Cancer Biology & Therapy 10:5, 416-421; September 1, 2010; © 2010 Landes Bioscience

EGFR expression variance in paired colorectal cancer primary and metastatic tumors

Nirit Yarom,[1,*] Celia Marginean,[2,3] Terence Moyana,[3] Ivan Gorn-Hondermann,[6] H. Chaim Birnboim,[6] Horia Marginean,[1] Rebecca C. Auer,[4,6] Michael Vickers,[1] Timothy R. Asmis,[5] Jean Maroun[5] and Derek Jonker[5,6]

[1]Ottawa Hospital Cancer centre; [2]Department of Cytopathology; [3]Department of Pathology; [4]General Campus; [5]University of Ottawa; [6]The Ottawa Hospital Research Institute; Ontario, Canada

Key words: Epidermal Growth Factor Receptor (EGFR), colorectal cancer, primary tumour metastatic, Cetuximab, Panitumumab, chemotherapy

Background: Previous studies indicate that drugs targeting the Epidermal Growth Factor Receptor (EGFR) signaling pathways can induce objective responses, prolong time to progression and improve survival of patients with metastatic colorectal cancer (mCRC). EGFR expression in the primary tumour may not predict response to these agents and data is conflicting regarding the correlation of EGFR expression in the primary tumour with the metastatic site. In other tumour sites, the presence of EGFR mutations was associated with efficacy in a subset of patients.

Objectives: The goal of this study is to correlate tumour EGFR expression between primary and liver metastatic sites, and to assess the mutational status in the EGFR kinase domain.

Methods: This is a single center retrospective study of patients who underwent surgical resection of CRC, for whom paired paraffin-embedded tissue blocks of primary tumours and resected liver metastases were available. EGFR immunostaining and mutation analyses were preformed.

Results: Fifty six paired colorectal primaries and metastases were available for analysis. EGFR was detectable in 96.6% of the primary samples and in 89.7% of the metastatic samples. Perfect concordance in the intensity score between the primary and the metastases was found in 46.5% of the cases. While individual pairs were poorly concordant for intensity, the proportion of primaries with intense staining was similar to the proportion with intense staining in the metastatic samples. Overall survival did not correlate with either EGFR expression in the primary tumour, or with EGFR expression in the metastasis. There were 2 cases with mutations in the EGFR kinase domain. Both mutations were found in exon21 C>T.

Conclusions: In this analysis, EGFR expression in the primary tumor site was not predictive of its level in the metastasis. EGFR expression levels in the primaries and in the metastases do not appear to be useful prognostic markers.

Background

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death in North America. Although CRC mortality rates have declined in recent years the burden of disease still remains high.[1,2] In the last decade the recent years the treatment of metastatic CRC has significantly improved with the median survival of the patients increasing from six months to 24 months.[3-6] Among these advances are the targeted biologic agents, including antagonists which target the Epidermal Growth Factor Receptor (EGFR).

The EGFR is a cell membrane receptor which, upon activation, initiates a series of intracellular signals that ultimately leads to cellular proliferation, induction of angiogenesis and tumor cell metastases.[7] The majority of human epithelial cancers are marked by functional activation of the EGFR family. In vitro human colorectal carcinoma cell line experiments indicate that EGFR mRNA plays a significant role during tumor progression,[8] while in-vivo EGFR levels are higher in the malignant zones of CRC specimens as compared to the surrounding mucosa.[9] In a clinical study, EGFR expression has been demonstrated to be associated with poor outcome in patients with CRC.[10]

There are two EGFR antagonists currently available for the treatment of metastatic CRC (mCRC), Cetuximab and Panitumumab.[11] Previous studies indicate that drugs targeting the EGFRsignaling pathways can induce objective responses, prolong time to progression and improve survival for patients with mCRC.[6,12,13] However EGFR expression in the primary tumor does not predict response in the metastatic location.[14] Published data regarding the correlation of EGFR expression between a primary tumor and the metastatic site is inconsistent. It has not yet been clarified whether the level of expression in the primary site predicts its level in the metastatic site. A difference in EGFR expression between the archival primary tumor and subsequent metastases might explain why patients without

