PIK3CA mutation in gastric cancer and the role of microsatellite instability status in mutations of exons 9 and 20 of the PIK3CA gene

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Abstract

Background. A better understanding of molecular gastric cancer (GC) entities may help in tailored treatments of that neoplasm. The PIK3CA mutation is one of the most important in many cancers.

Objectives. We performed a comparison of clinical and pathological data of the PIK3CA mutation in GC patients.

Material and methods. The analysis was done on 472 patients operated on in one center. Polymerase chain reaction (PCR) was used for the screening of PIK3CA (exon 9 and 20). For microsatellite instability (MSI) we used 5 quasi-monomorphic mononucleotide repeats − BAT-26, BAT-25, NR-24, NR-21, and NR-27. The clinical and pathological data was analyzed.

Results. PIK3CA mutation was observed in 10 out of 472 GC patients (2.1%). Nine out of 10 were MSI (9 of 111 MSI patients — 8.1%). Half of the 10 patients had mutations in exon 9 and the other half in exon 20. A majority of patients with the PIK3CA mutation had MSI (p < 0.001). The 5-year survival of MSI patients with the PIK3CA mutation was 40% and without the mutation, 70.4% (p = 0.309). For patients with the mutation in exon 9, the 5-year survival was 0%, and for those with the mutation in exon 20, 80% (p = 0.031). The Cox proportional hazards regression analysis did not show that PIK3CA is statistically correlated with a worse overall survival.

Conclusions. PIK3CA mutation in GC is a rare finding. It is strongly associated with the MSI molecular subgroup, presenting a worse outcome than other MSI patients. A completely different outcome is associated with the mutation in exon 9 compared to the mutation in exon 20, with the latter being more favorable.

Key words: gastric cancer, PIK3CA, mutation, microsatellite instability, exon
Introduction

Gastric cancer (GC) is a disease that is characterized by multiple molecular, genetic, and epigenetic events.\(^1\) Mutation in a signaling pathway is one such event and the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of the rapamycin pathway (PI3K/AKT/mTOR pathway) is one example of the mutation mechanism.\(^2\) A fundamental step in this pathway is the creation of phosphatidylinositol-3,4,5-triphosphate (PIP3), catalyzed by PI3K. This pathway is important in the cancer-related functions of cell proliferation, catabolism, cell adhesion, apoptosis, and autophagy.\(^3\) It also plays an important role in motility and glucose homeostasis.\(^4\) The mutations of this pathway have frequently been seen in cancers such as ovarian, breast, thyroid, and cervical. Many studies have revealed that this pathway is not of highest importance in GC patients. The PIK3CA gene is located on chromosome 3p26.\(^3,5\)

In the current literature, PIK3CA mutations in colorectal cancer (CRC) are associated with female gender, proximal position, well-differentiated tumors, and mucinous histology, but these findings are not consistent.\(^6\) Some studies have shown a significant coexistence of PIK3CA and KRAS mutations, while other studies have failed to show such a coexistence. Other contradictory data presented a link between the PIK3CA mutation and MSI or CpG island methylation phenotype (CIMP).\(^6\) Another inconclusive study showed worse outcome for early stage resectable disease, but other studies did not prove this finding.\(^6\) Such conflicting findings can be explained by important differences between cancers that have the mutation in exon 9 vs those with the mutation in exon 20.\(^6,7\) Exon 20 has a mutual relationship with BRAF mutation, CIMP high/low and MSI-H, and exon 9 is linked with KRAS mutations.\(^7\) In the study by Mao et al., authors showed that mutations in exon 20 are associated with resistance to anti-EGFR antibody therapy. This was not seen for mutations in exon 9.\(^8\) Tapia et al. reported that PI3K and AKT are overexpressed in GC with lymph node spread.\(^9\)

New molecular classifications of gastric cancer have recently been proposed.\(^1,10\) We are witnessing huge advances in our understanding of cancer from a molecular, immunological, diagnostic and even bioinformatic standpoint, all contributing to better tailored treatments for patients.\(^1,10−15\) In both classifications, microsatellite instability (MSI) is a distinct molecular subgroup of GC. In the available studies, MSI is associated with older age, female gender, intestinal histotype, non-cardia tumors, lower number of metastatic lymph nodes, and better survival.\(^16−18\) It seems that the MSI subgroup is not homogenous and other genetic and molecular factors may play an important role for these particular patients.

