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Review

Assessing epidermal growth factor receptor expression in tumours: What is the value of current test methods?

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Abstract

Over-expression of the epidermal growth factor receptor (EGFR) in tumours is associated with aggressive disease and poor clinical prognosis. In theory, the EGFR status of a tumour provides an indication of the likelihood of response to EGFR-targeted therapy. However, the clinical data do not support a relationship between EGFR expression and response to EGFR-targeted therapies cetuximab, gefitinib and erlotinib. Recently, patients who appear to lack EGFR expression have been shown to respond to cetuximab. Possible causes for this paradox include false negative results due to a lack of sensitivity in the detection system, heterogeneity of EGFR expression within the tumour and specific mutations that mediate response to the tyrosine kinase inhibitors. Immunohistochemistry is the most reliable assay for EGFR expression but its interpretation is confounded by the lack of non-standard techniques. Other approaches for measuring EGFR expression can be considered at best exploratory at this point. Further work is needed to identify how EGFR contributes to carcinogenic and metastatic processes. As tumours that appear to be EGFR negative can respond to cetuximab, there is some doubt as to the usefulness of immunohistochemistry as a screen to select patients for treatment. Histopathology will continue to be essential for unravelling the role of this enigmatic molecule and refining its status as a legitimate target in cancer therapy.

Keywords: EGFR; Expression; Immunohistochemistry; Prognosis; Response

1. Introduction

New molecular cancer therapies provide a way of delivering treatment directly to a specific molecular target with the aim of optimising efficacy while at the same time minimising the side effects associated with treatment. During the process of drug discovery, molecular targets are identified by the relationships between their expression, pathogenesis and clinical outcome. Consequently an accurate assessment of the degree of target-molecule expression in the clinical setting should provide an indication of both prognosis and whether or not a patient is likely to benefit from the targeted treatment. However, in the case of the epidermal growth factor receptor (EGFR), two major, interrelated obstacles have so far prevented the development of an accurate prognostic tool. The first of these is the lack of a standardised, reproducible assay for EGFR-expression that would allow a direct comparison of the results obtained from different laboratories. The second issue concerns whether or not an accurate assay for EGFR expression would actually provide valuable prognostic information given that its precise role in the processes of carcinogenesis and metastases has not been defined. This review takes a critical look at immunohistochemistry (IHC) and some of the newer techniques for assessing EGFR

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expression, and examines the recent histopathological studies that have investigated the role of EGFR in tumour biology.

2. Increased EGFR signalling

The EGFR plays an important role in regulating cellular processes such as proliferation, differentiation, survival and is central to the maintenance of normal epidermal tissues where its expression is highly regulated. When its function becomes deregulated, it contributes to the growth and survival of cancer cells and as such is recognised as an important target for cancer therapy. Indeed the EGFR is prominently expressed in a variety of human solid tumours, including colorectal cancer (CRC), head and neck squamous cell cancer (HNSCC) and non-small cell lung cancer (NSCLC) (Table 1) [1–3]. Although the relationship between EGFR status and prognosis is not completely understood, EGFR expression in tumours is usually associated with more aggressive disease, increased resistance to chemotherapy and radiotherapy, increased metastasis, poor clinical prognosis and decreased survival [4–6]. However, in some studies, EGFR expression in the tumour was found to have no prognostic significance [7,8].

Lack of a clear relationship between EGFR expression and prognosis is to be expected given that its activity, and therefore its influence on cancer cell survival, can be amplified by a number of mechanisms other than increased receptor expression [9]. These include an increase in ligand expression, interaction with human epidermal receptor-2 (HER2) to form highly stable and potent EGFR–HER2 heterodimers, and the expression of constitutively activated mutant EGFRs.

3. Assessing tumour EGFR status

Several standard techniques can be used for the detection of EGFR expression in tumours (Table 2), including protein expression assays (e.g., IHC, Western blot analysis, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS)), RNA transcript assays (e.g., Northern blot analysis, reverse transcriptase polymerase chain reaction (RT-PCR)) and DNA assays (e.g., quantitative PCR, Southern blot analysis and fluorescence *in situ* hybridisation (FISH)).

