Indian Journal of Forensic Medicine and Toxicology
Volume 16 No. 3, July-September 2022
DOI: http://dx.doi.org/xxxxx/ijfmt.xxxx.16322.12

The Expression of Neogene TIGD3 that Derived from DNA Transposons in Colorectal Cancer Cell lines

Ahmed Arnaoty[1], Hiba. M. Ahmed[2], Yves Bigot[3], Thierry Lecomte[4]

[1]Assistant Professor in Bilad AL-Rafidain University College Department of Anesthesia/Diyala/Iraq, [2]Lecturer in Al-Rasheed University College Department of Pharmacy/Baghdad/Iraq, [3]Director of research in UMR INRA-CNRS 7247, INRA Val de Loire-France, [4]Professor in Tours university, Department of Hepato-Gastro-Enterology; Tours/France.

How to cite this article: Ahmed Arnaoty, Hiba. M. Ahmed, Yves Bigot et al. The Expression of Neogene TIGD3 that Derived from DNA Transposons in Colorectal Cancer Cell lines. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):58-62.

Abstract

DNA transposons are exposed to a molecular domestication process, which results in the formation of neogenes, which may play a role in human genetic instability. TIGD3 (Tigger-derived [TIGD] family of proteins) is one of these Neogene, and its role in the human genome is unknown.

Aim: The expression of Neogene TIGD3 in colorectal cancer cell lines and its putative function in carcinogenesis are being investigated.

Method: The protein expression of the TIGD3 gene was investigated using the western blot method in twelve colorectal cancer cell lines (HCT116, SW48, LOVO, DLD1) that are microsatellite instable MSI, (SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463) that are microsatellite stable MSS, and in healthy colon tissue as a control in our study.

Results: The expression of the TIGD3 protein was found in all twelve colorectal cancer cell lines, with varying degrees of expression and numerous isoforms, which was not found in healthy colon tissue.

Conclusion: There may be a link between colorectal cancer evolution or progression and TIGD3 gene expression.

Keywords: DNA transposons; domestication; neogene**;** TIGD3**;** microsatellite instable; microsatellite stable.

Introduction

Carcinogenesis and tumor growth are linked to a breakdown in the genome's integrity, which is translated by genetic instability. Transposable elements, including DNA transposons that have had their mobility inactivated during evolution, make up half of the human genome.[1,2]

These DNA transposons usually appear as inactive DNA fragments that are epigenetically silenced by the host genome to inhibit transcription and further transposition.[3-5]

After then, such elements are subjected to low selective pressure and develop sequence variation (mutations) over time. However, it has recently been

Corresponding Author: Ahmed Arnaoty, Assistant Professor in Bilad AL-Rafidain University College Department of Anesthesia/Diyala/Iraq.

E-mail: ahmedarnaoty@yahoo.com

discovered that some transposable elements manage to evade host cell silencing and get domesticated by host genomes, resulting in the beginning of novel genes (neogenes) that encode proteins.[6-9]

Recent human genome investigations have revealed that several of these proteins are involved in a variety of biological processes that contribute directly or indirectly to genome stability (cellular proliferation, cellular cycle progression, chromatin modification, transcription control).[8,9]

These domesticated elements are also involved in placental development, viral resistance, chromatin structure, DNA recombination and repair, gene control, apoptosis, and brain development, among other cellular and developmental roles.[6]

RAG1, RAG2 are two proteins that play a role in recombination of genes for immunoglobulin and T cell receptor genes in vivo and can also serve as a transposase in vitro under certain conditions.[10] The TIGD3 neogene, which was chosen for investigation, has a DNA binding domain as well as a catalytic domain.[11]

In the current work, an in vitro model of human epithelial colorectal malignant cell lines was used to analyze the expression of TIGD3 neogenic protein using the western blot method, with the protein extracted from these cancer cell lines and antibodies produced by Arnaoty et al.[11], that allow us to investigate and analyze the numerous isoforms of neogenic recombinase corresponding to our TIGD3 neogene produced from DNA transposons.