The aim of the study was to compare the clinical and pathological data of PIK3CA mutation in GC patients. We divided PIK3CA mutations into 2 categories based on the hotspot mutation sites at exon 9 and exon 20. In addition, we investigated the coexistence of this mutation with MSI status and KRAS mutations.

Material and methods

Patients

The analysis was performed on a group of 472 GC patients treated in the General Surgery and Surgical Oncology Department, University of Siena, Italy. We used tissue material stored in our biobank collected from patients who were operated on between 1990–2011. None of these patients received neoadjuvant treatment. We used tumoral and healthy tissues for comparative analysis. All samples were collected just after resection in the operating theatre.

PIK3CA sample preparation

Genomic DNA was extracted by tumoral and constitutional fresh frozen sample tissues using a standard protocol (Genta Systems, Minneapolis, USA). The DNA concentration was calculated by spectrophotometry.

Polymerase chain reaction (PCR) is used for the screening of PIK3CA (exon 9 and 20).

To search for somatic alterations of the PIK3CA gene, exons 9 and 20 were sequenced according to the protocol described in detail by Velho et al.\(^19\) PCR reactions were carried out in a volume of 20 µL containing 100 ng/µL genomic DNA template, 1X Reaction Buffer, 0.5 µM of each PCR primer, MgCl\(_2\) 1.25 mM, 0.15 mM of each dNTPs, Taq polymerase 0.5 U/µL (Euroclone, Pero, Italy). The reactions were performed in programmable thermocyclers according to the standard protocol.

A 5 µL aliquot of each PCR reaction was run on a 2% agarose gel to confirm the size, quantity, and purity of each PCR product. The remaining 15 µL of PCR amplified bands were extracted from the gel with the Invisorb® Spin DNA Extraction Kit (Invitek, Stratec Biomedical Systems, Birkenfeld, Germany). Samples were then purified and 2 µL aliquot of purified PCR product was cycle sequenced using a BigDye Terminator Kit (Applied Biosystems, Foster City, USA) in a total volume of 20 µL. Samples were then purified and sequenced using an automated DNA sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Milan, Italy) according to the protocol of the manufacturer. Sequencing was performed in both strands. All sequence alterations in these genes were validated with a second independent PCR.

Pentaplex polymerase chain reaction and microsatellite analysis

A detailed description of MSI analysis was described in our previous paper.\(^18\) In short, we used 5 quasi-monomorphic
mononucleotide repeats, namely, BAT-26, BAT-25, NR-24, NR-21, and NR-27. Following the definition of the National Cancer Institute workshop on MSI for cancer, we considered a tumor as MSI when 2 or more markers showed instability on 5 loci (MSI-H).20

A detailed description of the pathological, clinical, surgical and follow-up data was also given in our previous publication.18

Statistical analysis

Statistical analysis was performed with the χ² test or Fisher exact test to compare categorical variables. The Mann-Whitney U test was used to compare continuous variables not normally distributed. Cumulative survival was calculated by the life table method of Kaplan and Meier, and the log-rank test was used to distinguish significant differences. Statistical significance was determined at p-value < 0.05.

Survival curves estimated using the Kaplan-Meier method were compared using a log-rank test, considering death from cancer as the end-point (cancer-related survival).

The Kaplan-Meier estimation was used to plot survival curves, and log-rank tests were used to calculate the difference of overall survival (OS) between groups.

Multivariate Cox proportional hazard regression analysis was used to investigate independent prognostic factors for overall survival between groups. The variables including PIK3CA status, age, sex, tumor location, MSI status, Lauren histotype, type of resection, T, N, M status, and adjuvant therapy were used as covariates. Statistical analysis was done using commercially available statistical software (SPSS 20.0 for Windows SPSS Inc., Chicago, USA).

