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# IGF-IR: a new prognostic biomarker for human glioblastoma

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**Background:** Glioblastomas (GBMs) are the most common malignant primary brain tumours in adults and are refractory to conventional therapy, including surgical resection, radiotherapy and chemotherapy. The insulin-like growth factor (IGF) system is a complex network that includes ligands (IGFI and IGFII), receptors (IGF-IR and IGF-IIR) and high-affinity binding proteins (IGFBP-1 to IGFBP-6). Many studies have reported a role for the IGF system in the regulation of tumour cell biology. However, the role of this system remains unclear in GBMs.

**Methods:** We investigate the prognostic value of both the IGF ligands' and receptors' expression in a cohort of human GBMs. Tissue microarray and image analysis were conducted to quantitatively analyse the immunohistochemical expression of these proteins in 218 human GBMs.

**Results:** Both IGF-IR and IGF-IIR were overexpressed in GBMs compared with normal brain ($P<10^{-4}$ and $P=0.002$, respectively). Moreover, with regard to standard clinical factors, IGF-IR positivity was identified as an independent prognostic factor associated with shorter survival ($P=0.016$) and was associated with a less favourable response to temozolomide.

**Conclusions:** This study suggests that IGF-IR could be an interesting target for GBM therapy.

Glioblastoma (GBM) is the most common malignant primary brain tumour in adults, accounting for approximately 12–15% of all intracranial neoplasms (Louis *et al*, 2007). Despite the progress made in surgery, radiotherapy and chemotherapy, the overall survival of patients with GBM remains poor, with a 5-year survival rate of 3.3% (Bondy *et al*, 2008).

Several studies identified subtypes of GBM associated with different prognoses or responses to treatment (Phillips *et al*, 2006; Verhaak *et al*, 2010; Le Mercier *et al*, 2012). To develop novel targeted therapies and improve patient outcome, it is imperative to better understand the molecular mechanisms involved in GBM pathogenesis and to identify new biomarkers associated with prognostic values and/or predictive of the response to treatment.

The insulin-like growth factor (IGF) system has a crucial role in tumorigenesis owing to its involvement in apoptosis, mitogenesis, cell migration, multidrug resistance and radioresistance (Guvakova, 2007; Samani *et al*, 2007). This system consists of soluble ligands (including IGFI and IGFII), cell surface transmembrane receptors (including IGFI receptor (IGF-IR) and IGFII receptor (IGF-IIR)) and soluble binding proteins (IGFBP1 (IGF binding protein-1) through IGFBP-6). The biological activities of IGFs are mediated by cell surface receptors and modulated by complex interactions with binding proteins (Le Roith, 2003; Denley *et al*, 2005; Sachdev and Yee, 2007).

Involvement of the IGF system in GBM pathogenesis is widely supported in the literature. The presence of IGF-IR and IGF-IIR in GBM cell lines has been demonstrated (Friend *et al*, 2001; Schlenska-Lange *et al*, 2008). *In vitro*, IGFs were shown to promote proliferation, survival and migration of GBM cell lines (Friend *et al*, 2001; Brockmann *et al*, 2003; Soroceanu *et al*, 2007;

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Rorive *et al*, 2008; Schlenska-Lange *et al*, 2008). However, reports on the expression of the members of the IGF system in human GBM samples are often limited to small series and thus have not yielded consistent results. Although IGFI expression was observed in the majority of GBMs (Antoniades *et al*, 1992; Sandberg-Nordqvist *et al*, 1993; Hirano *et al*, 1999) using *in situ* hybridisation (100%) or immunohistochemistry (75–100%), IGFII expression was less prevalent, detectable in only 6% of GBMs using *in situ* hybridisation (Soroceanu *et al*, 2007) and in 58% using immunohistochemistry (Suvasini *et al*, 2011). In contrast, Suvasini *et al* (2011) reported that IGFI and IGFII transcript levels evaluated by RT-qPCR do not change between normal brain samples and grade II astrocytomas, grade III astrocytomas and GBMs. Several studies demonstrated IGF-IR expression in the majority of GBM samples analysed by means of *in situ* hybridisation, binding assays or western blotting (Glick *et al*, 1989; Merrill and Edwards, 1990; Antoniades *et al*, 1992; Yin *et al*, 2010), whereas RT-qPCR demonstrated no significant difference in the transcript levels of normal brain samples and low- and high-grade astrocytomas (Suvasini *et al*, 2011). Literature data on IGF-IIR expression are scarce (Antoniades *et al*, 1992; Friend *et al*, 2001; Schlenska-Lange *et al*, 2008).

Considering the results of *in vitro* studies and the expression of IGF-IR in the majority of GBMs, it is surprising that little data are available concerning the clinical significance of the IGF system in GBM. To the best of our knowledge, only two studies have reported the prognostic value of the IGF system in GBM. Using gene expression analysis, Soroceanu *et al* (2007) identified a group of GBMs characterised by IGFII overexpression and belonging to a subclass associated with poor survival. Furthermore, a recent study showed an inverse correlation between IGF-IR gene and protein expression levels and survival (Zamykal *et al*, 2014). Therefore, our goal was to evaluate the prognostic value of the IGF ligands (IGFI and IGFII) and their receptors (IGF-IR and IGF-IIR) in a large series of GBMs using quantitative immunohistochemistry based on image analysis of tissue microarray (TMA) materials.

