PERGAMON

European Journal of Cancer 37 (2001) S9–S15

European Journal of Cancer

www.ejconline.com

EGFR and cancer prognosis

R.I. Nicholson*, J.M.W. Gee, M.E. Harper

Tenovus Cancer Research Centre, Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK

Received 7 March 2001; accepted 26 June 2001

Abstract

Elevated levels of the epidermal growth factor receptor (EGFR), a growth-factor-receptor tyrosine kinase, and/or its cognate ligands have been identified as a common component of multiple cancer types and appear to promote solid tumour growth. This article examines the relationship between EGFR expression and cancer prognosis based on literature compiled on PubMed between 1985 and September 2000. More than 200 studies were identified that analysed relapse-free-interval or survival data directly in relation to EGFR levels in over 20 000 patients. Analysis of the data showed that 10 cancer types both express elevated levels of EGFR relative to normal tissues and have been studied in sufficient depth to allow sound judgements to be made concerning the association between EGFR and patient outlook. The EGFR was found to act as a strong prognostic indicator in head and neck, ovarian, cervical, bladder and oesophageal cancers. In these cancers, increased EGFR expression was associated with reduced recurrence-free or overall survival rates in 70% (52/74) of studies. In gastric, breast, endometrial and colorectal cancers, the EGFR provided more modest prognostic information, correlating to poor survival rates in 52% (13/25) of studies, while in non-small cell lung cancer (NSCLC), EGFR expression only rarely (3/10 studies) related to patient outlook. However, it is likely that the true prognostic significance of the EGFR has been underestimated as the published studies only assessed total cellular EGFR levels, rather than the activated form of the receptor, and were not standardised with regard to patient populations or assay methods. Finally, it is important to stress that failure to detect a prognostic significance for EGFR in any one cancer type does not necessarily preclude patients from benefiting from anti-EGFR therapies.

Keywords: Neoplasms; Prognosis; Receptor; Epidermal growth factor; Receptor; ErbB-2

1. Introduction

The growth and development of cancer cells is thought to occur through multiple genetic events that cause fundamental changes in the pathways regulating cell differentiation, proliferation, survival and mobility. Activation of the proto-oncogene encoding the epidermal growth factor receptor (EGFR), a growth-factor-receptor tyrosine kinase, may contribute to the transformation of cellular phenotypes and provide tumour cells with substantial growth and survival advantages (reviewed in Ref. [1]). Over the last 20 years, elevated levels of the EGFR and its cognate ligands (which include EGF and transforming growth factor (TGF)-α) have been identified as a common component of numerous cancer types. In many cases aberrant EGFR activation, mediated primarily through changes in gene amplification and autocrine stimulation, appears to be an important factor in tumorigenesis, as well as an essential driving force for the aggressive growth behaviour of cancer cells [2]. Increased EGFR expression is therefore likely to be a strong prognostic feature in multiple tumour types, and the inhibition of its cellular actions appears to produce substantial therapeutic benefits.

2. Methodology

In this light, the current overview examines the association between EGFR expression and cancer prognosis. Relevant literature published between 1985 and September 2000 was identified on PubMed using the keywords epithelial growth factor, EGFR and EGF-R in combination with individual tissue or cancer types. Over 200 relevant references were identified from PubMed containing information about more than 20 000 patients. However, only general conclusions should be made from this retrospective investigation as the studies were not

* Corresponding author. Tel.: +44-292-087-5226; fax: +44-292-087-5152.

E-mail address: nicholsonri@cardiff.ac.uk (R.I. Nicholson).

0959-8049/01/$ - see front matter
PII: S0959-8049(01)00231-3

standardised with respect to patient populations and the assays used to determine tumour EGFR levels.

