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Research Article

Mutational Analysis of *PIK3CA* Gene from FFPE of Non-Small Cell Lung Cancer in Non-Smokers

Farah Bilal*, Abdulmohsen Alhejaily, Shahida Husnain

Department of Microbiology and Molecular Genetics, Quaid e Azam Campus, University of The Punjab, Lahore, Pakistan.

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FB did experiments and wrote the manuscript. AA presented the idea of the research and designed the project. SH upervised the study and reviewed the manuscript.

Keywords
NSCLC: Non-small Cell Lung Cancer, FFPE: Formalin Fixed paraffin Embedded tissue, *PIK3CA*: Phosphatidylinositol 3-kinase Catalytic Alpha Subunit

Abstract | *PIK3CA* is considered important component of PI3K pathway. Being a regulatory gene for RAS, PI3K and EGFR pathways, any change in *PIK3CA* gene sequence plays vital role in several cancers including lung cancer. Purpose of this study was to evaluate the prevalence of common nucleotide changes (E545K/E542K and H1047R/H1047L) occur in *PIK3CA* gene in non smoker patients of non-small cell lung cancer (NSCLC). Somatic mutational analysis was done by QMC-PCR following direct sequencing with sangers' method. Only one sample detected p.E545K mutation at c.1633G>A. Another most common mutation was observed in *PIK3CA* exon 20 which leads to change A>G at codon 1047. This transition converts amino acid histidine to arginine. Our study concluded p.H1047R most frequent while p.E545K rarely found mutations in *PIK3CA* gene in NSCLC population. Present study can be proved as road map in setting *PIK3CA* mutations as potential therapeutics target for NSCLC in non-smokers.

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Introduction

Single nucleotide changes which occur frequently in several cancers can be reason of tumor initiation and progression. These types of mutations trigger cancer called driver mutations and are vital target in therapeutics treatment of cancers (Pao and Girard, 2011). driver mutations lead to change in expressions of genes work up/down stream of signaling pathway which results in cancer by promoting malignant growth or switching off tumor suppressor genes (Thompson *et al.*, 2016). The signaling pathways promoting cell growth (Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways) involve several significant genes like *KRAS*, *BRAF* and *PIK3CA*. Mutations of these genes can drive cell towards cancer (Vakiani and Solit, 2011). Phosphatidylinositol-3-kinase (PI3K) protein has crucial role in cell proliferation and metabolism. Any abnormality in these kinases can results in cancers. PI3K has its two subunits, regulatory and catalytic subunit (Shull *et al.*, 2012). *PIK3CA* (Phosphoinositide-3-kinase

Corresponding authors: Farah Bilal
farry.fairy2006@gmail.com

catalytic alpha polypeptide) gene's catalytic unit has special importance in cancer study of being mutated in several cancers including lung cancer (Yamamoto *et al.*, 2008). *PIK3CA* mutations were found most frequently in exon 9 (helicase domain) and exon 20 (catalytic domain) which are called hot spot regions. The mutations E545K/E542K in exon 9 and H1047R/H1047L in exon 20, considered as vital oncogenic targets (Chaft *et al.*, 2012). Purpose of our study was to check the prevalence of most common mutations in *PIK3CA* exon9 and exon20 in non-small cell lung cancer (NSCLC) of non-smokers. *PIK3CA* mutations can be potential therapeutic target for treatment of anti-EGFR therapy resistant in never smokers and basic clinical characteristics can be proved best prognostic factors in diagnosis of NSCLC in non-smokers (McCubrey *et al.*, 2012).