The goal of this work is to demonstrate TIGD3 protein expression in colorectal cancer cell lines with two phenotypes, MSI and MSS, and to determine if this Neogene TIGD3 has a role in genetic instability and, as a result, in the initiation, promotion, or progression of cancer.

Materials and Methods

This work was carried out in the GICC (Genetics, Immunity, Chemistry, Cancer) unity of research department of CNRS (National centre of scientific research)/ Tours/ France.

Cell lines culture

Twelve colorectal cancer cell lines were included in this study, (HCT116, SW48, LOVO, DLD-1, SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463). These cell lines were grown in OptiMEM medium plus 10% FBS, streptomycin/penicillin 5.5µg/ml. Hela cell line was also used for achieving our transfection of our plasmids TIGD3. Culture conditions for all at 37 °C in a humidified 5% CO_2. All of theses cell lines were kindly provided by INSERM U915 /Tours/ France. Healthy gut tissue was taken from a healthy individual while achieving routine colonoscopy examination/ department of gastroenterology/ Trousseau Hospital/ France.

Cell lines proteins extraction and Dosing

Whole protein from all cell lines were extracted with using lyses buffer (SDS 20%, NaCl 100mM, BetaMercaptoEthanol 10mM, Protease inhibitor), heating at 65°C for 5 minutes then breaking the DNA by ultrasound wave for 20 seconds and centrifuging the tube in 15,000 rpm at 20°C for 10 minutes, taking the supernatant and the isolated protein was quantified by a commercially available modified Bradford assay by UV spectrophotometer.

Western blot assay

Western blot protein samples were prepared by boiling the isolated protein with denaturing sample, balanced amounts of cell proteins (40 µg) where placed in each well. The protein was then separated by SDS-PAGE on a 10% polyacrylamide gel and transferred to a PVDF (polyvinylidene difluoride membrane) (Bio-Rad, Richmond, USA). The membranes were blocked with 5% non fat dry milk in TBS and 0.5% Tween 20 for 1 hour and probed with the appropriate primary antibody that synthesized by us, for 2 hours at room temperature, then the membrane was washed 3 times with TBS and 0.1% Tween 20 for 10 minutes, and incubated with the appropriate horseradish peroxidase–conjugated anti anti mouse secondary antibody (Abcam) for 1 hour at room temperature. The membrane was then washed 3 times with TBS and 0.5% Tween 20 for 10 minutes and protein bands visualized by using a commercially available enhanced chemiluminescence kit (Amersham Biosciences) according to the manufacturer's instructions, the membrane was exposed to film for 1 and 30 min.

Results

Expression of the protein TIGD3 in colorectal cancer cell lines

The western blot analysis of the protein expression of the gene TIGD3 in 12 colorectal cancer cell lines

revealed four different products of expression of this gene, which correspond to four different TIGD3 isoforms (90, 60,52 and 50 kDa) (figure 1). Among the 12 colorectal cancer cell lines studied, no cell line shows the four isoforms of TIGD3 (90, 60, 52 and 50 kDa). The expression of three isoforms (90, 60 and 52 kDa) was observed only in two cell lines DLD1 and T84. The expression of two isoforms (60 and 52 kDa) was observed only in five cell lines HCT116, SW620, DLD1, SW1463 and T84. The expression of the isoform (50 kDa) was observed only in SW620 cell line. The isoform 52 kDa (a molecular weight equal to that of the *TIGD3* transposase) was common between them (all 12 colorectal cancer cell lines) and it was strongly expressed in all these cell lines except in SW480 (MSS). The most notable finding in this study is the lack of TIGD3 gene protein expression in sample C2, which corresponds to non-cancerous tissue collected from a healthy person. (Fig. 1,2,3). Figure 1 shows the signals of protein expression for the TIGD3 gene on protein extracts from colorectal cell lines, as captured by the PVDF membrane chemiluminescence reaction. By hybridizing the membranes with a particular monoclonal antibody, the amount of the housekeeping protein actin in each lane was determined. (Anti actin antibodies/ Abcam). As shown in figures 2 and 3, these levels were estimated using the Multigauge analysis program for signals taken from each cell line split by their contents or amount of protein actin.

