International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 05 (A); May 2019; Page No.18547-18551
DOI: http://dx.doi.org/10.24327/ijcar.2019.18551.3548

Research Article

ECTOPIC VIRAL INTEGRATION SITE-1 (EVI1)-MEDIATED REPRESSION OF CARBONIC ANHYDRASE III (CAIII) AND ITS IMPLICATIONS: ROLE IN ACUTE MYELOID LEUKAEMIA

Kanayo Eugene Ikeh*[1], Evi OgheneIretioluwa Osifo-Whiskey[2], and Sylvanus Akpak Upula[3]

[1]Department of Medical Laboratory Science, University of Jos, Nigeria
[2]Department of Biological Sciences, University of Glasgow, UK
[3]Department of Microbiology, Cross River University of Technology, Calabar, Nigeria

A R T I C L E I N F O

Article History:
Received 6th February, 2019
Received in revised form 15th March, 2019
Accepted 12th April, 2019
Published online 28th May, 2019

Key words:
Ectopic Viral Integration Site-1, Carbonic Anhydrase III, Transcriptional repression, Acute Myeloid Leukaemia

A B S T R A C T

Ectopic viral integration site-1 (EVI1) is a nuclear zinc finger protein whose increased expression has been associated with poor prognosis in cases of Acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). In this study, we showed that expression of EVI1 in Rat fibroblast cells represses carbonic anhydrase III (*CAIII*), which is involved in protecting cells from oxidative damage. Reporter assays showed that the *CAIII* promoter activity is 7 times stronger in the absence of enforced expression of EVI1. This demonstrated that the EVI1-mediated repression of *CAIII* occurred at the level of transcription and could either be due to a direct repression or an indirect repression of the *CAIII* gene promoter by EVI1. We also showed that CAIII protects cells from apoptosis induced by oxidative stress by knocking down *CAIII* with Dicer-substrate short inhibitory RNAs and treating *CAIII*-knockdown cells with hydrogen peroxide (H_2O_2). This suggests that EVI1-mediated repression of *CAIII* may expose cells to death due to oxidative damage by reactive oxygen species (ROS). We conclude that EVI1 represses expression of *CAIII* at transcriptional level and exposes cells to death by oxidative agents. Taken together, this study provides a possible therapeutic approach of using oxidizing drugs to target disease cells such as in AML with increased expression of EVI1.

INTRODUCTION

Ectopic viral integration site-1 (*EVI1*) is a proto-oncogene with a size of over 100kb and 12 exons located on chromosome 3q26.2 (Hinai and Valk, 2016). This gene codes for the nuclear protein, EVI1, having 1051 amino acids and an apparent size of 145kDa (Buonamici *et al*., 2003). EVI1 is able to recognize and bind to DNA using 2 zinc finger domains: an N-terminal/proximal domain of 7 zinc fingers (ZF1) and a C-terminal/distal domain of 3 zinc fingers (ZF2) (de Braekeleer *et al*., 2015).

Abnormal expression of the EVI1 gene has been observed in 5-10% cases of Acute Myeloid Leukaemia (AML), a disease characterised by clonal proliferation of haemopoietic progenitor cell; and elevated expression of EVI1 gene has been found in glioblastoma associated with poor prognosis (Yokogami *et al*., 2016).Microarray analysis shows that carbonic anhydrase III (CAIII) production is repressed in EVI1-expressing cells. This repression may be due to several factors and proteins and have nothing to do with EVI1. This may also be due to the EVI1-mediated repression of CAIII for the fact that the repression occurs in EVI1-expressing cells, which provides motivation for one of the aims of this study.

Corresponding author:* **Kanayo Eugene Ikeh
Department of Medical Laboratory Science, University of Jos, Nigeria

This would imply that in cases with overexpression of EVI1 gene, such as in AML and glioblastoma, there would be even further decrease in expression of CAIII.

CAIII is a cytosolic protein of about 30kDa (Harju *et al*., 2013). It is found primarily in skeletal muscles, the liver and adipocytes, with lower concentrations found in smooth muscle, lungs, heart, prostate, brain and red blood cells (Harju *et al*., 2013). CAIII belongs to the class of zinc metalloproteases that catalyses the hydration of CO_2 to produce bicarbonate and proton used in maintaining a constant pH and forming proton pump for transport of ions across membranes (Dai *et al*., 2008).It has been suggested that CAIII acts as an antioxidant and is able to protect cells from oxygen radical-induced damage that leads to apoptosis (Räisänen *et al*., 1998; Zimmerman *et al*., 2004).

