Alterations of insulin-like growth factor-1 receptor gene copy number and protein expression are common in non-small cell lung cancer

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ABSTRACT

Aims Insulin-like growth factor-1 receptor (IGF1R) is a tyrosine kinase membrane receptor involved in tumourigenesis that may be a potential therapeutic target. We aimed to investigate the incidence and prognostic significance of alterations in *IGF1R* copy number, and IGF1R protein expression in resected primary non-small cell lung cancer (NSCLC), and lymph node metastases.

Methods *IGF1R* gene copy number status was evaluated by chromogenic silver in situ hybridisation and IGF1R protein expression was evaluated by immunohistochemistry in tissue microarray sections from a retrospective cohort of 309 surgically resected NSCLCs and results were compared with clinicopathological features, including *EGFR* and *KRAS* mutational status and patient survival.

Results *IGF1R* gene copy number status was positive (high polysomy or amplification) in 29.2% of NSCLC, and 12.1% exhibited *IGF1R* gene amplification. High IGF1R expression was found in 28.3%. There was a modest correlation between *IGF1R* gene copy number and protein expression ($r=0.2$, $p<0.05$). Alterations of *IGF1R* gene copy number and protein expression in primary tumours were significantly associated with alterations in lymph node metastases ($p<0.01$). High *IGF1R* gene copy number and protein expression was significantly higher in squamous cell carcinomas (SCC) compared with other subtypes of NSCLC ($p<0.05$). There were no other associations between IGF1R status and other clinicopathological features including patient age, gender, smoking status, tumour size, stage, grade, *EGFR* or *KRAS* mutational status or overall survival.

Conclusions High *IGF1R* gene copy number and protein overexpression are frequent in NSCLC, particularly in SCCs, but they are not prognostically relevant.

INTRODUCTION

Lung cancer is still the leading cause of cancer death in the world despite significant progress over the past decade in the understanding of molecular mechanisms and the development of new targeted therapies for some subtypes of non-small cell lung cancer (NSCLC).[1 2] Insulin-like growth factor-1 receptor (IGF1R) is a member of the tyrosine kinase class of membrane receptors. IGF1R is a tetramer composed of two αß subunits that can also form hybrids with an αß subunit pair from the highly homologous insulin receptor.[3] These heterodimers have also been implicated in physiological and pathological insulin growth factor pathway signalling, but the exact role of IGF1R and hybrid receptors in malignancy is not completely understood.

The activated IGF1R is involved in cell growth and survival control.[3 4] Ligand binding activates the receptor kinase, leading to receptor autophosphorylation, and tyrosine phosphorylation of multiple substrates which lead to the activation of two main signalling pathways: the phosphoinositide 3-kinase (PI3K)- protein kinase B and Ras-mitogen-activated protein kinases (MAPK) pathways. Activation of the MAPK pathway results in cellular proliferation, whereas activating the PI3 K pathway inhibits apoptosis and stimulates protein synthesis.[5]

IGF1R plays an important role in tumour transformation and survival of malignant cells and is overexpressed in a variety of human malignancies[6] with frequent aberrations of *IGF1R* gene and protein expression in several common cancers such as prostate,[7] breast[8] and colorectal carcinomas.[9] As such, there is increasing interest in IGF1R as a therapeutic target for anticancer therapy, and several strategies for IGF1R inhibition are being investigated in phase II and III clinical trials in solid tumours.[3 10 11] Monoclonal antibodies directed against IGF1R have undergone early phase clinical trials in lung cancer in combination with chemotherapy or epidermal growth factor receptor (EGFR) inhibitors although most have shown disappointing results,[12 13] and results from one of the more promising phase II clinical trials have recently been retracted.[14]

Very few previous studies have investigated alterations of both *IGF1R* gene copy number (GCN) and protein expression in NSCLC,[15–17] and none have compared results in primary tumours and matched lymph node metastases. The aims of this study were to determine the incidence of alterations in *IGF1R* GCN and protein expression in primary and metastatic NSCLC in different histological subtypes and to investigate any correlations with clinicopathological features.

