UDC 575.
https://doi.org/10.2298/GENSR2103207O
Original scientific article

RUNX3 PROMOTER METHYLATION IS ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA LOCATION

Kenneth C. ONYEGBULA[1], Benjamin O. EMIKPE[2], Akinyele O. ADISA[3], Chiaka I. ANUMUDU[4,*]

[1]Department of Biomedical Laboratory Science, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria
[2]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria
[3]Department of Oral Pathology, Faculty of Dentistry, College of Medicine, University of Ibadan, Nigeria
[4]Department of Zoology, Faculty of Science, University of Ibadan, Nigeria

Onyegbula C.K., B. O. Emikpe, A. O. Adisa, C. I. Anumudu (2021). *RUNX3 promoter methylation is associated with oral squamous cell carcinoma location*. - Genetika, Vol 53, No.3, 1207-1217.

The aetiology of OSCC remains unclear, however, aberrant methylation of C_pG island promoters of tumor suppressor genes have been identified as contributory developmental pathways in several cancers. The aim of this study was to determine the presence of RUNX3 gene methylation and how its association with patients' demographic variables such as gender, age, histologic class and tumor location could be of diagnostic value for OSCC. Sixty-seven formalin-fixed paraffin-embedded (FFPE) solid tissue blocks of OSCC, and nine blocks of benign oral lesions of epithelial origin retrieved from the archives of the Department of Oral Pathology, University College Hospital, Ibadan, South-West Nigeria were used for the analyses. Frequency of C_pG island methylation in the promoter region of RUNX3 was determined by methylation-specific polymerase chain reaction (MSP). Association between gender, age, tumor location, histologic class and promoter methylation in RUNX3 was assessed with Pearson's χ^2 test. Overall, 45% (30/67) of OSCC demonstrated methylation in the RUNX3 promoter indicating a high frequency of methylation of the C_pG island promoter region of RUNX3. There was no association between gender, age, histologic class and promoter methylation in RUNX3 ($P > 0.05$), however a significant association was observed between tumor location and

Corresponding author Chiaka I. Anumudu, Department of Zoology, Faculty of Science, University of Ibadan, Nigeria, E-mail: cianumudu@yahoo.com; Tel.: 234-802-359-0478.

.

.

promoter methylation of RUNX3 ($P < 0.05$). Aberrant methylation of the C_pG island promoter region of RUNX3 together with tumor location could therefore be critical in the development and diagnosis of OSCC.

Key words: Diagnosis, risk factors, C_pG methylation, oral cancers, Ibadan

INTRODUCTION

Oral squamous cell carcinoma is the most common carcinoma affecting the oral cavity. It is capable of locally destructive growth and distant metastases. It is made up of three histological sub-types and constitutes about 94% of all oral carcinomas and 4% of all cancers irrespective of anatomic site (AL-RAWI and TALABANI, 2008; BHARGAVA *et al*., 2010; KHAN and KHAN, 2015). Worldwide, there are approximately 540,000 new cases annually, out of which 271,000 deaths occur, representing 50% mortality (CADONI *et al*., 2012). Approximately 4 - 6% of oral squamous cell carcinoma occurs in patients aged <40 years old, and are more prevalent in males than in females with a ratio of 3:1 (IAMAROON *et al*., 2004; DEL CORSO *et al*., 2016). Post-management recurrence and metastatic phenomena have been observed in 60% of patients and 15 - 20% of cases respectively (CHOI and CHEN, 2005) with a low survival of 5 years in 40 - 59% of patients (LEEMANS *et al*., 2011; CORDEIRO-SILVA *et al*., 2012). In Nigeria as in other African countries, there is a male preponderance with increasing incidence in younger populations, high morbidity and mortality rates and poor treatment outcome (LILLY-TARIAH *et al*., 2009).

The idea that epigenetic changes can be a mechanism for altering gene expression and driving tumorigenesis has been validated by works on breast cancer (SNIDER *et al*., 2019), lung cancer (NITSCHKOWOSKI *et al*., 2019), hepatocellular cancer (ZHENG *et al*., 2019), gastric cancer (FANG *et al*., 2019) and colorectal cancer (JENSEN *et al*., 2019). An important epigenetic pathway of transcriptional inactivation for many tumor suppressor genes is C_pG island methylation within promoter regions.