Even though the physiological functions of CAIII from previous studies are not very clear or definite, repression of *CAIII* may have many effects. This may include impairment of its putative role as an oxidative scavenger (Monti *et al*., 2017). This study investigated the possible role of EVI1 in reducing the expression of *CAIII* in Rat1 fibroblast cells, and if this repression is at the level of its promoter; indicating a transcriptional repression. We also investigated the significance of *CAIII* repression and its role in protecting cells from oxidative stress-induced apoptosis.

MATERIALS AND METHODS

Cell Source and Culture

Rat fibroblasts containing an empty vector control (Rat1neo1 cells) and Rat fibroblasts that contain the vector encoding and expressing the EVI1 gene (Rat5.6 cells) were maintained in Dulbecco's modified Eagle's medium (DMEM, Lonza, Slough, UK). *Escherichia coli* strain DH5α competent cells was used for transformation with plasmid DNA (Invitrogen Corporation, Paisley, UK).

Preparation of total cellular RNA, cDNA Synthesis and Real-time Quantitative PCR (qPCR)

RNA was prepared from Rat1neo1 and Rat15.61 cells using the NucleoSpin columns according to manufacturer's instructions (Machery-Nagel, Germany). The quantity and quality of RNA samples prepared from Rat1neo1 and Rat15.61 cells were determined using the Nanodrop spectrophotometer and RNA samples from Rat1neo1 and Rat15.61 diluted were to the same concentration. Structural integrity was assessed by running the RNA samples on agarose gel. We used 5µl each of total cellular RNA from Rat1neo1 and Rat5.6 cells, both at concentrations of 80.68ng/µl, was used to synthesise cDNA according to manufacturer's instructions (Invitrogen, UK). The cDNA samples were diluted by mixing 2µl of cDNA with 8µl of nuclease free water. The qPCR reaction was set up using 4.3µl of 5µM CAIII and GAPDH primers and 6.6µl of 1µM CAIII and GAPDH probes. Samples were amplified in the thermal cycler using the following parameters: 95°C 15 minutes for 1 cycle, 95°C 15 seconds, 60°C 60 seconds for 40 cycles. The Ct values were used to calculate the $2^{-\wedge\wedge Ct}$. The mean and standard deviations of $2^{-\wedge\wedge Ct}$ values were used to construct a histogram

Western Blot Analysis

Total cellular protein was prepared using NucleoSpin columns according to manufacturer's instructions (Machery-Nagel, Germany). A standard calibration curve was produced using dilution series of bovine serum albumin (BSA) and used to determine the protein concentrations in the 2 rat fibroblast cell lines. Protein gel electrophoresis, protein transfer to polyvinylidene difluoride (PVDF) membrane and Western blotting were performed using anti-EVI1 antibody, anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody and anti-CAIII antibody at 1/1000 dilution. IRDye800CW-conjugated anti-rabbit (LI-COR GmbH, Germany), IRDye680RD-conjugated anti-mouse (LI-COR GmbH, Germany) and IRDye800CW conjugated anti-mouse (LI-COR GmbH, Germany) were used as secondary antibodies for corresponding primary antibodies at a 1/15000 dilution. The activity of the fluorescent tags conjugated to the secondary antibody was measured using the Li-Cor Odyssey FC image analyser.

Dual Luciferase Reporter Activity

To determine if EVI1-mediated repression of CAIII occurs at the level of its transcription, a luciferase reporter assay was conducted. The Firefly luciferase activity was measured to determine relative CAIII promoter activity in Rat1neo1 and Rat15.61 cells. Renilla luciferase activity was used to normalise the data.Sub-cultured Rat1 fibroblast cells (50% confluent) were used for plasmid transfection using Fugene 6 (Promega Corporation, Madison, USA). Cells were transfected with pCAIIIuc recombined with Firefly luciferase gene and pRLCMV recombined with Renilla luciferase gene. pBluescriptkSII was used to maintain constant DNA concentrations, with pEGFPused as a transfection control. The plate with the transfected cells was incubated at 37°C in 5% CO_2 incubator for 48 hours. The cells were lysed and the luciferase activity was measured using a Fluostar OPTIMA luminometer (BMG LABTECH, Offenburg, Germany).