Results

PIK3CA mutation was observed in 10 of 472 GC patients (2.1%). Half of the 10 patients had mutations in exon 9 and the other half in exon 20. For exon 9 mutations, we found 2 mutations of ES42K, 1 mutation of ES45K, 1 of NS1SS, and 1 of ES45G. All 5 patients with a mutation in exon 20 had mutation of H1047R.

Interestingly, 9 of 10 patients were also MSI positive. In 2 patients (20%), we observed KRAS mutation and PIK3CA mutation in exon 20. Both patient showed KRAS mutation -12D and were associated with better prognosis.

A clinicopathological comparison of PIK3CA patients and wild-type (wt) PIK3CA patients is presented in Table 1. The only statistically significant factor associated with PIK3CA mutations was MSI status. We also performed an analysis of PIK3CA mutation on the MSI positive subgroup. The clinicopathological analysis is presented in Table 2. Here, the only statistically significant factor was tumor position.

| Table 1. PIK3CA wild type vs mutations in all GC group |
|-----------------------------|-----------------------------|-----------------------------|
| Clinico-pathological characteristic | PIK3CA wt | PIK3CA mut | p-value |
| Patient (n) | 462 | 10 |  |
| Age (years, median) | 70 | 75 | 0.058  |
| Sex (male, female) | 280.182 (60.6:39.4) | 3.7 (30:70) | 0.051  |
| pT | | | 0.657  |
| 1 | 42 | 9.1% | 0 | 0% |
| 2 | 74 | 16% | 1 | 10% |
| 3 | 95 | 20.6% | 3 | 30% |
| 4 | 251 | 54.3% | 6 | 60% |
| pN | | | 0.471  |
| 0 | 130 | 28.1% | 3 | 30% |
| 1 | 75 | 16.2% | 2 | 20% |
| 2 | 100 | 21.6% | 4 | 40% |
| 3a | 74 | 16% | 0 | 0% |
| 3b | 83 | 18% | 1 | 10% |
| Tumor site | | | 0.256  |
| Non cardia | 386 | 83.5% | 7 | 70% |
| Cardia | 76 | 16.5% | 3 | 30% |
| MSI status | | | <0.001  |
| MSS | 360 | 77.9% | 1 | 10% |
| MSI-H | 102 | 22.1% | 9 | 90% |
| Lauren | | | 0.078  |
| Diffuse/mixed | 153 | 33.1% | 0 | 0% |
| Intestinal | 305 | 66% | 10 | 100% |
| Unclassified | 4 | 0.9% | 0 | 0% |
| UICC-R | | | 0.345  |
| R0 | 339 | 73.4% | 6 | 60% |
| R+ | 123 | 26.6% | 4 | 40% |
| Stage | | | 0.389  |
| I | 74 | 16% | 1 | 10% |
| II | 108 | 23.4% | 3 | 30% |
| III | 194 | 42% | 6 | 60% |
| IV | 86 | 18.6% | 0 | 0% |
| M | | | 0.131  |
| M0 | 376 | 81.4% | 10 | 100% |
| M1 | 86 | 18.6% | 0 | 0% |
| WHO histological type* | | | 0.132  |
| Papillary | 15 | 3.2% | 0 | 0% |
| Poorly differentiated | 146 | 31.6% | 5 | 50% |
| Signet ring cell & mucinous | 138 | 29.9% | 0 | 0% |
| Tubular (well/mod. diff.) | 153 | 33.1% | 4 | 40% |
| Adjuvant | | | 0.406  |
| No | 216 | 46.8% | 6 | 60% |
| Yes | 246 | 53.2% | 4 | 40% |