METHODS

Patient cohort and tissue microarray construction

This retrospective study was conducted in a cohort of 309 resected NSCLC performed by the one cardiothoracic surgeon (BM) (266 consecutive tumours resected between 1994 and 2002 and 43 additional primary and matched nodal metastases resected between 2010 and 2012, the latter excluded from survival analyses and *EGFR* and

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KRAS mutation analyses). All patients undergoing surgical resection of primary lung cancer were entered into a clinical database managed by a dedicated database manager (CK) and including information on patient demographics, tumour histology, extent of resection, tumour, node, metastasis (TNM) staging, postoperative morbidity and survival. Data on survival were maintained by routine follow-up visits, incoming correspondence and contact with the patient's general practitioner. Median follow-up time was 40.4 months (IQR 18.6–99.4 months) for the cases available for survival analysis. Tissue microarrays (TMA) were constructed using a manual tissue arrayer (MTA-1, Beecher Instruments Tissue Arrayer) with cores of 1 mm diameter including 3–6 cores from all primary tumours as previously described.[18] Three to four cores from each tumour have previously been shown to be adequate for TMA studies in lung cancer.[19 20]

Tumour subtype was determined using the WHO 2004 classification[21] by an experienced lung pathologist (WAC). The 7th edition American Joint Committee on Cancer TNM classification[22] was used to retrospectively stage tumours for the purpose of the study. There were 138 adenocarcinomas (ADC), 114 squamous cell carcinomas (SCC), 54 large cell carcinomas