## MATERIALS AND METHODS

**Clinical and histopathological data.** Two normal brain TMAs were manufactured using formalin-fixed and paraffin-embedded samples from nine *post mortem* adult human brains (without neuropathological alterations) obtained within 24 h of death. Six tissue cores (diameter: 600 $\mu$m) were taken from six different areas per brain: grey and white matter from the cerebral hemispheres (frontal and occipital lobes), corpus callosum, and semioval center. A series of 218 GBMs was investigated in parallel. The series consisted of archival formalin-fixed and paraffin-embedded samples obtained from the Laboratory of Pathology of the Erasme University Hospital (Brussels, Belgium) that were collected between 1988 and 2006. All the samples were surgical specimens obtained by open surgical resection. Four TMAs that included three tissue cores (diameter: 600 $\mu$m) per case and that targeted the tumour bulk were produced. All of the tumours were classified by two pathologists (SR/IS) according to the 2007 revised World Health Organisation classification system (Louis *et al*, 2007). This study received the approval of the Ethics Committee of the Université Libre de Bruxelles Hôpital Erasme. According to the Belgian law of December 2008 'Loi relative à l'obtention et à l'utilisation de matériel corporel humain destine à des applications médicales humaines ou à des fins de recherche scientifique', no written informed consent was required. The Ethics Committee has thus waived the need for written informed consent from the participants.

The available clinical data for each patient included age, gender, multifocality of the tumour, date of surgery, extent of surgical resection, adjuvant treatment and follow-up (Table 1). The cancer-specific survival period was measured from the date of tumour surgery until the date of death due to tumour progression.

**Immunohistochemistry.** As previously described (Rorive *et al*, 2010), standard immunohistochemistry was performed on 5-$\mu$m-thick sections (one per antibody) to assess the expression of IGFI, IGFII, IGF-IR and IGF-IIR, using a mouse monoclonal antibody (anti-IGFI; sc-74116; dilution 1:25, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal antibodies (anti-IGFII; ab9574, dilution 1:400; Abcam, Cambridge, UK and anti-IGF-IIR; sc-25462; dilution 1:100; Santa Cruz Biotechnology), and a rabbit monoclonal antibody (anti-IGF-IR; 790–4346, RTU; Ventana Medical System, Tucson, AZ, USA).

Immunohistochemical staining was visualised using streptavidin–biotin–peroxidase complex kit reagents (BioGenex, San Ramon, CA, USA) with diaminobenzidine/$H_2O_2$ as chromogenic substrate. Counterstaining with hematoxylin concluded the processing. Negative controls were prepared by replacing the primary antibodies with non-immune serum (Dako, Glostrup, Denmark). As previously described (Battifora, 1991; Decaestecker *et al*, 2009), additional technical and fixative controls were carried out by staining the TMA slides with haematoxylin–eosin and anti-vimentin (V-9, dilution 1:100; BioGenex), respectively. The final validation stage (conducted by two pathologists (CM/SR)) aimed to confirm the adequacy of the specific tumour zones targeted and immunostaining compliance. Only the cores that satisfied all of the control staining tests were submitted for quantification (Decaestecker *et al*, 2009).

**Evaluation of immunohistochemical staining.** TMA core image acquisition and staining quantification were performed using SpotBrowser V2e (Alphelys, Plaisir, France) connected to a DXC-390 Sony camera and a motorised stage (Marzhaüser, Wetzlar-Steindorf, Germany) on a BX50 Olympus microscope (Olympus, Aartselaar, Belgium), as previously described (Decaestecker *et al*, 2009; Rorive *et al*, 2010). For each valid core, we measured the analysed (i.e., positive and negative) tissue area and the positive (i.e., stained) area. To characterise each normal or GBM case, we pooled all of the appropriate cores and computed the labelling index (LI), which is the percentage of the immunostained tissue area (Decaestecker *et al*, 2009). To discriminate between GBM showing no expression from those with expression, the cutoff value of 1% was used (i.e., negative LI $<1\%$ *vs* positive LI $\geqslant 1\%$). In addition, we took into account that using a higher cutoff decreases interobserver variability in the interpretation of immunohistochemical analysis and that 30% is a threshold relatively easy to interpret in clinical applications (Hameed *et al*, 2008). A refined three-class system was thus also used for IGF-IR LI: negative (LI $<1\%$), weakly positive ($1\% \leqslant$ LI $<30\%$), and strongly positive (LI $\geqslant 30\%$).

To evaluate colocation of ligands and receptors in GBM samples, we analysed the expression of the different proteins inside the same TMA core and across the different sections submitted to immunohistochemistry. For this purpose, we selected the cores showing ligand (IGFI or IGFII) expression and satisfying all of the control staining tests for the expression of the two receptors (IGF-IR and IGF-IIR) to be able to evaluate the proportion of cores showing receptor expression.