Table 1
EGFR expression in different tumour types

Tumour type (references)	Proportion of tumours expressing EGFR (%)
Colon [2,48]	25–77
Irinotecan-refractory colon [15,33,49]	72–82
Head and neck [48,50,51]	43–100
Pancreatic [48,52,53]	30–95
NSCLC [3,48,54]	32–84
Renal [17,48,55]	50–93
Breast [48,56]	14–91
Ovarian [45,48,57]	35–70
Glioma [48,58]	40–63
Bladder [59]	72

NSCLC, non-small cell lung cancer.

Table 2
Detection of EGFR in tumors

Method	Ideal specimen	Limitations
In situ methods		
IHC	Tissue section	Lack of standardisation
FISH	Tissue section	None
Extractive methods		
Western/Northern blot	Tissue extract or serum[a]	Technical complexity
RT-PCR	Tissue extract or serum[a]	Technical complexity
ELISA	Tissue extract or serum[a]	None
Flow cytometry	Tissue extract	None

[a] The relevance of serum EGFR in cancer patients is still not established. IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridisation; RT-PCR, reverse transcript-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

The most commonly used method for determining EGFR expression is IHC, which has been used in the majority of published studies for EGFR expression in human tumours. Its widespread use is mainly due to it being a relatively quick and simple technique that utilises commonly available reagents and equipment. Unlike extractive methods, IHC also has the benefit of preserving cellular morphology and tissue integrity and this can provide additional, important information regarding the distribution of the target molecule within the tissue sample.

The fidelity or quality of the information provided by IHC is dependent on the use of high-quality reagents, the optimal use of the methods and the careful assessment of the stained tumour sections by a suitably trained pathologist. On the other hand the reproducibility of IHC results that allows meaningful comparisons to be made between laboratories, is reliant on the use of highly standardised techniques and reagents at all stages of tissue handling. These include methods for tissue fixation (i.e., the storage period for cut tissue sections and the type of fixative used [10]), the supply of the primary and secondary antibodies, the chromogenic detection system, and the pathologist's analysis of the stained samples (see scoring system below).

A standardised IHC kit for EGFR (the DakoCytomation EGFR pharmDx™) has been developed. This has been approved by the Food and Drug Administration, USA in an attempt to provide consistent identification of patients with EGFR-expressing cancer

who would be eligible for inclusion in clinical trials investigating the anti-EGFR monoclonal antibody cetuximab (Erbitux®). The kit is validated for use on sections of routine, formalin-fixed, paraffin-embedded histological specimens. Whilst this approach certainly represents an important step in terms of improved standardisation, it obviously cannot make up for inconsistencies introduced by the many pre-analytical variables mentioned above. Some of these may be addressed in the future by, for example, the development of more sensitive and robust anti-EGFR antibodies for use in IHC.

4. IHC scoring

Immunohistochemistry has the potential to differentiate between patients on the basis of EGFR-expression in the tumour but does not really provide for a truly quantitative analysis. Nonetheless, many clinical studies have used IHC in an attempt to measure EGFR expression and correlate this parameter with clinical outcomes including tumour aggression (i.e., prognosis) and response to EGFR drug-inhibitors. To this end several investigators have developed algorithms that place a numerical value on IHC images of EGFR staining in tumour samples (Table 3) [11–15]. Although there is considerable variation between these methods, and a standard system has yet to be adopted, they are all based on an assessment of the proportion of positively stained cells and the intensity of the observed staining.

A major issue is the possibility of false negatives or disproportionately low staining that can occur from a lack of sensitivity with some methods, or the heterogeneity of EGFR expression within different tumour regions [2]. A specific drawback of the scoring systems is that they are not strictly quantitative and the interpretation of stained samples is a subjective process. While the subjective element within studies can be controlled to a degree using blinded independent assessments by two or three scorers, such measures do not normally control for centre-specific anomalies to allow direct intra-site analyses. A standardised scoring system is needed as a matter of urgency to allow direct comparison of the EGFR expression data from different studies [16]. A comparative study of different scoring methods for IHC data found assessments to be most consistent when the scoring was simple and included well defined, prescriptive descriptions and where a consensus was achieved following a panel discussion [16]. However, the picture is muddied further by a recent study which suggested that, for renal cell carcinoma at least, important prognostic information may be obtained by a detailed qualitative analysis to determine the precise location of EGFR over-expression [17]. In this study, exclusively cytoplasmic EGFR expression correlated with poor prognosis whereas immunoreactivity located on the plasma membranes either alone

