Mini-review

Clinical relevance of \textit{KRAS} mutations in codon 13: Where are we?

Tze-Kiong Er$^a$, Chih-Chieh Chen$^{b,c,d}$, Luis Bujanda$^e$, Marta Herreros-Villanueva$^{e,f,*}$

$^a$ Division of Molecular Diagnostics, Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
$^b$ Center for Lipid Biosciences, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
$^c$ Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsinchu, Taiwan
$^d$ Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan
$^e$ Department of Gastroenterology, Hospital Donostia/Instituto Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad del País Vasco UPV/EHU, San Sebastián, Spain
$^f$ Division of Oncology Research, Schulze Center for Novel Therapeutics, College of Medicine, Mayo Clinic, Rochester, MN 55905, USA

\textbf{ARTICLE INFO}

Article history:
Received 26 July 2013
Received in revised form 19 August 2013
Accepted 11 September 2013

\textbf{Keywords:}
Anti-EGFR therapies
Codon 12 and 13
Colorectal cancer
\textit{KRAS}

\textbf{ABSTRACT}

Recent advances in molecular diagnosis and the trend towards personalized medicine have made colorectal cancer one of the tumors where therapies have significantly improved patient survival after metastasis development. \textit{KRAS} mutations in codon 12 and 13 are recognized biomarkers that are analyzed in clinic previously for anti-EGFR therapies administration. Since originally mutations in both codons were considered as a predictor of lack of response to cetuximab or panitumumab, the European Medicines Agency and the US Food and Drug Administration suggested that patients harboring any of those mutations should be excluded from the treatment. However, subsequent retrospective analysis has shown that mutations in codon 12 and codon 13 of \textit{KRAS} gene could be different in their biological characteristics and as a result could confer variable effects in patients. In addition and increasing and sometimes contradictory number of solutions have been published demonstrating that patients with mutations in codon 13 could have worse outcome but could obtain a significant clinical benefit from anti-EGFR therapies. Here, we review and update the latest data on the biological role leading to a predictive outcome and benefit from anti-EGFR antibodies in patients with specific \textit{KRAS} mutations in codon 13.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide with an overall 5-year survival of 11% for patients with metastatic disease [1].

The immunoglobulins G1 monoclonal antibodies against EGFR, cetuximab (Erbitux$^\text{®}$, ImClone Systems) and panitumumab (Vectibix$^\text{®}$, Amgen) have been proven effective in combination with chemotherapy or as a single treatment of patients with metastatic colorectal cancer (mCRC) [2,3]. These monoclonal antibodies bind to the Epidermal Growth Factor Receptor (EGFR), preventing binding and activation of different EGFR downstream signaling pathways [4].

Mitogen-activated protein kinase (MAPK) signaling is one of the most important pathways situated downstream of EGFR, which is activated as a consequence of mutation in \textit{KRAS} gene.

It has been widely demonstrated that mutation in \textit{KRAS}, frequently in codon 12 and 13 and less common in codon 61, 63 and 146 are major predictive biomarkers for resistance to anti-EGFR treatment [5–7]. As a result of these findings, the European Medicines Agency and US Food and Drug Administration (FDA) advised that patients harboring mutations in codons 12 and 13 of \textit{KRAS} gene should not receive cetuximab or panitumumab.

\textit{KRAS} mutations in codon 12 and 13 are reported in approximately 40%–60% of all colorectal cancer specimens [7–10] what constitutes a significant part of the patients with reduced treatment options compared with those who are \textit{KRAS} wild type (WT). By contrast, mutations in codon 61, 63 and 146 occur at much lower prevalence (<5% of total \textit{KRAS} mutations) [11] and their clinical relevance has not been studied yet.

Taking together those data, only around 50% of patients with metastatic CRC could receive anti-EGFR therapies. Nevertheless, resistance to cetuximab is common and only 10–20% of patients truly benefits from the treatment. Several reasons have been pointed out as contributors to the fail therapy, i.e. mutations in BRAF, PI3K [12,13].