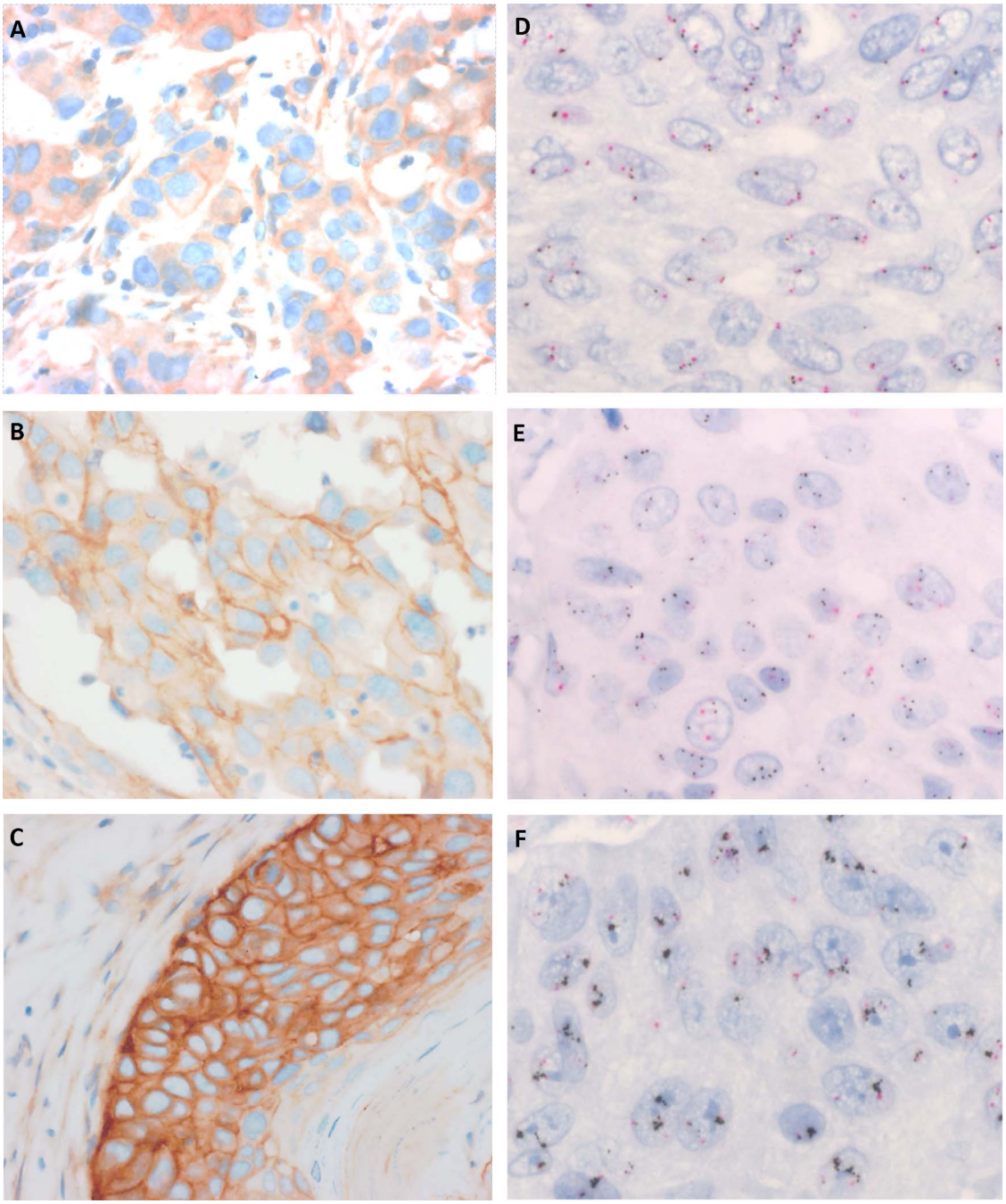

Figure 1 (A–C) Insulin-like growth factor-1 receptor (IGF1R) immunohistochemical membranous protein expression. (A) Weak (1)+ staining. (B) Moderate (2+) staining. (C) Strong (3+) staining. (D–F) *IGF1R* chromogenic in situ hybridisation with black *IGF1R* signals and red chromosome 15 signals. (D) Normal *IGF1R* gene copy number. (E) *IGF1R* high level polysomy with ≥40% of tumour cells containing ≥4 signals. (F) *IGF1R* amplification with tight gene clusters in ≥10% of cells.

(LCC) and 3 others (2 spindle cell carcinomas and 1 pleomorphic carcinoma). The study was approved by the Royal Prince Alfred Ethics Review Committee (X06-0167 and X10-0278) with all tissue specimens analysed anonymously.

Chromogenic silver in situ hybridisation (SISH)

Unstained 4 μm sections from each TMA were submitted to dual-colour in situ hybridisation (ISH) assay performed on BenchMark Ultra (Ventana) using a cocktail of *IGF1R* dinitrophenyl (DNP) probe (25 μg/mL) and Chromosome 15 digoxigenin (DIG) probe (25 μg/mL) (Ventana) and UltraView silver in situ hybridisation (SISH) DNP Detection Kit (Ventana).

Signals were enumerated in at least 50 non-overlapping tumour nuclei per core using a light microscope at 400×magnification. Tumours were classified into two groups: *IGF1R*-positive (amplification or high polysomy) and *IGF1R*-negative (low copy number). Cases were considered as *IGF1R* amplification if one of the following conditions was satisfied: IGF1R/CEP15 ratio ≥2.0; presence of tight gene clusters (innumerable clusters of IGF1R signals) in ≥10% of cells, or ≥10% of tumour cells containing ≥15 *IGF1R* signals. Cases were considered as high-level polysomy if they did not fulfil criteria for amplification but ≥40% of tumour cells contained ≥4 signals. Otherwise, a tumour was defined as low copy number.[23]

Immunohistochemical (IHC) staining

A 4 μm section of each TMA was stained on Benchmark Ultra autostainer (Ventana) with CONFIRM anti-IGF1R (G11) rabbit monoclonal primary antibody (Ventana) directed against the C-terminus of the β chain, using the manufacturer's instruction. Normal liver and kidney tissue cores were used as external IGF1R positive controls. No internal positive control was available. Staining was scored by determining the percentage of cells showing weak (1+), moderate (2+) or strong (3+) membranous staining. Scoring was undertaken independently by WAC and TNT, and any discrepant cases were re-evaluated and a consensus score reached. Tumours were considered IGF1R(+) if at least 10% of tumour cells showed cellular membrane staining at intensity 2+ or 3+.[24] Cytoplasmic staining alone was not considered positive.

Mutation detection

ADCs from the cohort of consecutively resected ADCs were analysed for *EGFR* or *KRAS* mutations using matrix-assisted laser desorption ionisation-time of flight mass spectrometry technology on the Sequenom MassArray platform using OncoCarta v1.0 Panel as previously described.[25]

Statistical analysis

R V.2.15.2 (The R Foundation for Statistical Computing) was used for all statistical analyses. The normality of variables was tested using Shapiro–Wilks test. Association with clinicopathological variables was tested using Pearson's χ^2 with Fisher's Exact test (categorical variables) and two-sample t test (continuous variables) or Cramer's V and Wilcoxon–Mann–Whitney tests for parametric and non-parametric testing, respectively. Overall survival estimation and comparison were calculated using the Kaplan–Meier method and log-rank tests. Statistical significance was set at $p<0.05$ for two sides. Multivariate analysis was performed using Cox proportional hazard regression model with a forward step-wise method.