**Statistical analyses.** All of the statistical analyses were performed using Statistica software (Statsoft, Tulsa, OK, USA). Comparisons between two independent groups of numerical data were performed using the non-parametric Mann–Whitney test. The association between two binary variables was assessed using the Exact Fisher test. Univariate survival analyses were performed

| Table 1. Clinical data for 218 patients | |
|---|---|
| **Age (years)** | |
| Median (range) | 64 (21–81) |
| **Gender** | |
| Female/male | 97/121 |
| **Multifocality** | |
| No | 162 |
| Yes | 45 |
| Missing | 11 |
| **Date of surgery** | |
| Median (range) | 1999 (1988–2006) |
| **Surgical resection** | |
| Complete | 63 |
| Partial | 140 |
| Missing | 15 |
| **Adjuvant therapy** | |
| No | 4 |
| Radiotherapy[a] | 142 |
| Standard protocol (dose (Gy)/number of fractions) (median (range)): 60 (55–66)/30 (28–40) | 81 |
| Incomplete protocol[b] (dose (Gy)/number of fractions) (median (range)): 32 (20–54)/10.5 (10–27) | 17 |
| Missing | 44 |
| Radiotherapy + temozolomide[a] | 26 |
| Radiotherapy | |
| Standard protocol (dose (Gy)/number of fractions) (median (range)): 60 (54–64)/30 (20–33) | 21 |
| Incomplete protocol[b] (dose (Gy)/number of fractions) (median (range)): 39.5 (39–40)/16.5 (13–20) | 2 |
| Missing | 3 |
| Temozolomide | |
| Concomitant (= every day during radiotherapy) | |
| Dose (mg m$^{-2}$ day$^{-1}$): 75 | 12 |
| Missing | 14 |
| Adjuvant | |
| Dose (mg m$^{-2}$ day$^{-1}$)/number of cycle (median (range)): 187.5 (100–200)/3 (1–9) | 15 |
| Missing | 11 |
| Others[c] | 11 |
| Missing | 35 |
| **Follow-up (months)** | |
| Median (range) | 8 (0–90) |
| **Death** | 77.1% |
| Median survival (months) | 10 |

The table displays the numbers (or percentage) of cases except when other features are indicated (such as median and range).
[a]Considered as standard therapies.
[b]For reasons such as clinical degradation of patients.
[c]Including non-standard therapies for GBM patients, such as chemotherapy alone or combined with radiotherapy or palliative management.

using the standard Kaplan–Meier analysis and the log-rank test (or its generalisation for $>2$ groups), except in cases of continuous variables, for which univariate Cox regression was used. We completed these analyses using multivariate Cox regression. Missing values were excluded from any analysis and $P$-values $<0.05$ were considered as being significant.

## RESULTS

**IGF-IR and IGF-IIR are overexpressed in GBMs compared with normal brain tissue.** Quantitative evaluations of the IGFI, IGFII, IGF-IR and IGF-IIR expression levels are shown in Figure 1, and the immunohistochemical stainings are illustrated in Figure 2.

The nine cases of normal adult brains (54 samples) presented negative expression (LI$<$1%) for each of the four investigated markers (Figures 1A–D), although we observed scattered staining in the cytoplasm of a few neurons, microglial or endothelial cells.

The majority of the GBMs showed no expression of IGFI (159 out of 212, i.e., 75%; Figure 2A) and/or IGFII (160 out of 204, i.e., 78%; Figure 2C). For the 53 cases characterised by positive expression of IGFI, the LI ranged between 1% and 35% (Figure 1A). Cytoplasmic IGFI staining was observed mainly in tumour cells and some endothelial cells (Figure 2B). IGFII expression was observed in 44 cases, with a LI ranged between 1% and 18% (Figure 1B). As was the case for the IGFI staining, cytoplasmic IGFII staining was detected in tumour cells (Figure 2D). These IGFII-positive tumour cells were often located in perinecrotic areas, that is, expressed by neoplastic cells just beside necrotic areas. In contrast to the IGFI staining results, we did not observe IGFII expression in endothelial cells. Given the small number of GBMs with positive staining for IGFI and/or IGFII, the IGFI and IGFII expression levels in the GBM tissues were not statistically different from those of normal brain tissue (Figures 1A and B).