Analysis of the data identified 10 major cancer types that both overexpress EGFR relative to normal tissues and have been studied in sufficient depth to allow sound judgements to be made concerning the relationship between EGFR and cancer prognosis. Only studies that directly analysed disease-free-interval and/or survival data in relation to EGFR were considered in determining the strength of the EGFR as a prognostic indicator in a particular cancer type, even though EGFR expression or gene amplification has also been correlated with parameters of known prognostic significance, such as tumour grade, stage and aneuploidy. Another factor taken into consideration was the number of studies with prognostic data relating to a particular neoplasm. For example, in colorectal cancer, although EGFR was associated with survival in 67% of studies and the majority of the patients had advanced cancers, the data was drawn from only three studies involving 207 patients. Thus, colorectal cancer was placed in the group in which EGFR is a 'modest prognostic indicator'.

It is likely that this report underestimates the true prognostic significance of the EGFR as the published studies only assessed the total cellular levels of EGFR rather than the activated form of the receptor, which is the only form thought to affect prognosis. Additionally, there is a great deal of variability with respect to the assay methods used, and even where similar assay methods were employed, the cut-off points defining elevated versus normal levels of EGFR expression were not standardised among studies. Finally, the inclusion of diverse patient populations with both early and late disease stages may also act to reduce the perceived impact of EGFR levels on patient outlook.

3. Results

3.1. EGFR as a strong prognostic indicator

In head and neck, ovarian, cervical, bladder and oesophageal cancer (e.g. [3–7], respectively), the association between elevated EGFR levels (Fig. 1) and poor patient outlook is particularly strong. Of the 74 studies in these cancer types, 70% showed that increased EGFR expression correlated with a reduction in recurrence-free survival or overall survival rates (Table 1).

In a number of investigations with these cancer types, the magnitude of the EGFR effect on survival was