RESULTS

IGF1R GCN and IGF1R protein expression

The median *IGF1R* GCN in all NSCLC was 2.3 (range, 1.4–8.9) and the median *IGF1R/CEN15* ratio was 1.6 (range, 0.8–4.5) in all NSCLC cases. Means of *IGF1R* signals and *IGF1R/CEN15* ratio were 2.8 and 1.7, respectively. There were two cases of NSCLC showing *IGF1R* clusters ≥10% of tumour cells, the majority of positive cases showing an increased number of separate signals (figure 1). Normal bronchial mucosa was *IGF1R*-negative and lacked IGF1R protein expression.

IGF1R GCN status was positive (high polysomy *or* amplification) in 77/264 NSCLC cases (29.2%), and 32/264 cases (12.1%) exhibited *IGF1R* gene amplification. Positive IGF1R expression was observed in 85/300 NSCLC cases (28.3%) (figure 1). Among 262 cases in which both *IGF1R* GCN and protein expression could be evaluated, a significant modest correlation was observed between *IGF1R* GCN positive (cases with *IGF1R* amplification *or* high polysomy) and protein overexpression (Cramer's V=0.2, $p<0.05$) (table 1).

The frequency of *IGF1R* ISH-positive cases was significantly higher in SCC (39.8%) than in ADC (23.5%) and LCC (22.2%) ($p<0.05$) (figure 2, table 1). Similarly, there were more cases with high IGF1R protein expression in SCC (43.2%) than in ADC (17%) and LCC (27.5%) ($p<0.05$). There was a significant association of *IGF1R* GCN alteration and IGF1R protein expression between primary and metastatic lymph node tumours (Cramer's V=0.7 and 0.5, respectively, $p<0.01$) (table 2).

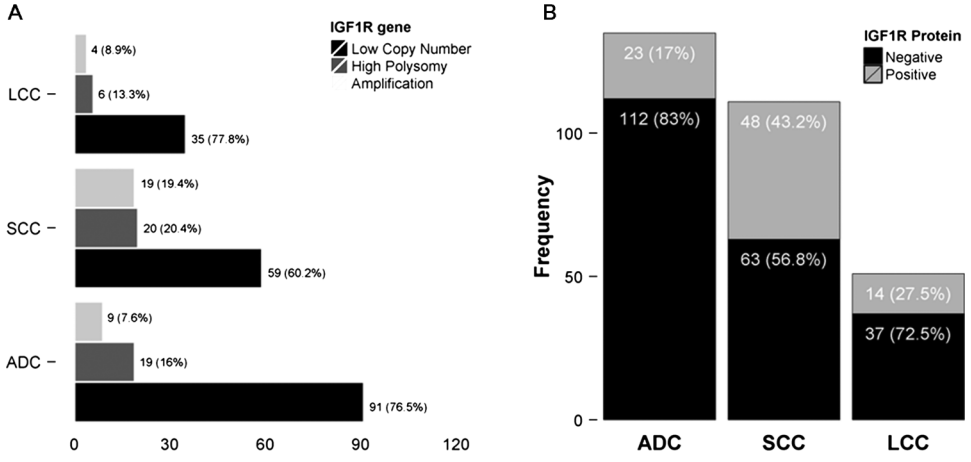

Figure 2 Frequencies of *IGF1R* gene alteration and protein expression in different subtypes of non-small cell lung cancer. (A) Gene copy number alterations. *IGF1R* positive (amplification or high-level polysomy) was more frequent in squamous cell carcinoma (SCC) than in adenocarcinoma (ADC) and large cell carcinoma (LCC) ($p<0.05$). (B) Insulin-like growth factor-1 receptor (IGF1R) protein overexpression was significantly higher in SCC than in ADC and LCC ($p<0.01$).

Correlation between IGF1R and clinicopathological features

There was no significant association between either *IGF1R* GCN status or immunohistochemical (IHC) protein expression and clinical features including patient age, gender or smoking status (table 1). Apart from the association with squamous histology, there was no association with other pathological features including tumour size, stage or grade (table 1). There was no correlation between *IGF1R* GCN or IGF1R protein expression and *EGFR* or *KRAS* mutational status (table 1). Overall survival of patients was not affected by *IGF1R* GCN or protein expression among all NSCLC patients or individual pathological subgroups (figure 3). In multivariable analysis, only stage, nodal status and lymphatic invasion were significant factors (HR 1.82, CI 1.55 to 2.09, p=0.03; HR 1.87, CI 1.7 to 2.04, p<0.001 and HR 1.92, CI 1.66 to 2.18 p=0.016, respectively).

