Open Journal of Pathology, 2012, 2, 71-80
Published Online July 2012 (http://www.SciRP.org/journal/ojpathology)
http://dx.doi.org/10.4236/ojpathology.2012.23014

Prognostic Significance of EGFR Gene Amplification and Overexpression in Diffuse Astrocytomas—A Literature Study*

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Received May 7th, 2012; revised June 8th, 2012; accepted June 15th, 2012

ABSTRACT

Epidermal growth factor receptor (EGFR) has been shown to play an important role in the malignant progression of diffuse astrocytomas. Numerous studies have investigated the clinical significance of overexpression and amplification of the EGFR gene in these tumours with ambiguous results. The aim of this study was therefore to review the literature to get the current prognostic and diagnostic status. The PubMed database was used to search for papers in English in the time period 1987-2010 using the following keywords: astrocytoma, glioma, c-*erb*B1, EGFR, glioblastoma, HER1 and prognosis. Other inclusion criteria were: 1) studies with more than 50 patients; 2) patient age over 18 years; 3) tumour grading according to the latest World Health Organization (WHO) classification (2007) and 4) EGFR overexpression assessed by immunohistochemistry. A total of 47 publications were included and several reported positive correlations between both EGFR gene amplification and overexpression with histological malignancy grade and survival. The results are, however, encumbered with elements of uncertainty involving for instance methodological diversities and small number of patients. Larger studies with standardized procedures are necessary to fully clarify the clinical role of EGFR in human astrocytomas.

Keywords: Brain Tumours; Diagnosis; erbB; Glioblastomas; Gliomas; Immunohistochemistry; HER; Receptor; Review; Survival

1. Introduction

Diffuse astrocytomas constitute the largest group of primary malignant human intracranial tumours. They are classified by the World Health Organization (WHO) into three histological malignancy grades: diffuse astrocytomas grade II, grade III (anaplastic astrocytomas) and grade IV (glioblastoma (GBM)), based on histopathological features such as cellular atypia, mitotic activity, necrosis and microvascular proliferation. Actually, grade II-IV astrocytomas can be regarded as a continuum along an axis of increasing malignancy. They have a considerable propensity to recur after surgery due to their infiltrative growth and to progress over time to more malignant forms with worse prognosis. Despite multimodal therapies, including surgical resection, radiotherapy and chemotherapy, less than half of the patients survive more than a year [1]. In each histological group, however, there are considerable differences in survival, partly due to heterogeneous tumour tissue and suboptimal classification systems. The histopathological diagnosis of these tumours is challenging, and various immunohistological and molecular genetic approaches have been introduced to improve the diagnostic accuracy.

Activation of oncogenes and inactivation of tumour-suppressor genes have been shown to be influential on development and growth of human astrocytomas. In this context the epidermal growth factor receptor (EGFR) is central. It is linked to the malignant transformation of these tumours by gene amplification, overexpression, and mutated variants, where EGFRvIII is the most common [2]. EGFR is a 170-kDa transmembrane tyrosine kinase receptor expressed in a variety of normal and malignant cells regulating critical cellular processes. When activated, EGFR triggers a number of signalling cascades via the Rasmitogen-activated protein kinase pathway and the phosphatidyl-inositol 3 kinase-protein kinase B (Akt) pathway, and thus behaves as a potent oncogene [3].

Amplification of the EGFR gene is a common genetic

*Conflict of Interest: Declared none.
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event in high-grade astrocytomas and occurs in about half of GBMs. It leads to overexpression at both the mRNA and protein levels, however, overexpression without this genetic event occurs as well [4,5]. Approximately 50% of GBMs with EGFR gene amplification also express EGFRvIII [6,7]. This is a mutated variant with truncated extracellular ligand-binding domain and is thus constitutively activated [8].

Overexpression of EGFR has proven to be a negative prognostic marker for multiple cancer types such as breast [9], bladder [10], oesophagus [11], cervix [12] and ovary [13]. Further, in carcinomas of the lung and colon EGFR-mediated molecular targeted therapy has been introduced [14]. In malignant gliomas, however, the prognostic and diagnostic role of EGFR has to date provided divergent results.

The aim of this study was therefore to survey the literature to get an updated collective status of EGFR's role in human astrocytomas with regard to diagnostic and prognostic aspects as well as to discuss some relevant methodological factors.