Whereas a large majority of the GBM cases were IGFI- and IGFII-negative, 64% (139 out of 218) of them showed IGF-IR expression, with a LI ranged between 1% and 73% (Figure 1C). IGF-IR staining was detected in the cytoplasm of the tumour cells and membranous staining was observed in a few of them, whereas the endothelial cells were negative (Figure 2F). Cytoplasmic IGF-IIR staining was also observed in tumour cells (Figure 2H) in 51% (109 out of 213) of the GBMs, with a LI ranged between 1% and 43% (Figure 1D). Interestingly, we also observed cytoplasmic dot-like staining in endothelial cells. This staining pattern was

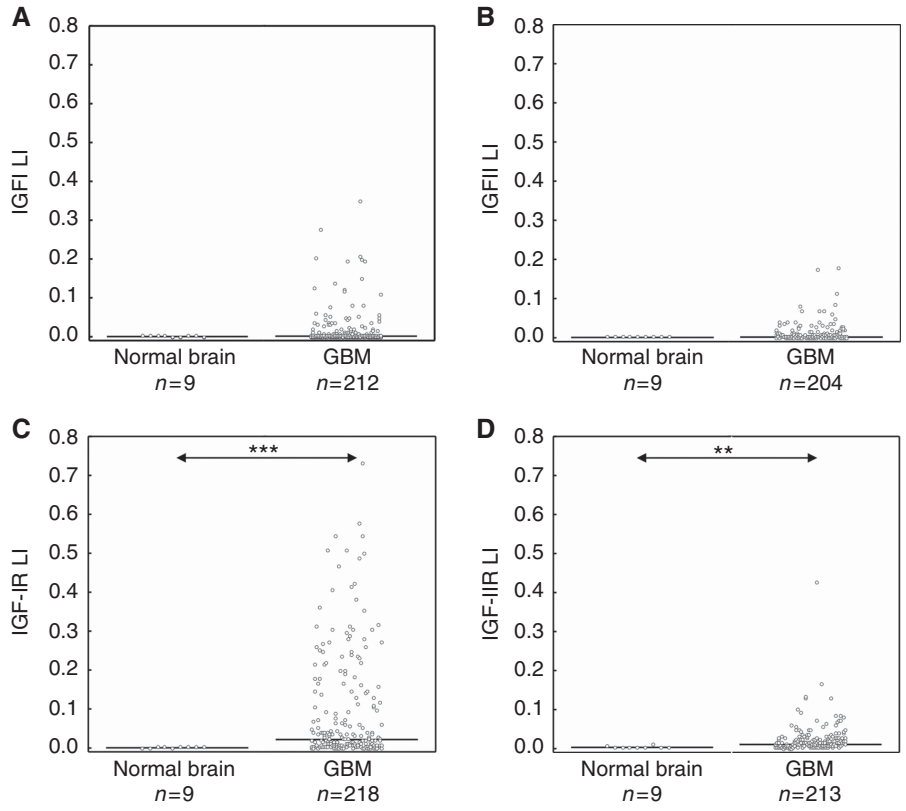


Figure 1. Quantitative evaluation of the tissue area exhibiting IGFI (**A**), IGFII (**B**), IGF-IR (**C**) or IGF-IIR (**D**) immunopositivity (LI, labelling index) in normal brain and glioblastoma samples. Each dot shows the value associated with one case. The horizontal line corresponds to the median. Only the significant differences are indicated as $^{**}P<0.01$ and $^{***}P<0.001$.

detected mainly in tumour microvessels exhibiting endothelial proliferation (Figure 2G). The IGF-IR and IGF-IIR expression levels in the GBMs were significantly greater than those of normal brain tissue ($P<10^{-4}$ and $P=0.002$, respectively; Figures 1C and D). Concerning the colocation of ligand and receptor expression in GBM samples, among the cores showing IGFI expression and analysable for receptor expression ($n=76$), we observed 82% IGF-IR-positive cores (i.e., 62 out of 76), 59% IGF-IIR-positive cores (i.e., 45 out of 76) and 51% cores positive for both receptors (39 out of 76). IGFII expression was detected in 63 cores where both receptor expression levels were analysable. Of them, 51% (i.e., 32 out of 63) exhibited IGF-IR expression, 65% (41 out of 63) IGF-IIR expression and 33% (21 out of 63) the expression of both.

**IGF-IR is a prognostic marker.** First, we analysed the impact of the clinical factors (listed in Table 1) on the cancer-specific survival by means of univariate analyses (Table 2). As expected, older age was associated with a reduced median survival ($P=0.0004$); macroscopically complete resection (based on radiology reports of first postoperative imaging) significantly improved the median survival of the patients (from 8.4 to 13.1 months; $P=0.002$), as did the addition of TMZ to radiotherapy (from 10.6 to 14.9 months; $P=10^{-5}$). No association was found between the quantitative immunostaining evaluation of the expression of IGFI, IGFII or IGF-IIR and the patient outcomes. In contrast, IGF-IR LI was negatively associated with cancer-specific survival ($P=0.046$). We also evaluated the prognostic impact of these four markers after binarising the data (negative/positive, as described in Materials and Methods). Similar to the results of the quantitative immunostaining evaluation, only positive expression of IGF-IR was associated with significantly reduced survival, as shown in Figure 3A ($P=0.02$). Interestingly, when the IGF-IR expression was categorised into three groups (i.e., negative, weakly positive and strongly positive), the median survival of patients with strong expression of IGF-IR was observed to be dramatically reduced (4.5 months) compared with that of the GBM IGF-IR-negative patients (11.6 months) (three-group comparison $P=0.01$; negative *vs* strongly positive $P=0.007$; Figure 3B). A multivariate Cox regression analysis was then performed to test the prognostic contribution of IGF-IR expression in the presence of the prognostic clinical factors, that is, those for which the univariate results were significant (see Table 2). This model was established using 167 cases (excluding cases with missing values and the non-standard treatment category, see Table 1). We previously verified that the univariate results shown in Table 2 remain valid with this reduced series (except that the quantitative IGF-IR LI variable slightly lost in significance with $P=0.057$), without impacting the selection of variables introduced in the Cox model. As detailed in Table 3, IGF-IR-positive staining ($P=0.016$) as well as older age ($P=0.003$), macroscopically partial resection ($P=0.039$) and radiotherapy alone ($P=0.003$) were independent prognostic factors associated with shorter survival.