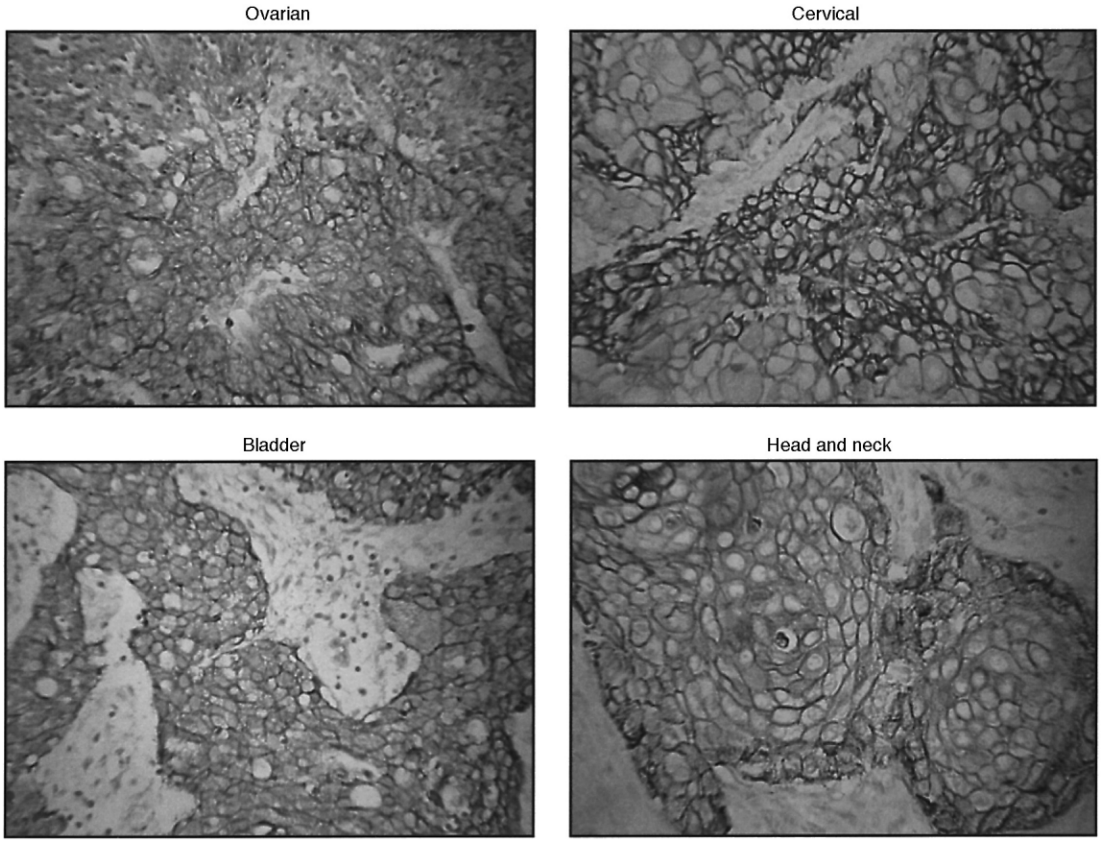

Fig. 1. Immunocytochemistry detecting the epidermal growth factor receptor (EGFR) in cancer types where receptor measurement is of strong prognostic value. Staining was performed using a mouse monoclonal primary antibody (clone 111.6) with enzymatic antigen retrieval of formalin-fixed, paraffin-embedded material. Original magnification: ×40.

highly significant. In a study of 140 patients with head and neck cancers, Maurizi and colleagues [3] found that EGFR levels determined by a radio-ligand receptor assay correlated with the risk of relapse and death. The 5-year survival rate for patients with EGFR-negative tumours was 81%, while patients with EGFR-positive tumours had a survival rate of 25%. Similarly, the 5-year relapse-free survival rate was 77% for patients with EGFR-negative tumours compared with 24% for patients with EGFR-positive tumours. Not all studies showed such a pronounced effect, and in some cases, overexpression of EGFR ligands was a more significant prognostic indicator than EGFR levels [8]. Parallel analysis of both EGFR and its cognate ligands also appeared to be a strong predictive tool in some cancer types. For example, in 40 patients with oesophageal cancer, the 5-year survival rate was 69% for patients with low levels of both EGF and EGFR, in contrast to 14% for patients with high levels of the same ligand and receptor [9]. These results show that increased levels of the EGFR and/or its ligands correlate with aggressive growth behaviour in head and neck, ovarian, cervical, bladder and oesophageal cancers. Since the growth of these tumours is not fully controlled by conventional treatments, as indicated by the survival patterns associated with EGFR-positive disease, these cancers are highly appropriate candidates for anti-EGFR therapies.

Earlier preclinical studies demonstrated that overexpression of EGFR and its cognate ligands may produce a transformed phenotype or enhance the tumorigenicity of cancerous cells, providing a potential rationale for the poor prognosis frequently associated with elevated EGFR levels in human malignancies. For example, co-expression of EGFR and EGF conferred a transformed phenotype to NIH-3T3 cells [10], and TGF-α overexpression in weakly tumorigenic rat bladder-cell xenografts significantly enhanced tumorigenicity, leading to a decrease in the latency period before tumour appearance, an accelerated tumour growth rate and increased tumour size [11]. Similarly, studies with transgenic mouse models targeting heterologous TGF-α to the mammary gland have shown that TGF-α overexpression can induce hyperproliferation, hyperplasia and occasional carcinoma, all of which are inhibited by agents that block the EGFR signalling pathway (reviewed in Ref. [12]).

3.2. EGFR as a modest prognostic indicator

There is also evidence linking EGFR levels to prognosis in gastric, breast, endometrial and colorectal cancers (e.g. [13–16], respectively), but the relationship is not as strong as in the first five cancer types (Table 1, Fig. 2). This is particularly true in terms of overall survival, where poor survival rates correlated with elevated EGFR levels in 52% of studies compared with over 70% in the other cancer types. However, EGFR levels were also associated with other clinically significant variables in gastric, breast, endometrial and colorectal tumours.