Caspase 3 Assay

TriFECTa™ siRNA kit (Integrated DNA technologies, Iowa) was used to knockdown expression of CAIII. Rat1neo1 cells were transfected with siRNAs in a 96-well plate: 2 wells were treated with a control siRNA;2 wells were treated with CAIII siRNA; 2 wells were left untreated serving as controls. The transfection agent used was siLENTFect® (BioRad, Hercules, USA). Cells were treated with Hydrogen peroxide (H_2O_2) and Caspase-Glo reagent according to manufacturer's instructions (Promega Corporation, UK). Luminescence was measured using Fluostar OPTIMA luminometer (BMG LABTECH, Offenburg, Germany).

RESULTS

The western blot results carried out to assess protein expression is shown in Fig 1. The band sizes for GAPDH after was approximately 37kDa for both Rat1neo1 and Rat15.61 lanes. Both band sizes were of approximate thickness indicating equal loading of protein during electrophoresis. The band sizes for EVI1 protein were at about 145kDa for both the Rat1neo1 and Rat15.61 cells. However, a thicker band was apparent in the Rat15.61 lane when compared to that in the Rat1neo1 lane (Fig 1). The band sizes for CAIII protein were about 28kDa in both the Rat1neo1 and Rat15.61 lanes as expected. The band size in the Rat15.61 lane was lighter than that in the Rat1neo1 lane (Fig 1).

cDNA generated from the purified RNA samples was used for the qPCR assay as described in Materials and Methods.Fig 2 shows the fold difference in expression of *CAIII* gene in Rat1neo1 cells and Rat15.61 cells. After normalization, the mean $2^{-\wedge\wedge Ct}$ in Rat1neo1 cells (lane 1) was 1 (s.d. = 0) and that for Rat15.61 cell (lane 2) was 0.246 (s.d. = 0.036). This indicates that CAIII is expressed approximately 4 times more in Rat1neo1 cells than in Rat15.61 cells.

Fig 3 shows the results of the dual luciferase assay carried out to ascertain if EVI1 represses CAIII at the level of transcription. Luminescence, indicating the expression of Firefly luciferase, was approximately 7 times more in Rat1neo1 cells (lane 1) than in Rat15.61 cells (lane 2). Caspase-Glo 3 assay was carried out to determine the significance of transcriptional repression of CAIII by EVI1. The results are presented in Fig 4. In the cells transfected with control siRNA, siRNA CAIII, and cells left untransfected, there was higher caspase 3 activity when they were treated with H_2O_2 (lanes 2, 4 and 6) than when left untreated (lanes 1, 3 and 5). The results showed a higher caspase 3 activity in CAIII-knockdown cells treated with H_2O_2(lane 6) than the other lanes, indicating a greater rate of cell death when compared with control cells expressing CAIII normally as shown in Fig. 4.

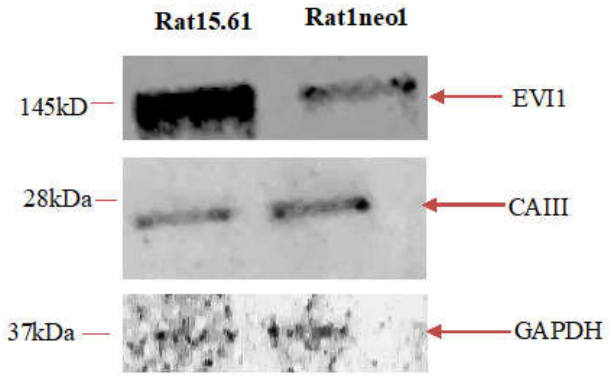

Fig 1 Western blot analysis of protein extracts from Rat15.61 and Rat1neo1 cells using EVI1, CAIII and GAPDH antibodies

