Low HER2-expressing glioblastomas are more often secondary to anaplastic transformation of low-grade glioma

Jean-François Mineo · Anne Bordron · Marc Baroncini · Claude-Alain Maurage · Carole Ramirez · Rose-Mary Siminski · Christian Berthou · Phong Dam Hieu

Abstract

Background Anti-Human Epithelial Receptor Type 2 (HER2) antibodies have the ability to induce in vitro apoptosis of glioblastoma (GBM) cells. This study was designed to evaluate the variability of HER2 expression in GBM and its role as a possible prognosis factor.

Methods Data of 57 patients with GBM and 16 patients with grade III gliomas were retrospectively analyzed. The expression of HER2 was determined by immunohistochemistry and intensity was noted from 0+ to 3+. We compared the HER2 expression in de novo GBM and in GBM resulting from anaplastic transformation of low-grade glioma ("secondary GBM"). Statistical analysis was performed using univariate analysis and the Kaplan–Meier method.

Findings All GBM expressing highly HER2 (2+ and 3+) were de novo GBM. All secondary GBM expressed HER2 with low intensity (0+ and 1+). Survival time was significantly longer when HER2 expression was low (Log Rank test \( P = 0.04 \)). The patterns of HER2 expression were similar between grade III gliomas and secondary GBM.

Conclusions To our best knowledge, our study showed for the first time a significant association between HER2 expression and the type of GBM, with subsequent influence on survival rate. GBM with low-HER2 expression are more likely to be secondary GBM, carrying a better prognosis than de novo GBM.

Keywords Glioblastoma · De novo glioblastoma · Secondary glioblastoma · HER2 · Survival

Abbreviations

BBB Blood Brain barrier
GBM Glioblastoma
HER2 Human Epithelial Receptor Type 2
IHC Immunohistochemistry
TMA Tissue Micro array
WHO World Health Organization
MGMT O6-methyl guanine-DNA methyl transferase

Introduction

Glioblastoma multiforme (GBM) is characterized by the World Health Organization (WHO) as an astrocytic tumor with nuclear atypia, mitosis and necrosis [1]. GBM is the most common malignant tumor of the central nervous system in adults, representing 50% of all gliomas and 20% of all operated intracranial solid lesions. With a mean incidence of 3 per year per 100,000 persons [2–3], it ranks fourth among the causes of death due to cancer in the middle-aged population [4].
Despite advances in therapeutic management of GBM, prognosis remains poor and the median overall survival rate is <1 year. Even in the most favorable case (young patients treated with radical surgery, radiotherapy and chemotherapy), death occurs in most cases within 2 years [5, 6].

Several factors, both clinical (age, performance status) and therapeutic (quality of surgery, radiotherapy, chemotherapy) have been established as prognosis factors [7–9], as well as specific tumor characteristics such as location and nature (de novo or secondary to a low-grade glioma; methylation of the MGMT gene promotor). Precisely identifying new prognosis factors is of high importance in managing patients with GBM in order to plan and optimize individual and specific immunotherapy for each patient.

Human Epithelial Receptor Type 2 or (HER2), (also called c-erbB2) could represent one of these new prognosis factors. The expression of tyrosine kinase receptors has been reported as having a prognostic value in GBM [10, 11] and other neoplasias such as medulloblastoma, breast and lung cancers [12–14]. Epidermal Growth Factor Receptor (EGFR) (also called c-erbB1) and HER2 are the most frequently expressed tyrosine kinase receptors in GBM cells. Targeting the HER2 receptors with specific antibodies (Trastuzumab®) is currently a well-established therapy for breast cancers [15].

In GBM cell lines, anti-HER2 antibodies have been recently demonstrated as being able to induce in vitro apoptosis and cellular-dependent cytotoxicity in human GBM [16, 17]. Along the same lines, a tyrosine kinase family inhibitor has been shown to prevent intracranial growth of GBM in rats [18]. Before considering therapeutic trials in humans, the prevalence of HER2 expression in human GBM must be precisely evaluated. Potential correlation between HER2 receptor expression and survival rates should also be assessed.