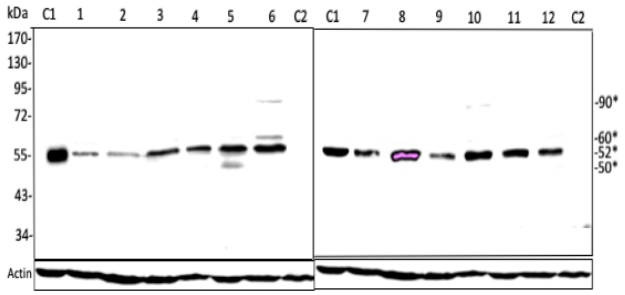

Fig. 1: Western blot analyses of TIGD3. Lanes 1 to 12 correspond to protein extracts from the human colorectal cancer cell lineages. C1 correspond to protein extracts from HeLa transfected with pVAX-TIGD3. C2 corresponds to an extract of human healthy gut. * indicates the 90, 60 and 50 kDa isoforms of TIGD3; **, indicates a 52 kDa isoform with a molecular weight equal to that of the TIGD3 transposase. Hybridizing the membranes with a specific monoclonal antibody actin in each lane. Molecular weights are indicated in the left margins. Molecular weights of the TIGD3 isoforms are indicated in the right margin.

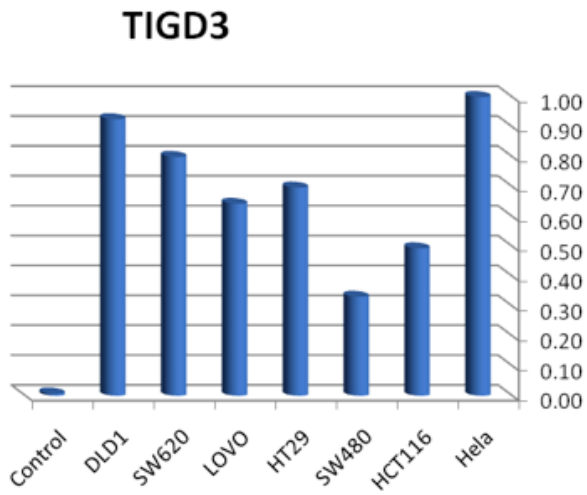

Fig. 2: TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3, HCT116, SW480, HT29, LOVO, SW620, DLD1 and Control(an extract of human healthy gut)) respectively.

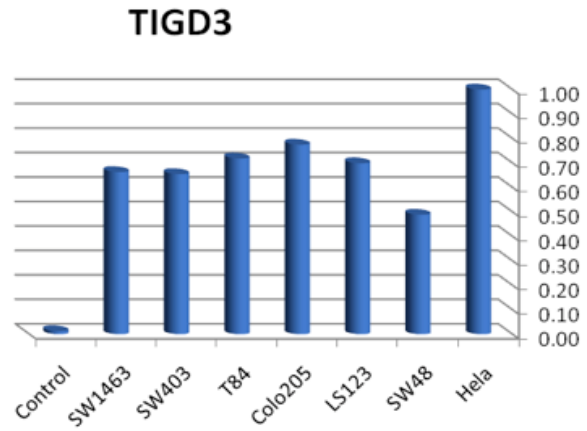

Fig. 3: TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3, SW48, LS123, COLO205, T84, SW403, SW1463 and Control (an extract of human healthy gut)) respectively.

