Anti-epidermal growth factor receptor monoclonal antibody-based therapy for metastatic colorectal cancer: a meta-analysis of the effect of PIK3CA mutations in KRAS wild-type patients

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Abstract

Introduction: We conducted a meta-analysis to dissect the association between PIK3CA mutations (exon 9 and exon 20) and resistance to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (MoAbs) in KRAS wild-type metastatic colorectal cancer (mCRC) patients.

Material and methods: In 11 previously published studies, 864 cancer patients were treated with cetuximab or panitumumab-based therapy. Primary outcomes included objective response (complete response + partial response vs. stable disease + progressive disease), progression-free survival (PFS), and overall survival (OS). We calculated the odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals (CIs) to estimate the risk or hazard. We found consistent and clinically substantial risk or hazard for objective response, PFS, and OS in the cetuximab or panitumumab-treated mCRC patients.

Results: PIK3CA mutations as a whole were associated with reduced response and poor PFS and OS in KRAS wild-type mCRC patients. PIK3CA exon 9 mutations had no effect, whereas exon 20 mutations were associated with a worse outcome compared with wild types, with an OR of 0.21 (95% CI 0.05–0.93).

Conclusions: PIK3CA mutations as a whole might be useful prognostic factors for assessing clinical outcomes of anti-EGFR MoAb-based chemotherapies in KRAS wild-type mCRC patients. In particular, PIK3CA exon 20 mutations were significantly associated with lack of response.

Key words: colorectal cancer, PIK3CA, monoclonal antibodies, meta-analysis.

Introduction

Colorectal cancer is one of the most common cancers worldwide and one of the leading causes of cancer mortality. There has been an increase in the incidence of colorectal cancer in Poland [1]. Despite ad-
vances in chemotherapy, the 5-year relative survival remains poor at just 11% for patients with metastatic colorectal cancer (mCRC) [2]. Currently, two monoclonal antibodies (MoAbs) targeted at epidermal growth factor receptor (EGFR), the chimeric IgG1 MoAb cetuximab and the fully humanized IgG2 MoAb panitumumab, have shown a relevant clinical effect in treatment of patients with chemotherapy-refractory mCRC [3–6]. Because of common resistance to anti-EGFR MoAbs, recent guideline recommendations suggest that anti-EGFR MoAbs be given only to patients with KRAS wild-type mCRC [7, 8]. However, even for non-carriers of KRAS mutations, the response rate to anti-EGFR MoAbs is not high, ranging from 17% to 60%, and only a subset of patients benefit from this treatment [9–18]. This heterogeneity suggests that there may be other predictive variables, besides KRAS, that determine responsiveness to anti-EGFR. Thus, the predictive value of additional mutations and deregulations of signaling pathways downstream of EGFR such as BRAF, PIK3CA or PTEN is currently under intensive investigation.

Specifically, what is highlighted in the exploration is PIK3CA. PIK3CA encodes the p110α catalytic subunit of the class IA phosphatidylinositol 3-kinases (PI3Ks). Tumor-derived PIK3CA mutations lead to constitutive activation of p110α enzymatic activity, stimulate the AKT pathway, and promote cell growth [19]. PIK3CA is frequently mutated in several malignancies such as colon, breast, brain, ovarian, liver, and lung cancers [20]. Mutation frequencies in CRC vary from 10% to 60% [17, 21]. The vast majority of activating PIK3CA mutations map to 3 sites: exon 9, codons 542 and 545 in the helical domain, and exon 20, codon 1047 in the kinase domain.

Emerging data have suggested that PIK3CA mutation is likely to be predictive of a lack of benefit from anti-EGFR therapy in mCRC [22–24], but the results are still inconclusive, partially due to the inclusion of molecularly unselected populations. For example, Prenen et al. suggested that PIK3CA mutations were not a major determinant of resistance to the anti-EGFR MoAbs in unselected mCRC patients [25], whereas De Roock et al. found that patients with PIK3CA mutations had a significantly lower response rate in KRAS wild-type patients [26]. Besides, the relatively small sample size of each study may have an effect. For example, Sartore-Bianchi et al. suggested that PIK3CA mutations were significantly associated with clinical resistance to anti-EGFR MoAbs [22] while Moroni et al. showed that PIK3CA alterations did not correlate with response [21]. Therefore, it is necessary to conduct a new meta-analysis to derive a more precise estimation of predictive value of PIK3CA mutations in KRAS wild-type mCRC patients treated with anti-EGFR MoAbs.