*Correspondence to: Nirit Yarom; Email: nirit.yarom@gmail.com
Submitted: 06/03/10; Accepted: 06/08/10
Previously published online: www.landesbioscience.com/journals/cbt/article/12610
DOI: 10.4161/cbt.10.5.12610

Table 1. Clinical data of patients

	All N = 56 (%)	Synchronous N = 28 (%)	Metachronous N = 28 (%)
Stage at diagnosis			
I	1 (1.7)	0	1 (3.6)
II	11 (19.6)	0	11 (39.3)
III	14 (25)	0	14 (50)
IV	28 (50)	28 (100)	0
Missing data	2		
Status post hepatic resection			
R0	40 (71.4)	18 (64.3)	22 (78.6)
R1	7 (12.6)	3 (10.7)	4 (14.3)
R2	6 (10.7)	5 (17.8)	1 (3.6)
Time in months between primary and hepatic resection median (range)	11.8 (0–76.8)	3.54 (0–9.3)	18.9 (1.6–76.8)
Adjuvant chemo for early disease	22 (39.3)	NA	5FU based 20 (71.4%) FOLFOX 2 (7.1%)

detectable EGFR expression in the primary still respond to anti EGFR therapy.

©2010 Landes Bioscience.
Do not distribute.

Objectives

This study was conducted to explore the role of EGFR in CRC primary and metastatic tumors. The analysis was done at multiple levels: (1) assessment of the expression of EGFR in the primary site as a predictor of its level in the metastatic lesion (using immunohistochemistry, IHC); (2) assessment of the clinical importance of EGFR expression level by correlating the above results with clinical outcome; (3) assessing the mutational status of the EGFR kinase domain and its clinical relevance.

Results

Patients. Clinical data was obtained on all 56 patients who had paired primary tumors and metastases. The median age at diagnosis was 58 (range: 31–80). Median follow up was 77 months (range 1.2 to 124). The primary and metastasis were synchronous in 29 patients (50%) and metachronous in the other 29 patients (**Table 1**). Following resection of the liver 47 patients (84%) of patients were clinically disease free (71.4% R0, 12.6% R1). Chemotherapy was received by 43 patients (74%) at some time in the course of their treatment. **Table 2** describes chemotherapy before and after metastasectomy as well as subsequent chemotherapy received upon recurrence or progression. All patients who received chemotherapy were treated with a TS inhibitor (74%), 52% received Irinotecan, 43% received Oxaliplatin, while only 9% received Bevacizumab or Cetuximab (5%).

EGFR IHC status. Fifty-eight paired primary and metastatic tumors were available for EGFR IHC analysis.

EGFR was detectable (1 to 3+) in 96.6% of the primary samples and in 89.7% of the metastatic samples (**Fig. 1**). A 1+ EGFR staining intensity score was most frequently observed, seen in 56.9% of primary tumors and in 54.9% of metastatic samples. A 3+ EGFR intensity score was identified in 16.1% of primary tumors and 14.3% of metastases.

Variation of intensity score in primary tumor samples versus metastatic samples. When a threshold of detectable (1 to 3+) vs. undetectable (score = 0) was used, there was no significant difference in the proportion of patients with detectable EGFR by IHC between primary tumors and metastases (Fisher's exact p = 0.162). Similarly, when a threshold of weak (0 to 1+) versus strong (2 to 3+) was used, there was no difference in the proportion of patients with weak versus strong EGFR intensity between primary tumors and metastases (Fisher's exact p = 1). If a stringent criteria of identical matching intensity between the primary and metastasis (e.g., concordant pairs, both with 2+ expression) was the measure of relative consistency, there was low concordance between paired samples (Goodman and Kruskal's gamma coefficient = 0.51). Better correlation was seen in the group with metachronous tumors (gamma = 0.61) vs. the group with synchronous tumors (gamma = 0.39).