**IGF-IR expression modulates the response to adjuvant treatment.** To examine whether IGF-IR expression correlates with the response to adjuvant treatment, we evaluated the efficacy of adding TMZ to radiotherapy in two distinct groups of GBM patients (IGF-IR-negative, i.e., LI<1% and IGF-IR-positive, i.e., LI⩾1%). As shown in Figures 3C and D, the addition of TMZ to radiotherapy significantly improved the survival of GBM patients compared with that of patients receiving only radiotherapy in both groups (IGF-IR-negative: $n=61$, $P=0.002$; IGF-IR positive: $n=107$, $P=0.007$). However, the benefit of TMZ seems more important in the IGF-IR-negative group (Figure 3C). Indeed, this latter group showed a mortality risk reduction of 83% associated with the addition of TMZ (hazard ratio of 0.17), whereas the

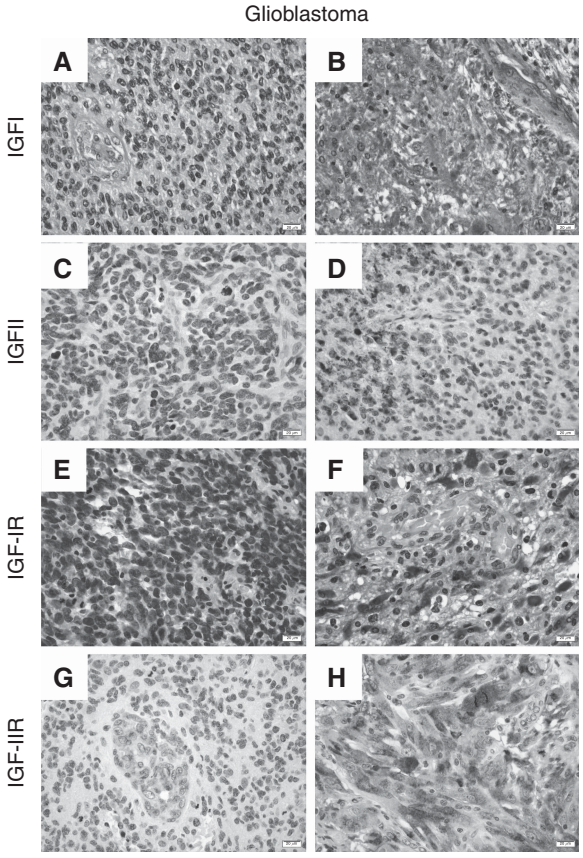


Figure 2. Immunohistochemical expression levels of IGFI (**A** and **B**), IGFII (**C** and **D**), IGF-IR (**E** and **F**) and IGF-IIR (**G** and **H**) in glioblastomas. Original magnification $\times 400$, scale bars $= 20\,\mu$m.

reduction was less (60%) in the IGF-IR-positive group (hazard ratio of 0.40). These data suggested that IGF-IR expression in GBMs might be associated with chemoresistance to TMZ.

**Table 2. Univariate survival analyses**

| | Median cancer-specific survival (months) | *P*-value |
|---|---|---|
| Age (years)* ($n = 218$) | | 0.0004 |
| Multifocality ($n = 207$) | | NS |
| No | 7.9 | |
| Yes | 10.6 | |
| Date of surgery* ($n = 218$) | | NS |
| Surgical resection ($n = 203$) | | 0.002 |
| Partial | 8.4 | |
| Complete | 13.1 | |
| Adjuvant therapy ($n = 168$) | | $10^{-5}$ |
| Radiotherapy | 10.6 | |
| Radiotherapy + temozolomide | 14.9 | |
| IGFI LI* ($n = 212$) | | NS |
| IGFII LI* ($n = 204$) | | NS |
| IGF-IR LI* ($n = 218$) | | 0.046 |
| IGF-IIR LI* ($n = 213$) | | NS |
| **Binary scores** | | |
| IGFI ($n = 212$) | | NS |
| Positive | 8.7 | |
| Negative | 10.5 | |
| IGFII ($n = 204$) | | NS |
| Positive | 8.8 | |
| Negative | 9.3 | |
| IGF-IR ($n = 218$) | | 0.020 |
| Positive | 9.0 | |
| Negative | 11.6 | |
| IGF-IIR ($n = 213$) | | NS |
| Positive | 10.3 | |
| Negative | 9.2 | |

Abbreviations: IGF = insulin-like growth factor; LI = labelling index; NS = not significant. Continuous variables were analysed using the univariate Cox regression (see asterisk (*)). The other binary variables were analysed using the log-rank test. For these latter variables, each category is characterised by the median cancer-specific survival time (in months). For IGFI, IGFII, IGF-IR and IGF-IIR, the cases labelled as positive correspond to LI $\geqslant$ 1% and those labelled as negative to LI $<$ 1%. The *n* values indicate the total number of cases taken into account in the univariate analyses (excluding the missing values and certain non-standard clinical categories that are detailed in Table 1).