This has been identified in several human cancers including head and neck cancers (LIYANGE *et al*., 2019; JASEK *et al*., 2019; KHATAMI *et al*., 2019; ELJABO *et al*., 2018). One of such gene is RUNX3, a transcription factor regulating developmental processes and localized on chromosome 1p36.1 (TSUNEMATSU *et al*., 2009; SUPIC *et al*., 2011). Non expression of this gene by epigenetic mechanism has been shown to inhibit the invasion of bone cells in oral squamous cell carcinoma (PARK *et al*., 2017).

The purpose of this study was to determine the pattern of RUNX3 gene methylation and its relationship with demographic variables such as gender, age, histology and location of tumor in OSCC patients in Ibadan, South-West Nigeria.

MATERIAL AND METHODS

Study samples

Between January 2004 and December 2015, blocks of 10% buffered FFPE solid tissues of OSCC samples (test samples) and benign oral lesions of epithelial origin (control samples) were retrieved from the archives of the Department of Oral Pathology, University College Hospital, Ibadan, South-West Nigeria. Genomic DNA extraction

Four tissue sections ($\leq$ 20 μm thick) were obtained from each block of tissue and transferred to 1.5 ml microcentrifuge tubes for deparaffinization (COURA *et al*., 2005). Samples

were subsequently digested with Protenaise-K followed by DNA isolation according to manufacturers' (ZYMO RESEARCH) instruction. The eluted DNA were then collected in sterile PCR tubes and stored at -30ºC for subsequent applications.

Bisulfite-modification of isolated DNA

50µl of DNA from each sample was used for sodium bisulfite treatment in order to convert all unmethylated cytosines to uracils whilst leaving methylated cytosines unaffected. For the denaturation of DNA, 50µl of genomic DNA from each sample was placed in sterile PCR tubes to which 0.2M NaOH was added and incubated for 10 minutes at 37ºC. Thereafter, 30µl of 10mM hydroquinone and 520µl of 3M sodium bisulfite at pH 5.0 both freshly prepared was added to each tube, mixed and incubated at 50ºC for 16 hours. Subsequently, the modified DNA was purified using DNA purification resin (Promega) and eluted into 50µl of water. Modification was completed by NaOH (0.3M) treatment for 5 minutes at room temperature, followed by ethanol precipitation (TSUNEMATSU *et al*., 2009).

Methylation-specific PCR amplification

Each modified DNA was thereafter amplified in a 12.5µl total reaction volume containing 2µl of modified DNA, 2.25µl of Mastermix, 2.0µl of RUNX3 gene primer and 6.25µl of sterile water after an initial denaturation step at 95°C for 3 minutes, subsequent denaturation at 95°C for 15 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 60 seconds and a final extension step at 72°C for 5 minutes. This was carried out for 40 cycles. Thereafter, the PCR products were loaded onto 2% agarose gel and visualized by ethidium bromide staining using 100-bp ladder (TSUNEMATSU *et al*., 2009). The following primer pairs were used for the detection of the methylated and unmethylated regions of the gene (F 5'-ATAATAGCGGTCGTTAGGGCGTCG-3'; R 5'- GCTTCTACTTTCCCGCTTCTCGCG-3' and F 5'- TTATGAGGGGTGGTTGTATGTGGG-3'; R 5'-AAAACAACCAACACAAACACCTCC-3'), respectively.

Statistical analysis

Data of promoter methylation of RUNX3 for gender, age, tumor location and histology was analyzed by descriptive statistics and presented as frequencies and percentages while Pearson's χ^2 test was used to assess association between gender, age, tumor location, histology and promoter methylation in RUNX3. For all analysis, $P \leq 0.05$ was considered significant.

Ethics

Ethical approval (reference number AD 13/479/4003A) for the study was obtained from the Ministry of Health, Oyo State, Nigeria.

RESULTS

Frequency of RUNX3 methylation in OSCC

On the basis of the presence of C_pG islands methylation in the 5′ region of RUNX3 gene, 45% (30/67) of the OSCC tumor samples were found to be methylated while 55% (37/67) were not methylated.

Association between RUNX3 methylation and patients' demographic variables

Association between promoter methylation of RUNX3 with gender, age, histologic class and tumor location in OSCC are shown in Figures 1-4 respectively and summarized in Table 1.

