PIK3CA GENE MUTATIONS AS POTENTIAL PREDICTIVE BIOMARKERS IN COLORECTAL CANCER

Elena Puerta García[1], Marisa Cañadas Garre[2], Cristina Pérez Ramírez[3], Maria Isabel Carrasco Campos[1], David Urbano Perez[1], Ana Segura Perez[1], and Miguel Ángel Calleja Hernández[4]

[1]Hospital Universitario Virgen de las Nieves
[2]Queen's University Belfast
[3]Universidad de Granada
[4]Complejo Hospitalario Universitario de Granada

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Abstract

Associations between colorectal cancer (CRC) survival and mutations in mediator of MAPK/ERK (RAS-BRAF-MEK-ERK) and PIK3CA/AKT signaling pathways have been reported. The objective of this study was to evaluate the influence of mutations in the EGFR pathway (KRAS, NRAS, BRAF and PIK3CA) on overall survival in CRC. We conducted a retrospective observational cohort study comprising 194 paraffin tumor samples from patients diagnosed with colorectal cancer were analyzed for KRAS codons 12, 13 and 61, NRAS codons 12, 13 and 61, BRAF and PIK3CA exons 9 and 20 gene mutations. Multivariate analysis confirmed that patients with ECOG of 0 presented lower risk of death (HR = 0.17, CI95%, 0.10 -0.31, p = 1656.10-9) compared to a higher ECOG (Table 4). The only independent genetic association was between PIK3CA20 mutation (H1047Y; rs121913281) and higher risk of death (HR = 8.93, CI95%, 1.20-66.57, p = 0.03268). No association was found between the rest of the mutations analyzed and overall survival. To explore the effect of mutations in patients with different degrees of ECOG, a stratified analysis was performed. Multivariate analysis in patients with ECOG 0 group confirmed the association between mutations of PIK3CA and an increased risk of death: E545K (HR = 5.49, CI95%, 1.28-23.51, p = 0.021720) and H1047Y (HR = 53.49; %, 4.63-617.40, p = 0.001429). In conclusion, our results show PIK3CA gene mutations may predict the overall survival of CRC patients, positioning PIK3CA as a potential biomarker for survival in CRC.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in the United States (1). From 2000 to 2014, CRC death rate has decreased by 34% in the population over 50 years of age, in part due to the implementation of screening techniques and improvement in the treatment of stages III (preoperative chemotherapy) and IV (treatment of metastases with monoclonal antibodies)(1). However, the decline in CRC mortality rates seems to be less important in patients who carried any mutation in the EFGR pathway. These mutations seem to be poor prognostic biomarkers, as recently reviewed (2,3). The epidermal growth factor receptor (EGFR) is widely expressed in CRC, among other tumors (1). Its ligand, the epidermal growth factor (EFG), activates MAPK/ERK (RAS-BRAF-MEK-ERK) and PIK3CA/AKT, two distinct signaling cascades implicated in cell proliferation and differentiation (1,2). Associations between CRC survival and mutations in some of these signaling pathway mediators have been reported (3–7). There are three oncogenes in*RAS* family: Harvey RAS (*HRAS)* , Kirsten RAS (*KRAS*) and neuroblastoma RAS (*NRAS*) (4). The

KRAS gene, whose mutation is the most frequent in CRC, mainly occurring in codons 12, 13 and 61 (7), is the one preferentially activated by the MAPK/ERK route (5,6). Patients who present mutations in *KRAS* have a 50% lower overall survival than patients with *KRAS wild type* and approximately 33% less progression-free survival (8). Mutation in another *RAS* gene, *NRAS* , has also been associated with approximately 50% loss of overall survival (8). V600E mutation in*BRAF* , described in 12% of CRC (9), is also considered a risk factor for progression and death, increasing the risk of death up to three times (8,10). Although these studies show a promising usefulness of mutations in EFGR pathway (*KRAS* , *NRAS* and *BRAF*) as biomarkers of survival in CRC (8,10), other studies of similar power have failed to find such association (12–14); therefore, the utility of EGFR pathway biomarkers on CRC survival is still under debate (2,3). Other signaling pathways, such as PIK3CA-AKT, involved in activation of*RAS* oncogenes (2,7), have also been proposed as potential biomarkers. Alterations on this signaling pathway have been observed in 60-70% of CRC cases (11). An approximate 33% decrease in overall survival has been reported in patients with a mutation in exon 20 of*PIK3CA* gene (8). Therefore, mutations in several components of different pathways may have a contribution in the survival of CRC patients.