Materials and Methods

This was a retrospective study. Histological confirmed NSCLC cases were selected from the department of pathology National Guard Hospital Biobank Riyadh. 50 tissue samples were selected from these confirmed NSCLC patients with no-smoking history. All samples were selected based on availability of formalin-fixed paraffin-embedded tissue samples (FFPE) (from lung cancer patients) and minimum 70% malignant appearance of tumor cells. Patient's baseline clinical characteristics (age, gender and family history of lung cancer) were noted. Tumor-rich area was micro dissected and DNA extraction was done by tissue by QIAamp DNA Mini Kit (Qiagen, Germany). DNA quantification was done by using Nano Drop ND-1000 spectrophotometer (Nano DropTechnologies, Wilmington, DE, USA). Quick multiplex consensus–polymerase chain reaction (QMC-PCR) was performed for DNA amplification followed by sanger's sequencing. Targeted outer primers for *PIK3CA* exon 9 (F 5'-CTGTGAATCCAGAGGGGAAA-3', R 5'-GCACTTACCTGTGACTCCATAGAA-3') and PIK3CA exon 20 (*PIK20F* 5'-TGAGCAAGAGGCTTTGGAGT-3', *PIK20R* 5'-CCTATGCAATCGGTCTTTGC-3') were used in first PCR reaction. final volume of 25 µl reaction mixture was prepared. Each reaction mixture consisted of DNA template (20ng), all outer primers (each concentration of 0.400 mM) and 13HotShot master mix. first PCR was performed using thermal cycle sequence: First cycle at 95°C for 10 minutes, at 95°C for 1 seconds 45 cycles and at 55°C last cycle for 1 second. Amplicon of this PCR was diluted (1:100) with PCR grade water and used as template for final specific diagnostic PCR. Reaction mixture of 10 µl total volume was used including all components with same concentration as in previous PCR. Internal primer (0.25µM) were used for *PIK3CA* exon9 (F 5'-AAGGGAAAATGACAAAGAACAG-3', R 5'-CACTTACCTGTGACTCCATAGAAA-3') and *PIK3CA* exon 20 (F 5'-GCAAGAGGCTTTGGAGTATTTC-3', R 5'-TTTTCAGTTCAATGCATGCTG-3') separately for final diagnostic PCR. Duplicate reaction for each target in specific diagnostic PCR was run on AB 7500 fast PCR thermal cycler. Direct sequencing reaction was done by using purified amplicon of last PCR reaction as template and universal M13 primers (M13F: TGTAAAACGACGGCCAGT, M13R: CAGGAAACAGCTATGACC). Sequencing was done by using commercial prepared kit (Big Dye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, CA). The sequence data was analyzed by DNA Sequence Analysis Software version 3.1.1 and POP 7 (Applied Biosystems, Life Technologies) on ABI 3700 BioAnalyzer.

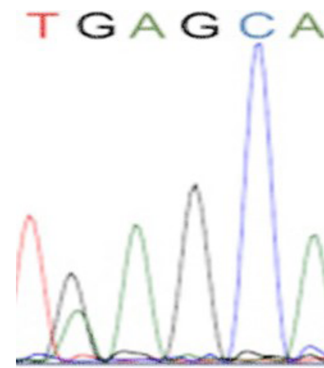

Figure 1: *PIK3CA* gene mutations exon9 c.1633 G>A in NSCLC population.

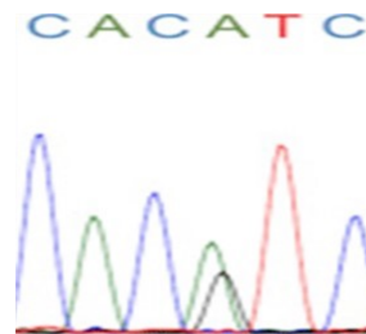

Figure 2: *PIK3CA* gene c.3140 A>Gin NSCLC population.