Table 1. Association between promoter methylation in RUNX3 and tumor location

Tumour site	Number of cases	methylated (%)	P-value
			0.010*
Tongue	13	6 (46.2)	
Antrum	2	1 (50.0)	
Facial mass	6	3 (50.0)	
Maxilla	10	5 (50.0)	
Lip	4	2 (50.0)	
Mandible	14	9 (64.3)	
Floor of mouth	2	2 (100.0)	
Commissure	1	1 (100.0)	
Buccal mass	2	1 (50.0)	
Palate	8	0 (0.0)	
Oropharynx	1	0 (0.0)	
Undisclosed	2	0 (0.0)	
Parotid	1	0 (0.0)	
Medial canter	1	0 (0.0)	

*significant at $p \leq 0.05$ using Pearson's χ^2 test

Out of a total of 43 male and 24 female OSCC samples, 21 male and 9 female samples respectively were methylated while 22 male and 15 female samples were not methylated. No association was established between promoter methylation of RUNX3 with gender (P = 0.157). Out of a total of 32 samples with ages in the range of ≥ 60 years, 13 were methylated while 19 were not methylated. Furthermore, out of the 32 samples with ages < 60 years, 16 were methylated and 16 were not methylated. Out of the 3 samples with undisclosed age, 1 sample was methylated while 2 were not methylated. No association was established between promoter methylation of RUNX3 with age (P = 0.223).

Out of the 17 well differentiated squamous cell carcinoma samples, 10 were methylated while 7 were not methylated. Furthermore, out of the 46 moderately differentiated squamous cell carcinoma samples, 19 were methylated while 27 were not methylated. And out of the 4 poorly differentiated squamous cell carcinoma samples used in the study, 1 was methylated while 3 were not methylated. No association was established between promoter methylation of RUNX3 with histology (P = 0.199). The total number of samples for each tumor location and the respective frequencies for methylated and unmethylated samples are also presented in Table 1. Strong association was established between promoter methylation of RUNX3 with tumor location (P = 0.010).

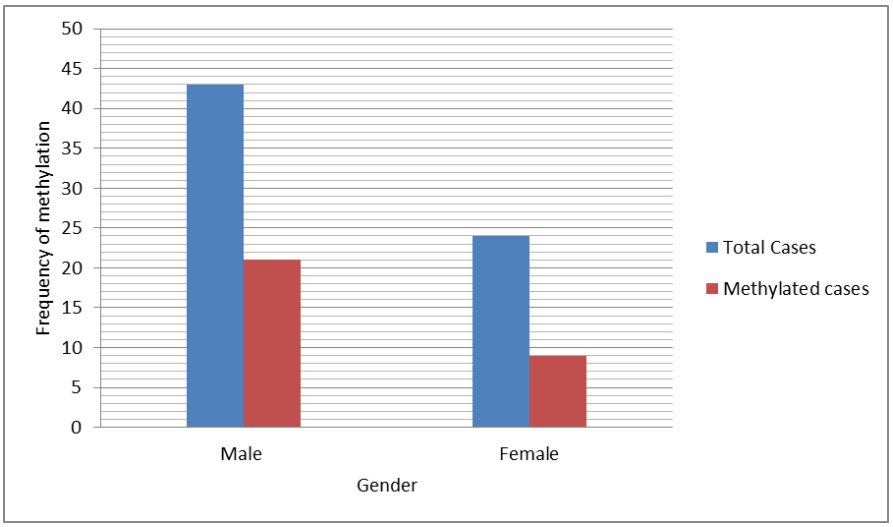

Figure 1. Association between promoter methylation in RUNX3 and gender (P-value = 0.157).

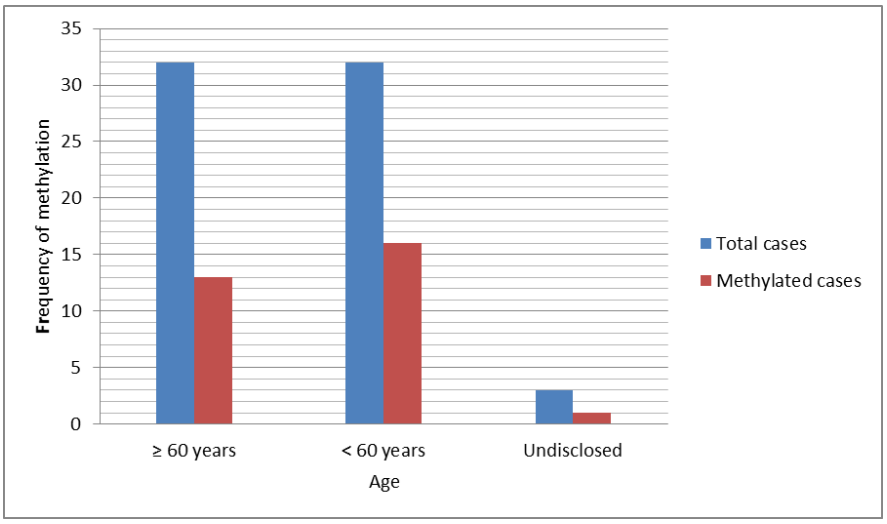

Figure 2. Association between promoter methylation in RUNX3 and age (P-value = 0.223).