In the case of gastric cancer, several studies have linked EGFR expression to advanced clinical stage [17–19] and the presence of lymph node metastasis [20–22]. Further evidence that EGFR may be an important prognostic indicator in gastric cancer comes from a number of small studies that examined concurrent expression of EGFR and its ligands [13,19,23,24]. In each of these four publications, co-expression of EGFR and either EGF or TGF-α was associated with a marked overall-survival or relapse-free-survival disadvantage.

Table 1
Frequency (%) of studies showing an association between increased EGFR levels and decreased survival

Cancer type	Association with RFS %	Association with OS %
EGFR as a strong prognostic indicator		
Bladder	60 ($n=5$)	63 ($n=11$)
Cervical	75 ($n=4$)	71 ($n=7$)
Oesophageal	0 ($n=1$)	69 ($n=13$)
Head and neck	75 ($n=8$)	82 ($n=11$)
Ovarian	80 ($n=5$)	67 ($n=9$)
Total[a]		70 ($n=51$)
EGFR as a modest prognostic indicator		
Breast	N/A	55 ($n=11$)
Colorectal	N/A	67 ($n=3$)
Gastric	N/A	50 ($n=6$)
Endometrial	N/A	40 ($n=5$)
Total[a]		52 ($n=25$)
EGFR as a weak prognostic indicator		
NSCLC	20 ($n=10$)	10 ($n=10$)

N/A, not available; NSCLC, non-small-cell lung cancer; OS, overall survival; RFS, relapse-free survival.
[a] Total = association with OS.