* 11 cases with unclassified WHO histotype are excluded; MSS – microsatellite stable; MSI-H – microsatellite unstable; M – metastases; pT – pathological tumor status; pN – pathological lymph node status.
Interestingly all ten patients showed Lauren intestinal histotype. We also analyzed cancer-related survivals. The first comparison was performed between all patients with or without PIK3CA mutation (Fig. 1). 5-year survival for PIK3CA wt patients was 43.2% and for PIK3CA mutation, 36% (p = 0.856). Secondly, we analyzed the group of MSI GC patients (Fig. 2). The 5-year survival of the MSI patients presenting with PIK3CA mutation was 40% and...
Cancer related survival p = 0.026

metastases status.

status; N parameter – pathological lymph node status; M parameter – microsatellite instability; T parameter – pathological tumor

and positive = 1; radicality of “R” resection expressed as R0 = 0 and R1–R2 = 1; “M” parameter expressed as negative = 0 and positive = 1; intestinal pathology. Also, our results show that mutation in exon 20 presented improved patient survival.

Radicality of resection

Lauren histotype

Gender

Tumor location

“T” parameter

“N” parameter

“M” parameter

Radicality of resection

Adjuvant therapy

Table 3. Cox proportional hazards regression analysis

Clinico-pathological characteristic | B | Exp (B) | 95% CI | p-value
----------------------------------|----|---------|--------|--------
PIK3CA status expressed as PIK3CA wt = 0 and PIK3CA mutation = 1; MS status expressed as MSS = 0 and MSI = 1; gender expressed as F = 0 and M = 1; Lauren histotype expressed as intestinal = 0 and non-intestinal = 1; tumor location expressed as non-cardias = 0 and cardias = 1; “T” parameter expressed as T1–T2 = 0 and T3–T4 = 1; “N” parameter expressed as N− = 0 and N+ = 1; “M” parameter expressed as negative = 0 and positive = 1; radicality of resection expressed as R0 = 0 and R1–R2 = 1; adjuvant therapy expressed as yes = 1 and no = 0; MS status – microsatellite instability; T parameter – pathological tumor status; N parameter – pathological lymph node status; M parameter – metastases status.

without the mutation, 70.4% (p = 0.309). We also checked the difference in survival of patients with the PIK3CA mutation in different exons (Fig. 3). For mutation in exon 9, the 5-year survival was 0% and for mutation in exon 20 – 80% (p = 0.031). For better visualization of these differences between the 2 mutation locations, we added a survival curve for MSI patients without PIK3CA mutation (p = 0.026) – Fig. 3.

The Cox proportional hazards regression analysis showed that age, sex, MSI status, “T” parameter, “N” parameter and type of “R” resection were the only prognostic factors statistically correlated with a worse overall survival (Table 3).

Discussion

In previous reports, the frequency of GC PIK3CA mutations varies from 4% to 13.2%. In our paper, PIK3CA mutation occurred in 8.1% of the MSI group. In total, only a frequency of 2.1% was observed, lower in comparison with previous studies. The debate about PIK3CA mutation and its prognosis for different cancers is unresolved. Some authors suggest PIK3CA mutation is associated with a better prognosis in the case of breast cancer, while other authors showed worse prognosis in cancers like colorectal, endometrial, and lung cancers. Although de Jong et al. presented interesting data for gastric cancer, showing worse survival in PIK3CA exon 20 mutation with intestinal histotype and better survival for the same mutation with diffuse histotype. These results were statistically significant. In our study, it was impossible to analyze this factor because we did not observe diffuse/mixed histotype in our sample. PIK3CA mutation in exon 20 was found to be an independent prognostic factor of survival for intestinal pathology. Also, our results show that mutation in exon 20 presented improved patient survival.

In the paper by Fang et al., they found PIK3CA mutation in 57/432 of patients (13.2%). They analyzed PIK3/AKT mutations together and found that in the intestinal histotype, patients presenting mutation in that gene showed tumors located mostly in the lower third of the stomach. In diffuse histotype, the location of the tumor was in the upper third of the stomach and patients showed a higher rate of hematogenous metastases. The authors did not find any difference in survival between patients presenting or not presenting PIK3/AKT mutation.