Table 3
Selected recent methods for EGFR immunohistochemistry evaluation

Reference	Proportion of stained cells	Intensity of staining	Overall score
Cunningham [15]	⩽10% >10–⩽20% >20–⩽35% >35%	Faint Weak or moderate Strong	None given
Hirsch [12]	Continuous variable from 0% to 100%	Negative or trace = 1 Weak = 2 Moderate = 3 Strong = 4	Proportion of positive cells multiplied by intensity to give range of 0–400, with expression classified as: • 0–200 negative or low • 201–300 intermediate • 301–400 high
Kersemaekers [11]	0 no positive tumour cells 1 (1–25%) 2 (26–50%) 3 (51–75%) 4 (76–100%)	0 no staining 1 weak staining 2 moderate staining 3 strong staining	Scores for proportion and intensity added to give range 0 and 2–7 and classified as: • 0 negative • 2–3 weak • 4–5 moderate • 6–7 strong
Scartozzi [13]	<1% of tumour cells with membrane staining ⩾1% of tumour cells with membrane staining	Weak, faint brown = 1+ Moderate, brown of intermediate darkness = 2+ Strong, dark brown/black = 3+	Positive or negative based only on the proportion of stained cells classification
Spano [14]	Grade 0 (none) Grade 1 (1–25%) Grade 2 (25–50%) Grade 3 (>50%)	Negative = 0 Weak = 1 Moderate = 2 Strong = 3	Grade multiplied by intensity to give range of 0–9: • <6 low expression • >6 high expression

or with cytoplasmic co-localisation did not. If corroborated, such findings would need to be considered in any proposed standardised method of assessment.

5. Other methods of measuring EGFR expression

In breast cancer, the over-expression of HER-2 protein occurs predominantly as a result of gene amplification. As a consequence, the detection of HER-2 gene amplification in archival formalin-fixed, paraffin embedded samples by fluorescence *in situ* hybridisation (FISH) correlates well with strong (3+) HER-2 protein expression. Compared to IHC, FISH is claimed to provide a more accurate prognostic indicator with the ability to segregate patients on the basis of low and high-risk breast cancers [18]. However, in consideration of both the high costs and technical complexity of FISH, a judicious approach in the clinical setting may limit the use of this technique to the category of 2+ HER-2 expressing breast cancers, in order to include the subset of cases exhibiting lower levels of HER-2 amplification. The situation with EGFR appears to be less clear and the relationship between gene amplification and protein expression may well turn out to vary with tumour type, or even stage of tumour development.

In oesophageal cancer, EGFR gene amplification (15%) or over-representation (increased copies of chromosome 7; 36%) correlated positively with EGFR protein expression which was seen in 49% of cases [19]. A similar, positive correlation between EGFR protein expression (as detected by IHC) and gene copy number (detected by FISH and resulting from gene amplification or over-representation) has been reported recently in NSCLC [12].

Not all investigations have found a clear relationship between EGFR protein expression and gene copy number. A comparative study investigated EGFR expression in 222 samples of invasive breast cancer and found that IHC could detect protein over-expression in 17.3% of cases [20]. Of these, only 13% had evidence for gene amplification as shown by FISH analysis and only 20% had increased first intron amplification (elements within the first intron can determine the level of gene transcription) as determined by quantitative RT-PCR. The authors concluded that mechanisms other than whole gene or first intron amplification must be considered to explain the majority (75%) of cases where EGFR over-expression is seen. Thus, in breast cancer at least, the lack of a relationship between gene copy number and protein expression means that FISH and RT-PCR are inferior to IHC for measuring EGFR. Similarly, a comparative study that analysed EGFR gene amplification by quantitative PCR in sections from CRC tumours with known EGFR expression status concluded that the two parameters were unrelated [21].