## DISCUSSION

Abundant data from cell cultures, animal models and human epidemiological studies suggest that the IGF system is implicated in the development of malignancies, including GBM (Pollak, 2004; Lonn *et al*, 2008). However, data on the expression of the members of the IGF system in GBM are limited and conflicting.

In the current study, we examined the expression of IGFI, IGFII, IGF-IR and IGF-IIR in the normal brain and in a large series of GBMs and correlated the results with clinical data. We detected IGFI expression in 25% of GBMs. This result is not consistent with those of previous studies (Antoniades *et al*, 1992; Sandberg-Nordqvist *et al*, 1993; Hirano *et al*, 1999). The discrepancy could be due essentially to the small number of immunohistochemically analysed cases in the other studies, that is, between 2 and 17 GBM samples, making the estimation of the proportions of positive cases less accurate. In contrast, we analysed 212 cases, that is, a series which better covers the known heterogeneity of GBMs and makes our estimation more accurate. Moreover, different primary antibodies were used across the different studies.

In the present work, while approximately 20% of the GBM cases were positive for the IGF ligands (IGFI and/or IGFII), most of them expressed the IGF receptors. IGF-IR and IGF-IIR staining was detected in the cytoplasm of the tumour cells. Although it would have been preferable to compare expression in the normal brain and GBM from the same patients, we noticed that IGF-IR and IGF-IIR are overexpressed in GBM compared with normal brain tissue. Interestingly, we observed cytoplasmic dot-like staining of IGF-IIR in endothelial cells, particularly in tumour microvessels exhibiting endothelial proliferation. This pattern of expression suggests that IGF-IIR could be involved in angiogenesis. This hypothesis, which is supported by several *in vitro* studies indicating a pro-angiogenic effect of IGF-IIR through interactions with G proteins (Groskopf *et al*, 1997; Herr *et al*, 2003; Maeng *et al*, 2009), will be investigated in future work.

With regard to the colocation of ligand and receptor expression in GBM samples, while the majority of IGFI-positive cores was IGF-IR positive, IGFII was more often located with IGF-IIR. This data can be related to the high affinity of IGF-IR for both IGFI and IGFII, whereas IGF-IIR binds IGFII with high affinity but IGFI with very low affinity (Denley *et al*, 2005; Sachdev and Yee, 2007). Moreover, it is interesting to note that many cores expressed both receptors and that most of the observed IGFII positivity was located in perinecrotic areas, consistent with reports that IGFII expression is upregulated by hypoxia (Feldser *et al*, 1999; Mohlin *et al*, 2013). Concerning the clinical impact, we observed that, among the different IGF members, only IGF-IR expression has a prognostic value, being negatively associated with cancer-specific survival. Various studies have evaluated the prognostic significance of IGF-IR expression in other cancers. Although conflicting data were reported concerning breast (Railo *et al*, 1994; Fu *et al*, 2011; Hartog *et al*, 2011) and lung (Ludovini *et al*, 2009;