2. Material and Methods

The PubMed database was used to search for publications using the following keywords: *glioma*, *astrocytoma*, *glioblastoma*, *c-erbB*1, *EGFR*, *HER*1 and *prognosis*. Other inclusion criteria were: 1) studies with more than 50 patients; 2) diffuse astrocytomas grade II, II, and IV according to the latest WHO classification scheme [1]; 3) EGFR overexpression (assessed by immunohistochemistry); 4) EGFR gene amplification; 5) articles written in English and published in the time period 1987-2010 and 6) adult patients (age > 18 years).

The following factors were recorded: number of patients, patient age, histological malignancy grade, pattern of EGFR expression (membranous and/or cytoplasmic), type of antibody (see **Table 1**: clone, monoclonal, polyclonal, epitope (external or internal EGFR domain)), EGFR gene dose and detection method (southern or slot blots, polymerase chain reactions, in situ hybridization), EGFR's clinical significance (relationship to prognosis (overall or progression free survival) or tumour grade), and statistical analysis applied (uni- or multivariate).

3. Results

A total number of 47 publications fulfilled the inclusion criteria, and the results are presented chronologically in **Table 2**. In **Figure 1** a typical EGFR immunostaining of a high-grade astrocytoma is shown.

Twenty-five enrolled publications that had analyzed EGFR gene amplification in human astrocytomas, displayed an approximate percentage range in grade II-IV tumours of 0% - 4%, 0% - 33%, and 34% - 64%, respectively. Six publications found that amplification correlated significantly to histological malignancy grade [15-20] and seven found amplification to be a significant prognostic factor on overall survival [7,15,16,21-25]. Some studies reported amplification to be associated with poorer prognosis in the younger and better prognosis in the elderly [15,21,25].

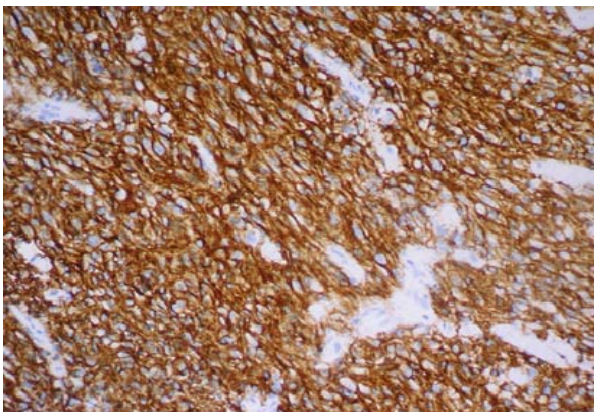

Figure. 1. Glioblastoma with strong immunoreactivity for EGFR (clone EGFR 113) (20× objective).

Overexpression of EGFR was examined in 28 publications. The percentage of astrocytomas overexpressing EGFR was in the range of 6% - 28%, 27% - 70%, and 22% - 89%, in grade II-IV tumours, respectively [4,7, 17,18,24,26-48]. Nine studies investigated the association between degree of expression and tumour grade, and such a constellation was demonstrated in only three studies [17,27,29]. Several publications showed prognostic significance on overall or progression free survival [7,24, 26,29,31,34-37,45-48].

Studies on EGFRvIII immunostaining revealed positivity only in high-grade astrocytomas with a frequency up to approximately 14% and 45% in anaplastic astrocytomas and GBMs, respectively [6,7,25,32,49]. In GBMs this expression was highly dependent on gene amplification, whereas it occurred approximately similarly in anaplastic astrocytomas with and without this genetic event [25]. Relations to survival showed that in anaplastic astrocytomas EGFRvIII was a significant predictor of unfavourable survival, especially with increasing patient age [6,25]. Among patients with GBMs, EGFRvIII positivity did not display any association with survival. In combination with EGFR gene amplification, however, other genetic subtypes, and patients surviving one year or more, it was a negative prognostic indicator [7,25,32,49].

4. Discussion

EGFR gene rearrangements and overexpression in human astrocytomas have been in the focus of scientists for

Table 1. Survey of antibodies used in the reviewed studies.

	Poly-/monoclonal	Intracellular domain	Extracellular domain	Publications
Clone E30	Monoclonal		X	[17,38,42,45]
Clone F4	Monoclonal	X		[35,48]
Clone 113	Monoclonal		X	[7,28,34]
Clone Ab-4	Polyclonal		X	[27,44]
Clone 528	Monoclonal		X	[25,26,32,37]
Clone 25	Monoclonal	X		[4,7]
Clone H11	Monoclonal		X	[43,45,46]
EGFR-Ab-10 (clone 111.6)	Monoclonal		X	[41]
Clone 31G7	Monoclonal		X	[30]
Clone 3C6	Monoclonal		X	[33]
mAb 425	Monoclonal		X	[18]

Table 2. Survey of findings in the reviewed publications.