This preliminary study was designed to evaluate the prevalence of HER2-receptor expression in human GBM and its possible role as a prognosis factor. We therefore compared HER2 expression with prognosis factors such as age, location, tumor type (de novo or secondary) and interval-free progression, already known to affect survival rate.

Inclusion criteria: patients over 16 years of age, having histologically confirmed GBM (WHO grading was used), with complete charts including pre-operative clinical evaluation, complete post-operative precise outcome and follow-up, and pre- and post-operative CT-scan and/or MRI. Clinical (e.g., age, performance status) and therapeutic data (type of surgery, radiotherapy, chemotherapy) as well as tumor characteristics (location, subtype) were studied. Tumors located in deep and/or eloquent areas that might have allowed only partial removal were qualified as “poor surgical locations” (corpus callosum, insulae, thalamus, basal ganglia, internal capsule, language areas, Rolandic scissure or multiple locations).

We strictly consider GBM as secondary to the anaplastic transformation of a low-grade glioma only when anterior histological sample demonstrating the presence of a low-grade-glioma was available. The expression of HER2 receptors in glioblastoma samples was determined via immunohistochemistry (IHC). To confirm our hypothesis of HER2 expression, we also analyzed 16 grade III gliomas via IHC (Table 1).

Tumor sections were obtained from frozen samples and assembled to form tissue micro array blocks (TMA). Three representative areas from each tumor were selected and stained using Haematoxylin & Eosin. Three cylinders of tumor tissue were included in the TMA blocks, each block containing 60 cylinders and having a diameter of 600 μm (Beecher instruments, Sun Prairie, WI, USA). Sections from the TMA blocks were processed (Ventana, Tucson, AZ, USA), and a solution containing anti-HER2 antibodies (Novocastra, clone CB11, final dilution 0.58 mg/l) was applied after heat incubation. As positive control, we used samples from patients who had undergone surgery for breast cancer in whom HER2 immunoreactivity had been previously established and graded before receiving a specific treatment. TMA blocks that had been processed without primary antibody were used as negative control.

Before sampling cylinders for the construction of the TMA blocks, unstained sample sections were prepared. Sections from ten patients and their TMA sections were simultaneously processed for immunohistochemistry. The HER2 immunoreactivity of these ten tumor samples was compared on hole-block immunostained sections and TMA sections: the pattern of immunoreactivity and its intensity were similar in all cases.

Human Epithelial Receptor Type 2 membranous density in tumor samples was evaluated via immunohistochemistry (Table 1 and Fig. 1). Immunostaining reflecting the expression of HER2 was evaluated as for breast cancer: 0 = no staining; 1+ = faint, incomplete membranous pattern; 2+ = moderate, complete membranous pattern; 3+ = strong membranous pattern [19]. We had previously

**Material and methods**

Data of all patients admitted to our academic neurosurgical departments between 2002 and 2003 with newly diagnosed GBM were retrospectively analyzed. Ninety-seven patients underwent surgery during this period, but only 57 patients were included in this study due to exclusionary factors, such as lack of frozen tumor samples and/or incomplete data (i.e., loss of patient contact or lack of complete imaging).
compared immunostaining with HER2 antibody to the density of membranar HER2 analyzed by flow cytometry [16].

Human Epithelial Receptor Type 2 expression intensity, clinical and therapeutic factors, and tumor characteristics were first analyzed using either an univariate Student t-test, $\chi^2$ classical, Yates’ chi-square test or Fisher exact test, depending on the number of patients. The factors that seemed to be determinant were subsequently evaluated by the Kaplan–Meier method [20] and by the Log-rank test. Statistical analysis was performed using SPSS® software.

