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Genetic alterations and PIK3CA gene mutations and amplifications analysis in cervical cancer by racial groups in the United States

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ABSTRACT

Introduction: A number of studies indicated racial differences in cervical cancer outcomes and several factors are associated with it such as stage, comorbidities, treatment pattern, and socioeconomic status. However, the associations of tumor genomic patterns such as phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) gene mutations and amplifications with cervical cancer racial disparities are largely unexplored.

Objectives: Therefore, the present investigation aimed to identify genetic alterations (mutations and copy number variations) in cervical cancer and determine whether the PIK3CA gene mutations and amplifications in cervical cancer differ across racial/ethnic groups in the United States.

Methods: This study made use of The Cancer Genome Atlas (TCGA) database. TCGA database is a publicly available database that was created by a joint effort between the National Cancer Institute and the National Human Genome Research Institute. The two-tailed Fisher's exact test was used to test for associations between the categorical variables, race, and PIK3CA gene mutation as well as PIK3CA gene amplification using the "Fisher test" function in R.

Results: There were 309 cervical cancer samples, and of these, 194 samples had gene mutations and 295 samples had copy number alteration data. The top five mutated genes in TCGA dataset were PIK3CA, MUC4, KMT2C, SYNE1, and KMT2D. The top five amplified genes in TCGA dataset were MECOM, TP63, PRKCI, PIK3CA, and TRFC. The PIK3CA gene had the highest number of mutations with 53 mutation counts. The mutation rates were 62.5%, 31.3%, 25.4%, and 21.1% for American Indian, African American, White, and Asian, respectively. The amplification rates were 28.6%, 21.1%, 18.9%, and 12.5% for African American, Asian, White, and American Indian.

Conclusions: There are many significantly mutated or amplified genes implicated in cervical cancer. Some of them are not grouped with the already known genes in relation to cervical cancer. For example, the KMT2C, SYNE1, KMT2D, EP300, RYR2, FLG, DMD, FBXW, MECOM, TRFC, RPL35A, LPP, TBL, FGF12, and SOX2 genes. They can be explored as therapeutic targets to improve cervical cancer treatment.

Keywords: Amplification, cervical cancer, ethnicity, MECOM, mutation, PIK3CA, race

Introduction

Despite the fact that cancer of the cervix is known to be a preventable cancer, it remains one of the major causes of cancer-related deaths in females under the age of 60.[1-3] Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related death in females worldwide with over 527,000 new cases,[1,2] and perhaps the second most common cancer among women in the third world countries.[3-5] In the United States, over 12,000 women were diagnosed with cervical cancer in 2015.[6]

The PIK3CA gene is the most commonly implicated gene in human cancer.[7-12] The PIK3CA gene is located on the long (q) arm of chromosome 3 at position 26.3 (3q26.3).[13] The PIK3CA gene provides information for making the p110 alpha (p110α) protein, which is the catalytic subunit of the phosphatidylinositol 3-kinase (PI3K) enzyme.[13] This is an

important enzyme in the PI3K pathway. The PI3K pathway is essential for several cellular activities such as cell metabolism, cell survival, cell growth, and proliferation.[13]

The PI3K enzyme phosphorylates phosphatidylinositol -4,5-bisphosphate (PIP2) to phosphatidylinositol -3,4,5- triphosphate (PIP3).[13] The PIP3 induces the activation of kinase enzyme, protein kinase B (PKB), or AKT. The activated PKB, in turn, regulates the function of enzymes involved in cell growth and division such as mammalian target of rapamycin complex 1 (mTORC1).[14] The mTORC1 serves as the main regulator of cell metabolism, growth, and survival. The mTORC1 regulates cell growth and division by encouraging various anabolic actions such as synthesis of proteins, organelles, and lipids and by preventing catabolic processes.[14] PIK3CA gene amplification and mutations are two common causes of excessive activation of this pathway in cancer.[13] PIK3CA pathway over activation has been associated both with higher rates of local recurrence after radiotherapy and with decreased survival.[7,8,12] In addition, PIK3CA gene mutations have been associated with decreased survival after radical chemoradiotherapy.[7]

A number of studies indicated racial differences in cervical cancer outcomes and several factors are associated with it such as stage,[15-23] comorbidities,[24] treatment differences,[15,16,18,20,24,25] and socioeconomic status.[16,17,24,26-32] However, the associations of tumor genomic patterns such as PIK3CA gene mutation as well as PIK3CA gene amplification with cervical cancer racial disparities are unknown. Thus, the central purposes of this study were to identify genetic mutation as well as copy number variations in The Cancer Genome Atlas (TCGA) cervical cancer data and to examine racial differences in both PIK3CA gene mutation and PIK3CA gene amplification. Identifying tumor genomic patterns across different racial groups can lead to the discovery of therapeutic targets and improved cervical cancer treatments that will contribute to alleviating cervical cancer outcomes.[33]