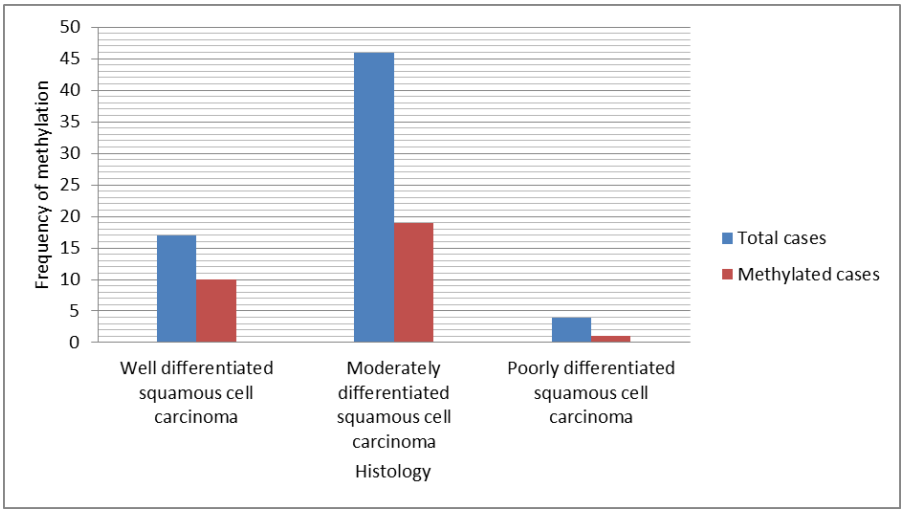

Figure 3. Association between promoter methylation in RUNX3 and histologic class (P-value = 0.199).

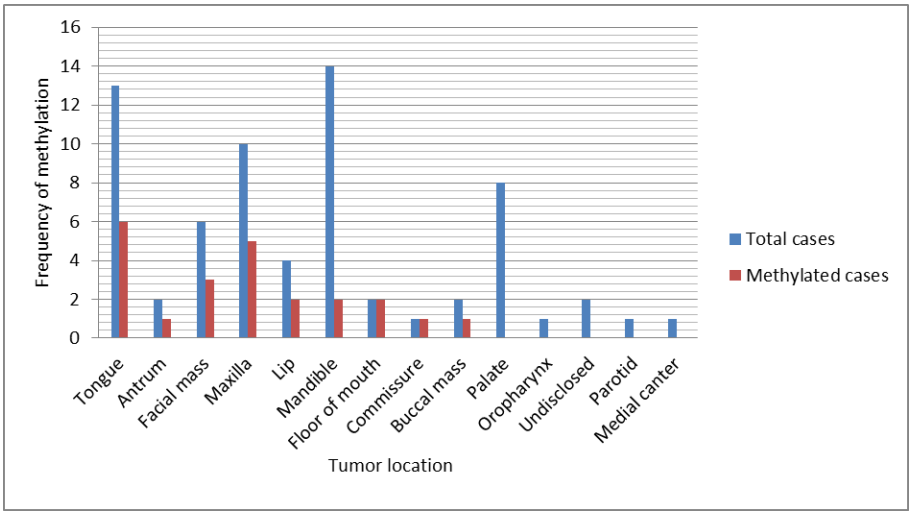

Figure 4. Association between promoter methylation in RUNX3 and tumor location (P-value = 0.010).

DISCUSSION

Aberrant C_pG island methylation of the promoter region is known to silence some genes as efficiently as mutations or deletions; genome scans for aberrant DNA methylation have shown that up to 10% of C_pG islands are methylated in human malignancies (COSTELLO *et al*., 2000). To this end, involvement of transcriptional silencing of several genes including p16 (SHI *et al*., 2015; NIKOLIC *et al*., 2015), MGMT (CHEN *et al*., 2018), ZNF471 (BHAT *et al*., 2017) and DAPK1 (JAYAPRAKASH *et al*., 2017) among others by DNA methylation in cancer development have been established.