Results and Discussion

The (PI3K)/AKT pathway plays vital role in cell sur-

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vival by regulating several cellular processes like differentiation, migration and proliferation. Any disturbance in this pathway's mechanisms can lead to cell malignancy and death. Variations in genes involved in (PI3K)/AKT pathway have been reported in many cancers (Steelman *et al.,* 2011). PI3K has two main subunits one is catalytic p110 and other is p85 which is called as regulatory subunit because of its involvement in regulation of cell division. Mutations in these subunits results in tumorigenesis (Chang *et al.,* 2003). The aim of our study was to detect mutations in hot spot region of *PIK3CA* gene in NSCLC of non-smokers. We used QMC-PCR for amplification of targeted sequence in *PIK3CA* gene which is relatively cheap, quick and précised technique. Basically it is a nested PCR which ensures the specificity of target amplification in FFPE tissue sample (Ebili *et al.,* 2017). Two common mutations were noted in *PIK3CA* gene. One mutation E545 (NM_006218.3) was observed in helicase domain shown in Figure 1. Another commonmutation H1047 (NM_ 006218.1) was also seen in kinase domain. Figure 2 showed A>G transition (p.H1047R) at codon 1047 in exon 20, observed in 16 samples (32%) of total 50 NSCLC samples while 34 NSCLC tumor showed wild type for the same position. This mutation (p.H1047R) results in histidine amino acid conversion to arginine amino acid. Second mutation was observed in only one sample (2%) from total NSCLC population. This mutation presented G>A change at codon 1633 in exon 9 of *PIK3CA*. The transition leads to substitute glutamic acid with lysine amino acid at position E545K. No co-existence of mutations was observed in *PIK3CA* exon 20 and exon 9 in any sample. both observed mutations in current study were also reported previous in pancreatic cancer and considered hotspot mutations which also effect the activation of Akt signaling (Schönleben *et al.,* 2006). The same mutations of *PIK3CA* gene were also reported in other cancers like colorectal (32%), gastric (25%) and in lung cancer (4%) (McCubrey *et al.,* 2012). But unfortunately, limited information published on NSCLC of nonsmokers. However, describing prevalence of *PIK3CA* mutations in non-small lung cancer of nonsmokers Barbareschi *et al.* (2007) reported high frequency of *PIK3CA* mutations in exon9 than exon20 in breast cancer, contrasting their data present study confirmed *PIK3CA* exon 20 mutations were more frequent than exon9 mutation in NSCLC population. We examined only one mutation E545K in helicase domain of one NSCLC tumor which is common mutation reported in many cancers specially in colorectal that was Glu545 to Lys545 (E545K) and thought to interrupt p85 N-terminal SH2 domain inhibitory interaction (Miled *et al.,* 2007). Contrasting our finding another study reported more frequent mutations of PIK3CA exon 9 in NSCLC (Yamamoto *et al.,* 2008). SNPs encoding K545E and H1047R have significant role in enhancing migrating and growth activity of epithelial cells (Okudela *et al.,* 2007). These observed factors predict mutations in *PIK3CA* exon 20 can be a potential targets for treatment of NSCLC in response of resistant NSCLC against anti-EGFR therapy hence can be recommended to identify better response to chemotherapies (Mao *et al.,* 2012). The small number of non-small lung cancer population limited mutational importance of *PIK3CA* gene. This issue can be resolved by increasing more patient's data to present study can lead to new drug therapy for NSCLC in future.

References

Barbareschi, M., Buttitta, F., Felicioni, L., Cotrupi, S., Barassi, F., Del Grammastro, M. and Marchetti, A., 2007. Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. *Clin. Cancer Res.,* **13**(20): 6064-6069.

Chaft, J.E., Arcila, M.E., Paik, P.K., Lau, C., Riely, G.J., Pietanza, M.C., Zakowski, M.F., Rusch, V., Sima, C.S., Ladanyi, M. and M. Kris, M.G., 2011. Coexistence of *PIK3CA* and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol. Cancer Ther.*, **11**:485-491. https://doi.org/10.1158/1535-7163.MCT-11-0692

Chang, F., Lee, J.T., Navolanic, P.M., Steelman, L. S., Shelton, J.G., Blalock, W.L., Franklin, R.A. and Mccubrey, J.A., 2003. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis and neoplastic transformation: a target for cancer chemotherapy. *Leukemia.*, **17**:590-603. https://doi.org/10.1038/sj.leu.2402824

Ebili, H.O., Hassall, J., Asiri, A., Ham-Karim, H., Fadhil, W., Agboola, A.J. and Ilyas, M., 2017. QMC-PCRx: a novel method for rapid mutation detection. *J. Clin. Pathol.*, **70**:702-711. https://doi.org/10.1136/jclinpath-2016-204264

Mao, C., Yang, Z.Y., Hu, X.F., Chen, Q. and Tang, J.L., 2011. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Ann. Oncol.*, **23**:1518-1525. https://doi.org/10.1093/annonc/mdr464