If less stringent criteria for concordant pairs are utilized, specifically that expression (1 to 3+) versus non-expression (0) between the primary and metastasis is the measure of agreement, then a trend towards concordance between paired samples was seen (two tailed p value calculated with McNemar's test = 0.07). There was perfect concordance in the intensity score between the primary and the metastasis in 27(46.5%) cases. An intensity score variance of ≤1 was seen in 25(43.1%) cases. A difference of 2 was seen in 6 m(10.3%) cases. No cases had a difference of 3. There were five discordant pairs where expression was lost in metastasis and no pairs where non-EGFR expression in the primary tumors was changed to expression in metastasis. In 13 cases the metastasis had a higher score and in 18 cases a lower score compared to the primary.

EGFR intensity and outcome. There was no correlation between overall survival and the EGFR intensity score in the primary tumor. The median survival was 56.1 vs. 57.1 months for patients whose primary tumor EGFR staining was 0 to 2+ versus 3+ respectively (HR = 1.25 [95% C.I. 0.52 to 3.11], log rank p = 0.60) (**Fig. 2A**).

Similarly, EGFR intensity score in the metastases did not predict for overall survival. The median survival was 56.1 vs. 62.8 months for patients whose metastatic tumor EGFR staining was 0 to 2+ versus 3+ respectively (HR = 1.09 [95% C.I. 0.43 to 2.8], log rank p = 0.86) (**Fig. 2B**).

EGFR mutation analysis. There were two patients with a mutation in the EGFR kinase domain. Both mutations were found in exon21 C>T. One patient had a mutation exclusively in the primary sample. This patient had EGFR IHC intensity of +1 both in the primary and metastasis. He was diagnosed at age 37 and had synchronous primary and metastatic disease. After R2 liver EGFR expression variance in paired colorectal cancer primary and metastatic tumors resection, he received Irinotecan based chemotherapy and had a remarkable PFS of

53.7 months and an overall survival of 62.2 months. The second patient had a mutation in both the primary and metastasis. The IHC EGFR intensity was +2 and +1 for the primary and metastasis respectively. She was diagnosed at age 75 years with a synchronous primary and metastasis. The progression free survival (PFS) after liver metastatectomy was 11.2 months and the overall survival was 25.9 months. Because of the limited number of patients with EGFR mutations (n = 2), no statistical analysis was preformed comparing these patients to those without mutation.

Discussion

We hypothesized that the EGFR expression levels would differ between primary and metastatic samples. Our results support this hypothesis. We have shown weak or an absence of concordance between the level of expression in the primary samples and their paired metastases.

Our data therefore reveal that it is possible that clinical correlative studies could find that evaluation of EGFR expression in metastatic samples may be more predictive than EGFR expression from the primary.

A number of other publications contradict our results and show a concordance between the EGFR expression in primary and metastatic samples of CRC. Italiano et al.[16] report that 94% of their 80 samples had concordant EGFR status when analyzed by IHC. A recent publication by Molinari et al.[17] analyzed 38 patients and found in all cases the same pattern of EGFR protein expression by IHC between primary tumor and related metastasis, either at distant sites or in lymph nodes ($k = 1$, $p < 0.0001$). Similarly, when mRNA levels of EGFR were analyzed by Kuramochi et al.[18] there was no significant difference in median EGFR mRNA between primary CRC and liver metastases (median value: 1.35 vs. 1.24, $p = 0.99$). This led the authors to the conclusion that prediction of mRNA levels of EGFR in liver metastases can be obtained by measuring those in the primary CRC.[18]

In contrast, our results are supported by Scartozzi et al.[19] who reported that EGFR expression by IHC (using the DAKO kit) in the primary did not correlate with EGFR expression in related metastatic sites. Bralet et al.[20] using a different EGFR antibody reported differences between primary tumors and related metastases, and also expression differences between lymph node and liver in several cases.