Publications	No. of cases	EGFR analysis	Statistical analysis	Findings and comment
[50]	54	Amplification	UVA MVA	No prognostic significance on overall survival. No significant correlation between amplification and histological malignancy grade.
[39]	88	Overexpression	NS	No prognostic significance on overall survival.
[42]	72	Overexpression	UVA	No prognostic significance on overall survival. No significant correlation between overexpression and histological malignancy grade.
[18]	75	Amplification and Overexpression	UVA	No prognostic significance on progression free survival Significant correlation between amplification and histological malignancy grade.
[17]	Exp.: 115 Amp.: 97	Amplification and overexpression	UVA MVA	No prognostic significance on overall survival. Significant correlation between amplification/ overexpression and histological malignancy grade.
[48]	71	Overexpression	UVA MVA	Significant negative prognostic factor on overall survival and progression free survival in UVA and MVA with EGFR as a continuous variable. No significant correlation between overexpression and histological malignancy grade.
[34]	51	Overexpression	UVA MVA	Significant negative prognosis on progression free survival. No significant correlation between overexpression and histological malignancy grade.
[27]	107	Overexpression	UVA	No prognostic significance on overall survival. Significant correlation between overexpression and histological malignancy grade.
[28]	63	Overexpression	UVA MVA	No prognostic significance on progression free survival and overall survival.
[31]	79	Overexpression	MVA	Significant predictor of shorter overall survival. Trend towards correlation between overexpression and histological malignancy grade.
[51]	143	Amplification	UVA MVA	No prognostic significance on overall survival. No significant correlation between amplification and histological malignancy grade.
[54]	75	Amplification	UVA	No prognostic significance on overall survival.
[38]	80	Overexpression	UVA	No prognostic significance on overall survival.
[19]	93	Amplification	UVA MVA	No prognostic significance on overall survival. Significant correlation between amplification and histological malignancy grade.

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[36]	168	Overexpression	UVA MVA	Statistical significant decreased overall survival.
[29]	81	Overexpression	UVA MVA	Statistical significant reduced overall survival. Significant correlation between overexpression and histological malignancy grade.
[26]	110	Overexpression	UVA MVA	Significant improved prognosis for patients ≥ 55 years, trend toward worse overall survival for patients < 55 years in UVA. Significant negative prognostic factor for p53wild-type-negative cases < 55 years.
[15]	174	Amplification	UVA MVA	Trend towards shorter overall survival for AA in UVA. Significant better prognosis when age ≥ 60 in MVA. Significant correlation between amplification and histological malignancy grade.
[35]	302	Overexpression	UVA MVA	Significant negative prognostic factor on overall survival.
[37]	59	Overexpression	UVA MVA	Significant shorter overall survival in overall population and high-grade astrocytomas in UVA. No significant correlation between overexpression and histological malignancy grade.
[53]	129	Amplification	UVA MVA	No prognostic significance on overall survival.
[7]	87	Amplification, overexpression and EGFRvIII expression	UVA MVA	Significant unfavorable predictor on overall survival for amplification in UVA/MVA, overexpression in UVA. EGFRvIII showed a trend towards shorter overall survival in UVA and significant shorter survival for EGFRvIII with amplification in MVA.
[43]	143	Overexpression	UVA MVA	No prognostic significance on overall survival.
[44]	95	Overexpression	UVA MVA	No prognostic significance on overall survival.
[20]	87	Amplification	UVA MVA	No prognostic significance on overall survival. Significant correlation between amplification and tumor grade.
[21]	133	Amplification	UVA MVA	Significant negative prognostic factor on overall survival when patient < 46 years. Significant positive prognostic marker on overall survival when patient > 46 years.
[25]	168	Amplification and EGFRvIII expression	UVA MVA	AA: amp.: significant negative prognostic factor on overall survival in UVA. AA: EGFRvIII: Significant negative prognostic factor on overall survival in UVA. No prognostic significance on overall survival with amp or EGFRvIII for GBM.
[55]	715	Amplification	UVA MVA	No prognostic significance on overall survival.
[41]	58	Overexpression	UVA MVA	No prognostic significance on progression free survival.
[46]	103	Overexpression	UVA MVA	Significant negative prognostic factor on overall survival for AII. No significant correlation between overexpression and histological malignancy grade.
[30]	155	Overexpression	UVA MVA	No prognostic significance on overall and progression free survival.
[32]	196	Overexpression and EGFR vIII expression	UVA MVA	No prognostic significance on overall survival. EGFRvIII: significant negative prognostic factor for patients who survive ≥ 1 year.
[22]	189	Amplification	UVA MVA	Significant unfavorable prognostic factor on overall survival in UVA.