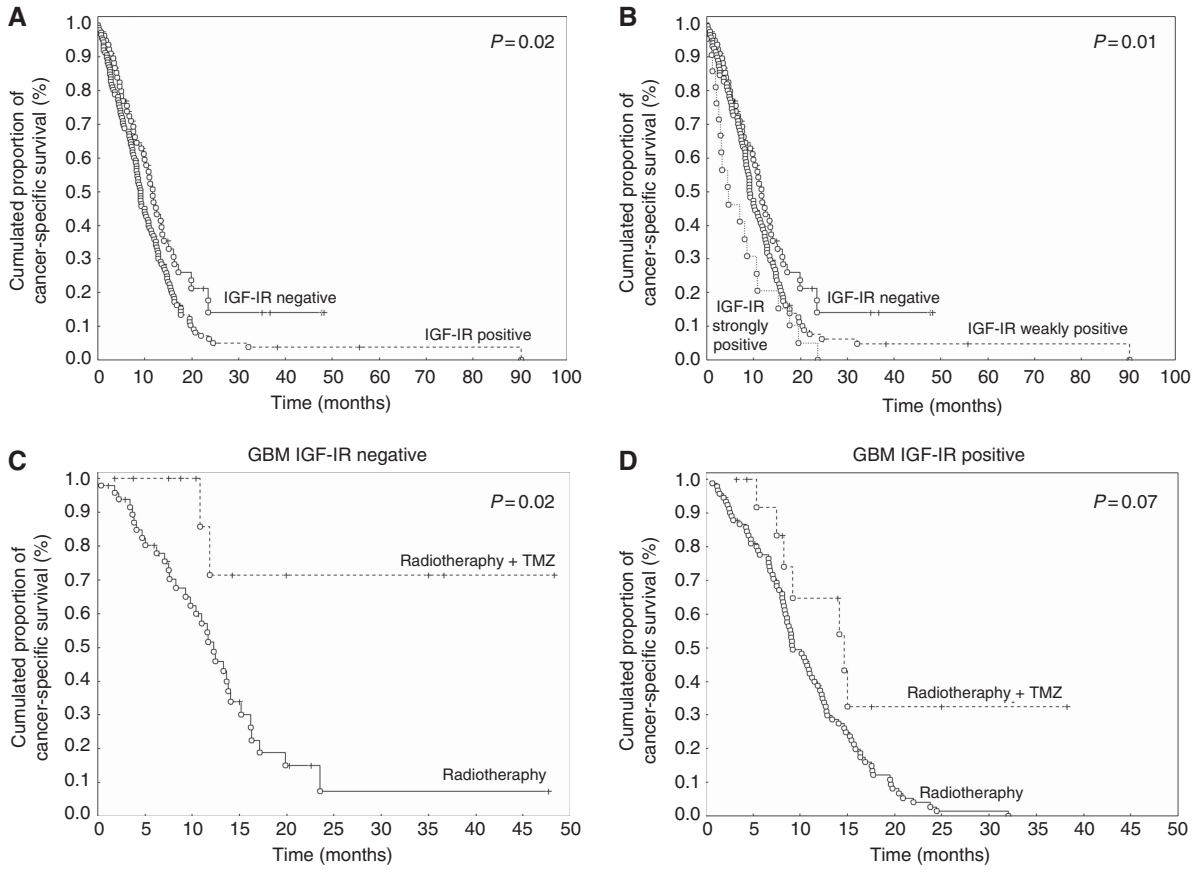


Figure 3. Kaplan–Meier survival curves of GBM patients according to the IGF-IR expression categorised as (**A**) negative (i.e., LI < 1%) or positive (i.e., LI ⩾ 1%); (**B**) negative (i.e., LI < 1%), weakly positive (i.e., 1% ⩽ LI < 30%) or strongly positive (i.e., LI ⩾ 30%), and Kaplan–Meier survival curves of GBM patients according to the adjuvant treatment in IGF-IR-negative (i.e., LI < 1%) (**C**) or IGF-IR-positive (i.e., LI ⩾ 1%) (**D**) cases. Each dot symbolises a death due to cancer and each cross indicates a survivor or a death not related to cancer (censured data).

**Table 3. Cox regression model ($n = 167$)**

| Model/*P*-value | Prognostic factors | Hazard ratio | 95% CI | *P*-value |
|---|---|---|---|---|
| $< 10^{-5}$ | Age | 1.02 | 1.01–1.04 | 0.003 |
| | Complete resection | 0.67 | 0.45–0.98 | 0.039 |
| | Radiotherapy + temozolomide | 0.35 | 0.17–0.70 | 0.003 |
| | IGF-IR positive | 1.65 | 1.10–2.47 | 0.016 |

Abbreviations: CI = confidence interval; IGF = insulin-like growth factor. The 'Model/*P*-value' indicates the overall level of significance of the multivariate model. With the exception of 'Age', which is a quantitative variable, all of the others are binary. Resection distinguishes between partial and complete, adjuvant treatment between radiotherapy and radiotherapy + temozolomide and IGF-IR between positive (LI ⩾ 1%) and negative (LI < 1%). The individual *P*-values represent the levels of significance of the independent contributions of each factor.

Cappuzzo *et al*, 2010) cancers, this biomarker is associated with a poor outcome in patients with oesophageal, gastric, oral or cervical carcinomas (Imsumran *et al*, 2007; Matsubara *et al*, 2008; Kalinina *et al*, 2010; Henriquez-Hernandez *et al*, 2011; Lara *et al*, 2011). So far, only one study has evaluated the prognostic impact of IGF-IR expression in GBM: using the Repository of Molecular Brain Neoplasia Data (REMBRANDT) of the National Cancer Institute, authors showed that GBM patients with upregulation of IGF-IR at the gene level carry a significantly worse prognosis than patients with relative downregulation of IGF-IR. They confirmed this inverse correlation between IGF-IR gene expression levels and survival at the protein level using a TMA of GBM samples (Zamykal *et al*, 2014). Our study confirms these data on a larger series of GBM. Furthermore, in our multivariate Cox regression, IGF-IR positivity was identified as an independent prognostic factor.