It seems that the preliminary data comparing EGFR gene copy number with protein expression show some promise in limited settings. However, a major problem with such studies is their dependence on inferior or non-standardised IHC methods, low sample numbers, and the potential for misrepresentation of protein expression due to intra-tumour heterogeneity [22]. As such, these studies should be considered investigational pending thorough validation [12,22]. Leaving aside the methodological problems, it seems that for many cancers in which EGFR is implicated, overt protein expression bears little relationship to gene copy number such that IHC remains the best current method for prognostic investigation.

6. EGFR expression and prognosis

It is known that EGFR expression is differentially controlled according to the stage of tissue development. *In vivo* studies of healthy mouse epithelial tissue show that EGFR expression is greatest in cells with proliferative capacity, whereas those which have started to differentiate or have lost their growth potential, display markedly reduced EGFR expression [23].

Although one might expect variability in the EGFR expression of tumours with different origins, such as the breast and lung, considerable variation is also reported by different investigators for tumours of the same type (Table 1). There are several possible sources for this variability. Firstly, whilst the traditional basis for the classification of solid tumours has relied on their typical histological features, the new molecular techniques have demonstrated neoplasms of the 'same' type to be heterogeneous with regards to cytogenetic and protein expression profiles. Secondly, as discussed earlier, such discrepancies will arise directly from the different methods that are in use for the measurement of EGFR expression [24]. Thirdly, a tumour sample may be considered as negative for EGFR expression on the basis of a non-representative tissue section or as a consequence of an IHC method with reduced detection threshold. In any case, this failure to determine the precise EGFR status makes it difficult to establish the exact nature of the relationship between EGFR expression and prognosis [9].

Nicholson and colleagues [5] attempted to overcome some of these issues and conducted a detailed investigation of EGFR expression and its relationship with clinical outcome. Their extensive, investigative review included more than 200 studies conducted between 1985 and 2000 and involved more than 20,000 patients. Suitable data were available for 10 major human tumour types that over-expressed EGFR compared with healthy, normal tissue. Of these, EGFR over-expression acted as a strong prognostic indicator in five; HNSCC,

ovarian, cervical, bladder, and oesophageal cancers. It had a more modest prognostic significance in another four (gastric, breast, endometrial and CRC), and little prognostic significance in one (NSCLC). These classifications of prognostic significance were based on the proportion of studies showing a relationship between EGFR and outcome with weighted adjustment according to the number of studies included in the analysis. For example, although 67% of studies in CRC indicated a relationship between EGFR status and survival, this was in effect only two out of three studies. Thus, the authors designated EGFR status as a 'modest prognostic indicator' in this tumour type.

The authors conceded that the true prognostic significance of EGFR was probably underestimated given that there were several problems inherent in such a retrospective analysis and that these would be confounded by the following issues surrounding the way in which the individual studies had been conducted. Firstly, there was considerable heterogeneity in the patient populations with regard to early and late stage disease. Secondly, the studies had determined tumour EGFR status using a variety of detection assays with further differences in the definitions that were used for high- or over-expression. Even where the same technique was used (e.g., IHC), there existed considerable variations between laboratories in the execution of methods, use of reagents, and assay cut-off points. Finally, the studies only measured cellular EGFR expression rather than the activated, phosphorylated form of the receptor.

A further meta-analysis involving 2185 patients from 11 studies was published in 2002 and looked specifically at the prognostic value of EGFR expression in lung cancer [25]. This study largely confirmed the finding of Nicholson and colleagues [5] in that EGFR expression was not found to be a statistically significant factor for survival when all studies were considered (hazard ratio [HR] 1.14, 95% confidence interval [CI] 0.94–1.39). Interestingly, when only those studies that utilised IHC were included the measure of inter-study heterogeneity became insignificant and the prognostic value reached statistical significance (HR 1.13, 95% CI 1.00–1.28). The authors concluded that any impact of EGFR was minimal in lung cancer but they could not rule out the possibility that even this may have been due to publication bias. In support of this, another recent study using both IHC and FISH concluded that EGFR over-expression, or a high gene copy number, had no significant effect on prognosis in NSCLC [12].