In addition, since personalized medicine has become more clinically relevant in the last few years, several studies have analyzed the significance of specific mutations. As a consequence, in colorectal cancer multiple results indicate that not all \textit{KRAS} mutations are equal in their biological characteristics and as a result they could confer variable effects.

Specifically, \textit{KRAS} codon 13 mutations showed greater transforming ability in vitro compared with mutations in codon 12.
KRAS effects. don and the amino acid changed conferring variable activated structural modifications will be different in case of each co-

with constitutive activation of the KRAS protein. Nonetheless, structural changes the EGFR signaling pathway is out of control binding pocket of the protein, interfering with the steric hindrance the projection of larger amino acid side chains into the GDP/GTP

binding pocket of the protein, interfering with the steric hindrance the projection of larger amino acid side chains into the GDP/GTP

The incorporation of other amino acids, most usually aspartate and valine at codon 12 and aspartate at codon 13[19], brings about and consequently patients who harbor this mutation could benefit from anti-EGFR therapy.

The purpose of this review is to provide an update of the latest information on the biological role leading to a predictive outcome and benefit from anti-EGFR antibodies in patients with specific KRAS mutations in codon 13.

2. Biological role of specific KRAS mutations in codon 13

Mutations in exon 2, codon 12 and 13 lead to alterations in encoded amino acids adjacent to the GDP/GTP binding pocket (Fig. 1), reducing or abolishing the GTPase activity of KRAS protein after guanine nucleotide activating protein (GAP) binding and locking the protein in an active, GTP-bound state [18]. Although in both cases, codons 12 and 13 in KRAS WT encode the glycine amino acid, the residue changed after mutation is frequently different. The incorporation of other amino acids, most usually aspartate and valine at codon 12 and aspartate at codon 13[19], brings about the projection of larger amino acid side chains into the GDP/GTP binding pocket of the protein, interfering with the steric hindrance in GTP hydrolysis [20]. Consequently to those conformational and structural changes the EGFR signaling pathway is out of control with constitutive activation of the KRAS protein. Nonetheless, these structural modifications will be different in case of each codon and the amino acid changed conferring variable activated KRAS effects.

In particular, retrospective studies in vitro suggested that the mutation in codon 13 of the KRAS (G13D), a glycine to aspartate, exhibit weaker transforming activity than codon 12 mutations [14]. Also some studies have demonstrated a reduced transforming capacity of the codon 13 mutation as compared with the codon 12 mutation in vitro and in vivo experimental systems [21]. A small number of available experimental data show that tumor clones carrying KRAS codon 13 mutations are less aggressive than those with codon 12 mutations because they show higher levels of apoptosis [22] but still the mechanisms are not fully understood. In addition, recent computational analysis demonstrated that KRAS mutations in codon 13 have similar behavior as KRAS wild type and consequently patients who harbor this mutation could benefit from the addition of anti-EGFR antibodies [23].

All of these data show that theoretically, codon 12-mutated KRAS remains in an active GTP-bound state longer than codon 13-mutated or WT KRAS. Herein, it seems that mutations in codon 13 can confer similar protein structure dynamics to WT KRAS and consequently possess reduced transforming capacity in tumoral cells.

In fact, these data support the clinical findings from Morelli and Kopetz [24] suggesting that although codon 12 and 13 KRAS mutations may be induced at a similar rate in precursor lesions in the colon, KRAS codon 12 mutations increase in frequency relative to KRAS codon 13 mutations during tumor progression.

In conclusion, the biological role of KRAS mutations in codon 13 described above supports its putative prognostic value and implications in the therapy setting. Therefore, multiple studies have been performed in this regarding during the last few years.

3. KRAS mutations in codon 13 as a prognostic marker

KRAS mutations, as a prognostic factor has been assessed in numerous studies and although the majority of them show those mutations as an adverse prognostic indicator, the results are still conflicting [25–28] (Table 1).

