IGF-1R expression in malignant tumours have yielded conflicting results. Although IGF-1R expression was found to have a

significant negative effect on prognosis in oral squamous cell carcinoma (Lara *et al.* 2011) and colorectal carcinoma (Takahari *et al.* 2009), other studies, in breast cancer (Papa *et al.* 1993; Hartog *et al.* 2011) and non-small cell lung cancer (Dziadziuszko *et al.* 2010), found it was associated with lower risk. IFG-1R expression is associated with well-differentiated tumours, suggesting that IGF-1R expression is lost when tumour cells progress to become more malignant (Schnarr *et al.* 2000; Kornprat *et al.* 2006). Liang *et al.* (2014) reported that IGF-1R expression was strong in the mitotic cHL cells and that inhibition of IGF-1R decreased proliferation and induced a G2/M cell-cycle arrest in cHL cell lines. As tumours with a high proliferation rate tend to respond better to chemotherapy (Paik *et al.* 2004; Uddin *et al.* 2010; Yerushalmi *et al.* 2010; Koh *et al.* 2013a,b), it is plausible that the superior survival rate of IGF-1R-positive patients with cHL is associated with this increased sensitivity to chemotherapy.

The most common approach to blocking IGF-1R downstream signalling is to use monoclonal antibodies against the receptor; they both inhibit ligand binding and down-regulate the receptor. Small molecule kinase inhibitors are a valid alternative. Anti-IGF-1R monoclonal antibodies have shown significant anti-tumour activity in Ewing's sarcoma (Olmos *et al.* 2010; Naing *et al.* 2011), adrenocortical carcinoma (Naing *et al.* 2011), multiple myeloma (Lacy *et al.* 2008) and pancreatic cancer (Kindler *et al.* 2012). The cyclolignan picropodophyllin, a small molecule kinase inhibitor, has been identified as an IGF-1R inhibitor (Girnita *et al.* 2004). It specifically blocks the phosphorylation of the Tyr1136 residue in IGF-1R and thus reduces kinase activity of the receptor (Girnita *et al.* 2004). Picropodophyllin inhibits the PI3K/AKT pathway, leading to apoptosis (Stromberg *et al.* 2006) and growth suppression of multiple myeloma cells (Menu *et al.* 2007), and Liang *et al.*, (2014) also found that it inhibits tumour proliferation in cHL cell lines.

The limitations of this study include its retrospective design, the short follow-up period of some of the more recent cases and the use of tissue microarray. The entire distribution cannot be reflected because of tissue heterogeneity in tissue microarray-based design.

In summary, our results suggest that IGF-1R may be a prognostic factor in cHL and may be useful for the identification of a subgroup of patients who may benefit from aggressive chemotherapy. Further studies, including prospective clinical trials, are needed to confirm the present findings and to investigate the effects of IGF-1R expression on clinical outcomes.

Acknowledgments

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Conflict of interest

There are no conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Comparison of survival rates according to IGF-1R/MET expression.