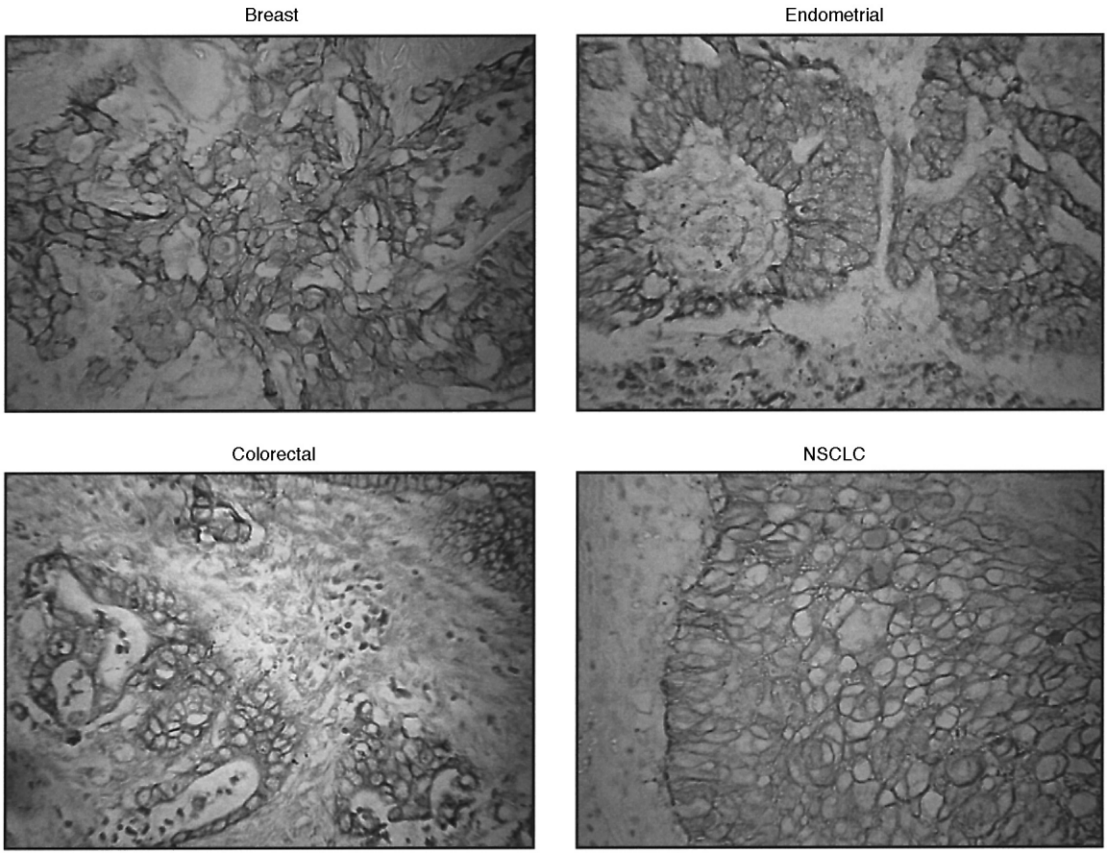

Fig. 2. Immunocytochemistry detecting the epidermal growth factor receptor (EGFR) in cancer types where receptor measurement is of moderate/weak prognostic value. Staining was performed as described in Fig. 1. Original magnification: ×40. NSCLC, non-small cell lung cancer.

For example, in a study of 167 patients with advanced gastric cancer, Yonemura and colleagues [13] found that patients with tumours expressing both TGF-α and EGFR had the poorest prognosis, with a 5-year survival rate of 12%. When tumour EGFR and TGF-α levels were normal or only the receptor or ligand was over-expressed, 5-year survival rates increased to 45 and 36%, respectively.

The situation appears to be similar in breast cancer. Just over half of the published studies (6/11) in this indication reported a relationship between increased EGFR expression and reduced patient survival. However, if only short-term (2-year) survival is examined rather than short-term and long-term (5-year) survival, then the EGFR appears to act as an early prognostic indicator [14]. This difference may be due to the presence of a small population of rapidly progressing cancer cells where the EGFR acts as a strong driving force for growth and development. Our own studies performed in collaboration with colleagues at the Nottingham City Hospital, Nottingham, UK, have produced evidence indicating a strong association between EGFR expression and loss of differentiation in breast cancer. Elevated EGFR levels have also been shown to correlate with advanced clinical stage, enhanced tumour-cell proliferation and the failure of patients to respond to endocrine therapy [25]. Furthermore, in a study with 95 breast cancer patients, the survival rates of patients with EGFR-positive tumours were significantly lower than those of patients with EGFR-negative tumours [26]. Interestingly, when tumour samples from oestrogen receptor (ER)-positive breast cancers were assayed for EGFR and TGF-α expression during relapse and response phases, levels of both the receptor and ligand were significantly higher during the relapse phase, indicating a possible causal relationship with the development of acquired endocrine resistance. Certainly, elevated expression of TGF-α is associated with a failure of ER-positive breast tumours to respond *in vivo* to endocrine therapy [27].

The situation in colorectal cancer is not entirely clear, as the data pertaining to EGFR and survival are currently very limited. Furthermore, the patient populations in the published studies were heterogeneous, including both early and advanced cases [16,28,29]. However, EGFR expression has been associated with tumour grade and stage [30,31], relapse-free survival [16] and overall survival [16,28]. In three studies, HER2 expression showed a better correlation with survival than did EGFR expression [32–34], while in another study TGF-α expression was found to be a prognostic indicator [16].

These data indicate that, although EGFR may not be as strong a prognostic indicator in breast, gastric, endometrial and colorectal cancers as it is in other carcinomas such as head and neck cancer, the EGFR signalling pathway does have some prognostic significance in these types of tumours.

3.3. EGFR as a weak prognostic indicator

In contrast, EGFR expression rarely relates to other clinical and prognostic indicators in non-small-cell lung cancer (NSCLC; Table 1). Only three studies out of ten showed an association between EGFR and either relapse-free or overall survival rates. However, there is some evidence suggesting that an EGFR autocrine loop may lead to a reduction in NSCLC patient survival rates. In a study with 131 NSCLC patients, Tateishi and colleagues [35] found that the 5-year survival rates of patients whose tumours did not express TGF-α or EGF were significantly higher than those with tumours positive for either EGFR ligand.