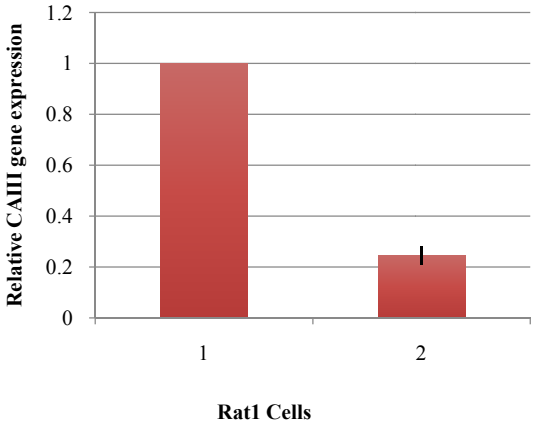

Fig 2 A bar graph showing the mean $2^{-\Delta\Delta Ct}$ values of CAIII in Rat1neo1 cells and Rat15.61 cells after qPCR. Bar 1 represents that for Rat1neo1 cells and Bar 2 represents that for Rat15.61 cells. CAIII expression in Rat1neo1 cells were used to normalise the date.

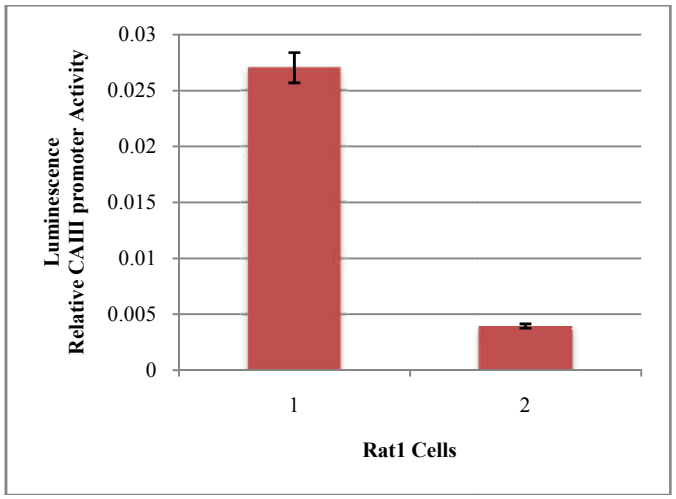

Fig 3 A bar graph showing the luminescence activity of firefly luciferase whose expression is driven by CAIII promoter in Rat1 cells. Each column shows the mean of the experiment done in duplicate, and the error bars represent the standard deviation. Bar 1 represents that for Rat1neo1 cells and Bar 2 for Rat15.61 cells.

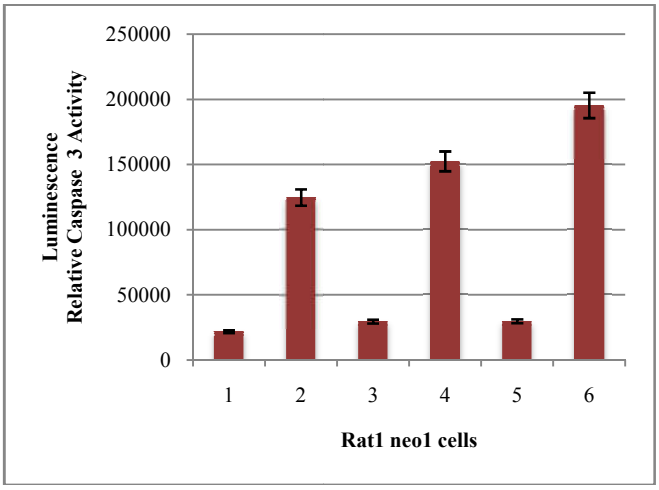

Fig 4 A bar graph showing relative Caspase 3 activity in Rat1neo1 cells. (1) Untransfected cells without treatment of H_2O_2 (2) Untransfected cells with treatment of H_2O_2 (3) Transfected control siRNA cells without treatment of H_2O_2 (4) Transfected control siRNA cells with treatment of H_2O_2 (5) Transfected siRNACaIII cells without treatment of H_2O_2 (6) Transfected siRNACaIII cells with treatment of H_2O_2.