Furthermore, methylation of TFP12, SOX17 and GATA4 genes (KIM *et al*., 2019); P15, P14, DAPK, P73, WIF1, E cadherin, hMLH1 genes (HEMA *et al*., 2017); APC, MGMT, TIMP3 and CDH1 genes (STRZELCZYK *et al*., 2018); P16, RASSF1A, TIMP3, PCQAP/MED15 genes (LIYANGE *et al*., 2019; ELJABO *et al*., 2018) to mention a few have been associated with OSCC development. In this study, we show the methylation status of RUNX3 in OSCC and investigate its association with patients' demographic variables such as gender, age, tumor location and histologic differentiation.

First, we observed the occurrence of a fairly high frequency of promoter methylation of RUNX3 in OSCC. Similar observations of elevated methylation of this gene had been made in colorectal cancer (SHIN *et al*., 2018); lung cancer (ZHAO *et al*., 2019); gastric cancer (LIN *et al*., 2017); bladder cancer (LEE and SONG, 2017); hepatocellular cancer (GAOFENG *et al*., 2017); esophageal cancer (REHMAN *et al*., 2020) and OSCC in a Brazillian study (CORDEIRO-SILVA *et al*., 2012).

In addition, SUPIC *et al*., (2011) did not only report a high rate of deviant RUNX3 methylation in tongue cancers but, also that this was positively connected with nodal metastases and tumour staging. On their part, LIU *et al*., (2016) also reported a high occurrence of RUNX3 methylation in laryngeal squamous cell carcinoma and that this was certainly associated with disease outcome and spread. It was also observed that epigenetic silencing of RUNX3 also contributes to invasion of bony structures and the development of osteolysis in OSCC (PARK *et al*., 2017). So was it also reported by SASAHIRA *et al*., (2011) that, down-regulation of RUNX3 gene correlates with poor prospects in both adenoid cystic and mucoepidermoid carcinomas of the salivary gland.

This suggests that aberrant methylation of C_pG islands in the promoter region of RUNX3 may be a mechanism for the development of OSCC through gene inactivation. Second, we also demonstrate that promoter methylation of RUNX3 in OSCC was not significantly associated with gender, age and histologic differentiation

This agrees with observations made in gastric cancer (HU *et al*., 2010; GARGANO *et al*., 2007); colorectal cancer (SHIN *et al*., 2018); and OSCC in a Brazillian study (CORDEIRO-SILVA *et al*., 2012). We however demonstrate a significant association between promoter methylation of RUNX3 in OSCC with tumor location which is in contrast with what was observed in the Brazillian study (CORDEIRO-SILVA *et al*., 2012).

CONCLUSIONS

We have shown that aberrant methylation of C_pG islands in the promoter region of RUNX3 (1) occurs frequently in OSCC (2) may be a possible mechanism for RUNX3

inactivation thus giving rise to OSCC development and that (3) tumor location together with promoter methylation may be an important factor in the development of OSCC, which should be further investigated on a larger cohort.

ABBREVIATIONS

OSCC: Oral squamous cell carcinoma; CpG: Cytosine-phosphate-guanine; FFPE: Formalin-fixed paraffin-embedded; MSP: Methylation-specific polymerase chain reaction; PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid

Received, June 12th, 2020
Accepted March 28th, 2021

REFERENCES

ARABI, Z. *et al*. (2017): Seed micromorphology and its systematic significance in tribe Alsineae (Caryophyllaceae). Flora, *234*: 41–59.

BI, D AL-RAWI, N.H., N.G., TALABANI (2008): Squamous cell carcinoma of the oral cavity: A case series analysis of clinical presentation and histological grading of 1425 cases from Iraq Clin. Oral Invest, *12*: 15-18.

BHARGAVA, A., S., SAIGA, M., CHALISHAZAR (2010): Histopathological grading systems in oral squamous cell carcinoma: A review. Journal of International Oral Health, *2*(4): 1-10.

BHAT, R., S., KABEKKODU, S.P., JAYAPRAKASH, C., RADHAKRISHNAN (2017): Gene promoter-associated CpG island hypermethylation in squamous cell carcinoma of the tongue. Virchows Archives, 470(4):445-454.

CADONI, G., S., BOCCIA, L., PETRELLI, P., DI GIANNANTONIO (2012): A review of genetic epidemiology of head and neck cancer related to polymorphisms in metabolic genes, cell cycle control and alcohol metabolism. ACTA Otorhinolaryngologia Italica, *32*(11):1-11.