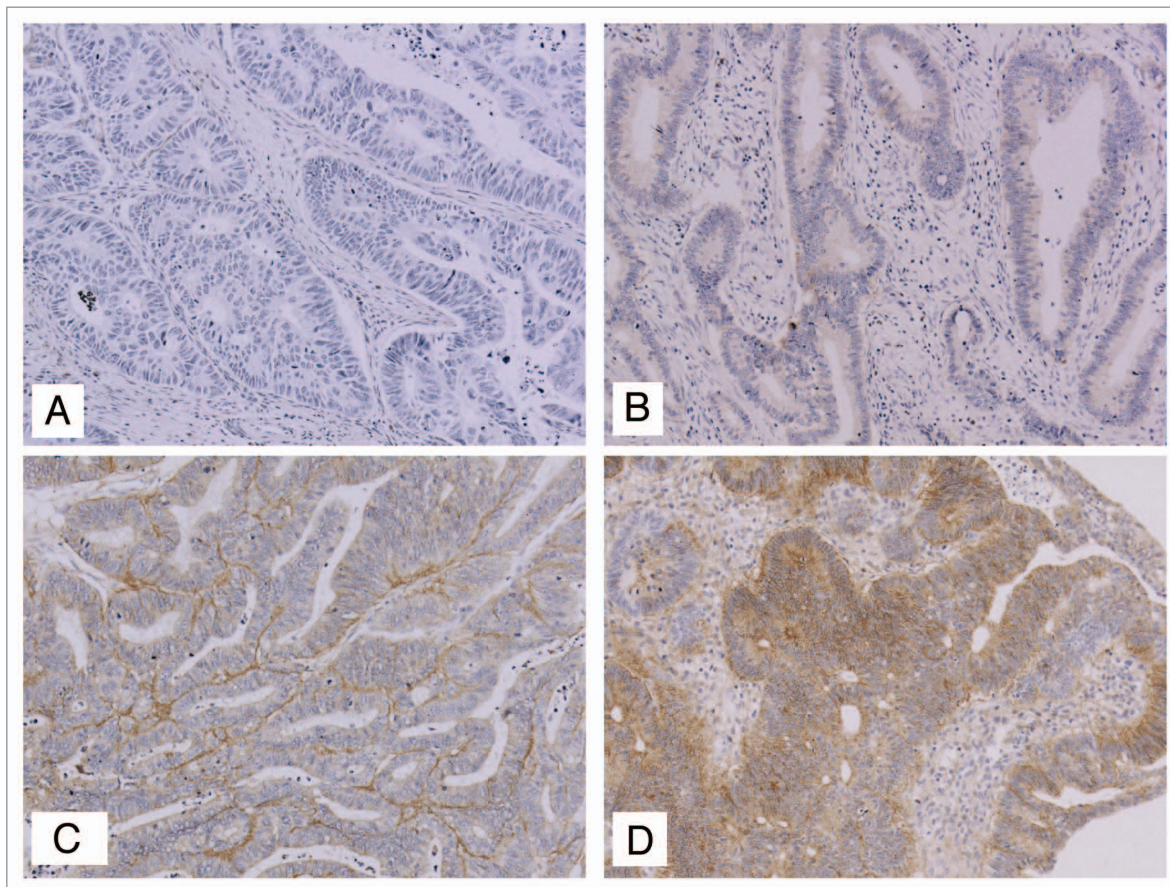
The explanation for the differences in findings between studies may relate to several factors. IHC intensity may be affected by the fixative and duration of fixation of the tissue, the time from fixation or cutting of slides until staining, the conditions of the IHC procedure including use of proteases, heat, titrations and the primary and secondary antibodies. Following staining, there are also differences in the method of scoring of IHC intensity, some using percentage of cells positive in combination with intensity scores and others assessing intensity alone. Variation may also occur due to the intrinsic subjectivity of individual pathologist review. Small sample size may also limit the power to identify small differences. It is arguable that the statistical tests used here (Goodman and Kruskal's or McNemer test) in analyzing individual paired samples provides a more rigorous assessment of correlation compared to tests such as Pearson or Spearmean as used by other authors. The retrospective nature of all studies to date could affect other confounding factors, such as patient selection.

