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# Coexpression of biological key modulators in primary colorectal carcinomas and related metastatic sites: implications for treatment with cetuximab

A. Barbier[1], J. Domont[2], N. Magné[3,4], J.-L. Goldmard[5], C. Genestie[1], C. Hannoun[6], J.-C. Vaillant[6], A. Bellanger[7], D. Khayat[4], F. Capron[1], J.-P. Spano[4]

[1]Département d'anatomopathologie, groupe hospitalier Pitié-Salpêtrière, Paris, France
[2]Département d'oncologie médicale, institut Gustave-Roussy, Villejuif, France
[3]Département de radiothérapie, institut Gustave-Roussy, Villejuif, France
[4]Département d'oncologie médicale, groupe hospitalier Pitié-Salpêtrière, Paris, France
<jean-philippe.spano@psl.aphp.fr>
[5]Département de statistiques médicales, groupe hospitalier Pitié-Salpêtrière, Paris, France
[6]Département de chirurgie viscérale, groupe hospitalier Pitié-Salpêtrière, Paris, France
[7]Département de pharmacie hospitalière, groupe hospitalier Pitié-Salpêtrière, Paris, France

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**Abstract.** **Background.** Recent studies suggested substantial differences between primary tumors and metastases for EGFR expression in colorectal cancer (CRC). The aim of the study was to correlate the expression of a panel of molecular markers between primary CRC samples and metastases. **Methods.** Expressions of EGFR, pEGFR, VEGF, pVEGF, PTEN, pAKT and p21 were analyzed in 28 primary tumors and 32 liver metastases by immunohistochemistry performed on formalin-fixed, paraffin-embedded sections from 46 CRC patients. The molecular profiles were evaluated by tissue micro-array. The correlation between tumor and metastasis biomarker expressions was tested. **Results.** Among 60 CRC samples, 25% were EGFR positive, 38% were pEGFR positive, 38% were VEGF positive, 48% were pVEGF positive, 70% were pAKT positive and 51% were p21 positive. PTEN was deleted in 39% of cases and absence of p21 expression was found in 49% of cases. A significant correlation was observed between primary tumors and metastases for pAKT (p = 0.037) and pEGFR (p = 0.0002) status. In patients treated with cetuximab-based therapy (n = 18), p21 appeared as a significant predictive factor of response (p = 0.036). **Conclusion.** Biomarkers status may change between primary and metastatic sites in CRC, with potential implications for the identification of patients who are likely to respond to anti-EGFR treatment. ▲

**Keywords:** colorectal cancer, EGFR, metastases, molecular markers, primary tumor, pAKT

## Introduction

During the past few years, the systemic treatment of colorectal cancer (CRC) has become a rapidly evolving field. For more than 40 years, 5-fluorouracil (5-FU) was the standard of care for patients with metastatic CRC (mCRC). The addition of effective newer cytotoxic agents, such as irinotecan and oxaliplatin to 5-FU-based therapies, the introduction of oral fluoropyrimidines and the recent development of targeted agents have prolonged the overall median survival time from one to two years [1-3]. Recently, three targeted agents were approved in the treatment of mCRC: the anti-vascular endothelial growth factor (VEGF) monoclonal antibody (mAb), bevacizumab in combination with first-line 5-FU-based chemotherapy regimens [4] and the human epidermal growth factor receptor (EGFR)-targeted mAbs, cetuximab as monotherapy or in combination with irinotecan as second-line therapy in refractory cancer [5, 6] and panitumumab after progression with 5-FU, oxaliplatin and irinotecan [7].

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Based on the importance of the EGFR axis in tumorigenesis and tumor progression, EGFR expression has been investigated as a possible prognosis indicator in CRC. EGFR expression was found in up to 82% of CRC [5, 6, 8]. In a review of 200 studies involving more than 20,000 patients and 10 cancer types, Nicholson *et al.* showed that increased EGFR expression was associated with reduced recurrence-free or overall survival rates in 52% of studies (13/25) with EGFR status considered as a modest prognostic indicator in CRC [9]. With the advent of EGFR targeted therapies, immunohistochemical screening strategies for EGFR expression were developed in clinical trials to select patients with EGFR-expressing tumors. However, the clinical data do not support a relationship between EGFR expression as assessed by immunohistochemistry (IHC) and response to EGFR-targeted mAbs [10-12].

Several technical and biological factors could be advocated to explain this lack of correlation. Recently, Francoual *et al.* pointed out the existence of a heterogeneous population of EGFR in CRC tumors with both one class (78% of tumors with physiologically relevant high-affinity binding sites) and two classes of binding sites (22% of tumors with a mixed presence of low- and high-affinity binding sites) and suggested that IHC could not be sensitive enough to quantify EGFR as high-affinity EGF binding sites [13]. From a biological point of view, the EGFR signaling pathway is complex and other molecular mechanisms such as activating EGFR mutations, increased ligand expression, alteration of downstream signaling pathways and cross-talk among different erB receptor family members are critically involved in the action of anti-EGFR mAbs, and therefore more predictive of treatment response than the total level of the receptor *per se* [14]. Currently, some biomarkers have been identified in CRC tumor samples as potential predictors of response to cetuximab or panitumumab therapy, i.e. activated EGFR [15], EGFR amplification [16], absence of KRAS mutations [17-21], PTEN (phosphatase protein homologue to tension) expression [22], low VEGF receptor expression [23], nuclear factor-κB tumor expression [24] or epiregulin and amphiregulin expression [20].

Another explanation suggested for the apparent discordance between EGFR status and clinical response to anti-EGFR mAb therapy in mCRC is a possible difference in EGFR status between primary tumor, which was usually assessed in the clinical trials, and related metastatic sites [25]. Scartozzi *et al.* showed that 36% of primary tumors expressing EGFR showed a loss of expression in the corresponding metastatic sites [25]. However, conflicting data were reported thereafter [26-28]. Moreover, very few data are available for other biological markers from major downstream signaling pathways that could emerge as new molecular predictive factors to anti-EGFR therapies [29-31].

The aim of the present study was to analyze and compare the expression of a panel of molecular markers, namely EGFR, phospho-EGFR (pEGFR), VEGF, PTEN, phospho-AKT (pAKT) and p21, assessed by IHC on tissue microarrays of primary colorectal tumor samples and/or liver metastases. In a subgroup of patients treated with cetuximab, the predictive value of these biomarkers on the response to treatment was also evaluated.

## Patients and methods

### Patients

Forty-six consecutive patients, who underwent surgical resection of the primary colon tumor and/or the liver metastases and treated at the Oncology Department of the Pitié Salpétrière Hospital (Paris, France), were selected from a pathological database of colorectal cancer cases. Eighteen patients were treated with cetuximab at an initial dose of 400 mg/m$^2$ intravenously followed by weekly doses of 250 mg/m$^2$ combined with irinotecan-based chemotherapy. Tumor response was evaluated by computerized tomodensitometry according to the Response Evaluation Criteria in Solid Tumors [32] and classified as complete (CR), partial response (PR), stable disease (SD) or progressive disease (PD). This retrospective study was approved by independent local ethics committee. The study was conducted in accordance with the Declaration of Helsinki (1996). All patients provided written informed consent.

### Tumor specimens and tissue microarray

Sixty-three paraffin-embedded specimens from primary CRC (designed as « T ») (29 samples) and liver metastases (« M ») (34 samples), resected before treatment, were available. For 14 cases, samples « T » and « M