Material and methods

Study selection

Systematic computerized searches of the PubMed and HuGENet databases (up to 25th February 2013) were performed. The following search terms were used: ‘cetuximab’, ‘panitumumab’, ‘colon cancer’, ‘rectal cancer’, ‘colorectal cancer’, ‘colorectal neoplasm’, ‘CRC’, ‘PIK3CA’, ‘phosphoinositide-3-kinase catalytic’, ‘α polypeptide’, ‘PI 3-kinases’. References of the retrieved articles were further screened for earlier original studies. The inclusion criteria were as follows: (i) the studies focused on mCRC patients; (ii) those exploring the relation between PIK3CA mutations and clinical outcomes of KRAS wild-type mCRC patients treated with anti-EGFR MoAbs; (iii) those using one or more of the following as outcomes to assess tumor response and survival: objective response, PFS, and OS.

Statistical analysis

For each outcome measure, we estimated effects separately for patients with wild-type PIK3CA and PIK3CA mutations. For overall response rate, an odds ratio (OR) was calculated from the reported number of objective response (complete response (CR) + (partial response (PR)) and no response (progressive disease (PD) + stable disease (SD)) in each arm, using the WHO criteria or RECIST (Response Evaluation Criteria in Solid Tumors) criteria [27]. In order to dissect the complicated relation between PIK3CA status and prognosis in relation to the treatment, we did stratified analyses and estimated the pooled OR according to KRAS mutation status. As different biological effects have been suggested for PIK3CA exon 9 (helical domain) and exon 20 (kinase domain) mutations [28–30], the OR or hazard ratio (HR) was also estimated for each type of mutation as well as for any PIK3CA mutations. Progression-free survival (PFS) and overall survival (OS) were evaluated by pooled Cox proportional HRs and 95% CIs published by published methods. The between-study heterogeneity was evaluated with $I^2$ and 50% in $I^2$ was regarded as the threshold. We carried out initial analyses with a fixed effect model and confirmatory analyses with a random effect model, if there was significant heterogeneity. We used inverted funnel plots and the Egger test to examine the effect of publication bias. All analyses were carried out using the Stata 9.2 and RevMan 5.0 software.

Results

The literature search (as of February 25, 2013) yielded 95 potentially assessable publications. Of these, 74 were excluded for the following reasons: not original studies, not human studies, not cohort
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95% CI 0.23–0.75; \( p = 0.003 \); Figure 2). In stratified analyses, the association remained significant in subgroups of exon 20 mutations (OR = 0.21; 95% CI 0.05–0.93; \( p = 0.04 \)), whereas exon 9 mutations had no effect (OR = 0.54; 95% CI 0.26–1.12; \( p = 0.10 \)). Given that all the ORs for the exon 9 subgroup were less than 1 except the study of Moniani et al., we performed an influence analysis by excluding the study. The result was similar to that of the former (OR = 0.46; 95% CI 0.21–1.01; \( p = 0.05 \); data not shown). No publication bias was detected by either the funnel plot or the Egger test (\( p = 0.065, p = 0.060, p = 0.185 \) for all exons, exon 20 and exon 9, respectively).

PIK3CA mutations and progression-free survival and overall survival in KRAS wild-type patients

Four studies including 545 patients were eligible for the final analysis. The PIK3CA mutations were associated with a significant increase of hazard for PFS in all patients (mutant vs. wild-type: HR = 1.54; 95% CI 1.13–2.09; \( p = 0.006 \); Figure 3). No significant between-study heterogeneity was detected.