**Results**

Results of HER2 expression are summarized in Table 1. HER2 was expressed in 82.5% of GBM patient samples. In more than half of these patients, the membranous density of HER2 expression was strong (2+ and 3+). The patterns of HER2 expression were associated with the nature of GBM. Low-HER2 expression was found in all patients having secondary GBM or grade III glioma. In the de novo GBM group, the HER2 expression was rather high, but without any significant specific pattern.

In GBM population, when HER2 expression was low (HER 0+ and 1+), survival rate was significantly better than when HER2 expression was high (Log-rank test $P = 0.04$) (Fig. 2). Mean and median survivals were longer in HER2 0+ and 1+ patients (17.8 and 12.5 months) than in HER2 2+ and 3+ patients (11.6 and 9.5 months). All surviving patients at the time of the study (four patients) were HER 1+. Mean survival in HER 3+ patients was only 6.25 months (four patients).

Glioblastoma were considered as de novo GBM excepted when patients have a low-grade glioma with secondary anaplastic transformation. For all these patients, a previous histological sample demonstrating the presence of a low-grade glioma was available. Survival time was calculated from the diagnosis of secondary GBM. In our study population, eight tumors (14%) were considered as

<table>
<thead>
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<th>Histology (OMS)</th>
<th>HER 0+</th>
<th>HER 1+</th>
<th>HER 2+</th>
<th>HER 3+</th>
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<tr>
<td>All glioblastomas (%)</td>
<td>17.5</td>
<td>31.5</td>
<td>43.8</td>
<td>7.2</td>
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<tr>
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<td>14</td>
<td>25</td>
<td>4</td>
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<td>0</td>
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<td>Astrocytoma III</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>Oligodendroglioma III</td>
<td>9</td>
<td>2</td>
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In the low-HER2 expression groups (0 or 1+), the GBM were more often secondary to a low-grade glioma than in the high HER2 expression groups (for HER2 0+ group $P < 0.01$; for HER2 0+ and 1+ group $P < 0.03$). Expression of HER 2 was lower in grade III gliomas than in de novo GBM ($P < 0.0001$). There was no statistical difference in HER2 expression when comparing grade III gliomas and secondary GBM.

![Fig. 1 Immunohistochemistry for HER2 intensity evaluation. Representative tumor samples were selected from Haematoxylin & Eosin stained sections. Three cylinders of tumor tissue were included in the tissue microarray blocks (diameter 600 μm). Anti-HER2 antibody was subsequently applied after heat incubation.](image)
secondary to the anaplastic transformation of low-grade glioma.

All secondary GBM expressed HER2 with low intensity (0+ or 1+). All GBM expressing HER2 with high intensity were de novo GBM. In our study, the mean survival was longer for secondary GBM (18.5 months) than for de novo GBM (13.5 months), but the difference in overall survival was insignificant (Table 2). Complete removal of the tumor was less frequent in cases of secondary GBM than in cases of de novo GBM (Table 2, χ² Yates’ chi-square test, P < 0.04).

Lastly, expression of HER2 was lower in grade III glioma than in de novo GBM (Table 2, χ² test, P < 0.01). Statistical analysis did not demonstrate any significant difference in HER2 expression when comparing grade III glioma and secondary GBM (Fisher exact test, P < 0.16: non-significant). HER2 expression was found to be similar between grade III gliomas and secondary GBM. No statistical relationship was established between HER2 expression and criteria that had been demonstrated to influence survival such age, tumor location, interval free-progression and performance status [4–7].

**Discussion**

We found that all secondary GBM expressed HER2 with low intensity and that all highly HER2-expressing GBM were de novo GBM. Prognosis in terms of survival rate was better in the group of low-HER2-expressing GBM. Low-HER2-expressing GBM are statistically more often secondary to the anaplastic transformation of a low-grade glioma than high HER2-expressing GBM. The prognosis of secondary GBM is more favorable than de novo GBM [3, 6, 21]. Subsequently, a low-HER2 expression could be considered as a positive prognosis factor.