While EGFR IHC in our study did possibly differentiate between primary tumors and metastases, other measures of EGFR activity may also not be concordant. Molinari et al.[17] found a difference in the deregulation of the EGFR gene between the primary and the metastasis in 22% of their cases, as measured by

Table 2. Systemic treatment of patients

	All	Synchronous	Metachronous
Patients receiving chemotherapy at stage IV			
Prior to metastesectomy			
Any chemotherapy	8	5	3
FOLFIRI	5	3	2
XELOX + Bevacizumab	1		1
5Fluorouracil	1	1	
Ralitrexed	1	1	
Post metastesectomy			
Any chemotherapy	31	16	15
FOLFIRI	15	9	6
XELOX + Bevacizumab	2	1	1
5Fluorouracil	8	2	6
Ralitrexed	1	1	
FOLFOX	2	1	1
IXO	1	1	
FOLFIRI + Bevacizumab	1	1	
Capecitabine	1		1
Chemotherapy upon recurrence or progression			
Any systemic therapy	37	20	17
FOLFOX	12	7	5
FOLFIRI	6	2	4
XELOX + Bevacizumab	1		1
5Fluorouracil	1	1	
Capecitabine	5	3	2
XELIRI	2	1	1
IXO	2	1	1
FOLFOX + Bevacizumab	1	1	
Irinotecan	3	1	2
Cetuximab	3	2	1
XELOX + Bevacizumab	1	1	
Patients ever exposed to systemic therapy	43	23	20
TS inhibitor based	43	23	20
Irinotecan	30	17	13
Oxaliplatin	25	13	12
Bevacizumab	5	4	1
Cetuximab	3	2	1

©2010 Landes Bioscience. Do not distribute.



Do not distribute.

Figure 1. Representative examples of EGFR intensity staining in colorectal adenocarcinoma: (A) Absent staining; Note weak positivity of stromal cells as internal control; (B) Weak intensity; (C) Moderate intensity; (D) Strong intensity; (All images at x200).

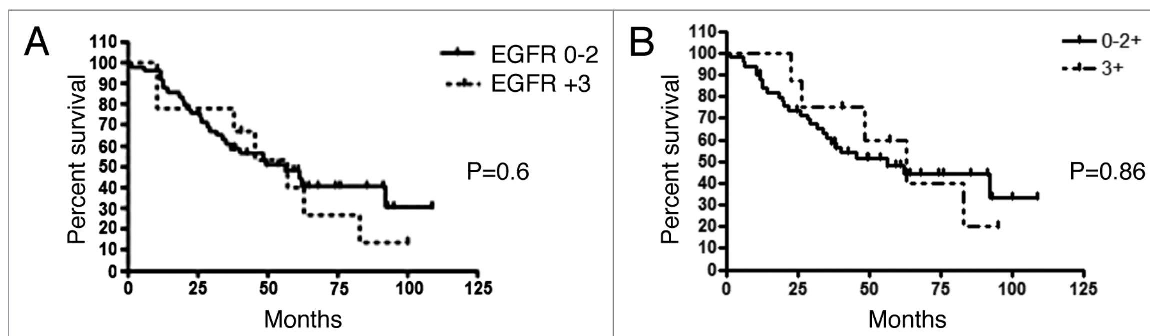


Figure 2. Correlation of EGFR expression with survival. (A) Correlation of primary tumors EGFR expression with survival. (B) Correlation of metastatic tumors EGFR expression with survival.