CAO, S., H., ZHENG, B., SUN, Z., WANG, T., LIU, D., GUO, Z., SHEN (2019): Long non-coding RNA are highly up-regulated in liver cancer promotes exosome secretion. World Journal of Gastroenterology, *25*(35): 5283-5299.

CHEN, L., Y., WANG, F., LIU, L., XU, F., PENG, N., ZHAO, B., FU, Z., ZHU, Y., SHI (2018): A systematic review and meta-analysis: Association between *MGMT* hypermethylation and the clinicopathological characteristics of non-small-cell lung carcinoma. Scientific Reports, *8*: 1439.

CHOI, P., C., CHEN (2005): Genetic expression profiles and biologic pathway alterations in head and neck squamous cell carcinoma. Cancer, *104*(6): 1113-1128.

CORDEIRO-SILVA, M., E., STUR, L., AGOSTINI, J., VASCONCELOS DE PODESTA *et al*., (2012): Promoter hypermethylation in primary squamous cell carcinoma of the oral cavity and oropharynx: A study of a Brazillian cohort. Molecular Biology Reports, *39*: 10111-10119.

COSTELLO, R., T., MALLET, F., GAUGLER, B., SAINTY (2000):Human acute myeloid LeukemiaCD34+/CD38promoter cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis,reduced immunogenicity and impaired dendritic cell transformation capacities. Cancer Res., *60*: 4403-4441.

COURA, R., J.C., PROLLA, L., MEURER, P. ASHTON-PROLLA (2005): An alternative protocol for DNA extraction from formalin fixed paraffin wax embedded tissue. J.Clinical Path., *58*(8): 894-895.

DEL-CORSO, G., A., VILLA, A., TARSITANO, A., GOHEL (2016): Current trends in oral cancer: A review. Cancer Cell and Microenvironment, *3:* e1332

ELJABO, N., N., NIKOLIC, J., CARKIC, D., JELOVAC, M., LAZAREVIC, N., TANIC, J., MILASIN (2018): Genetic and epigenetic alterations in the tumour, tumour margins, and normal buccal mucosa of patients with oral cancer. Int. J. Oral and Maxillofacial Surgery, *47(8)*:976-982.

FANG, X., J., WEN, M., SUN, Y., YUAN, Q., XU (2019):CircRNAs and its relationship with gastric cancer. J. Cancer, *10*(24): 6105-6113.

GAOFENG, S., C., ZANG, F., MIN, L., WENSHENG, X., HUIFANG, Q., QIN, E., ZHAO, L., WAN (2017): Methylation analysis of *P16*, *SLIT2*, *SCARA5* and *RUNX3* in hepatocellular carcinoma. Medicine, *96*(41): 8279.

GARGANO, G., D., CALCARA, S., CORSALE, V., AGNESE, C., INTRIVICI, F., FULFARO, G., PANTUSO, M., CAJOZZO, V., MORELLO, R.M., TOMASINO, L., OTTINI, G., COLUCCI, V., BAZAN, A., RUSSO (2007): Aberrant methylation within *RUNX3* CpG island associated with the nuclear and mitochondrial microsatellite instability in sporadic gastric cancers. Ann. Oncology, *18*(6): 103-109.

GUIU, J., E., HANNEZO, S., YUI *et al*. (2019): Tracing the origin of adult intestinal stem cells. Nature, *570*: 107-111.

HEMA, K.N., T., SMITHA, H.S., SHEETHAL, S.A., MIRNALINI (2017): Epigenetics in oral squamous cell carcinoma. J. Oral and Maxillofacial Pathology, *21*(2): 252-259.

HU, S., X., KONG, Z., CHENG, *et al.* (2010): Promoter hypermethylation of *p16*, *RUNX3*, *DAPK* and *CHFR* genes are frequent in gastric carcinoma. Tumori, *96*: 726-733.

IAMAROON, A., S., PATTANAPORN, S., PONGSIRIWET *et al*. (2004): Analysis of 587 cases of oralsquamous cell carcinoma in Northern Thailand with focus on young people. International Journal of Oral and Maxillofacial Surgery, *33*: 84-88.

JASEK, K., P., KUBATKA, M., SAMEC, A., LISKOVA, K., SMEJKAL (2019): DNA methylation in cancer disease: modulations by plant-derived natural compounds and dietary interventions. Biomolecules, *9*(7): 289.