Epidermal Growth Factor Receptor and HER2 are the most frequently expressed tyrosine kinase receptors in GBM [22]. The HER2 receptor is not expressed in the normal adult central nervous system [23], but appears in abnormal astrocytes. Its expression increases with the degree of anaplasia [24] becoming frequent in GBM [25]. Therefore, some authors speculate that observation of increased HER2-receptor protein expression could allow the distinction of malignant astrocytomas from low-grade astrocytomas [24].

Moreover, because HER2 seems to be specific to gliomas, immunotherapy with HER2 targeting could be considered as a new therapeutic approach, as targeting HER2 could partially modulate the activity of other tyrosine kinase receptors. The major action of HER2 results from heterodimerization of HER2 with other tyrosine kinase family activated receptors such as EGFR [26]. Ligand-receptor complexes that include HER2 appear to be more potent than other receptor complexes and have a higher

![Fig. 2 Overall survival and HER2 expression in glioblastoma. Kaplan-Meier survival curve demonstrated better survival for low-HER2 expression (0+ or 1+), Log-rank test: P = 0.04). Continuous line: HER2 intensity graded 0 or 1+; dotted line: HER2 intensity graded 2 or 3+. HER2 expression was evaluated by immunostaining (Table 1).](image-url)
ligand affinity, a lower rate of internalization and degradation, and higher tyrosine kinase activity [27].

New therapeutic strategies are undergoing study to assess their suitability for human trials: immunotherapy using anti-HER2 monoclonal antibodies has been reported to induce apoptosis and cellular-dependent cytotoxicity in GBM cell lines [16]. Normal Brain Blood Barrier (BBB) is not permeable to antibodies. However, intravitral fluorescence via microscopic approach demonstrated that glioma microvascularization produces abnormal BBB. The intravitral approach [28] showed an intra-tumoral homogenous extravasation of a 150-kDa protein corresponding to the weight of IgG. To overcome a. permeability of the BBB to antibodies, anti-Her2 antibodies could be directly injected into the CSF.

Upon our analysis of the literature regarding HER2 and GBM, we found a wide range of results reported for HER2 in vivo detection (10–90% of tumor biopsies). These differences can be explained by the modality of evaluation of HER2 expression. Some authors assessed HER2 expression using a quantitative mode, whereas others used a qualitative mode. Some authors classified a tumor as positive for HER2 upon detection of HER2, but without analysis of receptor density, thus reporting a positive tumor rate of over 80% [25, 29, 30]. Other authors considered only the 2+ and 3+ tumors as positive, thus reporting only a 15–30% prevalence of positive tumors [10, 11, 19]. In our study, we found that 43% of tumors could be classified as 2+ and only 7% as 3+. The variation in the percentage may express the fluctuation of HER2 expression in different GBM population samples (i.e., percentage of de novo GBM could be slightly higher in our study than in other studies). A part of the variation could also be explained by inter-individual variation in IHC intensity evaluation. Fluorescence In Situ Hybridization (FISH) or reverse transcriptase polymerase chain reduction (RT-PCR) are available methods for HER2 assessment; both are semi-quantitative methods and could be helpful to improve sensitivity of HER2 determination.

In this study, we found a more favorable prognosis in terms of survival rate in the group of low HER2-expressing GBM (HER2 value 0 or 1+; Fig. 2 Kaplan Meier curve). Our result must be confirmed by a larger study. Moreover, the prognosis value of HER2 over-expression has been suggested by previous reports [10, 11]. The prognosis value of HER2 over-expression in GBM was expected for two reasons:

First, for each cancer with an HER2 over-expression reported, the consequence was a negative prognosis value [12–15]. Second, an over expression of a tumor inductor gene (as HER2) is known to favor the growth ability of tumors [12–15].