EGFR gene copy number and EGFR gene amplification. Twelve cases (33%) showed a discordant pattern. Eight cases were found with a trend in favor of EGFR gene deregulation from primary tumor to metastasis and four cases with the opposite trend. These data reflect those obtained by Italiano et al.[21] in non-small-cell lung cancers. Several recent studies have demonstrated that patients showing EGFR gene deregulation defined either by Chr7 polysomy or EGFR gene amplification (cumulatively named CNG) could benefit from Cetuximab treatment.[9,22-25]

We failed to show any correlation between the levels of expression in either the primary or metastatic samples and survival. Although some reports did find correlation with histology and stage most reports failed to show that the level of expression of EGFR correlated with survival.[26] The predictive value of EGFR expression was examined in patients treated with monoclonal antibodies against EGFR (Cetuximab, Panitumumab).

Giralt et al.[27] found that EGFR predicted for a lower response rate to neoadjuvant radiotherapy in rectal cancer. Their study

also found that patients whose tumor expressed EGFR had a shorter disease-free survival (DFS). A study of rectal cancer patients undergoing brachytherapy demonstrated that patients whose tumors had EGFR staining in <85% of cells had a significantly longer median survival [87.0 months (69.0–103.0)] compared to patients whose tumors overexpressed the protein [35.0 months (23.0–58.0)] ($p < 0.001$). A multivariate survival analysis adjusting for T stage, N stage, tumor grade, vascular invasion and age, found that EGFR overexpression was independently associated with worse survival time ($p < 0.001$) [HR (95% CI) = 1.93 (1.44–2.57)].[28] These two studies evaluated a distinct population of patients with rectal tumors undergoing radiotherapy. However, even in this distinct population a contradicting result was reported by Fernbero et al.[29] who immunostained rectal tumors for EGFR and did not find any correlation with prognosis. We note that the studies which reported a prognostic value were those which reported overexpression and not expression at any level.

EGFR mutations in CRC are reported to be a rare event.[30] EGFR mutations are found primarily in the tyrosine kinase domain of the receptor; affecting (in lung cancer) responsiveness to EGFR inhibitors.[31] This prompted us to investigate specifically the tyrosine kinase domain of the EGFR. Like previous reports our analysis showed that the EFGR mutation is a rare event in CRC. However in one of the patients an exon21 C>T mutation was detected interestingly, solely, in the primary tumor and not in the metastasis. This patient presented with a very favorable course of disease exceeding the median overall survival of patients with metastatic CRC. Since this is a single case the predictive or prognostic value of the mutation cannot be evaluated. The loss of a mutated EGFR gene found in the primary tumor in the metastatic lesion was reported in lung cancer. In a series of 18 patients who were with EGFR mutation-positive by direct sequencing of the primary lung tumors 50% of the patients' tumors had lost the mutations in metastases. For 26 lung cancer patients who were EGFR mutation positive in the metastatic tumors, 17 (65%) were negative in the primary tumors.[32] We did not find any corresponding data in colon cancer. EGFR deregulation is emerging as a biomarker with more prognostic and predictive value in patient treated with anti EGFR therapy.[17] Their EGFR IHC and gene analysis in primary tumors, lymph nodes and distant metastases demonstrated variations in the EGFR pathway signaling between primary tumors and related metastasis. These results may have implications for clinical practice and the design of clinical trials using anti-EGFR therapies.

While there has been extensive interest in the value of EGFR intensity as a biomarker for response or survival in colorectal cancer, and while its value as a target of therapy has been established, its measurement in tumors via IHC remains controversial and results are conflicting. With such poor consistency between studies, it is difficult to envision EGFR IHC as a useful biomarker in the near future. Far more important will be the analysis of biomarkers with other methods (such as mRNA levels or specific mutations), as demonstrated in the successful development of KRAS, BRAF and MSI as colorectal biomarkers in recent years.