While most authors agree that different KRAS gene mutations have different impacts on the outcome [19], it has to be indicated that the initial analysis considered both mutations, in codon 12 and 13, as a whole. Only a small and very recent detailed analysis estimated the effect of KRAS mutations when codon 12 and 13 are counted separately.

In fact, initial data from clinical trials including those obtained from RASCAL study concluded that KRAS status is an important factor for progression and outcome in the CRC and glycine to valine in codon 12 was associated with poorer prognosis (shorter disease-free interval and lower survival rate) [19,29,30]. This study, has been broadly accepted around the world since more than four thousand patients from 21 different countries were included and data analysis was very robust.

Regarding to mutations in codon 13, Pajkos et al. [31] conducted a study in 88 Hungarian patients and concluded that mutations in codon 13 appear more frequently in cases of local recurrence and Samowitz et al. [32], based on a population study of 1413 patients including black, white and Hispanic subjects, that mutation 13G-A portends worse prognosis. Bazan et al. [33] analyzed 160 patients in Italy and showed that the combination of KRAS mutation in codon 13 are also associated with worse prognosis, advanced

![Fig. 1. Structural analysis of the (A) WT, (B) G12D and (C) G13D KRAS proteins. Cluster of GTP-binding residues are shown in electrostatic potential surfaces mode in the same orientation. Electrostatic potential surfaces in blue for positive, white for hydrophobic, and red for negative. High negative potentials are found at the residues 12 and 13 for the structures of G12D and G13D, respectively. All images were generated by the PyMOL software. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)
Dukes’ stages and lymph-node metastasis. These authors, in agreement with Smowitz et al. found that codon 13 mutation, most common G to A transitions, had a stronger predictive value than any KRAS mutations (considering all mutations in codon 12 separately). Previous findings also showed that compared with KRAS codon 12 mutations, codon 13 mutated CRC present as a more aggressive disease frequently associated with local and distant metastases at first diagnosis [34]. By contrast, more recent analysis done by Imamura et al. using a molecular epidemiology database with 1261 patients (1075 for KRAS), show that compared with KRAS wild type, KRAS codon 12 mutations, but not codon 13 mutations are associated with inferior survival in colorectal cancer [35]. Considering the outcome of patients as responders to therapies, data from different randomized studies have shown that both KRAS codon 12 and 13 mutations have a negative prognostic value in mCRC, receiving different chemotherapies on the basis of 5FU and Oxaliplatin [36]. Patients with codon 13 mutations seem to benefit more in terms of progression free survival (PFS) and (overall survival) OS from the oral capicetabine although the objective response rate (ORR) was lower [37]. In addition, De Roock et al. [38] and Tejpar et al. [39] reported that patients with codon 13 mutations in KRAS exhibit a worse overall prognosis with short overall survival times under standard chemotherapy.

In conclusion, since the majority of studies demonstrated that KRAS mutation in codon 13 confers worse prognosis and outcome for patients, several clinical investigations considered several therapeutic options including those that take into account anti-EGFR antibodies and its effectivity has been evaluated.

### 4. KRAS mutations in codon 13 and response to anti-EGFR

Several findings indicated that KRAS G13D mutation may have a unique behavior of colorectal cancer patients, survival and different effects on anti-EGFR therapies responses. Messner et al. [40] demonstrated in different cell lines with a G13D mutation that cetuximab and panitumumab treatment induce significant growth inhibition in contrast to cell lines with mutations at codon 12 or 61.

In fact, a few authors reported that patients with G13D mutation could obtain benefit from cetuximab or panitumumab although the clinical relevance of these data has not been fully clarified and there is not consensus in the oncology field. The first data from Benvenuti et al. analyzing 48 patients enrolled into clinical trials of panitumumab or cetuximab, indicated that a small percentage of patients, around 10% (1 harboring G13D mutation of 11 responders) with KRAS mutated tumors could respond to anti-EGFR therapy [41]. Then, KRAS mutational analysis of 113 patients with irinotecan refractory therapy treated with cetuximab in clinical trials (EVEREST, BOND, SALVAGE and BABEL from 4 Belgian centers) demonstrated that about 15% of patients with KRAS mutations in codon 12 have long term disease stabilization [42]. However, regarding to the efficacy of anti-EGFR therapies, very few studies have been done considering separately mutations in codon 13 from those in codon 12. To date, only 6 analysis have been published (Table 2).