EGFR expression in CRC was judged by Nicholson and colleagues [5] to have moderate prognostic influence and this has not yet been investigated by further meta-analysis. The prognostic role of EGFR in this setting is currently controversial with several recent studies observing no significant impact on survival [8,14,26], a finding that contrasts with others who found it to be associated with reduced life expectancy [27,28].

The outcome of head and neck cancers was considered to be influenced strongly by EGFR expression in the review of Nicholson and colleagues [5], but again this has not yet been corroborated by further meta-analysis. A correlative study in 155 patients with HNSCC found wide variation in the expression of EGFR [6]. Investigation in respect to clinical outcome showed significant correlation between increased EGFR expression and reduced overall survival ($P = 0.0006$), reduced disease free survival ($P = 0.0016$) and increased local relapse rate ($P = 0.0031$), when compared to patients with low-EGFR expressing tumours [6]. However, whilst EGFR over-expression correlated with poor local control in early glottic cancer [29], it was not found to affect disease-free, or overall survival in undifferentiated carcinoma of the larynx [30].

Although EGFR expression is seen commonly in many solid tumours (Table 1) its ability to provide consistent prognostic information for a given tumour type has not yet been demonstrated. The last few years have seen an increase in the molecular classification of traditional tumour types and this is set to continue as more molecular probes become available to the histopathologist. NSCLC provides a good example of heterogeneity within a conventional tumour classification and interestingly the degree of EGFR over-expression appears to vary with the different NSCLC histological subtypes from 33% for large cell carcinoma, and 40% for adenocarcinoma to around 80% for squamous cell or bronchoalvaeolar carcinomas [12]. Even within such histopathological subdivisions, the simple involvement of EGFR is not absolute where even in this example, 20% of bronchoalvaeolar tumours clearly did not appear to over-express EGFR. Accordingly, it may become increasingly inappropriate to determine the prognosis for a tumour on the basis of anything other than the actual protein expression in each individual case. For example, a recent study in 60 HNSCC samples identified four distinct subtypes on the basis of molecular analysis of cDNA microarrays [31]. The tumours exhibited statistically significant differences in recurrence-free survival but interestingly, only one of the subtypes appeared to be dependent on EGFR-pathway signalling.

7. Tumour EGFR status and response to EGFR inhibition

The rationale for selecting EGFR as a target for cancer therapy is based soundly on its ability to induce tumorigenic effects such as cell proliferation and survival, and the massed data showing that its expression in many different human solid tumours is associated with aggressive disease and poor clinical outcome

(reviewed by Baselga [32]). This rationale has been extended such that tumour EGFR status has been pursued as both an indicator of suitability for treatment with, and likelihood of response to, EGFR-targeted therapies. However, the ability of EGFR expression in the tumour to predict a response to therapy is currently unclear. The available data from clinical trials suggest that there is no relationship between either the number of EGFR-expressing cells, or the intensity of EGFR-staining, and a response to treatment. Data from the phase II randomised BOND study showed that in patients with metastatic CRC receiving second-line treatment with cetuximab plus irinotecan the likelihood of a response was independent of the level of EGFR expression (Table 4) [15]. This result may have been influenced by the co-administration of irinotecan, however, data from the cetuximab monotherapy arm of the same study also found no correlation between EGFR expression and response. The lack of a relationship between the degree of EGFR expression and response to second-line cetuximab monotherapy in patients with metastatic CRC was also noted by Saltz in a separate phase II study [33]. A retrospective follow-up investigation of 16 patients with EGFR-negative metastatic CRC tumours found that 25% responded to cetuximab and irinotecan [34]. Clearly IHC evaluation of EGFR expression is an unreliable way to screen for patients with CRC who may be suitable for cetuximab therapy [35].