Nevertheless, our study demonstrated for the first time a relationship between GBM subtype (secondary to a low-grade glioma or de novo) and the intensity of HER2 expression. It is well-established that the genetic alterations observed in de novo GBM and secondary GBM are different [4, 21, 32]. In low-grade gliomas and secondary GBM, the tumor suppressor TP53 gene is frequently mutated [33]. In de novo GBM, an amplification of the tumor’s gene inductor is frequently reported. One of the most frequent amplifications implicates the tyrosine kinase receptor family. EGFR, especially its variant III [34], and HER2 amplification are often reported [10, 19]. However, the relevance of HER2 expression intensity in determining the origin of the GBM had not previously been reported.

Here we showed that the chance of discovering a GBM secondary to a low-grade glioma was inversely proportional to the intensity of HER2 expression. Glioblastomas are most likely secondary GBM in the HER2 0+ (non-expressing) and HER2 1+ groups. No secondary GBM was found in HER2 2+ or 3+ groups. The HER2 expression of secondary GBM was always noted as 0 or 1+. Moreover, the percentage of non-expressing HER2 tumors is higher in secondary GBM than in de novo GBM.

To confirm the hypothesis that GBM nature was an important factor influencing HER2 expression, we measured the HER2 intensity via IHC in 16 grade III gliomas (Table 2). In all samples, only low expression of HER2 was found (HER2 0 or 1+). Infrequent over-expression in low-grade glioma has already been reported [35]. The intensity of HER2 expression between grade III gliomas and GBM was compared by χ² or Fisher tests. The grade III gliomas expressed HER2 with a lower intensity than de novo GBM, but no difference was found between grade III gliomas and secondary GBM. The patterns of HER2 expression in grade III gliomas, which will always degenerate into grade IV gliomas, confirm the existing relationship between HER2 expression and GBM origin.

In our study, HER2 expression for secondary GBM was always valued as 0+ or 1+. This relationship between the intensity of HER2 expression and tumor origin could be helpful for the pathologist in determining the subtype of GBM. In WHO classification, a HER2 2+ or 3+ GBM is more likely de novo rather than secondary GBM. The pathological examination is largely similar in both secondary and de novo GBM. However, the nature of GBM (secondary or de novo) is a widely accepted prognosis factor [21].

We found that complete removal of the tumor was less frequent in cases of secondary GBM than in cases of de novo GBM (Table 2). Moreover, total removal of GBM is a widely accepted prognosis factor influencing survival rate [7, 36]. For this reason, a shorter survival prognosis should be expected for this subgroup of patients having secondary GBM.
But here we report overall survival as being even longer in the secondary GBM group than in the de novo GBM group (Table 2) confirming results of a previous study performed in a larger population [6]. We assume that this habitual prevalence of longer survival is due to the different genetic pathways between de novo and secondary GBM. These genetic differences could induce different tumor kinetics and particularly a slower tumoral growth in the secondary GBM group. Genetic differences could also explain the relatively better prognosis in the secondary GBM group by means of a better response to both radiotherapy and chemotherapy. In our study as well as in the literature, we did not find evidence of differing efficacy of radio and/or chemotherapy relative to GBM type.

In our study, we found a relationship between the intensity of HER2 expression and tumor origin. For our group, one of major conclusion of this preliminary study is that HER2 determination is worthwhile when designing a prospective follow-up study including several molecular determinants as HER2, P53, EGFRvIII, PTEN, VEGF, O6-MGMT, and others. We hope that the current molecular determinants as HER2, P53, EGFRvIII, PTEN, VEGF, O6-MGMT, and others. We hope that the current determination of HER2, P53, PTEN, and EGFRvIII in a large study will confirm our current result.

Acknowledgments  The tissue arrayer is a gift of M. Copin “Ligue contre le Cancer,” Nord-Pas-de-Calais, France; We are grateful to Mrs Tracey Montagnon for her English editorial assistance.

References