Discussion

For the first time, we were able to demonstrate the protein expression of the TIGD3 gene in these colorectal cancer cell lines, which reflects the expression of a domesticated DNA Neogene on such cell lines. No other study achieves the same level of success as this one, owing to the lack of a particular antibody aimed against this type of domesticated DNA Neogene.[11] Despite the fact that commercial antibodies available for testing the protein expression of the TIGD3 gene in these cell lines are unable to provide any of these results.[11] Also, there is no data in the previous bibliography that shows the exact molecular weight of TIGD3 protein by western blot method, but our prior work revealed the molecular weight of this protein by western blot to be 52 kDa.[12]

Multiple isoforms of protein expression of the TIGD3 gene, which were discovered in our work by western blot in different cell lines and could be explained by the possibility of post translational alteration of this protein, were previously unknown. The findings, which were acquired using the anti-TIGD3 antibody that we developed in partnership with In Cell Art, reveal new information on TIGD3 gene expression.[11]

All of the colorectal cancer lines tested expressed the 52 kDa isoform. It's worth noting that the expression of this isoform was higher in cell lines (SW620, DLD1, LOVO, HT29, LS123, Colo205, T84, SW403, SW1463) that had either metastatic or advanced colorectal cancer, with the exception of the LS123 cell line, which had non-advanced colorectal cancer (Dukes B). No expression was discovered in the C2 for any of the four isoforms identified in our study (protein extract from healthy gut tissue; figure 1).

All of these data could point to a link between this gene's level of expression and the stage of cancer progression, with high expression in cell lines associated with advanced cancer or metastasis. This could be due to either the gene playing a role in cancer progression or the highly advanced tumour expressing more of this gene; however, more research and confirmation is needed.

All these cell lines (SW620, DLD1, LOVO, HT29, LS123, Colo205, T84, SW403, SW1463) which were highly express the 52 kDa isoform of TIGD3 gene represent a status of MSS (Micro Satellite Stable) except for the DLD1 and LOVO which are status of MSI (Micro Satellite Instable). This link between gene expression and cell line MSS status could indicate that either nucleotide stability (MSS) has a favorable effect on TIGD3 gene expression or that this gene plays a function in nucleotide stability inside the nucleus of these cell lines. The possibility of an inverse relationship between nucleotide instability (MSI) and gene expression level, on the other hand, may support the idea that nucleotide stability has a favorable effect on gene expression. Unfortunately, there is no material in the bibliography that attempts to demonstrate this probable link between MSS status at the nucleotide level and TIGD3 gene expression. We need more research and work on this gene before we can approve this possible link.

As well as, the isoform which correspond to the molecular weight of 60 kDa which is observed in cell lines (HCT116, SW620, DLD1, SW1463) was highly expressed in the cell line DLD1 than other cell lines. Also the isoform 90 kDa which is observed in only two cell lines (DLD1 and T84) was higher in DLD1 (MSI) than T84 (MSS). The high expression of these two isoforms (90, 60 kDa) in DLD1 with a status of MSI may suggest a relation between the micro satellite instability and the level of expression which is seen only in these two isoforms (90, 60 kDa) but not seen in the isoform 52 kDa which was highly expressed in most cell lines with a status of MSS. This could suggest a link between nucleotide stability and the expression of distinct isoforms of this gene. In other words, the expression of distinct isoforms for the same gene depends on whether the cell line is MSS or MSI, and the development of these four TIGD3 isoforms in these cell lines could be related to nucleotide stability or instability.

Our research was carried out on cell lines that have the chromosomal region 11q13.1, which contains the TIGD3 gene and is not deleted, implying that our gene expression analysis is complete.[13-19]

In addition, the data observed in colorectal cancer series indicate that chromosomal deletions type LOH (Loss of heterozygoty) are less common on the 11q chromosome arm than the other arm.[20,21] Therefore, the expression of a TIGD3 gene is not related to allelic loss on chromosome 11q.

To our knowledge, no studies have been reported on the study of gene expression TIGD3 in these human cancer cell lines.