Continued

[6]	221	Amplification and EGFR vIII expression	UVA MVA	Trend towards decreased postoperative survival with amp or EGFRvIII for AA in UVA.
[56]	107	Amplification	UVA MVA	No prognostic significance on overall survival.
[23]	220	Amplification	UVA MVA	Significant positive prognostic factor on overall survival in MVA (adjusted for age).
[16]	338	Amplification	UVA MVA	Significant worse overall survival for AA in UVA. Significant correlation between amplification and histological malignancy grade.
[47]	595	Amplification and overexpression	UVA MVA	AA: exp.: Significant negative prognostic factor on overall survival in UVA. AA: amp.: Trend towards negative prognostic factor on overall survival in UVA. GBM exp.: trend to positive prognostic factor on overall survival in UVA and MVA.
[49]	649	EGFRvIII expression	UVA MVA	EGFRvIII negative GBMs together with genetic changes implied poorer overall survival.
[40]	51	Overexpression	MVA	No prognostic significance on overall survival.
[33]	99	Overexpression	UVA	No prognostic significance on postoperative survival.
[24]	194	Amplification and overexpression	UVA MVA	Significant poorer overall survival in UVA when EGFR were amplified/overexpressed and p53-immunopositive.
[45]	54	Overexpression	UVA MVA	Significant negative prognostic factor on overall survival in MVA.
[57]	301	Amplification	UVA MVA	No prognostic significance on overall or progression free survival.
[52]	112	Amplification	UVA MVA	No prognostic significance on overall or progression free survival.
[4]	99	Amplification and overexpression	UVA MVA	Amplification showed a trend towards worse overall survival in MVA, when analysis was done with FISH.
[71]	149	Amplification	MVA	No prognostic significance on overall survival.

Abbreviations: UVA, univariate analysis; MVA, multivariate analysis; Amp., EGFR amplification; Exp., EGFR overexpression; GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; AII, grade II astrocytoma.

several years because of the receptor's pivotal role in gliomagenesis, and, accordingly, for potential use in prognostic, diagnostic and therapeutic aspects. Still, the clinical significance is controversial. We therefore decided to survey relevant literature to get an overview of the current status of EGFR's clinical role as well as to discuss some sources of errors. In general, frequent overexpression and amplification of the EGFR gene occur predominantly in high-grade astrocytomas, whereas the clinical aspects still appear unclarified.

Studies have shown that the frequency of EGFR gene amplification increases significantly with increasing tumour grade. In grade II astrocytomas amplification is a rare event and was only found in one study with a frequency of 4% [16]. Up to one third of anaplastic astrocytomas were shown to harbour an amplified EGFR gene [6,15-17,19,25,50,51], whereas in GBMs it was encountered in about 60% of the cases [4,6,7,15-19,21-25,47, 50-57]. These results highlight the significance of amplification as a central process in the molecular pathway not only for primary GBMs but for secondary GBMs as well. The high frequency of EGFR amplification in GBMs can be used in the distinction between small cell GBMs and anaplastic oligodendrogliomas [58].

EGFR gene amplification was detected by different methods with varying sensitivity, thus providing possible variations in the frequency of this phenomenon. For instance, one study investigated the association between amplification determined by FISH and PCR, and found amplification in 47.4% and 63.6% of the tumour samples, respectively [4]. This clearly illustrates the need for standardization of such methods. Additionally, the well-known heterogeneity of glioblastoma tissue may be influential on tumour sampling and thus explain varying results.

