1. ABSTRACT

The role of EGFR in cancer development and progression has been recognized for long time in a variety of human malignancies including lung, head and neck, colon, breast, ovary and glioma. Recently its role as a target of antineoplastic agents has also been identified and a variety of EGFR-targeted drugs is already being used in a clinical setting and others are at present under investigation. Many data involving EGFR protein expression are now available for the choice of anti-EGFR monoclonal antibodies in colorectal cancer and with regard to EGFR gene mutations for the choice of tyrosine kinase inhibitors in lung cancer. Other EGFR-related molecular factors, including the EGFR gene copy number, are currently under investigation. This review summarizes both preclinical and clinical available data regarding EGFR genomic alterations as prognostic and predictive factors.

2. INTRODUCTION

The epidermal growth factor receptor (EGFR) and members of its family are important targets for cancer treatment due to their ability to stimulate cell proliferation, survival and migration in normal and cancerous cells (1). The EGFR gene is located on chromosome 7p12-13 and encodes a tyrosine kinase receptor composed of four functional domains: an extra-cellular cysteine-rich ligand-binding domain, which can be further divided into four additional sub-domains (EGFR-I, EGFR-II, EGFR-III, EGFR-IV); a trans-membrane domain; an intracellular tyrosine kinase (TK) domain and a C-terminal domain which functions as a regulator (2). The binding of the epidermal growth factor (EGF) or other ligands, such as betacellulin, epiregulin, TGF-α, amphiregulin, and heparin-binding EGF-like growth factor (HB-EGF), to the extracellular domain, induces homodimerization of two
EGFR genomic alterations

EGFRs or heterodimerization of EGFR with other members of its family, particularly with HER2. In this way the tyrosine kinase domain becomes active, causing the auto-phosphorylation of C-terminal domain (3). The phosphorylated tyrosine residues are the binding sites for adapter proteins and this activates different pathways, including that of MAPK, PI3K and STAT3/5 (4). This explains how EGFR overexpression or aberrant activation induces proliferation, invasion and metastatization, all mechanisms associated with tumor phenotype.

Since EGFR is involved in a variety of human cancers, including those involving the lung, head and neck, colon, breast and ovary and also gliomas (5), and has been linked to poorer outcomes (6), EGFR inhibitors have improved the range of treatments for various solid tumors. Different clinical studies have been conducted with these agents alone or in combination with other anticancer drugs and it has been seen that their action depends not only on tumor type but also on other factors, such as EGFR gene alterations. In the light of the evidence which has emerged in recent years, the aim of this review is to find out whether EGFR protein expression, mutation status and gene copy number can be considered as predictive and prognostic biomarkers for the efficacy of EGFR target therapy, in particular with regard to anti-EGFR monoclonal antibodies and tyrosine kinase inhibitors (TKI).

3. EGFR PROTEIN EXPRESSION

EGFR expression and cancer prognosis have been investigated in several types of human cancers and although there are some conflicting results, patients with EGFR over-expression tend to have poor prognosis. The tissue expression of the EGFR protein is estimated between 60 and 85% in colorectal cancer (CRC). Its increased expression seems to be correlated with a higher stage, aggressiveness, presence of metastases and poorer prognosis. In the first clinical studies with anti-EGFR monoclonal antibodies, only patients affected by mCRC expressing EGFR protein were included. This limitation derived from the supposition that these agents could be effective only when their target is present in cancer cells. Subsequently retrospective analysis of these clinical trials suggested a less clear role of EGFR positivity for the prediction of the response to these monoclonal antibodies. In fact, patients with EGFR-negative mCRC also showed benefit from this treatment (7) (Table 1).

Three different clinical trials have evaluated the relationship between the response rates in patients treated with anti-EGFR monoclonal antibodies and the EGFR tissue expression levels tested by immunohistochemistry (IHC) and no correlation was found in any of these studies (8-10) (Figure 1).