Figure 1. Flow chart for the process of selecting eligible publications
| References          | Country of origin | Year  | Mutation analysis methods | No. of KRAS wild-type patients | Age | Previous treatment protocols | Study treatment protocols | Outcomes | Criteria          |
|---------------------|-------------------|-------|---------------------------|--------------------------------|-----|-----------------------------|----------------------------|----------|------------------|
| Soeda et al. [40]   | Japan             | 2012  | DS                        | 31                             | 57  | ≥ 1 Chemotherapy            | C alone; or C + I         | CR, PR, SD, PD | RECIST           |
| Molinari et al. [41]| Italy, Switzerland| 2011  | DS                        | 67                             | 60  | I and/or O                  | C alone, P alone, or C + Chem | PR, SD, PD | RECIST           |
| Spindler et al. [39]| Denmark           | 2011  | DxS                       | 53                             | 62  | ≥ 1 Chemotherapy            | C + I                      | PFS, OS, CR, PR, SD, PD | RECIST           |
| Wong et al. [24]    | USA               | 2011  | DxS                       | 19                             | 56  | ≥ 1 Chemotherapy            | C + capecitabine, C+ Q, C+B | CR, PR, SD, PD | RECIST           |
| Spindler et al. [39]| Denmark           | 2011  | DxS                       | 53                             | 62  | ≥ 1 Chemotherapy            | C + I                      | PFS, OS, CR, PR, SD, PD | RECIST           |
| Wong et al. [24]    | USA               | 2011  | DxS                       | 19                             | 56  | ≥ 1 Chemotherapy            | C + capecitabine, C+ Q, C+B | CR, PR, SD, PD | RECIST           |
| Saridaki et al. [18]| Greece            | 2011  | Sanger sequencing         | 75                             | 66  | ≥ 1 Chemotherapy            | C + I based, or C + O based | TTP, OS | NR               |
| De Roock et al. [26]| 7 countries       | 2010  | DxS                       | 339                            | 61  | ≥ 1 Chemotherapy            | C + Chem                   | PFS, OS, CR, PR, SD, PD | RECIST or WHO |
| Perkins et al. [16] | France            | 2010  | DS                        | 23                             | 61.8| I based                     | C alone, P alone, C + I, or C + FOLFIRI | CR, PR, SD, PD | RECIST           |
| Prenen et al. [25]  | Belgium           | 2009  | AD + DS                   | 122                            | 61  | I based                     | C + I, or C alone          | PFS, OS, CR, PR, PD, SD | RECIST           |
| Saridaki et al. [18]| Greece            | 2011  | Sanger sequencing         | 75                             | 66  | ≥ 1 Chemotherapy            | C + I based, or C + O based | TTP, OS | NR               |
| Peronne et al. [23] | Italy             | 2009  | DS                        | 18                             | 57.3| I based                     | C + I                      | PFS, PR, SD, PD | RECIST           |
| Moroni et al. [21]  | Italy             | 2005  | DS                        | 21                             | 65.6| ≥ 1 Chemotherapy            | C alone, P alone, or C + I based | PR, SD, PD | RECIST           |

AD – allelic discrimination, C – cetuximab, Chem – chemotherapy, DS – direct sequencing, DxS – DxS PI3K Mutation Test Kit, FOLFOX – fluorouracil, folinic acid, and oxaliplatin, FOLFIRI – fluorouracil, folinic acid, and irinotecan; I – irinotecan, NR – not reported, C – cetuximab, P – panitumumab, B – bevacizumab, O – oxaliplatin, PFS – progression-free survival, OS – overall survival, OR – odds ratio, TTP – time to tumor progression, CR – complete response, PR – partial response, SD – stable disease, PD – progressive disease.
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found in the initial fixed model, so a random model did not need to be performed ($I^2 = 36.6\%$). Additionally, Egger’s test did not show publication bias ($p = 0.894$), and no significant outcome of influence analysis was observed (data are not shown).

Three studies including 527 patients were eligible for the final analysis. There was a substantial effect of PIK3CA mutations on death hazard in all patients (mutant vs. wild-type: HR = 1.40; 95% CI 1.02–1.91; $p = 0.036$; Figure 4). No significant between-study heterogeneity was observed in the initial fixed model ($I^2 = 0\%$). Moreover, no publication bias was detected with a $p$ value of 0.552 in Egger’s test, and no significant outcome of influence analysis was observed (data are not shown).

Discussion

This meta-analysis used objective response, PFS, and OS as primary parameters to assess the influence of PIK3CA mutations on clinical outcomes of anti-EGFR MoAb chemotherapy in mCRC with KRAS wild-type. Our results imply that PIK3CA mutations were a biomarker of low objective response as well as short PFS and OS in KRAS wild-type patients with anti-EGFR MoAb chemotherapy.

Our study has some strengths although a similar meta-analysis was conducted before. The previous meta-analysis suggested that PIK3CA exon 20 mutations might be a potential biomarker for resistance to anti-EGFR MoAbs in KRAS wild-type mCRC [38], even though it failed to reach stati-
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...most likely due to the small sample size. Moreover, flaws in the previous meta-analysis were that PFS and OS data were not sensibly combined. Because of the different ways and the incompleteness of reported data, the previous authors stated that the results could not be combined. But we believe that it is possible to combine the survival data if they were made full use of. For example, there were two duplicate studies, and we included a study [9] which provided survival data in wild-type KRAS patients, while the previous authors excluded it. In addition, a study was available because original data information was provided by the authors [23]. Recently, 6 studies with a larger sample size have been reported [32, 36, 37, 39–41]. Several studies have suggested that PIK3CA mutation was likely to be predictive of a lack of benefit from anti-EGFR therapy in mCRC. Among them, two studies [39, 40] provided data in KRAS wild-type patients and two studies [36, 37] were in patients unselected by KRAS mutation status. However, one study suggested that PIK3CA alterations were not associated with response [41]. Considering the inconsistent conclusions, we updated the previous meta-analysis and confirmed its finding that PIK3CA exon 20 mutations were a potential biomarker for a low objective response in KRAS wild-type patients treated with anti-EGFR MoAbs.