Methods

This study made use of TCGA database. TCGA database is a publicly available database that was created by a joint effort between the National Cancer Institute and the National Human Genome Research Institute. TCGA database has comprehensive key genomic changes in 33 types of malignancy, including cervical cancer.[34] The two CESC genomic profiles used were mutation data from whole exome sequencing and putative copy-number alteration data from GISTIC 2.0. Clinical profiles including race were also accessed. The CESC clinical and genomic profiles were submitted to TCGA between 2011 and 2014. These were accessed through the cBioPortal for Cancer Genomics analytic tool. The cBioPortal is an open-access analytic tool that allows visualization, downloading, and analyzing of TCGA datasets.[35,36] The R statistical software was also utilized for the analysis. The two-tailed Fisher's exact test was used to test for associations between the categorical variables, race, PIK3CA gene mutation, and PIK3CA gene amplification using the "Fisher test" function in R. The two-tailed Fisher's exact test was selected considering the small sample size. Statistical significance was defined as $P < 0.05$. The analysis excluded variables that have no available values or sample values < 5.

Results

There were 309 cervical cancer samples, and of these, 194 samples had gene mutations and 115 samples did not have gene mutations. There were 295 samples with gene copy number variations and 14 samples without gene copy number variations. There were many significantly mutated genes. The top 10 mutated genes in TCGA dataset were PIK3CA, MUC4, KMT2C, SYNE1, KMT2D, EP300, RYR2, FLG, DMD, and FBXW7.

Table 1 shows the top 10 mutated genes in 194 profiled samples. The PIK3CA gene has the highest number of mutations with 53 mutation counts and 23.7%. The FBXW7 gene, the tenth frequent mutated gene has 19 mutation counts and 9.8%. The 53 PIK3CA gene mutations were all missense point mutations. Table 2 shows the common PIK3CA gene mutations in the dataset. Out of the 53 PIK3CA missense mutations, 48 mutations were known to be oncogenic (i.e., the mutated PIK3CA proteins have augmented catalytic action, leading to increased downstream signaling and oncogenic alteration). Thus, the percentage of PIK3CA mutations with oncogenic effect was about 91%.

The white race has the highest number of samples (211), followed by Black or African American (31), Asian (20), American Indian or Alaska Native (8) while native Hawaiian or other Pacific Islander close off with having the lowest number of samples. Table 3 shows the comparison of PIK3CA gene mutation status by race. The racial group with the highest mutation rate was the American Indian or Alaska Native (62.5%), followed by Black or African American (31.3%), white (25.4%), and Asian (21.1%). The

Table 1: Ten 10 mutated genes in 194 profiled samples

Gene symbol	Mutation count (%)
PIK3CA	53 (27.3)
MUC4	37 (19.1)
KMT2C	29 (14.9)
SYNE1	23 (11.9)
KMT2D	22 (11.3)
EP300	21 (10.8)
RYR2	21 (10.8)
FLG	20 (10.3)
DMD	20 (10.3)
FBXW7	19 (9.8)

Table 2: Types of mutation in PIK3CA gene

PIK3CA: Mutation (AA change)	Clinical implication	Mutation type
MUT: E726K	Oncogenic	Missense
MUT: E545Q	Oncogenic	Missense
MUT: E542K	Oncogenic	Missense
MUT: E545K	Oncogenic	Missense
MUT: E81K	Oncogenic	Missense
MUT: R93W	Oncogenic	Missense
MUT: H1047Q	Oncogenic	Missense
MUT: E545K	Oncogenic	Missense
MUT: H1047R	Oncogenic	Missense
MUT: K111E	Oncogenic	Missense
MUT: H1047L	Oncogenic	Missense
MUT: Q546R	Oncogenic	Missense
MUT: G106V	Oncogenic	Missense
MUT: R38H	Oncogenic	Missense
MUT: G118D	Oncogenic	Missense
MUT: E542K	Oncogenic	Missense

Table 3: PIK3CA gene mutation status by race

Race	Count (%)		Total
	PIK3CA mutation present	PIK3CA mutation absent	Sample profile
White	35 (25.4)	103 (74.6)	138
Asian	4 (21.1)	15 (78.9)	19
Black or African American	5 (31.1)	11 (68.7)	16
American Indian or Alaska Native	5 (62.5)	3 (37.5)	8

Fisher's exact result was 0.0792 for the PIK3CA gene mutation status by race.