De Rook et al. were the first studying retrospectively the association between KRAS mutation in codon 13 (G13D) versus mutations in codon 12 evaluating response and survival in patients with chemotherapy refractory treated with cetuximab [38]. On the basis of their observations obtained from 579 patients with chemotherapy-refractory included in C0.17, BOND, MABEL, EMR202600, EVEREST, BABEL or SALVAGE clinical trials, mutations in codon 13 were associated with better outcome after cetuximab treatment observed by a longer OS and longer PFS. In particular, patients harboring G13D KRAS mutation who received cetuximab in monotherapy or combined with chemotherapy, presented a OS of 7.6 months and PFS of 4.0 months while these values were 5.7 and 1.9 months respectively for those with other KRAS mutations. No significant difference in OS or PFS was observed between patients with G13D and KRAS wild-type tumors. Then, Tejpar et al. [39] analyzed the association between KRAS status (wild type, G13D, G12V or other mutations) in 533 patients from CRYSTAL and OPUS studies and concluded that the addition of cetuximab to first-line chemotherapy seemed to benefit patients harboring mutations in codon 13. Cetuximab plus chemotherapy versus chemotherapy alone significantly improved PFS (7.4 versus 6 months) but non survival (15.4 versus 14.7 months) in G13D patients. Patients with G12V and other mutations did not benefit from this treatment combination and those with G13D receiving chemotherapy alone had worse outcomes than those with other mutations. Band et al. [43] could not find significant differences in progression or survival between patients with mutations in both codon but conclude that the disease control rate was higher in KRAS G13D mutant patients. Analyzing 109 Japanese patients refractory or intolerance to fluoropyrimidines, oxaliplatin and irinotecan the authors suggested that cetuximab showed some activity in KRAS p.G13D-mutant colorectal cancer patients. Specifically, for wild type, G13D and all other KRAS mutations the response rate was 30%, 14% and 0% respectively; the PFS was 4.6, 4.1 and 2.1 months and the OS 11.2, 8.5 and 6.8 months respectively. Finally, Mao et al. [44] performed a meta-analysis with 1487 patients and concluded that mCRC patients harboring mutations in KRAS G13D may benefit from cetuximab treatment. In this systematic review they showed that patients with G13D receiving cetuximab had a higher ORR and longer PFS and OS than patients with codon 12 mutations. By contrast, those parameters are worse if they do not get the anti-EGFR treatment.

By contrast, recent retrospective analysis including 110 patients in Spain by Gajate et al. [45] reported that patients (with cetuximab or cetuximab in combination with chemotherapy in the first and subsequent lines of treatment) whose tumor harbored a KRAS G13D allele did not benefit from cetuximab treatment and had a trend toward lower OS compared with patients whose tumors harbored either wt KRAS or one of the other KRAS mutations (8.2, 14.9 and 19 months respectively).

In addition, Peeters et al. [46] in a retrospective analysis of three randomized phase III studies evaluated the impact of panitumumab and concluded that patients with mutations in codon 12 and 13 are unlikely to benefit from this therapy. They did not find any specific KRAS mutation was consistently associate with...
PFS or OS in patients receiving panitumumab treatment. Only in one of the three studies, 20050203, a significant negative interaction was interaction was found in G13D patients associated with OS.

This result analyzing Panitumumab are in contrast with reported cetuximab data [38,39,43]. Although the number of studies carried out with Panitumumab are very limited, the differences could be attributed to the different epitopes recognized by the two different antibodies and not equal abilities to bind to EGFR [47]. However, this important conflict reported between the both different antibodies should be clarified with further studies.

5. Conclusion

On the basis of the revised literature most of the publications seem to demonstrate that KRAS codon 13 mutations occur in a unique molecular and clinical context.

