The Prognostic Impact of Epidermal Growth Factor Receptor (EGFR) in Patients with Acute Myeloid Leukaemia

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Abstract Expression of Epidermal Growth Factor Receptor (*EGFR*), an important proto-oncogene, regulates cell differentiation, proliferation, cell migration and survival in most of the cancer types. *EGFR* expression has been reported in Acute Myeloid Leukaemia (AML), however, many other reports nullified *EGFR* expression in AML. These contradictory data prompted us to reevaluate the expression of *EGFR* in AML and carry out a comparative survival analysis between *EGFR* expressing and non-expressing AML patients (Children and Acute Promyelocytic Leukaemia patients excluded). Bone marrow and/or peripheral blood samples were collected from 60 adult patients with AML with written informed consent. PCR, Real-Time Taqman gene expression assays were used for the detection of genetic alterations. Statistical analysis was conducted using SPSS software (IBM SPSS 20). In our study, *EGFR* expression was detected in 21 out of 60, in 35% (95% C.I. 23.45–48.48) AML patients. Overall survival was significantly shorter in patients with *EGFR* ($p = < 0.01$), with an average survival of 18.57 months (95% C.I. 12.42–24.73 months) compared with

31.27 months (95% C.I. 28.19–34.33 months) in patients without *EGFR*. *EGFR* expression was significantly higher in female patients compared to male ($p = 0.037$). This study confirms the presence of *EGFR* in AML and indicates that *EGFR* expression confers poor prognosis in AML. However, the underlying cause of this adverse prognostic effect has not been identified. Further clinical studies are warranted to determine the exact mechanism through which *EGFR* activity might contribute to AML progression and identify the potential therapeutic target for the reversal of resistance to conventional chemotherapeutics.

Keywords AML · *EGFR* expression · Real-time PCR · Prognostic impact

Introduction

Acute Myeloid Leukaemia (AML) is a genetically heterogeneous clonal disorder that results in disruption of self-renewal, proliferation, and differentiation. Molecular and cytogenetic studies have reported a number of genetic alterations in AML such as Runt-related transcription factor 1/*RUNX1* Partner Transcriptional Co-Repressor 1 (*RUNX1-RUNX1T1*), Core-Binding Factor Beta/Myosin Heavy Chain 11 (*CBFβ-MYH11*) and Fms-like Tyrosine Kinase 3 (*FLT3*), *TP53*, *IDH* mutations having varied prognostic impacts [1]. Expression of Epidermal Growth Factor Receptor (*EGFR*), an important proto-oncogene that regulates cell differentiation, proliferation, cell migration, and survival has been reported in AML. *EGFR* is a cell surface receptor belonging to the *ERBB* family of proteins. Binding of ligand (*EGF*) activates *EGFR* through dimerization leading to activation of other downstream pathways such as Ras/Raf/Mitogen-activated protein kinases (*Ras/*

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Raf/MAPK), phosphatidylinositol 3-kinase/*Akt* (*PI3K/Akt*) through signal transduction [2]. Mutations in *EGFR* lead to constitutive receptor activation. Overexpressed *EGFR* results in aberrant signaling that induces uncontrolled cell growth and oncogenesis [3]. The expression of *EGFR* is not uniform across all the cell types. It has been shown that *EGFR* is expressed in epithelial, mesenchymal and neuroectodermal cells but not in hematopoietic cells [4]. Recent studies have reported *EGFR* gene expression in AML and emphasized on the fact that *EGFR* inhibitors in combination with other potential drugs may prove to be useful against the haematological malignancy [5], while others have reported no *EGFR* expression in AML cells [6].

The expression of *EGFR* in AML is poorly defined and the role of *EGFR* in AML remains unclear which prompted us to reevaluate the expression of *EGFR* in patients with AML and carry out a comparative survival analysis between *EGFR* expressing and non-expressing AML patients (Children and Acute Promyelocytic Leukaemia patients excluded).

Materials and Methods

Sample Collection

Bone marrow aspirate or peripheral blood samples were collected in 2 ml K2EDTA tubes (Becton–Dickinson, Franklin Lakes, NJ, USA), from 60 de novo adult AML patients with written informed consent. These tubes were mixed well and transported to the laboratory on ice as soon as possible and processed within 24 h of collection. Ethics committee clearance was obtained from the institutional ethics committee to conduct the study.