Conclusion

The presence of protein expression for the TIGD3 gene in all colorectal cancer cell lines, with higher expression in cell lines that had progressed or metastasized, and absence in healthy tissue, could indicate a strong relationship between cancer evolution or progression and gene expression, which could lead to a possible role for this gene in colorectal cancer.

Source of Funding: This work was funded by the C.N.R.S, the I.N.R.A., the Groupement de Recherche CNRS 2157, and the Ministère de l'Education Nationale, de la Recherche et de la Technologie. It also received funding from a research program grant from the Cancéropôle Grand-Ouestand grants from Amgen and the French National Society of Gastroenterology.

Ethical Clearance: Samples were taken from the department of histopathology/Trousseau hospital/ Tours/France. Patients had been informed of using their samples for research purposes and their consents to participation in this type of research had been collected.

Conflict of Interest: There is no conflict.

References

1. Loeb LA. "Mutator Phenotype May Be Required for Multistage Carcinogenesis." *Cancer Res*. 1991;51:3075–3079. Cited by Cancers.2021Apr23;13(9):2042. doi:10.3390/cancers13092042.
2. Hartwell L. "Defects in a Cell Cycle Checkpoint May Be Responsible for the Genomic Instability of Cancer Cells." *Cell*. 1992;71:543–546. Cited by Cancers. 2021 Mar6;13(5):1134.doi:10.3390/cancers13051134.
3. Kidwell MG, Lisch DR. "Perspective: Transposable Elements, Parasitic DNA, and Genome Evolution." *Evolution*. 2001;55:1–24. Cited by Genome Biol Evol. 2021 Jul 6;13(7):evab135. doi: 10.1093/gbe/evab135.
4. O'Donnell KA, Boeke JD. Mighty Piwis defend the germline against genome intruders. Cell 2007 ; 129(1)**:**37-44. Cited by Cancer Cell Int. 2021 Jun 30;21(1):328. doi: 10.1186/s12935-021-02034-3.
5. Miller WJ, McDonald JF, Nouaud D, Anxolabehere D: Molecular domestication--more than a sporadic episode in evolution. Genetica 1999;107(1-3):197-207. Cited by R Soc Open Sci. 2019 Sep 25;6(9):190304. doi: 10.1098/rsos.190304. eCollection 2019 Sep.
6. Volff JN. Turning junk into gold: domestication of transposable elements and the creation of new genes in eukaryotes. Bioessays 2006;28(9):913-922. Cited by Cells. 2021 Jul 6;10(7):1707. doi: 10.3390/cells10071707.
7. Kalitsis P, Saffery R .Inherent promoter bidirectionality facilitates maintenance of sequence integrity and transcription of parasitic DNA in mammalian génomes**.** Research article BMC Genomics 2009;10:498. Cited by Mob DNA. 2021 Jan 6;12(1):1. doi: 10.1186/s13100-020-00229-5.
8. Feschotte C, Pritham EJ . DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet. 2007;41:331-68. Cited by Nat Rev Genet. 2021 Aug 5. doi: 10.1038/s41576-021-00385-1.
9. Sinzelle L, Izsvk Z,Ivics Z. Molecular domestication of transposable elements: from detrimental parasites to useful host genes. Cell Mol Life Sci. 2009;66:1073-93. Cited by Genes. 2021 Jun 15;12(6):918. doi: 10.3390/genes12060918.
10. Agrawal A, Eastman QM, Schatz DG. Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. Nature. 1998;394: 744–751. Cited by Biology. 2021 Feb 10;10(2):140. doi: 10.3390/biology10020140.