3.4. HER2 as a prognostic indicator

In addition to considering EGFR and its cognate ligands as prognostic indicators, the expression of EGFR heterodimerisation partners may also provide valuable prognostic information. The EGFR is known to form heterodimers with the HER2, HER3 and HER4 members of the EGFR family [36]. HER2 is unique within the EGFR family of receptor tyrosine kinases as none of the known EGFR family ligands activates HER2 homodimers. Instead, HER2 appears to function primarily as a heterodimerisation partner for other EGFR family members [37], making it an ideal candidate for use as a prognostic indicator in combination with the EGFR. Analysis of the literature revealed that 45 out of 56 studies (80%) demonstrated an association between HER2 overexpression and poor survival in ovarian, cervical, bladder, head and neck, endometrial, colorectal, gastric, NSCLC, oesophageal and breast cancer (Table 2). Interestingly, in a study with 119 NSCLC patients, EGFR expression alone was not a significant predictor of 5-year survival rates, but the expression of both HER2 and EGFR did identify patients with a poorer prognosis [38]. The 5-year survival rates of patients with EGFR-positive tumours were 51%, compared with 42% for patients with EGFR-negative tumours. In contrast, of the total patient population with EGFR-positive tumours, 5-year survival rates of patients with tumours that co-expressed EGFR and HER2 were 33%, while those that expressed only EGFR were 59%. These results suggest that considering multiple aspects of the EGFR signal transduction pathway may provide a more accurate prediction of patient outlook.

Table 2
Frequency (%) of studies that correlate HER2 overexpression with poor survival

Cancer type	Correlation with survival % (n)
Bladder	100 ($n=5$)
Breast	75 ($n=8$)
Cervical	83 ($n=6$)
Colorectal	100 ($n=2$)
Endometrial	80 ($n=5$)
Oesophageal	50 ($n=2$)
Head and neck	60 ($n=5$)
Gastric	83 ($n=12$)
NSCLC	75 ($n=4$)
Ovarian	86 ($n=7$)
Total	80 ($n=56$)

NSCLC, non-small-cell lung cancer.

4. Discussion

Analysis of the existing literature on EGFR and prognosis clearly indicates that elevated levels of EGFR are correlated with poor patient outlook in many different cancer types. As mentioned above, this analysis is likely to underestimate the true predictive significance of the EGFR due to inconsistencies in the assay methods used and the heterogeneity of the patient populations between studies. The lack of a standardised assay for determining tumour EGFR status is particularly problematic. Tumour EGFR status can be evaluated by more than ten different methods designed to detect gene amplification, gene mutation or elevated levels of either mRNA transcripts or protein. The studies included in this overview determined tumour EGFR status using many of these assays, each defining EGFR overexpression in a slightly different manner. Furthermore, even when a single technique, such as immunohistochemistry (IHC), was used by several laboratories to evaluate tumour EGFR levels, differences in reagents, detection methods or assay cut-off points led to different results, increasing the variability between studies.

Future studies should address these issues by adopting a universal method to evaluate tumour EGFR status. Given that increased EGFR expression may occur as a result of gene amplification or transcriptional events, evaluating protein expression levels is the most comprehensive approach, as it detects the consequences of either gene amplification or transcriptional events. Of the range of techniques that can be used to detect aberrant EGFR protein expression, IHC is probably the most appropriate method for clinical use. However, standardisation of antibody preparations and detection methods, as well as a scoring system is necessary to produce an assay for EGFR evaluation that provides consistent, comparable results.

Additionally, future studies should consider the biology of the EGFR network to a greater extent. Measuring

tumour EGFR levels in combination with the expression of EGFR ligands and dimerisation partners, in addition to the activation states of both receptors and downstream EGFR signalling components, may greatly improve our understanding of the prognostic role of this signal transduction network. This type of analysis may also identify patient populations that would benefit from anti-EGFR therapies. Finally, it also has the potential to provide an accurate means of monitoring the efficacy of anti-EGFR treatments and lead to significant improvements in cancer therapy.