Cytomorphology and Karyotyping

As per standard techniques bone marrow and peripheral blood, smears were stained following French-American-British (FAB) and World Health Organization (WHO) criteria [7]. Karyotyping was done at diagnosis according to the International System for Human Cytogenetic Nomenclature [8].

Molecular Assays

Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) extraction and cDNA preparation, *FLT3* (*FLT3-ITD* and *FLT3-D835*), *NPM1* mutation detection were performed as discussed earlier [9]. *FLT3-ITD* was detected by PCR amplification (Veriti, Thermo Fisher Scientific) whereas *FLT3-D835* mutation was detected by PCR amplification

and amplified products were then digested with EcoRV restriction endonuclease (HiMedia Laboratories Pvt. Ltd, Mumbai, India). *NPM1* mutations were detected using PCR amplification followed by Sanger sequencing (sequence outsourced). The primers for screening *TP53* mutations in exons 5–9 were used as described before [10]. *TP53* mutations were detected using PCR amplification followed by Sanger sequencing. IDH mutation detection was performed as described earlier [11]. *IDH* mutations were detected using PCR amplification followed by Sanger sequencing. Taq-man assays (Taqman Universal Master Mix II with UNG from Thermo Fisher Scientific Waltham, MA USA) were used in Real-Time PCR to detect RUNX1-RUNX1T1 (ID: Hs03024752_ft), CBF β -MYH11 (ID: Hs03460064_ft) translocations and *EGFR* (ID: Hs01076078_m1) expression in a StepOnePlus (Thermo Fisher Scientific Waltham, MA USA) Real-Time PCR. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) served as endogenous control (ID: Hs99999905_m1) with all assays.

Statistical Analysis

The main focus of our statistical analysis was to determine the significance of the overall survival between the *EGFR* expressing and non-expressing AML patients. Kaplan–Meier survival curve was constructed to explain the survival status between *EGFR* positive and negative patients. For statistically significant, *p* value < 0.05 was considered. SPSS software (IBM SPSS 20) was used for the analysis.

Treatment Protocol

At present, the standard treatment paradigm for AML is induction chemotherapy with an anthracycline/cytarabine combination followed by either remission consolidation therapy or allogeneic stem cell transplantation. Patients received remission induction therapy “7 + 3 regimen” of cytarabine (100 mg/m²) for 7 days plus daunorubicin (60 mg/m²) for 3 days. As a consolidation 3 cycles of high dose cytarabine 3 g/m² every 12 h on days 1, 3 and 5 were given.

Results

In our study group, we detected *EGFR* expression in 21/60, 35% (95% C.I. 23.45–48.48) AML patients. Comparison of 21 *EGFR* expressing and 39 non expressing AML did not reveal any statistically significant difference in clinical parameters such as age, white blood cell (WBC) count, platelet, haemoglobin count, blast count, and prevalence of common genetic alterations at diagnosis (Table 1). CBF β -

Table 1 Clinical characteristic of *EGFR* positive and *EGFR* negative patients

Comparison between <i>EGFR</i> positive (n = 21) and <i>EGFR</i> negative (n = 39)			
Demographic, laboratory and clinical characteristics	+ve	-ve	P value
Gender	n (%)	n (%)	< 0.05
Male	7 (22.6%)	24 (77.4%)	
Female	14 (48.3%)	15 (51.7%)	
Sex ratio (M:F)	0.5	1.6	
	<i>EGFR</i> (+ve) Mean $\pm$ SD [median(range)]	<i>EGFR</i> (-ve) Mean $\pm$ SD [median(range)]	P value
Age (years)	36 $\pm$ 8.64 [34 (28–48)]	44.37 $\pm$ 13.84 [42(25–84)]	> 0.05
WBC count, $\times 10^9$ /L	116911.43 $\pm$ 219703.64 [25,000 (10,640–957,000)]	47843.95 $\pm$ 56765.63 [25,830(5600–224,500)]	> 0.05
Platelet count, $\times 10^9$ /L	58714.29 $\pm$ 117558.56 [30,000 (5000–567,000)]	42962.05 $\pm$ 47151.34 [26,000 (4000–215,000)]	> 0.05
Haemoglobin level, $\times$ g/dL	6.86 $\pm$ 1.91 [7.0 (4–10)]	7.10 $\pm$ 2.42 [7.0 (2–13)]	> 0.05
Blast count (%)	44.14 $\pm$ 20.29 [39 (20–80)]	47.05 $\pm$ 20.44 [45 (20–85)]	> 0.0
	<i>EGFR</i> (+ve) n (%)	<i>EGFR</i> (-ve) n (%)	P value
<i>FLT3-ITD</i>	2 (9.5)	2 (5.1)	> 0.05
<i>RUNX1-RUNX1T1</i>	4 (19)	6(15.4)	> 0.05
<i>NPM1</i>	8 (38.1)	15 (38.5)	> 0.05
<i>IDH</i>	0 (0)	0 (0)	–
<i>TP53</i> mutation	1 (4.8)	2 (5.1)	> 0.05
Dead patients	12 (57.1)	5 (12.8)	< 0.01