The authors also searched for a link between the PIK3CA mutation and Epstein-Barr virus (EBV) infection. The rate of EBV infection was higher only in the situation where the tumor was situated in the middle part of the stomach for GC patients with PIK3CA mutation. In a paper by Barbie et al., the authors also analyzed PIK3CA mutation in GC and its association with GC and MSI. Only 8 of 39 MSI GC cases harbored the H1047R mutation. They found that this finding did not correlate with survival or any other clinical or pathological features linked with MSI GC. This is similar to our results on the exon 20 mutation in MSI patients, in which similar survival results were observed. A worse outcome was only observed in the case of exon 9 mutation, which was not observed in the above-mentioned study.
In our study, we observed that PIK3CA patients were older and showed intestinal histotype more often but without statistical significance. Analyzing the subgroup of MSI GC patients, the only differences were tumor position (p = 0.008) and Lauren histotype (p = 0.069) – PIK3CA mutations were more commonly seen in the upper third of the stomach and also showed only intestinal histology. We did not observe any difference in survival between wild-type and mutated PIK3CA GC patients; however, in the MSI GC subgroup of patients, those with PIK3CA mutation had a worse 5-year survival rate (40%) than those without the mutation (70.4%).

PIK3CA mutations are also observed in head and neck squamous cell carcinoma (HNSCC) for 6–21% of patients. Interestingly, this mutation was absent across German, Vietnamese, and Greek patients. Likely, some ethnic, environmental, and/or other unknown factors are associated with this finding. A paper by Seiwert et al. pointed out that PIK3CA mutations are more commonly associated with human papillomavirus (HPV) positive HNSCC cancers. This finding did not reach statistical significance but can be an example of a factor that may play an important role in developing this mutation. In our study, we observed one of the smallest incidences of PIK3CA mutation in GC patients – 2.1%.

In a CRC study by Day et al., it was found that PIK3CA exon 20 mutations were associated with proximal tumors and a sessile-serrated pathway (MSI-H/CIMP high/BRAF mutations), and PIK3CA exon 9 mutations were linked with the traditional serrated pathway of tumorigenesis (CIMP-low/KRAS mutations). PIK3CA mutations were significantly associated with older age, proximal tumor site and mucinous histology, and KRAS mutation. Comparison between wt PIK3CA and either exon 9 or 20 mutations showed some significant results. Mucinous histology was associated with exon 20, and for exon 9, factors like older age and KRAS mutations were associated. The direct comparison of exon 9 and 20 mutations did not reach statistical significance. In a study by Sukawa et al., MSI status was observed in 50% of PIK3CA GC patients. Similarly, our results show a statistically significant link between PIK3CA mutation and MSI status. In fact, almost all of our GC patients presenting with a PIK3CA mutation were also MSI positive. KRAS mutation was observed only in the exon 20 mutation group (2 of 5 patients – 40%).

In breast cancer, PIK3CA mutations are seen in 40% of cases. The presence of this mutation is associated with better prognosis in this cancer. Also, clinicopathological factors show higher rates of small tumor size, low grade, and positive estrogen receptor much more frequently in this group of patients. Importantly, these patients also showed better survival. In other cancers like colorectal, endometrial, or lung cancer, PIK3CA mutation is associated with worse prognosis. We did not find any difference in survival between patients with and without PIK3CA mutations. The difference was observed when we analyzed subgroups according to the type of exon mutation.

Our study was limited by the small number of patients with PIK3CA mutations in our GC patient pool. We presented a link between different PIK3CA exon mutations and MSI GC that present completely different prognoses depending on the type of mutation. The MSI subtype of GC is a relatively new molecular subgroup and requires further analysis of different mutations that may have a positive or negative impact on patient outcome.

Our research leads to some important conclusions about PIK3CA mutations. Firstly, PIK3CA mutations in GC is rare. It is strongly associated with the MSI molecular subgroup, presenting a worse outcome than wt PIK3CA MSI GC patients. A completely different outcome is associated with mutation in exon 9 vs exon 20, with the latter being more favorable. The role of this mutation must be further studied with larger groups of patients.

References