DISCUSSION

Several strategies to target IGF1R are being evaluated for clinical use against NSCLC.[12 13] Biomarkers that assess IGF1R in tumours may be clinically useful to identify patients most likely to benefit from IGF1R inhibition, and there is in vitro evidence that high IGF1R protein levels and GCN predict sensitivity to IGF1R inhibition by monoclonal antibodies.[24] Very few studies have investigated the incidence of both *IGF1R* GCN change and

Table 1 *IGF1R* gene copy number, IGF1R protein expression and clinicopathological characteristics

	IGF1R gene copy number (GCN)*			IGF1R protein expression*		
Patient characteristics	**Negative**	**Positive (amplification or high polysomy)**	**p Value**	**Low**	**High**	**p Value**
n	187	77		215	85	
Age (years)						
Median (range)	68 (41–87)	66 (47–83)	1†	68 (41–87)	66 (42–83)	0.21†
Gender, n (%)						
Male	120 (71)	49 (29)	1‡	140 (73)	52 (27)	0.59‡
Female	67 (71)	28 (29)		75 (69)	33 (31)	
Tumour size (mm)						
Median (range)	35 (6–120)	38 (12–150)	0.54†	35 (6–150)	37 (7–120)	0.6†
Stage group, n (%)						
I–II	161 (72)	64 (28)	0.57‡	182 (72)	72 (28)	1
III	26 (67)	13 (33)		33 (72)	13 (28)	
Smoking, n (%)§						
Former/present	120 (72)	46 (28)	1‡	134 (71)	55 (29)	0.18‡
Never	8 (73)	3 (27)		11 (92)	1 (8)	
Histology, n (%)						
SCC	59 (60)	39 (40)	**<0.01‡**	63 (57)	48 (43)	**<0.01‡**
Non-SCC	128 (77)	38 (23)		152 (80)	37 (20)	
Grade, n (%)						
I–II	105 (70)	44 (0.30)	0.89‡	121 (72)	48 (28)	1‡
III	82 (71)	33 (29)		94 (72)	37 (28)	
Vessel invasion, n (%)§						
Absent	162 (73)	60 (27)	0.1‡	183 (74)	66 (26)	0.2‡
Present	25 (60)	17 (40)		28 (64)	16 (36.4)	
Perineural invasion, n (%)§						
Absent	172 (70)	73 (30)	0.6‡	196 (72)	77 (28)	1‡
Present	15 (79)	4 (21)		15 (75)	5 (25)	
Lymphatic invasion, n (%)§						
Absent	159 (73)	60 (27)	0.2‡	181 (74)	65 (26)	0.21‡
Present	28 (62)	17 (38)		30 (64)	17 (36)	
IGF1R GCN						
Negative				141 (76)	44 (24)	**<0.01¶** (r=0.2)
Positive				47 (61)	30 (39)	
EGFR mutation**						
Wild-type	66 (81)	16 (19)	1	76 (83)	16 (17)	1
Mutant	7 (88)	1 (12)		9 (90)	1 (10)	
KRAS mutation**						
Wild-type	51 (80)	13 (20)	0.77	62 (85)	11 (15)	0.56
Mutant	22 (85)	4 (15)		23 (79)	6 (21)	

**IGF1R* gene copy number was able to be evaluated in 264 cases and IHC in 300 cases.
†Wilcoxon–Matt–Whitney Test.
‡Fisher's Exact Test.
§Smoking status was available for IGF1R GCN and IHC correlation analyses in 177/264 and 201/300 cases, respectively, while the vessel, perineural and lymphatic invasion parameters were available for IGF1R IHC correlation in 293/300 cases.
¶Cramer's V χ^2 Test.
**EGFR and KRAS mutation testing was available for in situ hybridisation and IHC IGF1R correlation in 90 and 102 cases, respectively.
IGF1R, insulin-like growth factor-1 receptor; IHC, immunohistochemical; SCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor; KRAS, Kirsten Ras.

Table 2 Correlation of IGF1R status between primary tumours and corresponding lymph node metastases

	Primary tumours					
	IGF1R gene copy number			IGF1R protein expression		
	Negative	Positive	p Value	Low	High	p Value
Lymph node metastases						
IGF1R gene copy number						
Negative	39 (91)	4 (9)	**<0.01***			
Positive	6 (25)	18 (75)	(r=0.7)			
IGF1R protein expression						
Negative				48 (81)	11 (19)	**<0.01***
Positive				6 (24)	19 (76)	(r=0.5)

*Cramer's V χ^2 Test.
IGF1R, insulin-like growth factor-1 receptor.