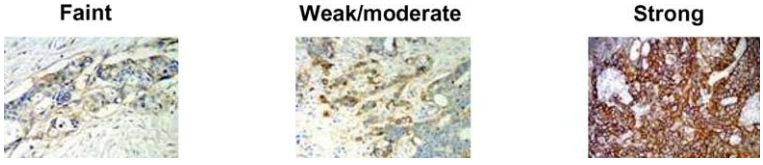
Correlative data between clinical outcome following EGFR-targeted therapy and EGFR expression are also available for the small molecule tyrosine kinase inhibitors gefitinib (Iressa®) and erlotinib (Tarceva™), both of which have been approved for patients with pre-treated NSCLC. In both cases EGFR expression in NSCLC tumours could not predict response (or survival) to the EGFR-inhibitor therapy [36,37]. Preliminary, phase II data for the unlicensed monoclonal antibody ABX-EGF also found no relationship between EGFR expression and progression-free survival in renal cell cancer [38]. These findings are supported, at least for the tyrosine kinase inhibitors, by several reports that specific mutations in the tyrosine kinase domain of the EGFR molecule confer sensitivity to gefitinib and erlotinib and may define a small subset of patients with lung cancer who have 'never-smoked' [39–41]. Under such circumstances, the target is not EGFR *per se* but rather its mutated form. Whether similar aberrations in the EGFR, the other growth factor receptors with which it interacts, or its downstream signalling pathways exist and contribute to the heterogeneity in response seen with cetuximab or ABX-EGF is currently unknown. However, this example serves to illustrate that a simple reliance on protein expression alone may be insufficient to identify patients who may respond to a given therapy such that all available avenues should be explored.

8. EGFR expression in context

Several IHC studies have shown the distribution of EGFR to be heterogeneous within the tumour with increased expression at the invading edge [2,42]. Furthermore, Goldstein and Armin [2] compared EGFR staining within sub-tumour locations with clinical outcome and observed that an increased number of cells

Table 4
Response to cetuximab appears to be independent of the degree of tumour EGFR expression (after Cunningham [15])

Subgroups/results from the BOND study (n = 329)	Cetuximab/irinotecan		Cetuximab	
	n/N	(%)	n/N	(%)
% EGFR-expressing cells				
⩽10%	25/109	23	4/56	7
>10–⩽20%	4/20	20	5/16	31
>20–⩽35%	6/27	22	0/7	0
>35%	15/62	24	3/32	9
EGFR-staining intensity				
Faint	11/53	21	1/21	5
Weak/moderate	22/89	25	7/55	13
Strong	17/75	23	4/34	12



with moderate (2+) to strong (3+) reactivity in the regions of the tumours that had penetrated furthest into the normal structures of the gut correlated with decreased survival ($P = 0.052$) in patients with metastatic CRC. These observations suggest that EGFR may have a highly specific role in tumour growth and invasion, a notion that is supported by the known distribution and function of EGFR in normal tissues [23]. Such observations require corroboration, which if found to be consistent, could have profound implications for the preparation of histopathological specimens and their evaluation as guidance for treatment options.

If EGFR is implicated in the growth and invasive spread of tumours, then one could postulate that it may also be critical for the development of metastases. There are limited data comparing expression in the primary tumour with that at metastatic sites, and the data from four studies in colorectal cancer are equivocal [2,8,13,43]. Goldstein and Armin [2] noted that the intense IHC EGFR staining they observed in the most invasive portions of the tumour correlated with EGFR reactivity at metastatic sites. Preliminary data from a study which used RT-PCR showed that levels of EGFR mRNA were similar at the primary and corresponding metastatic sites, although actual EGFR protein expression was not compared directly [43]. In contrast to the findings from these studies, McKay and colleagues [8] reported no significant association in IHC-determined EGFR status between primary colorectal tumours and paired lymph node metastases. Scartozzi and colleagues [13] also found no correlation between the EGFR status of primary CRC tumours and metastases at several sites including liver, lung, brain and bone. In addition, they also found seven EGFR-positive metastases (15% of their sample) in patients with EGFR-negative primary tumours. The authors concluded that the EGFR status of the primary tumour alone could be inadequate for planning EGFR-targeted therapy in a considerable proportion of CRC cases.