Methods

A retrospective study of patients who underwent surgical resection for both a primary and metastatic CRC (synchronous or metachronous) was undertaken at The Ottawa Hospital Cancer Center (TOHCC) between 1999 and 2005. Paired paraffin-embedded tissue blocks of primary tumor and resected liver metastases were available for inclusion in the study. The tissues were collected as part of the Ottawa Colorectal Tumor Bank and all patients signed informed consent prior to the collection of the samples. This study was approved by the Ottawa Hospital Research Ethics Board.

IHC Staining. Fifty six paired samples were identified. EGFR immunostaining was performed using the DakoCytomation EGFR pharmDx kit (DAKO) following manufacturer guidelines in the Department of Pathology, Faculty of Medicine, University of Ottawa. Two pathologists, blinded to the clinical information, independently evaluated EGFR staining in both primary tumors and corresponding metastases. EGFR stains the cell membrane and an intensity score from 0 (absent staining) to 3+ was given. A score of 1+ was given when there was weak and focal staining of tumor cells, 2+ when there was moderate staining and 3+ when there was strong staining of tumor cells. When there was significant discrepancy between the scores of the two pathologists, the case was reviewed by both at a multiheaded microscope and a consensus was reached.

©2010 Landes Bioscience. Do not distribute.

EGFR mutation analysis. To evaluate EGFR mutations, genomic DNA was extracted from the formalin-fixed, paraffin-embedded tissue. Ten-micron histological sections were deparaffinized with Hemo-De (Scientific Safety Solvents, TX). The sections were digested for 18 hours at 55°C with proteinase K (1 mg/mL) in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Residual fragments were removed by centrifugation. DNA was precipitated from thesupernatant with 2 volumes of ethanol. The DNA pellet was collected by centrifugation, washed with 80% ethanol, air-dried and dissolved in TE buffer. PCR was performed targeting exons 18, 19 and 21 encompassing most of the tyrosine kinase domain. Nested PCR was performed in a 50 μL vol. containing 100 ng genomic DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM each of deoxynucleotide trisphosphate, 2.5 units of Taq DNA polymerase (Invitrogen, CA) and 10 pmol each of EGFR primers (Exon specific primer sequences and cycling conditions can be found in Cappuzzo et al. 2005[15]). PCR products were purified by polyethylene glycol 8,000 precipitation. PCR products were separated on a 6% polyacrylamide gel. Separation was done at 150 V in Tris-borate-EDTA buffer for approximately 45 min. Gels were stained with ethidium bromide to visualize the fragments. Purified PCR amplicons were sequenced at the DNA Sequencing Facility in the Ottawa Hospital Research Institute.

Statistical analysis. The correlation between primary tumors and related metastatic sites for EGFR expression by IHC was evaluated by means of the Goodman and Kruskal's or McNemer test, appropriate for the assessment of the concordance between two categorical measurements of the same individual. All statistical tests were two sided. Significance levels were set at $p < 0.05$. All statistical analyses were carried out using Stata software version 8.0 (http://www.stata.com).