11. Arnaoty A, Gouilleux-Gruart V, Casteret S, Pitard B, Bigot Y, Lecomte T. Reliability of the nanopheres-DNA immunization technology to produce polyclonal antibodies directed against human neogenic proteins. Mol Genet Genomics. 2013;288(7-8):347-63. Cited by Mol Ther Nucleic Acids. 2019 Jun 7;16:186-193.doi: 10.1016/j.omtn.2019.02.016. Epub 2019 Feb 26.
12. Arnaoty A, Pitard B, Bateau B, Bigot Y, Lecomte T. Novel Approach for the Development of New Antibodies Directed Against Transposase-Derived Proteins Encoded by Human Neogenes. Methods Mol Biol. 2012;859:293-305. Cited by Mol Genet Genomics. 2013 Aug;288(7-8):347-63. doi: 10.1007/s00438-013-0754-8. Epub 2013 Jun 7.
13. Knutsen T, Padilla-Nash HM, Wangsa D, et al. "Definitive Molecular Cytogenetic Characterization of 15 Colorectal Cancer Cell Lines." Genes Chromosomes Cancer. 2010;49:204–223. Cited by Pharmacogenomics J. 2021 Jun;21(3):390-401. doi: 10.1038/s41397-021-00217-9. Epub 2021 Mar 17.
14. Abdel-Rahman WM, Katsura K, Rens W, et al. "Spectral Karyotyping Suggests Additional Subsets of Colorectal Cancers Characterized by Pattern of Chromosome Rearrangement." Proc Natl Acad Sci U S A. 2001;98:2538–2543. Cited by Nat Rev Genet. 2021 Apr;22(4):251-262. doi: 10.1038/s41576-020-00299-4. Epub 2020 Nov 30.
15. Tsushimi T, Noshima S, Oga A, Esato K, Sasaki K. "DNA Amplification and Chromosomal Translocations Are Accompanied by Chromosomal Instability: Analysis of Seven Human Colon Cancer Cell Lines by Comparative Genomic Hybridization and Spectral Karyotyping." Cancer Genet Cytogenet. 2001;126:34–38. Cited by Radiat Environ Biophys. 2019 Aug;58(3):417-424. doi: 10.1007/s00411-019-00797-y. Epub 2019 May 24.
16. Kawai K, Viars C, Arden K, et al. "Comprehensive Karyotyping of the HT-29 Colon Adenocarcinoma Cell Line." Genes Chromosomes Cancer. 2002;34:1–8. Cited by Oncol Rep. 2021 Feb;45(2):680-692. doi: 10.3892/or.2020.7882. Epub 2020 Dec 3.
17. Kuechler A, Weise A, Michel S, et al. "Precise Breakpoint Characterization of the Colon Adenocarcinoma Cell Line HT-29 Clone 19A by Means of 24-color Fluorescence in Situ Hybridization and Multicolor Banding." Genes Chromosomes Cancer. 2003;36:207–210. Cited by Genes Chromosomes Cancer. 2010 Mar;49(3):204-23. doi: 10.1002/gcc.20730.
18. Kleivi K, Teixeira MR, Eknaes M, et al. "Genome Signatures of Colon Carcinoma Cell Lines." Cancer Genet Cytogenet. 2004;155:119–131. Cited by Genes (Basel). 2021 Feb 9;12(2):245. doi: 10.3390/genes12020245.
19. Melcher R, Steinlein C, Feichtinger W, et al. "Spectral Karyotyping of the Human Colon Cancer Cell Lines SW480 and SW620." Cytogenet Cell Genet. 2000;88:145–152. Cited by Oncol Lett. 2020 Dec;20(6):389. doi: 10.3892/ol.2020.12252. Epub 2020 Oct 29.
20. Choi SW, Lee KJ, Bae YA, et al. "Genetic Classification of Colorectal Cancer Based on Chromosomal Loss and Microsatellite Instability Predicts Survival." Clin Cancer Res. 2002;8:2311–2322. Cited by Elife. 2021 Jun 23;10:e70339. doi: 10.7554/eLife.70339.
21. Ogino S, Goel A. "Molecular Classification and Correlates in Colorectal Cancer." JMD.2008;10:13–27. Cited by Int J Mol Sci. 2021 May 15;22(10):5246. doi: 10.3390/ijms22105246.