References

1. Huang SM, Harari PM. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. *Invest New Drugs* 1999, **17**, 259–269.
2. Salomon DS, Bradt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Critical Rev Oncol/Hematol* 1995, **19**, 183–232.
3. Maurizi M, Almadori G, Ferrandina G, *et al*. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. *Br J Cancer* 1996, **74**, 1253–1257.
4. Fischer-Colbrie J, Witt A, Heinzl H, *et al*. EGFR and steroid receptors in ovarian carcinoma: comparison with prognostic parameters and outcome of patients. *Anticancer Res* 1997, **17**, 613–620.
5. Kersemaekers AM, Fleuren GJ, Kenter GG, *et al*. Oncogene alterations in carcinomas of the uterine cervix: overexpression of the epidermal growth factor receptor is associated with poor prognosis. *Clin Cancer Res* 1999, **5**, 577–586.
6. Mellon K, Wright C, Kelly P, Horne CH, Neal DE. Long-term outcome related to epidermal growth factor receptor status in bladder cancer. *J Urol* 1995, **153**, 919–925.
7. Inada S, Koto T, Futami K, Arima S, Iwashita A. Evaluation of malignancy and the prognosis of esophageal cancer based on an immunohistochemical study (p53, E-cadherin, epidermal growth factor receptor). *Surg Today* 1999, **29**, 493–503.
8. Wen QH, Miwa T, Yoshizaki T, Nagayama I, Furukawa M, Nishijima H. Prognostic value of EGFR and TGF-alpha in early laryngeal cancer treated with radiotherapy. *Laryngoscope* 1996, **106**, 884–888.
9. Hirai T, Kuwahara M, Yoshida K, *et al*. Clinical results of transhiatal esophagectomy for carcinoma of the lower thoracic esophagus according to biological markers. *Dis Esophagus* 1998, **11**, 221–225.
10. Di Fiore PP, Pierce JH, Fleming TP, *et al*. Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. *Cell* 1987, **51**, 1063–1070.
11. Kawamata H, Kameyama S, Oyasu R. In vitro and in vivo acceleration of the neoplastic phenotype of a low-tumorigenicity rat bladder carcinoma cell line by transfected transforming growth factor-alpha. *Mol Carcinog* 1994, **9**, 210–219.
12. Humphreys RC, Hennighausen L. Transforming growth factor alpha and mouse models of human breast cancer. *Oncogene* 2000, **19**, 1085–1091.
13. Yonemura Y, Takamura H, Ninomiya I, *et al*. Interrelationship between transforming growth factor-alpha and epidermal growth factor receptor in advanced gastric cancer. *Oncology* 1992, **49**, 157–161.
14. Klijn JG, Berns PM, Schmitz PL, Fockens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. *Endocr Rev* 1992, **13**, 3–17.
15. Niikura H, Sasano H, Matsunaga G, *et al*. Prognostic value of epidermal growth factor receptor expression in endometrioid endometrial carcinoma. *Hum Pathol* 1995, **26**, 892–896.
16. De Jong KP, Stellema R, Karrenbeld A, *et al*. Clinical relevance of transforming growth factor alpha, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. *Hepatology* 1998, **28**, 971–979.
17. Tsugawa K, Yonemura Y, Hirono Y, *et al*. Amplification of the c-met, c-erbB-2 and epidermal growth factor receptor gene in human gastric cancers: correlation to clinical features. *Oncology* 1998, **55**, 475–481.
18. Takita M, Onda M, Tokunaga A. Immunohistochemical demonstration of angiogenic growth factors and EGF receptor in hepatic metastases and primary human gastric cancer. *Nippon Ika Daigaku Zasshi* 1998, **65**, 358–366.
19. Yasui W, Hata J, Yokozaki H, *et al*. Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. *Int J Cancer* 1988, **41**, 211–217.