protein overexpression in the same cohort of NSCLC and this is the first study to compare IGF1R alterations in primary tumours and matched lymph node metastases although the number of cases with nodal metastases was relatively low. Our study has demonstrated that IGF1R alterations are common in NSCLC with *IGF1R* amplification occurring in 12.1%, high GCN (high polysomy or amplification) in 29.2% and protein overexpression in 28.3%. This is consistent with another study of NSCLC by Dziadziuszko *et* al[15] whereby 27% of NSCLC showed high *IGF1R* GCN using similar scoring criteria although only 3% of their cases showed gene amplification. Others have reported high *IGF1R* GCN in 40% of NSCLC[16] although they had a higher proportion of cases with SCC than in our cohort (50.4% vs 37%). One study by Tsuta *et al*[17] found a lower proportion of NSCLC with high *IGF1R* GCN (8.4%) in a Japanese population using similar scoring criteria suggesting there may be racial differences in *IGF1R* alteration. However, the differences may also relate to a different range of NSCLC subtypes between the studies or other differences between the patient cohorts.

We found IGF1R overexpression correlated significantly with *IGF1R* GCN alterations, suggesting GCN alterations are responsible for high protein levels in many cases. However, there was incomplete agreement between the two techniques which may relate at least in some cases, to a potentially more complex regulation of the IGF1R signalling pathway and the existence of several IGF1R isoform hybrids.[26] Others have found a correlation between IGF1R IHC score and GCN in NSCLC[16] and in small cell lung carcinomas.[27] Similar to our results, others have reported high IGF1R protein expression in 30.5%[28] and 39.3%[24] of NSCLC, using the same scoring criteria as in our study.

To our knowledge, our study is the first to compare *IGF1R* GCN and protein expression in primary tumours and matched lymph node metastases, and we found a significant strong association between primary and metastatic tumours although the results were not completely identical in all cases. This suggests that if these biomarkers were used in clinical practice, then it would be adequate to assess IGF1R in biopsies from either primary or metastatic tumours.

As in our study, most previous reports have consistently identified higher levels of IGF1R (protein expression and/or GCN) in lung SCC compared with other histological subtypes suggesting this subtype of NSCLC may be most likely to benefit from IGF1R inhibition.[15 17 28 29] However, a few studies have not identified such an association with histological subtype.[30 31] The lack of significant association between IGF1R and smoking status in our study suggests factors other than tobacco smoke may contribute to higher levels of IGF1R in SCC, however, it is not possible to draw conclusions, as smoking status was not available for all patients in our cohort.

In vitro studies have shown an intriguing relationship between mutational status of NSCLCs and response to IGF1R small molecule inhibitors. A screening study of small molecule inhibitors found that *KRAS* mutant NSCLC cell lines were more sensitive to inhibition of IGF1R compared with wild-type tumours. They showed that KRAS mediated signalling via PI3 K was dependent on IGF1R activity suggesting cooperative activity of IGF1R and oncogenic *KRAS* mutations, which could be exploited for therapeutic inhibition. Interestingly, we did not find any relationship between IGF1R status and *KRAS* mutations among the lung ADCs in our study, similar to a previous study.[17]

Overexpression of IGF1R has been implicated in acquired resistance to EGFR tyrosine kinase inhibition in *EGFR* mutant ADCs.[16 32] Cotreatment with an anti-IGF1R monoclonal antibody and an EGFR tyrosine kinase inhibitor improved anticancer response in a cell line with high IGF1R and an activating *EGFR* mutation.[24] Interestingly, we found only two tumours with high *IGF1R* GCN, or protein expression also harboured an activating *EGFR* mutation in this treatment-naive population although there was no statistically significant correlation between *EGFR* mutation status and IGF1R alterations in our ADC cohort or in previous studies.[17]

Although alterations of *IGF1R* GCN and protein expression are frequent in NSCLC, they appear to have no impact on prognosis as shown in this study as well as in several previous