Mccubrey, J.A., Steeman, L., Chappell, W., Abrams, S., Montalto, G., Cervello, M., Nicoletti, F., Fagone, P., Malaponte, G., Mazzarino, M., Candido, S., Libra, M., Basecke, J., Mijatovic, S., Maksimovic-Ivanic, D., Milella, M., Tafuri, A., Cocco, L., Evangelisti, C., Chiarini, F. and Martelli, A., 2012. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades. *Oncotarget.*, **3**. https://doi.org/10.18632/oncotarget.652

Miled, N., Yan, Y., Hon, W.C., Perisic, O., Zvelebil, M., Inbar, Y., Schneidman-Duhovny, D., Wolfson,

Links Researchers

H.J., Backer, J.M. and Williams, R.L., 2007. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science*, **317**:239-242. https://doi.org/10.1126/science.1135394

Okudela, K., Suzuki, M., Kageyama, S., Bunai, T., Nagura, K., Igarashi, H., Takamochi, K., Suzuki, K., Yamada, T., Niwa, H., Ohashi, R., Ogawa, H., Mori, H., Kitamura, H., Kaneko, T., Tsuneyoshi, T. and Sugimura, H., 2007. PIK3CA mutation and amplification in human lung cancer. *Pathol. Int.*, **57**:664-671. https://doi.org/10.1111/j.1440-1827.2007.02155.x

Pao, W. and Girard, N., 2011. New driver mutations in non-small-cell lung cancer. *Lancet Oncol.*, **12**:175-180. https://doi.org/10.1016/S1470-2045(10)70087-5

Schonleben, F., 2006. PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin. Cancer Res.*, **12**:3851-3855. https://doi.org/10.1158/1078-0432.CCR-06-0292

Shull, A.Y., Latham-Schwark, A., Ramasamy, P., Leskoske, K., Oroian, D., Birtwistle, M.R. and Buckhaults, P.J., 2012. Novel somatic mutations to PI3K pathway genes in metastatic melanoma. *PLoS One.*, **7**:e43369. https://doi.org/10.1371/journal.pone.0043369

Steelman, L.S., Chappell, W.H., Abrams, S.L., Kempf, C.R., Long, J., Laidler, P., Mijatovic, S., Maksimovic-Ivanic, D., Stivala, F., Mazzarino, M.C., Donia, M., Fagone, P., Malaponte, G., Nicoletti, F., Libra, M., Milella, M., Tafuri, A., Bonati, A., Bäsecke, J., Cocco, L., Evangelisti, C., Martelli, A.M., Montalto, G., Cervello, M. and Mccubrey, J.A., 2011. Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. *Aging.*, **3**:192-222. https://doi.org/10.18632/aging.100296

Thompson, J.C., Yee, S.S., Troxel, A.B., Savitch, S.L., Fan, R., Balli, D., Lieberman, D.B., Morrissette, J.D., Evans, T.L., Bauml, J., Aggarwal, C., Kosteva, J.A., Alley, E., Ciunci, C., Cohen, R.B., Bagley, S., Stonehouse-Lee, S., Sherry, V.E., Gilbert, E., Langer, C., Vachani, A. and Carpenter, E.L., 2016. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by Next-Generation Sequencing of cell-free circulating tumor DNA. *Clin. Cancer Res.*, **22**:5772-5782. https://doi.org/10.1158/1078-0432.CCR-16-1231

Vakiani, E. and Solit, D.B., 2010. KRAS and BRAF: drug targets and predictive biomarkers. *J. Pathol.*, **223**:220-230. https://doi.org/10.1002/path.2796

Yamamoto, H., Shigematsu, H., Nomura, M., Lockwood, W.W., Sato, M., Okumura, N., Soh, J., Suzuki, M., Wistuba, I.I., Fong, K.M., Lee, H., Toyooka, S., Date, H., Lam, W.L., Minna, J.D. and Gazdar, A.F., 2008. *PIK3CA* mutations and copy number gains in human lung cancers. *Cancer Res.*, **68**:6913-6921. https://doi.org/10.1158/0008-5472.CAN-07-5084