The aim of this study was to evaluate the influence of mutations in the EGFR pathway (*KRAS* , *NRAS* , *BRAF* and *PIK3CA*) on overall survival in CRC.

MATERIAL AND METHODS

Study design

A retrospective observational cohort study was conducted.

Study population

We studied 194 patients diagnosed with colorectal cancer (stages I-IV). All patients were over 18 years old and had been treated by the Oncology Unit of the Virgen de las Nieves University Hospital (HUVN) between 2000 and 2016. Patients with other types of colorectal cancer, synchronous neoplasms and those whose Clinical histories were incomplete were excluded. The study was approved by the HUVN Ethics and Research Committee, and was conducted according to the Declaration of Helsinki.

Clinical and sociodemographic variables

Clinical and sociodemographic data were collected from clinical histories: sex, diagnosis, date and age of diagnosis, surgery, tumor size, stage, ECOG (Eastern Cooperative Oncology Group) Performance Status, histopathological type, date of death and scheme of chemotherapy treatment. Tumor stage was determined according to the TNM classification of the AJCC (American Joint Committee of Cancer)(15).

Genetic variables

DNA was extracted from paraffin tumor samples from the Biobank of Andalusia (Collection Code: 19150007). The QIAamp DNA Mini Kit was used following the manufacturer's instructions and stored at -40 ° C.

Gene mutations were analyzed by Real-Time PCR using TaqMan® probes:*KRAS* codons 12, 13 and 61 (rs121913529, rs121913530, rs121913535, rs112445441, rs17851045, rs121913238, rs121913240), *NRAS* codons 12, 13 and 61 (rs121913237, rs121434596, rs11554290, rs121913254),*BRAF* (rs113488022), *PIK3CA* exons 9 and 20 (rs121913273, rs104886003, rs121913281, rs121913279). Positive samples were confirmed by Sanger DNA Sequencing.

Outcome variables

Overall survival was the primary endpoint, and was defined as time from the diagnosis of CRC until death from any cause. The end date of the study was used when the death event had not occurred.

Statistical analysis

The normal distribution of the variables was determined using the Shapiro-Wilks test. Quantitative non-normally distributed variables were described by median and percentiles (25 and 75). Qualitative variables were expressed as frequencies.

The Kaplan-Meier method and the log-rank test were used to evaluate the influence of clinical, genetic and sociodemographic variables on survival (bivariate analysis). Multivariate Cox proportional hazard regression model was used to obtain the adjusted hazards ratio (HR) and 95% confidence interval (CI95%) for potential prognostic factors for survival.

The significance level for all the tests was $p < 0.05$. The software used for the survival analysis was EZR 1.35 (R 2.3.0)(16).

RESULTS

Clinical and sociodemographic characteristics of patients

Baseline characteristics of the 194 Caucasian patients are summarized in Table 1. Seventy-two of the 194 patients were women (37.1%). Median age at diagnosis was 62 years [57-69] and median tumor size was 4.6 cm [3.5-6.0]. Number of recorded deaths was 62 (31.9%), with a median overall survival of 41.4 months [30.8-55.9]. Approximately 85% of the patients presented ECOG 0 (157/185) at the time of diagnosis. One patient (0.5%) had a stage I tumor at the time of diagnosis; the rest of the patients were stages II (25.8%), III (49.0%) and IV (24.8%). The most frequent histopathology was adenocarcinoma (90.2%) and the most common location was in the sigmoid colon (38.1%). Regarding the first line of chemotherapy treatment, most of the schemes were based on pyrimidines in combination with oxaliplatin (85.57%) or monotherapy (11.34%).