References

1. Rim SH, Seeff L, Ahmed F, King JB, Coughlin SS. Colorectal cancer incidence in the United States, 1999–2004: an updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology and End Results Program. Cancer 2009; 115:1967-76.
2. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin 2009; 59:366-78.
3. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; 18:2938-47.
4. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000; 355:1041-7.
5. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335-42.
6. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007; 357:2040-8.
7. Sporn MB, Todaro GJ. Autocrine secretion and malignant transformation of cells. N Engl J Med 1980; 303:878-80.
8. Tong WM, Ellinger A, Sheinin Y, Cross HS. Epidermal growth factor receptor expression in primary cultured human colorectal carcinoma cells. Br J Cancer 1998; 77:1792-8.
9. Messa C, Russo F, Caruso MG, Di Leo A. EGF, TGF-alpha and EGF-R in human colorectal adenocarcinoma. Acta Oncol 1998; 37:285-9.
10. Goldstein NS, Armin M. Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. Cancer 2001; 92:1331-46.
11. Capdevila J, Elez E, Macarulla T, Ramos FJ, Ruiz-Echarri M, Tabernero J. Anti-epidermal growth factor receptor monoclonal antibodies in cancer treatment. Cancer Treat Rev 2009; 35:354-63.
12. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351:337-45.
13. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007; 25:1658-64.
14. Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005; 23:1803-10.
15. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 2005; 97:643-55.
16. Italiano A, Saint-Paul MC, Caroli-Bosc FX, Francois E, Bourgeon A, Benchimol D, et al. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors correlates with EGFR expression in related metastatic sites: biological and clinical implications. Ann Oncol 2005; 16:1503-7.
17. Molinari F, Martin V, Saletti P, De Dosso S, Spitale A, Camponovo A, et al. Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may be clinically relevant. Br J Cancer 2009; 100:1087-94.
18. Kuramochi H, Hayashi K, Nakajima G, Kamikozuru H, Yamamoto M, Danenberg KD, Danenberg PV. Epidermal growth factor receptor (EGFR) mRNA levels and protein expression levels in primary colorectal cancer and corresponding liver metastases. Cancer Chemother Pharmacol 2010; 65:825-31.
19. Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Fabris G, Cascinu S. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. J Clin Oncol 2004; 22:4772-8.
20. Bralet MP, Paule B, Adam R, Guettier C. Loss of epidermal growth factor receptor expression in lymph node and liver metastases of colon carcinoma. J Clin Oncol 2005; 23:5844.
21. Italiano A, Vandenbos FB, Otto J, Mouroux J, Fontaine D, Marcy PY, et al. Comparison of the epidermal growth factor receptor gene and protein in primary non-small-cell-lung cancer and metastatic sites: implications for treatment with EGFR-inhibitors. Ann Oncol 2006; 17:981-5.
22. Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005; 6:279-86.
23. Cappuzzo F, Toschi L, Finocchiaro G, Ligorio C, Santoro A. Surrogate predictive biomarkers for response to anti-EGFR agents: state of the art and challenges. Int J Biol Markers 2007; 22:10-23.
24. Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007; 97:1139-45.
25. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 2007; 67:2643-8.
26. Spano JP, Fagard R, Soria JC, Rixe O, Khayat D, Milano G. Epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives. Ann Oncol 2005; 16:189-94.
27. Giralt J, de las Heras M, Cerezo L, Eraso A, Hermosilla E, Velez D, et al. The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis. Radiother Oncol 2005; 74:101-8.
28. Zlobec I, Vuong T, Hayashi S, Haegert D, Tornillo L, Terracciano L, et al. A simple and reproducible scoring system for EGFR in colorectal cancer: application to prognosis and prediction of response to preoperative brachytherapy. Br J Cancer 2007; 96:793-800.
29. Fernebro E, Bendahl PO, Dictor M, Persson A, Ferno M, Nilbert M. Immunohistochemical patterns in rectal cancer: application of tissue microarray with prognostic correlations. Int J Cancer 2004; 111:921-8.
30. Nagahara H, Mimori K, Ohta M, Utsunomiya T, Inoue H, Barnard GF, et al. Somatic mutations of epidermal growth factor receptor in colorectal carcinoma. Clin Cancer Res 2005; 11:1368-71.
31. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350:2129-39.
32. Gow CH, Chang YL, Hsu YC, Tsai MF, Wu CT, Yu CJ, et al. Comparison of epidermal growth factor receptor mutations between primary and corresponding metastatic tumors in tyrosine kinase inhibitor-naive non-small-cell lung cancer. Ann Oncol 2009; 20:696-702.

©2010 Landes Bioscience.
Do not distribute.