Currently, the standard treatment for GBM consists of maximal surgical resection, radiotherapy and concomitant and adjuvant TMZ chemotherapy (Stupp *et al*, 2009). TMZ is an alkylating agent that induces the formation of $O^6$-methylguanine in DNA, which mispairs with thymidine during the following cycle of DNA replication, leading to the activation of apoptotic pathways (Darkes *et al*, 2002). Other mechanisms of action have also been described such as the induction of G2-M arrest or autophagy (Hirose *et al*, 2001; Kanzawa *et al*, 2004). Although the improvement in median survival caused by the addition of TMZ to radiotherapy is significant, it remains modest (Stupp *et al*, 2009). Indeed, there are inherent and acquired resistances conferred by multiple mechanisms (Zhang *et al*, 2012) such as the lack of expression of the DNA repair enzyme $O^6$-guanine-DNA-methyl transferase, deficiencies in DNA mismatch repair and initiation of the base excision repair

system (Johannessen and Bjerkvig, 2012). In this context of resistance to TMZ, our study suggests that IGF-IR expression in GBM could be correlated with the response to adjuvant treatment. Nevertheless, it should be noted that these results might be interpreted with caution because of the small patients number in this subgroup analysis. Anyway other therapeutic modalities are needed.

IGF-IR is considered as a potential therapeutic target in cancer (Hewish et al, 2009). As reviewed by Trojan *et al* in 2007, multiple investigations targeting IGF-IR in GBM demonstrated antineoplastic activity in *in vitro* and *in vivo* models (Trojan *et al*, 2007). In the *in vivo* models, downregulation of IGF-IR using an antisense strategy (Resnicoff *et al*, 1994), triple-helix strategy (Rininsland *et al*, 1997), inhibitors such as picropodophyllin (Yin *et al*, 2010) or a dominant-negative mutant (D'Ambrosio *et al*, 1996) resulted in inhibition of tumour growth. Inhibition of IGF-IR causes apoptosis of tumour cells, inhibition of tumorigenesis and an immune antitumour response. All of these data motivated the first clinical trial involving the use of an antisense IGF-IR strategy for 12 patients with recurrent GBM or anaplastic astrocytoma (Andrews *et al*, 2001). This treatment was associated with a rather high rate of clinical and radiological improvement with two complete responses and four partial responses achieved. More recently, Zamykal *et al* (2014) investigated the effect of the IGF-IR blocking antibody IMC-A12 on *in vivo* GBM growth. They confirmed that IGF-IR may be an interesting therapeutic target in GBM.

Currently, there is a phase I/IIa study to investigate the safety, tolerability and antitumour efficacy of AXL1717 (picropodophyllin as an active agent formulated in an oral suspension) in patients with recurrent malignant astrocytomas (www.clinicaltrials.gov). Furthermore, studies in other tumour types have demonstrated that NVP-AEW541, a pyrrolo [2,3-d]pyrimidine derivative small molecular weight kinase inhibitor of the IGF-IR (with a high selectivity: $IC50 = 0.086\,\mu M$) (Garcia-Echeverria *et al*, 2004), produces synergistic growth inhibition when combined with other chemotherapeutic agents (Gotlieb *et al*, 2006; Mukohara *et al*, 2009).

Literature data provide clear evidence that GBMs constitute a heterogeneous group of tumours. In 2006, Philipps *et al* used gene expression to divide GBMs into 3 groups (i.e., proneural, proliferative and mesenchymal), which are associated with different prognoses (Phillips *et al*, 2006). In 2007, Soroceanu *et al* showed that IGFII is overexpressed in the proliferative group, which is characterised by a poor survival (Soroceanu *et al*, 2007). Verhaak *et al* (2010) proposed classifying GBMs into four groups (i.e., classical, mesenchymal, proneural and neural) based on genomic abnormalities such as IDH1 mutation, EGFR amplification, p53 mutation, NF1 deletion or mutation and PDGFRA amplification. These subtypes were associated with different responses to therapy. A recent study conducted in our laboratory defined a simplified classification based on immunohistochemistry. With this method, we identified two clinically relevant subtypes of GBM: the 'Classical-like subtype' (CL) characterised by EGFR-positive, p53-negative and PDGFRA-negative staining and the 'Proneural-like subtype' (PNL) characterised by p53- and/or PDGFRA-positive staining. The addition of TMZ to radiotherapy significantly improved the survival of patients with GBMs of the CL subtype but did not affect the survival of patients with GBMs of the PNL subtype (Le Mercier *et al*, 2012). Because 70 patients were common between the previous study and the present work, we evaluated whether IGF-IR expression is related to this recent classification system. Interestingly, the proportion of IGF-IR-positive cases was significantly higher in the PNL subtype (for which the addition of TMZ was evidenced as being ineffective), compared with the CL subtype (PNL: 31 out of 44, i.e., 70% *vs* CL: 12 out of 26, i.e., 46%; $P = 0.04$).

In conclusion, IGF-IR is overexpressed in the majority of GBMs compared with the normal brain. With regard to standard clinical factors, this overexpression is associated with an independent prognostic value in terms of cancer-specific survival and a less favourable response to TMZ. Our data suggest that IGF-IR could be an interesting target for GBM therapy. Additional studies are, however, needed to investigate the role of IGF-IR in the chemoresistance of GBMs and to determine which patients could benefit from combination therapy with TMZ and an IGF-IR inhibitor.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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