Genetic characteristics of tumors

Table 2 details the frequency of mutations in *KRAS, NRAS, BRAF*and *PIK3CA* genes. The most frequent mutations were found in codon 12 of the *KRAS* gene (9.28% for SNP rs121913530 and 5.67% for SNP rs121913529). All patients analyzed for *KRAS* (rs112445441, rs121913238, rs121913240), *NRAS* (rs11554290, rs121913254) and*PIK3CA* (rs121913279) were found to be *wild-type* , so they were not included in the statistical analysis.

Influence of the clinical, histopathological and genetic characteristics on overall survival

Bivariate analysis

Patients with ECOG of 0 presented a greater overall survival (92.93 vs 28.76 months, $p = 3.4 \cdot 10^{-11}$) in bivariate analysis (Table 3) (Figure 1). There was no association between overall survival and the rest of clinical-pathological characteristics analyzed.

Patients with mutated *BRAF* gene (V600E; rs113488022) had lower overall survival (29.90 vs 77.43 months, $p = 0.049$). Same happened with the *PIK3CA9* gene (E545K; rs104886003) (37.35 vs 77.43months, p =

0.0024). Patients with the *PIK3CA20* H1047Y mutation (rs121913281) showed a trend towards an increased risk of death compared to *wild type* (30.40 vs 77.43 months, p = 0.055).

Multivariate analysis

Multivariate analysis confirmed that patients with ECOG of 0 presented lower risk of death (HR = 0.17, CI95%, 0.10 -0.31, p = 1656.10-9) compared to a higher ECOG (Table 4). The only independent genetic association was between *PIK3CA20* mutation (H1047Y; rs121913281) and higher risk of death (HR = 8.93, CI95%, 1.20-66.57, p = 0.03268). No association was found between the rest of the mutations analyzed and overall survival.

Statistical analysis stratified by ECOG

To explore the effect of mutations in patients with different degrees of ECOG, a stratified analysis was performed.

Group of patients with ECOG 0

The bivariate analysis in patients with ECOG 0 group showed that overall survival was lower in those with mutated *PIK3CA* gene, both for E545K mutation (rs104886003) in exon 9 (37.35 vs 92.93 months; = 0.003) and H1047Y mutation (rs12191328) in exon 20 (30.40 vs 92.93 months, p = 0.0048) (Table 5) (Figure 2). Lower overall survival was also observed in patients whose tumor was located in colon (76.9 vs> 50 months, p = 0.012). Multivariate analysis confirmed the association between both mutations of *PIK3CA* and an increased risk of death: E545K (HR = 5.49, CI95%, 1.28-23.51, p = 0.021720) and H1047Y (HR = 53.49; %, 4.63-617.40, p = 0.001429) (Table 6). In addition, patients diagnosed with colon cancer had a higher risk of death than those diagnosed with rectal cancer (HR = 5.95, CI95%, 1.42-25.02, p = 0.014870).

Group of patients with ECOG 1-2

In the group of patients with ECOG greater than 0, those diagnosed with adenocarcinoma had greater overall survival (32.02 vs 22.95 months, HR = 0.27, CI95%, 0.08-0.93, p = 0.033) (Table 5), an association that was not confirmed in multivariate analysis (Table 6) (Figure 2).

DISCUSSION

EFG, when bound to its receptor EFGR, activates two main signaling pathways, RAS-BRAF-MEK-ERK and PIK3CA-AKT, which are responsible for initiating processes of cell progression and differentiation, apoptosis and DNA repair (2,11,17). Mutations in *KRAS, BRAF, NRAS* genes (via MAPK/ERK) and *PIK3CA* (via PIK3CA/AKT) have been linked in numerous studies to monoclonal antibodies (Cetuximab and Panitumumab) response, progression and overall survival in CRC (3,8,10,13,14,18–27).