DISCUSSION

This study was carried out to determine if EVI1 represses CAIII and to understand the nature of tis repression if so.We also sought to understand the implication of an EVI1-mediated CAIII repression, particularly in acute myeloid leukaemia. The Western blot analysis showed that the quantity of CAIII is reduced in EVI1-expressing Rat15.61 cells when compared to CAIII in Rat1neo1 cells (Fig 1). qPCR also showed that *CAIII* mRNA transcripts was more expressed in Rat1neo1 cells than in Rat15.61 cells expressing EVI1 (Fig 2). These results taken together indicate that EVI1 suppresses expression of *CAIII*. Similar results have been described stating that *CAIII* expression was repressed up to 97% in cells overexpressing EVI1 (Roy et., 2010).The inference from this study affirms that overexpression of EVI1 is possibly responsible for suppressing the expression of *CAIII*. However, it may be possible that some isoforms of EVI1 are involved in repressing expression of *CAIII* while others either have no effect or possibly opposing roles.

The dual luciferase assay showed that the *CAIII* promoter activity is almost 7 times stronger in Rat1neo1 cells than in Rat15.61 cells, characterised by increased expression of firefly luciferase gene in Rat1neo1 cells, as shown in Fig 3. Not only does this confirm that EVI1 represses expression of *CAIII*, it also shows that this repression occurs at the level of transcription involving the *CAIII* promoter, confirming similar reports (Roy *et al*., 2010). However, this does not exclude the chance of translational repression, such as mRNA repression, as some genes are known to be both transcriptionally and translationally repressed such as p53 (Fernando *et al*., 2013). Therefore, further investigations are required to determine if EVI1-mediated repression of *CAIII* is strictly transcriptional or both transcriptional and translational.

MATINSPECTOR analysis of the *CAIII* murine promoter sequence revealed multiple potential binding sites for EVI1 suggesting that EVI1 directly binds to *CAIII* to repress its transcription (Roy *et al*., 2010).Repression of CAIII expression could also be indirect as EVI1 has been shown to be involved in either repressing or activating signalling pathways such as Transforming growth factor-β (TGFβ) and Jak-Stat signalling

pathways (Liu *et al*., 2006; Glass *et al*., 2013). EVI1 is also thought to be able to alter gene expression by recruiting and binding to transcriptional/epigenetic regulators such as CBP, HDAC, HMT, and GATA1/2; and also influences transcriptional repression by recruiting CTBP1/2, indicating that this may be an underlying mechanism in its role in inducing TGFβ signalling-pathway suppression (de Braekeleer *et al*., 2015). It is therefore possible that EVI1 represses expression of *CAIII* by its involvement in these signalling pathways or by binding to a transcription factor that is important for the expression of *CAIII*.

Knowledge of this EVI1-mediated repression of CAIII can be considered in designing future experiments exploiting some of the functions of CAIII, though putative, to advance medical innovations. It has been demonstrated that leptin is able to decrease expression of *CAIII*, and that the decreased expression of *CAIII* in obesity may be due to hyperlectinaemia (Alver *et al*., 2004). Though this possibility may be correct, it should also be taken into consideration that, by increasing the levels of peroxisome proliferator-activated receptor γ2 (PPARγ2), EVI1 is able to convert nonadipogenic cells to adipocytes (Ishibashi *et al*., 2012).As evidenced in our study, the decreased expression of *CAIII* seen in obesity may therefore also be as a result of elevated expression of EVI1, whose gene product represses expression of *CAIII* as opposed to hyperlectinaemia.

In a remarkable discovery at a time when detection of myoepithelial cells by light microscopy was difficult, it was demonstrated that CAIII present in myoepithelial cells could be used as a diagnostic marker for some rare forms of myoepithelial breast cancer (Vaananen and Autio-harmainen, 1987). In light of results from our study, it can be seen that in conditions leading to increased amounts of EVI1, diagnosis of myoepithelial breast cancer using CAIII as a diagnostic marker could lead to false negative results. Also, it was demonstrated that CAIII increases the invasiveness of hepatocellular carcinoma by elevating focal adhesion kinase activity (Dai *et al*., 2008). Therefore, research focussed on gene therapeutic approach using EVI1 in a viral or non-viral vector should, in theory, lead to repression of *CAIII* and most likely reduce the invasiveness of hepatocellular carcinoma cells, bearing in mind however that EVI1 is an oncogene.