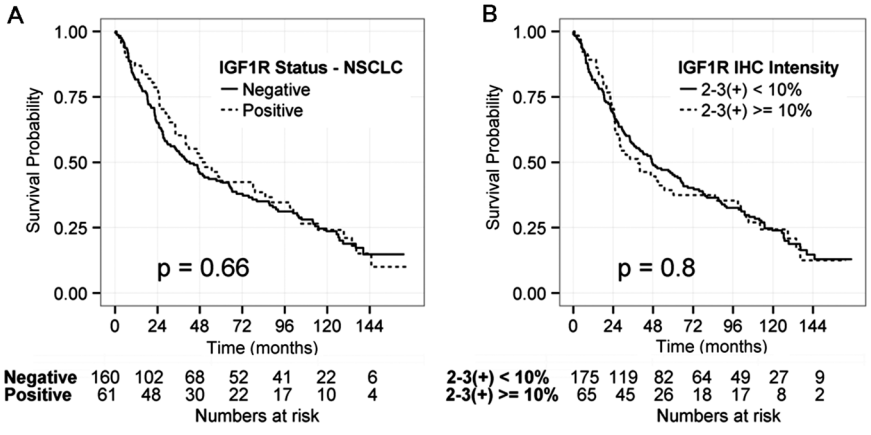

Figure 3 Overall survival of patients with non-small cell lung cancer (NSCLC) by (A) *insulin-like growth factor-1 receptor (IGF1R)* gene copy number status (positive=amplification or high polysomy, negative=low gene copy number) and (B) protein expression. There was no significant difference of overall survival in terms of *IGF1R* gene copy number status and protein expression (p>0.05).

studies.[15 17 29] One previous study by Dziadziuszko *et al*[15] demonstrated a favourable prognostic value of high *IGF1R* GCN but not *IGF1R* gene expression evaluated by quantitative reverse transcription PCR, or IGF1R protein expression. In another study, lung ADCs with high IGF1R protein expression (cut-off value of 10% positive cells) showed an independent increased risk of postoperative recurrence but not in SCCs or all NSCLC.[33] Another study with multivariate analysis showed high expression of IGF1R, and EGFR was a worse prognostic factor of disease-free survival.[30]

In summary, *IGF1R* high GCN and protein expression are frequent in NSCLC, particularly in SCCs, but they are not prognostically relevant. A thorough understanding of IGF1R alterations in NSCLC is essential to guide therapeutic approaches that target IGF1R. Evaluation of IGF1R protein expression and GCN is feasible on histological specimens of NSCLC and further evaluation of these biomarkers as possible predictive markers is recommended in future clinical trials investigating IGF1R inhibitors.

Take home messages

- High insulin-like growth factor-1 receptor (IGF1R) gene copy number and protein overexpression are frequent in non-small cell lung cancer (NSCLC), particularly in squamous cell carcinomas.
- IGF1R is not a prognostic marker in NSCLC.
- *IGF1R* gene copy number and IGF1R protein expression are readily detectable in histological specimens of NSCLC and could potentially be used as biomarkers when evaluating patient response to IGF1R inhibitors.