The binding of a ligand to EGFR invokes a complex series of molecular events within the cell including direct activation of the intracellular signalling pathways and co-activation of other membrane-bound tyrosine kinase receptors. Given the diversity of this signalling network, it is possible for unregulated EGFR activation to have an effect on tumour cell growth that is disproportionate to the detectable level of protein. Examination of all four EGFR family members by IHC in patients with HNSCC found the expression of EGFR, HER-2 and HER-3 to be associated with the occurrence of lymph node metastases and all four with distant metastases and shortened survival [44]. When EGFR, HER-2 and HER-3 were considered together, the prognostic value was improved significantly. Similarly, in borderline ovarian cancer the combination of HER-2 and EGFR over-expression significantly improved the prognostic accuracy [45]. The independent expression of metalloproteinase-9 (MMP-9) and perinuclear carbonic anhydrase IX (pCA IX) have both been associated with poor prognosis in NSCLC ($P = 0.001$ and $P = 0.03$, respectively), whereas EGFR alone has not [46]. Multivariate analysis demonstrated that the co-expression of MMP-9 with EGFR was significantly associated with a worse prognosis than MMP-9 alone ($P < 0.001$) and, similarly, the prognosis for patients with co-expressed pCA IX and EGFR was worse than for pCA IX expression alone ($P = 0.05$). A study to examine the molecular determinants of response to gefitinib in NSCLC confirmed that the expression of EGFR, or even its activation status (i.e., phosphorylated EGFR [p-EGFR]) would not predict response [47]. By comparison, the expression of activated, phosphorylated, key EGFR-downstream signalling molecules gave a good indication of response to gefitinib. The response rate in patients whose tumours stained positive for p-Akt and negative for p-Erk was 60% compared with 0% in the opposite case (i.e., p-Akt negative and p-Erk positive tumours). Intense nuclear staining of p-Akt was associated with prolonged time to progression ($P = 0.018$) and overall survival ($P = 0.008$).

Taken together these data illustrate that any assessment of EGFR expression for the purposes of determining patient prognosis, treatment options, or the likelihood of response to EGFR-inhibitor therapy must consider the wider context of both the complex multistage carcinogenic process and the rich milieu in which EGFR functions.

9. Conclusions

This review set out to determine whether, given the limitations of the current technology, there was any value in routinely testing individual patients for EGFR expression when considering cancer therapy with anti-EGFR drugs. The definitive answer to such a question is perhaps some way off. In the case of the tyrosine kinase inhibitors (erlotinib and gefitinib) it appears that specific mutations within the ATP binding site of EGFR mediate the response to these agents. Under these circumstances, gross data for EGFR expression may have less clinical meaning than detection of the specific mutations. The monoclonal antibody cetuximab inhibits EGFR signalling by interacting with the extracellular ligand-binding domain and it is therefore likely that its activity is independent of mutations in the intracellular domain. However, even in this setting the precise value of EGFR testing cannot yet be determined due to the lack of a standardised method for its detection and the lack of understanding around the precise role of this enigmatic molecule in the carcinogenic process. Indeed, a recent investigation showing that patients who

appeared to have EGFR-negative metastatic CRC responded to cetuximab, throws serious doubt on the use of IHC for the screening of such patients for this targeted therapy [34,35].

The relationship between the EGFR gene copy number and expression is not straightforward such that, despite the drawbacks, IHC remains the best method for investigating its presence and distribution within the tumour. However, the lack of a standardised method, including evaluation of stained sections (e.g., the choice of cut-off for positive staining) must be addressed urgently. The development of a standardised IHC method for EGFR expression should be relatively straightforward with the rate-limiting step being the level of cooperation between the global community of investigators and pathologists rather than technological innovation. However, even when such a method has been agreed and refined, its integration into the diagnostic armoury will depend on a clearer understanding of precisely how the EGFR contributes to the development of tumours. Advances are needed in at least two key areas. Firstly, optimal methods are needed to detect the different ways in which the EGFR-dependent pathways become dysfunctional, for example mutations within the receptor itself, or changes in the way it interacts with other receptors, or effectors of downstream signalling. Secondly, a greater understanding is needed as to which of the critical stages of cancer development are most affected or even dependent on this aberrant activity. With the current limitations, it is not possible to state with certainty that a tumour which appears to be negative for EGFR expression will be unresponsive to therapy with an anti-EGFR therapy such as cetuximab.

Conflict of interest statement

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