P values computed using Pearson Chi square, Unpaired and Mann–Whitney U tests

MYH11, *FLT3-D835* and *IDH* mutations were not detected in the study group.

From the KM-curve (Fig. 1) it has been observed that *EGFR* positive patients survive on the average 18.57 months (95% C.I. 12.42–24.73 months) whereas *EGFR* negative patients survive on the average 31.27 months (95% C.I. 28.19–34.33 months). The result of the Log-rank test shows that the survival time between *EGFR* positive and negative patients is significantly different (*p* value: 0.000178). The proportion of *EGFR* positive among males was 22.6% whereas in the case of females every 48 females out of 100 females were *EGFR* positive. The proportion of *EGFR* positive female was significantly higher than the male counterpart (*p* value: 0.037).

Fig. 1 Survival analysis between *EGFR* positive and negative patients in AML

Discussion

Despite the critical role of *EGFR* in maintaining the normal cellular function, regulation of *EGFR* signaling pathways is important since dysregulation of *EGFR* signaling pathways triggers the development of malignancy via effects on cell-cycle progression, inhibition of apoptosis, induction of angiogenesis, and promotion of tumor-cell migration [12]. *EGFR* plays a pivotal role in the pathogenesis and progression of various cancers, mainly in solid tumors [13]. In cancers such as breast cancer, bladder cancer, glioblastoma multiforme, non-small cell lung cancer the involvement of *EGFR* is already been studied [14]. Aberrant expression of the *EGFR* gene leads to poor prognosis in different cancer types [15]. On the contrary, the view on *EGFR* expression in AML is highly debatable and poorly defined which prompted us to carry out this study.

To the best of our knowledge, this is the first report from India relating to the clinical outcome of *EGFR* in patients with AML. Our study shows that the frequency of *EGFR* in AML is almost 35% and confers a poor prognosis. The presence of an *EGFR* mutation does not respond well to conventional chemotherapy. Targeted therapies to *EGFR* offers the promise of better treatment for different types of solid tumors. Despite *EGFR* inhibitors such as gefitinib (Iressa), erlotinib, 4, 5-dianilinophthalimide (DAPH1) were found to induce myeloid maturation and inhibit viability in AML cell lines, as well as in primary patient AML blasts, at low micromolar concentrations [5], there have been no clinical trials published evaluating the efficacy of *EGFR* inhibitors in patients with AML and hence a potential therapeutic target for the reversal of resistance to conventional chemotherapeutics needs to be identified.

Conclusion

This study confirms the presence of *EGFR* in AML and also indicates that *EGFR* expression confers poor prognosis in AML. There are not enough clinical data at present to make an evidence-based clinical decision for AML patients with *EGFR* and conventional chemotherapeutics is not sufficient enough to accurately represent all the resistance mechanisms. Further clinical studies are warranted to determine the exact mechanism through which *EGFR* activity might contribute to AML progression and identify the potential molecules to target to overcome drug resistance.

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Author's Contribution All authors listed have contributed to the work and agreed to submit the manuscript for publication

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Compliance with Ethical Standards

Conflict of interest Sukanta Nath, Jina Bhattacharya, Partha Pratim Sarma, Renu Saxena, Sudha Sazawal, Manash Pratim Barman and Kandarpa Saikia declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study methodology was approved by the institutional ethics committee of Gauhati University, Guwahati, Assam, India (GUEC-12/2015).

Informed Consent Informed consent was obtained from all individual participants included in the study

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