Conflicting findings exist with regard to the association between prognosis and EGFR gene amplification. Intriguingly, it seems to be a positive relationship between the prognostic value of amplification and age. This is illustrated by the fact that older GBM patients with amplified gene have significantly increased survival compared with younger age groups [7,15,21-23,25]. Further, amplification has been found to be significantly more

frequent in GBMs in older patients than in younger ones [16,22,59]. These observations may reflect age-related different molecular pathways in gliomagenesis [21]. Another explanation could be that the astrocytomas with EGFR gene amplification in younger patients are a subgroup of astrocytomas with a more aggressive biology. Some studies also found that amplification conferred a negative influence on survival in patients with anaplastic astrocytomas [6,16,25,47]. This may indicate a subgroup with a more aggressive behaviour. One cannot, however, exclude that some of these tumours represent small-cell GBMs.

Regarding EGFR overexpression, all studies demonstrated an increasing frequency of overexpression with increasing tumour grade. Noteworthy is the use of different antibodies with immunoreactivity against either the external or the internal domain of the receptor. This makes comparison of studies difficult. We have for instance found that the latter unveiled higher number of EGFR immunoreactive astrocytomas. It is therefore possible that the external domain is more vulnerable to fixation or tissue processing than the internal one [60,61]. Since most studies used antibodies directed against the external domain, it may be that EGFR expression in astrocytomas is more abundant than expected. Actually, in previous studies using binding assays with biotinylated EGF on glioma tissue, we found that this technique was superior in detecting EGFR overexpression than antibodies [62]. Hence, due to methodological heterogeneity of determination of this receptor protein in tumour samples, there is a need for standardization. Promising is the introduction of EGFR immunohistochemical kits, such as the commercial kits for c-erbB2/HER2 testing in breast cancer.

Studies have shown that reactive astrocytes express EGFR, whereas normal astrocytes do not [62,63]. This is demonstrated by an upregulation of EGFR in astrocytes after brain injury with release of epidermal growth factor [64]. Thus, EGFR serves as a marker of astrocyte activation. For this reason EGFR immunoreactivity cannot be used uncritically to distinguish between reactive and neoplastic astrocytes. A recent study has, however, reported a stronger immunological EGFR reactivity in the latter [65].

Most studies have showed that overexpression of EGFR is a result of gene amplification [4,7,17,24,47]. There are, however, reports in which overexpression occurs in the absence of this genetic event [16,17,66]. In these cases, overexpression may be a result of changes in transcription, protein stability and/or protein modification unrelated to amplification.

As only 13 out of 28 studies found EGFR overexpression to be a significant prognostic factor, its role in this context is debatable. One study found overexpression to be significantly associated with better prognosis in older patients and a trend toward worse overall survival amongst the younger [26]. Another study showed a significant shorter overall survival for patients with overexpression and p53 mutations [24]. In respect to analysis using immunohistochemistry, it can be argued that overexpression used as a continuous variable, as opposed to dichotomous, can be more useful to predict outcome in EGFR positive patients [48].

The mutant EGFRvIII has gained much interest because of its selective expression on neoplastic astrocytes, and thus represents a promising goal in molecular targeted therapy [67,68]. It is detected immunohistochemically predominantly in anaplastic astrocytomas and GBMs, most frequent in the latter [6,7,25,32,49]. Its expression is tightly linked to EGFR gene amplification with some exceptions [7,25]. Furthermore, the antibodies used may also influence the results of EGFRvIII expression [61,69,70]. Concerning prognosis, the data appear somewhat complex due to additional genetic and clinical factors. For instance, its expression seems only to have prognostic value in patients with anaplastic astrocytomas, and especially with increasing patient age [6,25]. In GBMs EGFRvIII expression combined with molecular genetic findings, such as EGFR gene amplification, co-expression of PTEN (phosphatase and tensin homologue deleted in chromosome 10) tumour-suppressor protein and activation of the AKT/MAPK pathways and YKL-40, was shown to be associated with poorer prognosis [7,49, 68].

This review has no intention to pose as a meta-analysis or to declare the definite clinical role of EGFR in human astrocytomas, just to present an update of the current status. There are several limitations of such a study, including its retrospective issue, small series of patients and various detection systems making comparisons problematic. In any case, the literature is conflicting regarding this receptor protein as a biological marker, pointing to the fact that it does not behave as an independent marker in the gliomagenesis. Still, one cannot rule out EGFR as a promising and potential tool in various clinical aspects as far as astrocytomas are concerned. Larger studies are, however, necessary to predict the clinical role of EGFR in these tumours, and not least, to establish feasible, robust and reproducible detections systems for overexpression and amplification of the EGFR gene in astrocytic tumour tissue.

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