JAYAPRAKASH, C., V.K., VARGHESE, R., BELLAMPALLI, R., RADHKRISHNAN *et al*. (2017): Hypermethylation of death-associated protein kinase (*DAPK1*) and its association with oral carcinogenesis - an experimental and meta-analysis study. Archives of Oral Biology, *80*: 117-129.

KHAN, N.R., A.R., KHAN (2015): Insight into squamous cell carcinoma of head and neck region: Current statistics. J. Dental App., *2*(6): 232-239.

KHATAMI, F., B., LARIJANI, S., NIKFAR, M., HASANZAD, K., FENDERESKI, S.M., TAVANGAR (2019): Personalized treatment options for thyroid cancer: current perspectives. Pharmacogenomics and Personalized Medicine, *12*: 235-245.

KIM, S.Y., Y.K., HAN, J.M., SONG *et al*. (2019): Aberrantly hypermethylated tumor suppressor genes were identified in OSCC. Clinical Epigenetics, *11*: 116.

LEE, K., C.G., SONG (2017): Epigenetic regulation in bladder cancer: development of new prognostic targets and therapeutic implications. Translational Cancer Research, *6*(4): S677–S688.

LEEMANS, C.R., J.M., BRAAKHUIS, R.H., BRAKENHOFF (2011): Molecular biology of head and neck cancer. Nature Reviews Cancer, *11*: 9-22.

LILLY-TARIAH, O., A., SOMEFUN, W., ADEYEMO (2009): Current evidence on the burden of head and neck cancers in Nigeria. Head and Neck Oncology, *1*: 14.

LIN, Z., M., LUO, X., CHEN, X., HE, Y., QIAN, S., LAI, J., SI, S., CHEN (2017): Combined detection of *plasmaZIC1*, *HOXD10* and *RUNX3* methylation is a promising strategy for early detection of gastric cancer and precancerous lesions. J. Cancer, *8*(6): 1038-1044.

LIU, Z.H., J.J., LIU, S.S., LI, X.M.,YANG (2016): Association of RUNX3 methylation with clinical outcome and cell migration/invasion in laryngeal squamous cell carcinoma. Cancer Investigations, *34(2)*: 105-113.

LIYANAGE, C., A., WATHUPOLA, S., MURALEETHARAN, K., PERERA *et al*., (2019): Promoter hypermethylation of tumour suppressor genes *P16*, *RASSF1A*, *TIMP3*, *PCQAP/MED15* in salivary DNA as a quadruple biomarker panel for early detection of oral and oropharyngeal cancers. Biomolecules, *9*(4): 148.

NIKOLIC, N., ANICIC, B., CARKIC, J., SIMONOVIC, J., TOLJIC, B., N., TANIC (2015): High frequency of p16 and p14 promoter hypermethylation and marked telomere instability in salivary gland tumors. Archives of Oral Biology, *60*:1662-1666.

NITSCHKOWOSKI, D., S., MARWITZ, S., KOTANIDOU *et al*., (2019): Live and let die: epigenetic modifications of surviving and regucalcin in non-small cell lung cancer tissues contribute to malignancy. Clinical Epigenetics, *11*: 157.

PARK, J., H.J., KIM, K.R., KIM, S.K., LEE, H., KIM, K.K., PARK, W.Y. CHUNG (2017): Loss of RUNX3 expression inhibits bone invasion of oral squamous cell carcinoma. Oncotarget, *8*:9079-9092.

REHMAN, A.U., M.A., IQBAL, R.S.A., SATTAR, S., SAIKIA, M., KASHIF, W.M., ALI, S., MEDHI, S.S., SALUJA, S.A., HUSAIN (2020): Elevated expression of RUNX3 co-expressing with EZH2 in esophageal cancer patients from India. Cancer Cell International, *20*: 445.

SASAHIRA, T., M., KURIHARA, K., YAMAMOTO, U.K., BHAWAL, T., KIRITA, H., KUNIYASU (2011): Downregulation of runt-related transcription factor 3 associated with poor prognosis of adenoid cystic and mucoepidermoid carcinomas of the salivary gland. Cancer Sci., *102*: 492-497.

SHI, H., X., CHEN, C., LU, C., GU, H., JIANG, R., MENG, X., NIU, Y., HUANG, M., LU (2015): Association between $p16^{INK4a}$ promoter methylation and HNSCC: A meta-analysis of 21published studies. PLOS one, *10*(4): e0163257.