20. Yasui W, Sumiyoshi H, Hata J, *et al*. Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. *Cancer Res* 1988, **48**, 137–141.
21. Prakash I, Mathur RP, Kar P, Ranga S, Talib VH. Comparative evaluation of cell proliferative indices and epidermal growth factor receptor expression in gastric carcinomas. *Indian J Pathol Microbiol* 1997, **40**, 481–490.
22. Yang A, Lu X, Chen Y. The role of endoscopic ultrasonography and epidermal growth factor receptor assay in preoperative staging for gastric carcinoma. *Chung Hua Nei Ko Tsa Chih* 1995, **34**, 743–746 (in Chinese).
23. Tokunaga A, Onda M, Okuda T, *et al*. Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric cancer. *Cancer* 1995, **75**, 1418–1425.
24. He SW, Guo LY, Li JQ. Clinical study on epidermal growth factor and its receptor in human gastric cancer. *Chung Hua I Hsueh Tsa Chih* 1994, **74**, 413–415, 455 (in Chinese).
25. Nicholson RI, Gee JMW, Jones H, *et al*. erbB signalling and endocrine sensitivity of human breast cancer. In Lichtner RB, Hawkin RN, eds EGF, eds. *Receptor and Tumour Growth and Progression. Ernst Schering Research Foundation, Workshop 19*. Springer-Verlag, Berlin, 1997, 105–128.
26. Nicholson RI, McClelland RA, Gee JM, *et al*. Epidermal growth factor receptor expression in breast cancer: association with response to endocrine therapy. *Breast Cancer Res Treat* 1994, **29**, 117–125.
27. Nicholson RI, McClelland RA, Gee JM, *et al*. Transforming growth factor-alpha and endocrine sensitivity in breast cancer. *Cancer Res* 1994, **54**, 1684–1689.
28. Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor and mdr gene expression in colorectal cancer. *Cancer* 1993, **71**, 2454–2460.
29. Takemura K, Obara T, Okano S, *et al*. Immunohistochemical study on the expression of epidermal growth factor receptor (EGFR) in human colorectal carcinomas. *Nippon Shokakibyo Gakkai Zasshi* 1991, **88**, 1177–1183 (in Japanese).
30. Kluftinger AM, Robinson BW, Quenville NF, Finley RJ, Davis NL. Correlation of epidermal growth factor receptor and c-erbB2 oncogene product to known prognostic indicators of colorectal cancer. *Surg Oncol* 1992, **1**, 97–105.
31. Steele RJ, Kelly P, Ellul B, Eremin O. Epidermal growth factor receptor expression in colorectal cancer. *Br J Surg* 1990, **77**, 1352–1354.
32. Kay EW, Mulcahy H, Walsh CB, Leader M, O'Donoghue D. Cytoplasmic c-erbB-2 protein expression correlates with survival in Dukes' B colorectal carcinoma. *Histopathology* 1994, **25**, 455–461.
33. Osako T, Miyahara M, Uchino S, Inomata M, Kitano S, Kobayashi M. Immunohistochemical study of c-erbB-2 protein in

colorectal cancer and the correlation with patient survival. *Oncology* 1998, **55**, 548–555.
34. Kapitanovic S, Radosevic S, Kapitanovic M, *et al*. The expression of p185 (HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. *Gastroenterology* 1997, **112**, 1103–1113.
35. Tateishi M, Ishida T, Mitsudomi T, Kaneko S, Sugimachi K. Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. *Cancer Res* 1990, **50**, 7077–7080.
36. Lenferink AE, Pinkas-Kramarski R, van de Poll ML, *et al*. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. *Embo J* 1998, **17**, 3385–3397.
37. Earp HS, Dawson TL, Li X, Yu H. Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer research. *Breast Cancer Res Treat* 1995, **35**, 115–132.
38. Tateishi M, Ishida T, Kohdono S, Hamatake M, Fukuyama Y, Sugimachi K. Prognostic influence of the co-expression of epidermal growth factor receptor and c-erbB-2 protein in human lung adenocarcinoma. *Surg Oncol* 1994, **3**, 109–113.