Both objective response and stable disease rates in EGFR-negative cases appeared similar to EGFR-positive patients (9, 11) and these results were confirmed in mCRC patients treated with panitumumab (12, 13). Several studies have shown that there is no significant correlation between EGFR expression by IHC and efficacy of TKIs even in NSCLC patients (14-26). Two clinical trials comparing erlotinib and gefitinib with a placebo for non–small-cell lung cancer both demonstrated a survival benefit (26, 27), while phase II studies and three other phase III randomized studies (16, 19, 28) did not find a significant relationship (Table 1). Thus, EGFR protein expression cannot be considered as a useful biomarker of activity for anti-EGFR TKIs. Some authors have attempted to explain these findings (7, 29). They found possible reasons in the biological and technical problems related to the limitations and nonquantitative nature of conventional IHC.

The following limits of IHC have been recognized for some considerable time:

- Malignant cells of a tumor are heterogeneous, so the EGFR expression is variable within a neoplastic mass.

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Tumor</th>
<th>No. of patients</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saltz LB, et al.</td>
<td>II</td>
<td>CRC</td>
<td>57</td>
<td>Cetuximab</td>
<td>No correlation of response rate with EGFR expression levels</td>
</tr>
<tr>
<td>Cunningham D, et al.</td>
<td>II</td>
<td>CRC</td>
<td>474</td>
<td>Cetuximab + irinotecan Vs Cetuximab alone</td>
<td>No correlation of response rate with EGFR expression levels</td>
</tr>
<tr>
<td>Lenz HJ, et al.</td>
<td>II</td>
<td>CRC</td>
<td>346</td>
<td>Cetuximab</td>
<td>No correlation of response rate with EGFR expression levels</td>
</tr>
<tr>
<td>Berlin J, et al.</td>
<td>II</td>
<td>CRC</td>
<td>43</td>
<td>Panitumumab + IFX or FOLFIRI</td>
<td>No correlation of response rate with EGFR expression levels</td>
</tr>
<tr>
<td>Hecht JR, et al.</td>
<td>II</td>
<td>CRC</td>
<td>388</td>
<td>Panitumumab</td>
<td>Clinical responses found also in patients with negative EGFR</td>
</tr>
<tr>
<td>Douillard JY, et al.</td>
<td>III</td>
<td>NSCLC</td>
<td>380</td>
<td>Gefitinib vs docetaxel</td>
<td>No predictive role of EGFR protein expression</td>
</tr>
<tr>
<td>Herbst RS, et al.</td>
<td>III</td>
<td>NSCLC</td>
<td>1079</td>
<td>Erlotinib vs placebo</td>
<td>No correlation between EGFR expression levels and clinical outcomes</td>
</tr>
<tr>
<td>Tsao MS, et al.</td>
<td>III</td>
<td>NSCLC</td>
<td>525</td>
<td>Erlotinib vs placebo</td>
<td>Correlation of EGFR protein expression with an objective response</td>
</tr>
<tr>
<td>Hirsch FR, et al.</td>
<td>III</td>
<td>NSCLC</td>
<td>379</td>
<td>Gefitinib vs placebo</td>
<td>Correlation of EGFR protein expression with clinical outcomes</td>
</tr>
</tbody>
</table>

Table 1. The most important studies about correlation of EGFR protein expression and efficacy of anti-EGFR drugs
EGFR genomic alterations

Figure 1. Differences of response rates to Cetuximab according to the EGFR staining intensity (A) and the percentage of the EGFR expressing cells (B).

- EGFR protein includes both high and low affinity binding domains. Only the high affinity domains, which are usually poorly expressed, exert an effective biological function. The authors supposed that IHC could not be sensitive enough to recognize the high affinity binding sites. IHC allows the overall evaluation of EGFR protein, but is not able to distinguish the various receptor types with different biological activity

- the IHC-applying laboratories use different methods regarding the management of the specimens, and involving the tests and data reporting.

- the primitive site of the tumor and its metastases seem to express the EGFR protein differently.

At present, an effective scoring system and clear guidelines involving the standardization of EGFR protein evaluation by IHC are required.