SHIN, E., H., KIM, M., SON, T., AHN *et al*. (2018): Epigenetic inactivation of *RUNX3* in colorectal cancer. Annals of Surgical Treatment and Research, *94*: 19-25.

SNIDER, H., B., VILLAVARAJAN, Y., PENG, L., SHEPHERD, A., ROBINSON, C., MUELLER, (2019): Region specific glucocorticoid receptor promoter methylation has both positive and negative prognostic value in patients with estrogen receptor-positive breast cancer. Clinical Epigenetics, *11*:155.

STRZELCZYK, K.J., L., KRAKOWCZYK, A., OWCZAROK (2018): Aberrant DNA methylation of the*P16*, *APC*, *MGMT*, *TIMP3* and *CDH1* gene promoters in tumors and the surgical margins of patients with oral cavity cancer. J. Cancer, *9*(11): 1896-1904.

SUPIC, G., R., KOZOMARA, N., JOVIC, K., ZELJIC, Z., MAGIC (2011): Hypermethylation of RUNX3 but not WIF1 gene and its association with stage and nodal status of tongue cancers. Oral Discoveries, *17(8)*:794-800.

TSUNEMATSU, T., Y., KUDO, S., LIZUKA, I., OGAWA *et al*. (2009):*RUNX3* has an oncogenic role in head and neck cancer. PLos one, *4*(6): e5892.

ZHAO, J., X., CUI, X., HUANG, R., LU, P., CHEN, Z., ZHANG (2019): Methylation of *RUNX3* and *RASSF1A* and the risk of malignancy in small solitary pulmonary nodules. J. Cancer Research and Therapeutics, *15*(4): 899-903.

RUNKS3 PROMOTER METILACIJA JE POVEZANA SA LOKACIJOM ORALNIH SKVAMOCELULARNIH KARCINOMA

Kenneth C. ONYEGBULA[1], Benjamin O. EMIKPE[2], Akinyele O. ADISA[3], Chiaka I. ANUMUDU[4,*]

[1]Departman za biomedicinske laboratorijske nauke, Fakultet za osnovne medicinske nauke, Koledž za medicinu, Univerzitet Ibadan, Nigerija
[2]Departman za veterinarsku patologiju, Fakultet veterinarske medicine, Univerzitet Ibadan, Nigerija
[3]Departman za oralnu patologiju, Stomatološki fakultet, Koledž za medicinu, Univerzitet Ibadan, Nigerija
[4]Departman za zoologiju, Prirodno-matematički fakultet, Univerzitet Ibadan, Nigerija

Izvod

Etiologija OSCC-a ostaje nejasna, međutim, aberantna metilacija CpG ostrva promotera tumorskih supresorskih gena identifikovana je kao dodatni razvojni put kod nekoliko karcinoma. Cilj ove studije je bio da se utvrdi prisustvo metilacije gena RUNX3 i kako njegova povezanost sa demografskim varijablama pacijenata kao što su pol, starost, histološka klasa i lokacija tumora može biti od dijagnostičkog značaja za OSCC. Šezdeset sedam formalinom fiksiranih parafinom ugrađenih (FFPE) čvrstih blokova tkiva OSCC-a i devet blokova benignih oralnih lezija epitelnog porekla preuzetih iz arhive Odeljenja za oralnu patologiju, Univerzitetske bolnice, Ibadan, jugozapadna Nigerija koristi su se za analize. Učestalost metilacije CpG ostrva u promotorskom regionu RUNX3 određena je lančanom reakcijom polimeraze specifične za metilaciju (MSP). Povezanost između pola, starosti, lokacije tumora, histološke klase i metilacije promotera u RUNX3 procenjena je Pearsonovim χ^2 testom. Sve u svemu, 45% (30/67) OSCC je pokazalo metilaciju u promotoru RUNX3 što ukazuje na visoku učestalost metilacije regiona promotera CpG ostrva RUNX3. Nije bilo veze između pola, starosti, histološke klase i metilacije promotera u RUNX3 ($P > 0,05$), međutim uočena je značajna povezanost između lokacije tumora i metilacije promotera RUNX3 ($P < 0,05$). Aberantna metilacija regiona promotora CpG ostrva RUNX3 zajedno sa lokacijom tumora mogla bi stoga biti značajna u razvoju i dijagnozi OSCC-a.

Primljeno 12.VI.2020.
Odobreno 28. III. 2021.