Notably, the result is different if mutations on exon 9 and exon 20 are considered separately, as confirmation of the fact that the two exons have different mechanisms of action and related effects [42]. To induce transformation exon 20 mutants depend on binding with the regulatory subunit p85α, whereas exon 9 mutants circumvent p85α binding but depend on RAS binding instead [43, 44]. Regarding objective response, exon 20 mutations alone significantly decrease the chance of achieving an objective response, whereas exon 9 mutations are unable to reproduce these data (Figure 2). Unfortunately, the survival results could not be divided into exon 9 and exon 20 mutations in a meta-analysis so far. Only one study by De Roock et al. [26] provided survival data according to the PIK3CA exon of the mutations. It reported that PIK3CA exon 20 mutations were statistically significantly associated with shorter PFS and OS.
in KRAS wild-type patients. It seems that the predictive power of exon 20 mutation is greater than that of any exon mutations and exon 9 mutations. A recent study in which stage χ CRC patients were present in 151 (13%) cases found that coexistence of PIK3CA exon 9 and 20 mutations, but not PIK3CA mutation in either exon 9 or 20 alone, was associated with poor prognosis of CRC (not mCRC) patients [45]. So far, it is not certain whether exon 20 mutation could be a potential biomarker for resistance to anti-EGFR MoAbs in KRAS wild-type mCRC. Taking lessons from studies in which the distinctive prognostic role of exon 9 and exon 20 mutations has been described, additional larger studies are still warranted to investigate the possibilities since the ultimate aim of the treatment of mCRC is to prolong the survival without affecting health-related quality of life parameters.

Despite our efforts to make an accurate and comprehensive analysis, limitations of our meta-analysis need to be addressed. First, most of the included studies were retrospective and not all the methods of PIK3CA mutation determination were consistent. This may have caused heterogeneity between studies. Second, not all treatment arms used anti-EGFR MoAbs as a single compound but in combination with irinotecan, oxaliplatin or 5-Fu, which could cause some bias in our estimates but was unlikely to change our major conclusions, because no studies showed that chemotherapy correlated with PIK3CA. Third, only 4 studies presented data on HR with 95% CI for PFS and only three studies presented data on HR with 95% CI for OS. The relatively small sample size might not have enough statistical power to detect the real association. Fourth, our analysis largely used unadjusted estimates, because not all published studies presented adjusted estimates, or when they did, the estimates were not adjusted by the same potential confounders. Fifth, we were unable to analyze the association between PIK3CA mutations and cetuximab or panitumumab toxicities, because few studies provided this information or used different toxicity profiles. Finally, our study was restricted to mCRC patients with KRAS wild-type, so we failed to assess the influence of other mutations. BRAF mutations have been associated with a poor prognosis in colorectal cancer [46] and might therefore confound analyses of PIK3CA mutations and survival in colorectal cancer. In contrast, Ulivi et al. concluded that BRAF and PIK3CA mutations would seem to be independent predictors of anti-EGFR therapy effectiveness [37]. PIK3CA exon 9 mutations in primary tumors and loss of PTEN nuclear expression in metastases correlated with KRAS mutations [47]. We were unable to investigate potential interactions between PIK3CA mutations on exon 9 and exon 20 due to the limited publications available on this topic in which only one sample carried a mutation in both exon 9 and exon 20 [25].

Overall, our meta-analysis showed that PIK3CA mutations as a whole might be useful prognostic factors for assessing clinical outcomes and further confirmed that PIK3CA mutation on exon 20 decreases the response rate of anti-EGFR MoAb-based chemotherapies in wild-type KRAS mCRC patients. But we could not exclude the potential confounding by the interaction effect of other mutations which frequently associated with PIK3CA exon 20 mutations. We also strongly recommend that exon 9 and 20 mutations be studied separately. And future prospective studies with a larger sample size are required to confirm our findings.

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Lulu Huang and Zhenfang Liu have contributed equally to this work and should be considered as co-first authors.

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