4. EGFR MUTATIONS IN THE TYROSINE KINASE DOMAIN

EGFR mutations in the tyrosine kinase domain have been classified “as activating mutations” because they are all responsible for a ligand-independent activation of TK activity. Such mutations have been found in lung adenocarcinoma and more frequently among East Asian female patients with this type of cancer but without a smoking history (30, 31). The genomic region most affected by TK domain mutations is between exons 18 and 21 and the majority of EGFR mutations are deletions in exon 19 (over 20 variant types) and point mutation which substitutes an arginine with a leucine at codon 858 (L858R) in exon 21 (30). As minor EGFR mutations, there have also been reports of mutations at codon 719 (exon 18), 765 and 783 (exon 20) and in-frame insertion mutations in exon 20. Activating mutations have been found to confer sensitivity to the TKIs gefitinib and erlotinib (31-33) as the compromised ATP affinity of the EGFR mutants renders them susceptible to inhibition (34, 35). In fact, for patients harboring those mutations in the TK domain of EGFR, the response rate with erlotinib and gefitinib is approximately 70% (36, 37). Several reports have suggested that patients with exon 19 deletions have a longer PSF and overall survival compare to those with the L858R mutation after treatment with erlotinib and gefitinib (38, 39). Furthermore, no significant differences in response rate, median PFS and survival between the two TKIs have been observed (40). The INTEREST study has shown gefitinib to be a valid treatment option for patients with pretreated advanced non-small-cell lung cancer, proving to be non-inferior in overall survival and similar in tumor response and progression-free survival to docetaxel (41).

The IPASS trial compared first-line gefitinib with carboplatin/paclitaxel in Asian patients with advanced NSCLC and with no history of substantial smoking. Patients harboring EGFR mutations had a significantly longer PFS with gefitinib (HR, 0.48; P<0.001), whereas those with wild-type EGFR had a better PFS with chemotherapy (HR, 2.85; P<0.001) (42). Not all tumors having activating mutations are associated with greater response to treatment with gefitinib and erlotinib; their clinical efficacy is limited by the development of acquired drug resistance such as mutation of the substitution of threonine 790 with methionine (T790M), which has been detected in 50% of clinically resistant patients (43, 44) and has been associated with a short PFS (7.7 months in patients with the T790M mutation and 16.5 months in those without the mutation) (45). Nevertheless, the T790M mutant remains sensitive to irreversible inhibitors, such as EKB-569 and HKI-272 (43, 46, 47). In vitro studies have shown that EGFR T790M restores the affinity for ATP to the same levels of WT EGFR (48). In NIH 3T3 cells the T790M with the L858R mutant increases EGFR activity and enhances the transformed phenotype (49) and transgenic mice expressing T790M mutant develop lung adenocarcinomas (50), although with a longer latency than those harboring the L858R or combined L858R and T790M mutations (51, 52).

The T790M mutation has also been detected in a small fraction of tumor cells before drug treatment, supporting the idea that tumor cells harboring this mutation might be present from the beginning of the treatment with gefitinib or erlotinib and be enriched over time (52). Genetic heterogeneity of tumors is an issue to be considered when interpreting EGFR mutation data. In recent years, it has been suggested that, in order to detect a small fraction of mutant alleles among a large number of wild-type alleles in clinical samples, it is advisable to use a sensitive assay, such as SARMS technology, rather than direct DNA sequencing (53). Contrary to what was observed in non-small lung cancer, mutations in the catalytic domain of EGFR are rare in CRC, suggesting that gefitinib is unlikely to be effective in patients with these tumors (54-56).
5. EGFR MUTATIONS IN THE EXTRACELLULAR DOMAIN

In the extracellular domain of EGFR, three different types of deletions have been found, defined as EGFR variant I, II and III (EGFR vI, EGFR vII and EGFR vIII). The most common of the three mutants found in human cancer is EGFRvIII, consisting of in-frame deletion from exons 2 through 7 (amino acids 6–273) in the extracellular domain, resulting in ligand-independent constitutive activation of EGFR (57). This mutation has not been observed in normal tissue (58) and is expressed in a number of cancers, including breast cancer, ovarian cancer, prostate cancer, and lung cancer (58) most notably glioblastoma (59-61), where it is reported to occur at a frequency of 20%-30% and in 50%-60% of tumors the amplification of wt EGFR is observed (62, 63).