CAIII is involved in maintaining a constant pH (Dai *et al*., 2008). This role of CAIII was further demonstrated in another study showing that CAIII levels are markedly increased after episodes of GORD (Min *et al*., 2016). This correlates with a study in which microarray analysis indicated higher levels of expression of EVI1 in other forms of high grade dysplasia when compared to Barrett's oesophagus (Quante *et al*., 2012). Barrett's oesophagus is a complication of GORD (Liu *et al*., 2013) and the increased levels of CAIII in GORD may be due to decreased expression of EVI1 leading to a decreased-repression of *CAIII*.

In this study, we chose to assess the significance of EVI1-mediated repression of *CAIII* in its role acting as an antioxidant, protecting cells from H_2O_2-induced apoptosis as suggested by Räisänen *et al*., (1998). Fig. 4 shows higher caspase 3 activity in CAIII knock-down cells treated with H_2O_2 when compared to control cells. This confirms the role of CAIII in protecting cells from oxygen-induced cell death. Knowledge of this EVI1-mediated repression of CAIII and its role in H_2O_2-induced apoptosis may have a very useful application in treatment of AML. Perhaps in almost all types of cancer, the ability of cancerous cells to expand lies not only in their acquired proliferative abilities but also in their ability to avoid the normal cell death program, apoptosis, making 'evasion of apoptosis' one of the hallmarks of cancer (Hanahan and Weinberg, 2011). EVI1 has been shown to be markedly elevated in AML and is used as a prognostic marker for the disease (Hinai and Valk, 2016). Several studies have shown that EVI1 protects cells from apoptosis. EVI1 inhibits the gene coding for the anti-apoptotic protein, B-cell lymphoma-extra large (Bcl-xL), thereby preventing release of cytochrome c that activates caspase 3 (Pradhan *et al*., 2011). EVI1 has been shown to repress the pro-apoptotic effects of interferon-α (INFα) and TGFβ signalling pathway (Buonamici *et al*., 2005; Elliott and Blobe, 2005). These previous studies demonstrate ways in which EVI1 helps cancerous cells evade apoptosis. However, this present study illustrates EVI1-mediated repression of *CAIII* and its significance in H_2O_2 apoptosis, suggesting that oxidising agents may be attractive chemotherapeutic agents in cancers mediated by EVI1, taking advantage of the repression of CAIII, whose gene-product protects cells from oxidative stress and ultimately cell death.

CONCLUSION

We conclude that EVI1 represses *CAIII*at the level of its promoter and that this repression exposes acute myeloid leukaemic cells to death by oxygen-induced stress, providing a novel means for AML therapy.

Acknwoledgement

The authors wish to acknowledge and thank Dr Catherine Wright and other staff of the Department of Life Sciences, Glasgow Caledonian University for the support rendered throughout the workshop.

References

1. Alver A, Uçar F, Keha EE, Kalay E, Ovali E. 2004.Effects of leptin and insulin on ca iii expression in rat adipose tissue. *Journal of enzyme inhibition and medicinal chemistry*., 19(3):279-281.
2. Buonamici S, Chakraborty S, Senyuk V, Nucifora G. 2003.The role of evi1 in normal and leukemic cells. Blood cells, molecules and diseases., 31(2):206-212.
3. Buonamici S, Li D, Mikhail FM, Sassano A, Platanias LC, Colamonici O, Anastasi J, Nucifora G. 2005. Evi1 abrogates interferon-alpha response by selectively blocking pml induction. The journal of biological chemistry., 280(1) pp. 428.
4. Dai H, Hong C, Liang S, Yan M, Lai G, Cheng A, Chuang S. 2008. Carbonic anhydrase iii promotes transformation and invasion capability in hepatoma cells through faksignaling pathway. Molecular carcinogenesis., 47(12):956-963.
5. de Braekeleer M, Le bris M, de Braekeleer E, Basinko A, Morel F, Douet-guilbert N. 2015. 3q26/evi1 rearrangements in myeloid hemopathies: a cytogenetic review. Future oncology.,11(11):1675-1686.