We analyzed the overall survival in 194 CRC patients according to the status of different mutations in components of the EGFR pathway. Our results showed association of the PIK3CA/AKT pathway, but not the MAPK/ERK cascade on CRC overall survival. In particular, we observed an association between risk of death and presence of H1047Y mutation in exon 20 of *PIK3CA* (HR = 8.93, CI95%, 1.20-66.57, p = 0.03268). We also observed a strong effect of ECOG 0 on greater overall survival (HR = 0.17, CI95%, 0.10 -0.31, p = 1656.10-9). ECOG PS is a scale of 0 to 5 published in 1982 by the Eastern Cooperative Oncology Group that measures how the disease affects the patient's daily activities. It seems logical to think that patients with ECOG 0 (full functionality) have more chances to survive than those with ECOG 1-2. Given that the effect of ECOG could be masking the effect of other variables on overall survival, we performed a analysis stratifying the sample by ECOG status, which confirmed that patients with *PIK3CA20* H1047Y mutation had a higher risk of death (HR = 8.93, CI95%, 1.20-66.57, p = 0.03268) and showed a new association between the E545K

mutation of exon 9 of*PIK3CA* (HR = 5.49, CI95%, 1.28-23.51, p = 0.021720) and overall survival in patients with ECOG 0.

Our findings are comparable to those obtained by Sartore-Bianchi (19) and De Roock (8). Sartore-Bianchi and colleagues investigated the influence of loss PTEN, *PIK3CA* exon 9 and 20 and *KRAS* exon 2 mutations on objective response and survival in 110 metastatic CRC patients treated with anti-EFGR monoclonal antibodies (Panitumumab or Cetuximab). Patients with at least one alteration in the *PIK3CA*and *PTEN* genes had a higher risk of progression (HR = 1.86, CI95%, 1.16-2.96, p = 0.009). As observed in our patients, *KRAS*mutations could not predict progression-free survival (HR = 1.50, CI95%, 0.89-2.52, p = 0.128). They also failed to find association with overall survival for any gene (*PIK3CA* , p = 0.2518; *KRAS* , p = 0.1127), while in our patients, *PIK3CA* exon 20 (H1047Y) mutation was associated with lower survival. De Roock explored the possible effect of *KRAS, NRAS, BRAF* and *PIK3CA* mutations on objective response, overall survival and progression-free survival of 773 CRC chemotherapy-refractory patients treated with Cetuximab plus chemotherapy. As our results, they found that mutations of exon 20 were capable to predict survival (progression-free and global), but only in patients with *KRAS* wild-type tumors (HR = 2.27, CI95%, 1.10-4.66, p = 0.042 / HR = 3.30, CI95 %, 1.46-7.45, p = 0.012).

Wendy De Roock, in addition, suggests that *PIK3CA* exons 9 and 20 mutations should be studied separately, since the alterations they produce are different and can lead to biased results (8). Exon 9 (helical domain) mutation produces the activation of the RAS protein, while exon 20 (kinase domain) mutation induces a gain in function independent of RAS (28). In our study, as De Roock suggests, and unlike the Sartore-Bianchi study, we separately analyzed *PIK3CA* exons 9 and 20 mutations.

The limited size of our sample did not allow us to explore the effect of the mutations on response to the treatment, since the patients were in different stages of disease and lines of treatment at the time of our study, and the follow-up period (two years) was also insufficient for study progression-free survival in CRC. The statistical power proved to be insufficient to investigate the influence on CCR survival of some of the mutations in the EFGR pathway mutations *KRAS* (rs121913529, rs121913530, rs121913535, rs112445441, rs17851045, rs121913238, rs121913240), *NRAS* (rs121913237, rs121434596, rs11554290, rs121913254) and *BRAF* (rs113488022)] and no mutations were observed on those genes. Therefore, further studies with larger size will be necessary to properly explore and elucidate those genes as potential biomarkers in CRC survival. Despite the limited sample size, we clearly demonstrated the influence of *PIK3CA* gene mutations on CCR survival.