In glioblastoma cell lines, EGFRvIII confers resistance to gefitinib with constitutive activation of EGFR and persistence of phospho-Akt (64). In lung cancer, contrary to the other deletions and mutations of EGFR, EGFRvIII expression is relatively resistant to gefitinib and erlotinib and more sensitive to treatment with irreversible EGFR TKIs such as HKI-272 (65). In mCRC this variant is rare and does not play an important role (66).

6. EGFR GENE COPY NUMBER

Amplification or high polysomy of EGFR have been detected in a variety of solid malignant tumors, and have been associated with poor prognosis (67). In NSCLC, the gene copy number appears to be a promising biomarker for predicting a survival benefit with EGFR-TKI therapy in both second line, (68) and third line clinical trials (26, 27) and is more predictive of patient survival after gefitinib treatment than EGFR mutations (69). However, the predictive value of EGFR gene amplification for TKI sensitivity has proved to be lower in Japanese cohorts than in Western NSCLC cohorts. These findings suggest a possible difference mechanism of EGFR pathway activation in NSCLC between Asian and Caucasian populations (70, 71). A high EGFR copy number is frequently correlated with EGFR somatic mutations, (72-77) although, since it is difficult to obtain sufficient amounts of tumor tissue for genetic analysis from patients with advanced NSCLC, the relationship between these two types of EGFR alterations has remained unclear. In CRC, discrepant results have emerged from recent studies. As emerged from two large studies, the percentage of CRC with increased EGFR copy number is probably only 10% to 15% of tumors (29, 78) but despite the low incidence it seems to be an interesting predictor of response to anti-EGFR (55, 79, 80). Contrary to these authors, Lenz et al. (9) showed a positive relationship between increased EGFR gene copy number (determined by quantitative PCR) and OS of patients, but not with PFS or response to cetuximab. Finally Khambata-Ford S et al., did not find any correlation between increased EGFR copy number and response to cetuximab (81). So we need further data to conclude about its applicability in clinical practice for decision making of anti-EGFR monoclonal antibodies.

7. PERSPECTIVE

The EGFR-related pathway is recognized as one of the main molecular mediators of tumor development and progression. When this pathway becomes abnormally activated, tumor cells acquire independence from mitogenic extracellular signals. Constitutive activation of the EGFR pathway induces prognostic worsening. For this reason, some researchers have developed new molecules to target both the extracellular and the intracellular domain of the EGFR. Since the cost of these agents is often higher than conventional chemotherapeutic drugs, research is continually underway in order to discover predictive factors leading to the clearer identification of potential responders.

At the present time, the anti-EGFR monoclonal antibodies, cetuximab and panitumumab, are frequently used in advanced colorectal cancer management. EGFR protein expression by IHC was initially considered a mandatory requirement to deliver these drugs but later some authors have highlighted the absence of relationship between EGFR expression and clinical response in these patients. To date others parameters are required for the selection of patients to be treated with these monoclonal antibodies. The EGFR gene copy number and mutations have not proved to have any role as predictive factors in this setting of patients. KRAS and BRAF mutation testing has now become fundamental to assess responsiveness in colorectal cancer patients. Activating mutations in the EGFR intracellular domain are however, related to the clinical response to EGFR TKIs, gefitinib and erlotinib, in patients with advanced NSCLC. Other genomic alterations of EGFR, even in other malignancies, have been identified through various preclinical studies, although they have not yet been applied in clinical practice. Further clinical investigations are needed to clarify the relationship between genomic changes and therapeutic efficacy.

8. ACKNOWLEDGEMENT

Giuseppe Bronte and Marianna Terrasi contributed equally to this work

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