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REFERENCES

1 Jemal A, Bray F, Center MM, *et al*. Global cancer statistics. *CA Cancer J Clin* 2011;61:69–90.
2 Li T, Kung H-J, Mack PC, *et al*. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. *J Clin Oncol* 2013;31:1039–49.
3 Haisa M. The type 1 insulin-like growth factor receptor signalling system and targeted tyrosine kinase inhibition in cancer. *J Int Med Res* 2013;41:253–64.
4 Cooper WA, Lam DCL, O'Toole SA, *et al*. Molecular biology of lung cancer. *J Thorac Dis* 2013;5:S479–90.
5 Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* 2006;441:424–30.
6 Sell C, Rubini M, Rubin R, *et al*. Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. *Proc Natl Acad Sci USA* 1993;90:11217–21.
7 Hellawell GO, Turner GDH, Davies DR, *et al*. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res* 2002;62:2942–50.
8 Law JH, Habibi G, Hu K, *et al*. Phosphorylated insulin-like growth factor-insulin receptor is present in all breast cancer subtypes and is related to poor survival. *Cancer Res* 2008;68:10238–46.
9 Hakam A, Yeatman TJ, Lu L, *et al*. Expression of insulin-like growth factor-1 receptor in human colorectal cancer. *Hum Pathol* 1999;30:1128–33.
10 Avnet S, Sciacca L, Salerno M, *et al*. Insulin receptor isoform A and insulin-like growth factor II as additional treatment targets in human osteosarcoma. *Cancer Res* 2009;69:2443–52.
11 Yee D. Insulin-like growth factor receptor inhibitors: baby or the bathwater? *J Natl Cancer Inst* 2012;104:975–81.
12 Pillai RN, Ramalingam SS. Inhibition of insulin-like growth factor receptor: end of a targeted therapy? *Transl Lung Cancer Res* 2013;2:14–22.
13 Langer CJ, Novello S, Park K, *et al*. Randomized, Phase III trial of first-line figitumumab in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin alone in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2014;32:2059–66.
14 Karp DD, Paz-Ares LG, Novello S, *et al*. Phase II study of the anti-insulin-like growth factor type 1 receptor antibody CP-751,871 in combination with paclitaxel and carboplatin in previously untreated, locally advanced, or metastatic non-small-cell lung cancer. *J Clin Oncol* 2009;27:2516–22.
15 Dziadziuszko R, Merrick DT, Witta SE, *et al*. Insulin-like growth factor receptor 1 (IGF1R) gene copy number is associated with survival in operable non-small-cell lung cancer: a comparison between IGF1R fluorescent in situ hybridization, protein expression, and mRNA expression. *J Clin Oncol* 2010;28:2174–80.
16 Ludovini V, Flacco A, Bianconi F, *et al*. Concomitant high gene copy number and protein overexpression of IGF1R and EGFR negatively affect disease-free survival of surgically resected non-small-cell-lung cancer patients. *Cancer Chemother Pharmacol* 2013;71:671–80.
17 Tsuta K, Mimae T, Nitta H, *et al*. Insulin-like growth factor-1 receptor protein expression and gene copy number alterations in non-small cell lung carcinomas. *Hum Pathol* 2013;44:975–82.
18 Tran TN, Selinger CI, Kohonen-Corish MRJ, *et al*. Fibroblast growth factor receptor 1 (FGFR1) copy number is an independent prognostic factor in non-small cell lung cancer. *Lung Cancer* 2013;81:462–7.
19 Karlsson C, Bodin L, Piehl-Aulin K, *et al*. Tissue microarray validation: a methodologic study with special reference to lung cancer. *Cancer Epidemiol Biomark Prev* 2009;18:2014–21.
20 Schmidt LH, Biesterfeld S, Kümmel A, *et al*. Tissue microarrays are reliable tools for the clinicopathological characterization of lung cancer tissue. *Anticancer Res* 2009;29:201–9.
21 World Health Organization. *International agency for research on cancer, International Association for the Study of Lung Cancer, et al. Pathology and genetics of tumours of the lung, pleura, thymus and heart*. Lyon: Oxford: IARC Press, Oxford University Press (distributor), 2004.
22 American Joint Committee on Cancer. *AJCC cancer staging manual*. 7th edn. New York: Springer, 2010.
23 Cappuzzo F, Hirsch FR, Rossi E, *et al*. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–55.
24 Gong Y, Yao E, Shen R, *et al*. High expression levels of total IGF-1R and sensitivity of NSCLC cells in vitro to an anti-IGF-1R antibody (R1507). *PloS ONE* 2009;4:e7273.
25 Yip PY, Yu B, Cooper WA, *et al*. Patterns of DNA mutations and ALK rearrangement in resected node negative lung adenocarcinoma. *J Thorac Oncol* 2013;8:408–14.
26 Belfiore A, Frasca F, Pandini G, *et al*. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev* 2009;30:586–623.
27 Badzio A, Wynes MW, Dziadziuszko R, *et al*. Increased insulin-like growth factor 1 receptor protein expression and gene copy number in small cell lung cancer. *J Thorac Oncol* 2010;5:1905–11.

28 Nakagawa M, Uramoto H, Oka S, *et al*. Clinical significance of IGF1R expression in non-small-cell lung cancer. *Clin Lung Cancer* 2012;13:136–42.
29 Cappuzzo F, Tallini G, Finocchiaro G, *et al*. Insulin-like growth factor receptor 1 (IGF1R) expression and survival in surgically resected non-small-cell lung cancer (NSCLC) patients. *Ann Oncol* 2010;21:562–7.
30 Ludovini V, Bellezza G, Pistola L, *et al*. High coexpression of both insulin-like growth factor receptor-1 (IGFR-1) and epidermal growth factor receptor (EGFR) is associated with shorter disease-free survival in resected non-small-cell lung cancer patients. *Ann Oncol* 2009;20:842–9.
31 Lee CY, Jeon JH, Kim HJ, *et al*. Clinical significance of insulin-like growth factor-1 receptor expression in stage I non-small-cell lung cancer: immunohistochemical analysis. *Korean J Intern Med* 2008;23:116–20.
32 Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. *Cancer Res* 2002;62:200–7.
33 Nakagawa M, Uramoto H, Shimokawa H, *et al*. Insulin-like growth factor receptor-1 expression predicts postoperative recurrence in adenocarcinoma of the lung. *Exp Ther Med* 2011;2:585–90.

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