6. Elliott RL, Blobe GC. 2005.Role of transforming growth factor beta in human cancer. Journal of clinical oncology., 23(9):2078-2093.
7. Fernando JL, Philippe G, Sri KB, Jianxin Z, Sonia VR, Charles S, Thea DT. 2013.Coordinate transcriptional and translational repression of p53 by tgf-β1 impairs the stress response. Molecular cell., 50(4):552--564.
8. Glass C, Wuertzer C, Cui X, Bi Y, Davuluri R, Xiao Y, Wilson M, Owens K, Zhang Y, Perkins A. 2013.Global identification of evi1 target genes in acute myeloid leukemia. Plos one., 8(6): pp. E67134.
9. Hanahan D, Weinberg R. 2011. Hallmarks of cancer: the next generation. Cell., 144(5):646-674.
10. Harju A, Bootorabi F, Kuuslahti M, Supuran CT, Parkkila S. 2013.Carbonic anhydrase III: a neglected isozyme is stepping into the limelight. *Journal of enzyme inhibition and medicinal chemistry*.,28(2):231-239.
11. Hinai AA, Valk PJM. 2016.Review: aberrant evi1 expression in acute myeloid leukaemia. British journal of haematology.,172(6):870-878.
12. Ishibashi J, Firtina Z, Rajakumari S, Wood KH, Conroe HM, Steger DJ, Seale P. 2012. An evi1-c/ebpβ complex controls peroxisome proliferator-activated receptor γ2 gene expression to initiate white fat cell differentiation. Molecular and cellular biology.,32(12): pp. 2289.
13. Liu X, Wong A, Kadri SR, O'donovan M, Lao-sirieix P, Burnham R, Fitzgerald R. 2013.Ptu-173 gord symptoms and demographic factors as a pre-screening tool for barrett's oesophagus. Gut.,62, pp. A119.
14. Liu Y, Chen L, Ko TC, Fields AP, Thompson EA. 2006.Evi1 is a survival factor which conveys resistance to both tgfbeta- and taxol-mediated cell death via pi3k/akt. Oncogene., 25(25): pp. 3565.
15. Min HJ, Hong SC, Yang HS, Mun SK, Lee SY. 2016. Expression of caiii and hsp70 is increased the mucous membrane of the posterior commissure in laryngopharyngeal reflux disease. *Yonsei medical journal*., 57(2):469-474.
16. Monti DM, de Simone G, Langella E, Supuran CT.2017. Insights into the role of reactive silfhydryl groups of carbonic anydrase iii and vii during oxidative damage. *Journal of enzyme inhibition and medicinal chemistry*.,32(1): 5-11.
17. Pradhan AK, Mohapatra AD, Nayak KB, Chakraborty S. 2011.Acetylation of the proto-oncogene evi1 abrogates bcl-xl promoter binding and induces apoptosis. Plos one.,6(9): pp. E25370.
18. Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, Lee Y, Friedman R, Asfaha S. 2012.Bile acid and inflammation activate gastric cardia stem cells in a mouse model of barrett-like metaplasia. Cancer cell., 21(1):36-51.
19. Räisänen S, Lehenkari P, Tasanen M, Härkönen P, Väänänen K. 1998. Carbonic anhydrase iii protects cells from hydrogen peroxide induced apoptosis. Pathophysiology.,5:118-118.
20. Roy P, Reavey E, Rayne M, Roy S, Abed el Baky M, Ishii Y, Bartholomew C. 2010.Enhanced sensitivity to hydrogen peroxide-induced apoptosis in evi1 transformed rat1 fibroblasts due to repression of carbonic anhydrase iii. Febs journal., 277(2):441-452.
21. Vaananen KH, Autio-harmainen, H. 1987.Carbonic anhydrase iii: a new histochemical marker for myoepithelial cells. *The journal of histochemistry and cytochemistry*.,35(6):683-686.
22. Yokogami K, Mizuguchi A, Yamashita S, Morishita K, Takeshima H. 2016.P01.06 function of Ectopic viral integration site 1 (evi1) gene in human glioma cells. Neuro-oncology., 18(suppl_4): pp. Iv19-iv19.
23. Zimmerman U, Wang P, Zhang X, Bogdanovich S, Forster R. 2004.Anti-oxidative response of carbonic anhydrase iii in skeletal muscle. Iubmb life.,56(6):343-347.

How to cite this article:

Kanayo Eugene Ikeh *et al* (2019) 'Ectopic Viral Integration Site-1 (Evi1)-Mediated Repression of Carbonic Anhydrase Iii (Caiii) and its Implications: Role in Acute Myeloid Leukaemia', *International Journal of Current Advanced Research,* 08(05), pp. 18547-18551. DOI: http://dx.doi.org/10.24327/ijcar.2019.18551.3548

****