CONCLUSIONS

In conclusion, our results show that mutations in *PIK3CA* gene may predict the overall survival of CRC patients, positioning *PIK3CA*as a potential biomarker for survival in CRC. In particular,*PIK3CA* exon 20 (H1047Y) mutation was associated with lower survival in CRC patients, while both exon 9 (E545K) and exon 20 (H1047Y) mutations were also associated with lower survival in patients with ECOG 0.

The PIK3CA/AKT signaling pathway could play an important role in CRC survival and should be subject of further studies in the future.

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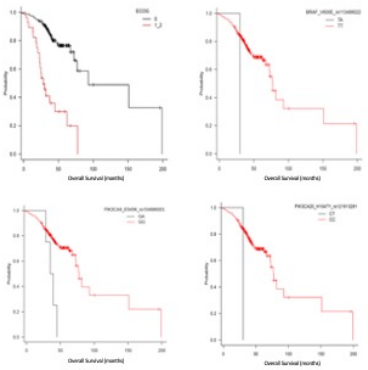
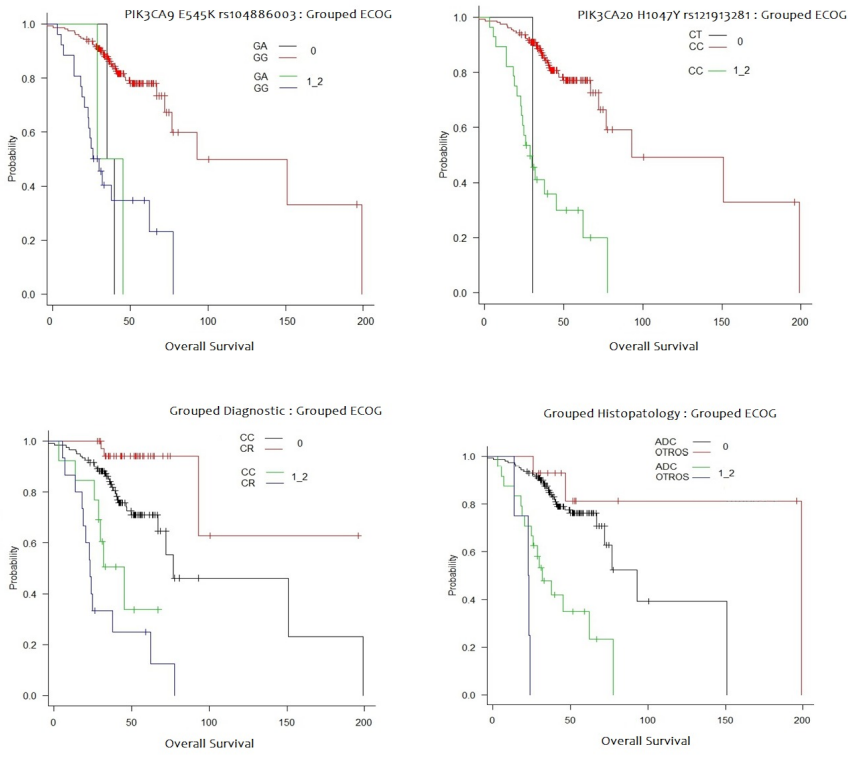
FIGURE LEgENDS

Figure 1 . Overall survival of colorectal carcinoma patients according to BRAF, PIK3CA mutations and ECOG.

Figure 2 . Influence of PIK3CA gene, diagnosis and histopathology on overall survival stratified by ECOG





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