Table 4 shows the top 10 amplified genes in 295 profiled samples. The top 10 amplified genes in TCGA dataset were MECOM, TP63, PRKCI, PIK3CA, TRFC, RPL35A, LPP, TBL, FGF12, and SOX2. The MECOM gene has the highest numbers of amplification with 62 counts and 20.3%. The PIK3CA gene, the fourth frequent amplified gene has 60 counts and 20.3%. The 60 PIK3CA copy number variations were all due to the amplification of PIK3CA gene. There was no PIK3CA copy number variation that was due to deletion.

Table 5 shows the comparison of PIK3CA gene amplification status by race. The racial group with the highest rates of PIK3CA amplification was the Black or African American (28.6%), followed by the Asian (21.1%), white (18.9%), and American Indian or Alaska Native (12.5%). The Fisher's exact result was 0.234 for the PIK3CA gene amplification status by race.

Table 4: Ten 10 CNA genes in 295 profiled samples

Gene symbol	Cytoband	CNA	Count (%)
MECOM	3q26.2	AMP	62 (21.0)
TP63	3q28	AMP	62 (21.0)
PRKCI	3q26.3	AMP	60 (20.3)
PIK3CA	3q26.3	AMP	60 (20.3)
TRFC	3q29	AMP	59 (20.0)
RPL35A	3q29	AMP	59 (20.0)
LPP	3q28	AMP	58 (19.7)
TBL	3q26.32	AMP	58 (19.7)
FGF12	3q28	AMP	57 (19.3)
SOX2	3q26.3	AMP	57 (19.3)

Table 5: PIK3CA amplification status by race

Race	Count (%)		Total
	PIK3CA amplification present	PIK3CA amplification absent	Profiled samples
White	38 (18.9)	163 (81.1)	201
Asian	4 (21.1)	15 (78.9)	19
Black or African American	8 (28.6)	20 (71.4)	28
American Indian or Alaska Native	1 (12.5)	7 (87.5)	8

Discussion

There are many significantly mutated or amplified genes implicated in cervical cancer. Some of them are not well-known genes in relation to cervical cancer. For example, the KMT2C, SYNE1, KMT2D, EP300, RYR2, FLG, DMD, and FBXW genes are not commonly implicated gene mutations in cervical cancer, but they are significantly mutated in TCGA cervical cancer dataset. Similarly, MECOM, TRFC, RPL35A, LPP, TBL, FGF12, and SOX2, are not already well-established gene amplifications implicated in cervical cancer, but they are significantly amplified in TCGA dataset.

The Fisher's exact test showed that there were no significant differences in PIK3CA gene mutation status and amplification status by race. This study has helped us to understand that PIK3CA gene mutation and PIK3CA gene amplification do not differ across racial groups with cervical cancer, unlike with breast cancer in which racial differences in certain genes contribute to racial disparities in mortality and survival.[37] However, there are many other significantly mutated/amplified genes such as KMT2C, SYNE1, KMT2D, EP300, RYR2, FLG, DMD, and FBXW that can be explored as therapeutic targets to improve cervical cancer treatment. The finding that PIK3CA is the most frequently mutated gene in this study is similar to the result of the study conducted by Wright *et al*.;[12] however, in addition to the PIK3CA gene, they also found the

KRAS and EGFR genes.[12] Similarly, Xiang *et al*. and Lou *et al*. found the PIK3CA gene as the highest mutated gene in cervical cancer.[11,38]

Limitations of the study and future perspectives

As with any study, this investigation has limitations. The available TCGA dataset for cervical cancer was small; thus, the generalization of this study must be done with caution. Further evaluation with a larger dataset will be required to validate these findings. Focusing on different gene mutations and amplifications that may be implicated in racial differences are highly encouraged.

Conclusions

There are many significantly mutated or amplified genes implicated in cervical cancer. Some of them are not grouped with the already known genes in relation to cervical cancer. They can be explored as therapeutic targets to improve cervical cancer treatment. PIK3CA gene mutation and amplification in cervical cancer do not differ significantly across the various racial groups. The findings of this study are important as they will allow scientists to focus on different gene mutations/amplifications that may be implicated in racial differences. Furthermore, this can push for the creation of targeted prevention and treatment programs that will address other attributed causes, other than those related to genomics.

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