The majority of in vitro studies at molecular level demonstrate that KRAS codon 13 mutations confers weaker transforming capacity of the cells [15]. By computational analysis it has been seen that KRAS protein with mutation in codon 13 has a similar structure and dynamics to WT KRAS protein, compared with mutations in codon 12 [23].

These differences may result in a subtle but important difference in how these mutant forms of KRAS activate downstream effectors and/or may reflect differences in the molecular context in which these tumors develop.

By contrast to the molecular data, most of the clinical studies show that patients harboring mutations in KRAS codon 13 appear to have worse outcomes and prognosis. Compared with tumors with codon 12 mutations, tumors that contain a codon 13 mutation are more likely to have spread to lymph nodes at the time of diagnosis, have fewer dendritic cell immune infiltrates, and are less likely to arise in the proximal colon [32,34]. However, a biological explanation is not showed in this clinical analysis and still we can not fully understand the different behavior of tumors with different mutations and further outcome in CRC patients. Further detailed molecular analysis of patients is needed to clarify this important issue.

Since KRAS codon 13 mutations are present in approximately 5% of tumors from patients with metastatic colorectal cancer, decision regarding treatment are important from the ethical, economic and social point of view.

Although original clinical trials have indicated that mCRC with KRAS mutations are resistant to anti-EGFR monoclonal therapy [48] and consequently anti-EGFR therapy is used only for patients with wild-type mCRC patients, numerous studies indicate that patients with codon 13 KRAS mutation benefit from anti-EGFR therapy. In particular, there is a consensus showing Cetuximab increases OS, PFS and ORR [39,43,44]. Regarding to the efficacy of anti-EGFR therapies, here we presented 6 studies where mutations in codon 13 has been analyzed separately from those in codon 12. From them, 5 have been made with cetuximab and only one with panitumumab. Four of the five studies with cetuximab demonstrated that this therapy provides some advantage to KRAS G13D patients, mostly due to a higher disease control and PFS. Advantage in OS is controversial since half of the studies (2 of 4) found a positive correlation with G13D mutation and the other half did not show significative differences. Contrary, the only analysis by Gajate et al. did not find any differences in OS or PFS comparing patients mutation in codon 13 with either WT KRAS or one of the other KRAS mutations. In summary, the majority of studies here reported, show an advantage for patients harboring G13D mutation after cetuximab administration and none of them reported worse outcome than those with wild type or other KRAS mutations.

Nevertheless and although the trend seems positive, since there is not total agreement and the number of studies is small, further evaluation should be done taking into account economic and ethical considerations.

On the other hand, the only study done with panitumumab [46] cannot demonstrate benefit from the treatment. It is clear that more and bigger analysis should be done with this therapy, before make further conclusions and support opinions for patients’ treatment.

In our opinion a large and comprehensive clinical trials are needed to clarify whether patients with mCRC harboring a KRAS G13D mutation respond to anti-EGFR therapies in the near future, also differentiating cetuximab from panitumumab. There is an urgent need to make efforts in molecular characterization that could increase the hope for those patients that first, have a poorer prognosis than WT or codon mutant 12 patients and second, can obtain clear benefit from Cetuximab.

Here we add some insight to the controversies in anti-EGFR therapy in mCRC [49] but still, further studies are necessary to implement KRAS G13D as a robust biomarker that could be integrated into clinical practice.

Because the mutations in KRAS codon 13 represent a substantial proportion of the population suffering from CRC, the issue regarding the possible response of this mutated form of the KRAS to anti-EGFR therapies should be considered relevant from biological, ethical and economical perspectives.

Disclosure

The authors declare no competing financial interests associated with this work.
Funding

MHV is supported by Universidad del Pais Vasco, Instituto Biodonostia, San Sebastian and CIBERehd (red de enfermedades hepáticas y digestivas).

Conflict of Interest

None declared.

References

[3] US Food and Drug Administration, FDA approves Vectibix (panitumumab) to treat metastatic colorectal carcinoma, [www.fda.gov].

5