Study the expression of Neogene TIGD3 that derived from DNA transposons in colorectal cancer cell lines

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Abstract
The process of molecular domestication that occurred on DNA transposons and give what is called neogenes that may play an important role in the human genetic instability. One of these Neogene is TIGD3 (Tigger-derived (TIGD) family of proteins) which is of unclear role in human genome.
Aim: Study the expression of Neogene TIGD3 in colorectal cancer cell lines and its possible role in carcinogenesis.
Method: By western blot method we study the protein expression of TIGD3 gene in twelve colorectal cancer cell lines (HCT116, SW48, LOVO, DLD1) which are microsatellite instable MSI, (SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463) which are microsatellite stable MSS and in healthy tissue of colon as a control in our study.
Results: We obtained a protein expression of TIGD3 gene in all these 12 colorectal cancer cell lines with variable degree of expression with multiple isoforms which was not seen in healthy colon tissue.
Conclusion: TIGD3 may have a role in colorectal cancer either in initiation, promotion or progression which need further research and confirmation.
Key words: DNA transposons, Domestication, Neogene**,** TIGD3**,** microsatellite instable, microsatellite stable.

الخلاصة

الهدف: عملية التدجين الجزيئي التي وقعت في ترانسبوزونات الحامض النووي والتي أنتجت ما يسمى ب النيوجينات التي من الممكن أن تلعب دور مهم عدم الاستقرار الجيني البشري.

واحدة من هده النيوجينات هو TIGD3 (عائلة من البروتينات) والدي دوره غير واضح في الجينوم البشري.

طرق العمل: بطريقة western blot حاولنا دراسة التمثيل الجيني للبروتين TIGD3 في اثنا عشر نوع من الخلايا السرطانية للقولون والمستقيم (DLD1،LOVO،SW48،HCT116) والتي تعتبر غير مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي و
(SW1463،SW403،T84،COLO205،LS123،HT29،SW620،SW480)والتي تعتبر مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي و في عينة من نسيج القولون الماخود من شخص غير مصاب بسرطان القولون في هده الدراسة.

النتائج: من خلال هده الدراسة لقد تم الحصول على تمثيل جيني للبروتين TIGD3 في كل من الاثنا عشر نوع من الخلايا السرطانية للقولون والمستقيم بدرجات متفاوتة من هدا التمثيل الجيني مع ظهور عدة أسوية في هده الخلايا السرطانية والتي لم تظهر في نسيج القولون الغير مصاب بالسرطان.

الاستنتاج: إن البروتين THGD3 قد يكون له دور في سرطان القولون وسرطان المستقيم إما في الشروع، الترويج أو في تطور وتقدم (تفاقم) السرطان والتي تحتاج إلى المزيد من البحوث والتأكيد على هدا الدور.

الكلمة المفتاح:

ترانسبوزونات الحامض النووي، عملية التدجين، النيوجين، TIGD3، غير مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي، مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي.

Introduction

The process of carcinogenesis and tumoral progression are associated to a loss of the integrity of the genome being translated by a genetic instability. Half of the human genome is established by transposable elements, and among them the DNA transposons in which their mobility was inactivated during the evolution. [1,2]

These DNA transposable elements generally occur as neutrally emerging inactive DNA remnants that are epigenetically silenced by the host genome to prevent transcription and subsequent transposition. [3-5]

Such elements are then submitted to little selective pressure and after that acquire sequence variation (mutations) over time. Nevertheless, it has recently been shown that some transposable elements escape host cell silencing to become domesticated by host genomes resulting in the initiation of novel genes (neogenes) that encode proteins. [6-9]

The recent studies realized on the human genome showed that some of these proteins are implied in diverse biological processes which participate directly or indirectly in the stability of the genome (cellular proliferation, progress of the cellular cycle, the modification of the chromatin, the regulation of the transcription. [8,9]

Also theses domesticated elements are implicated in many cellular and developmental functions involving placental development, viral resistance, chromatin structure, DNA recombination and repair, gene regulation, apoptosis and brain development. [6]

An example on these proteins are RAG1, RAG2 that have a role for recombination of genes for immunoglobulin and T cell receptor genes in vivo and can function as a transposase under some conditions in vitro. [10] TIGD3 neogene that was chosen for study its expression is contain a DNA binding domain and catalytic domain . [11]

In the present study the model retained for the study of the expression of TIGD3 neogenic protein by the western blot method was an in vitro model of human epithelial colorectal cancerous cell lines, using the protein extracted from these cancer cell lines and by the antibodies synthesized by Arnaoty et al [11], that allow the study of the expression and the analysis of the various isoformes of neogenic recombinase corresponding to our TIGD3 neogene derived from DNA transposon.

The aim of this study is to show the protein expression of TIGD3 in these colorectal cancer cell lines with two phenotypes MSI, MSS and to reveal if truly this Neogene TIGD3 have a role in the genetic instability and in turn a role in the process of initiation, promotion or progression of cancer.

2. Materials and Methods

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

2.1 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% CO2. 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.

2.2 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.

2.3 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 30min.

3. Results

Expression of the protein TIGD3 in colorectal cancer cell lines

The study of the protein expression of the gene TIGD3 in the 12 colorectal cancer cell lines by western blot method highlighted four different products of expression of this gene corresponding to four different isoforms of TIGD3 (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 important thing we see in this study is the absence of protein expression of TIGD3 gene in the sample C2 which correspond to non cancerous tissue which is taken from healthy person (Fig 1,2,3). The signals of protein expression for TIGD3 gene on protein extracts of colorectal cell lines were appeared in figure 1 exactly as taken by chemiluminescence reaction of PVDF membrane. The amount of the housekeeping protein, actin, in each lane was checked by hybridizing the membranes with a specific monoclonal antibody (Anti actin antibodies/ Abcam). These amounts were calculated by using the programme of Multigauge analyses for the signals taken from each cell line divided on their contents or amount of protein actin as represented in figure 2 and 3.

If we compare the expression in all these colorectal cancer cell lines and healthy tissue, we can suggest that the expression of this gene may have an association with cancer. This may indicate either TIGD3 gene has a role in the cancer initiation, promotion, progression or the cancer increase the expression of this gene. This suggestion will require more research to confirm the possible relationship between gene expression and colorectal cancer because no data available concerning this gene expression and its role in cancer.

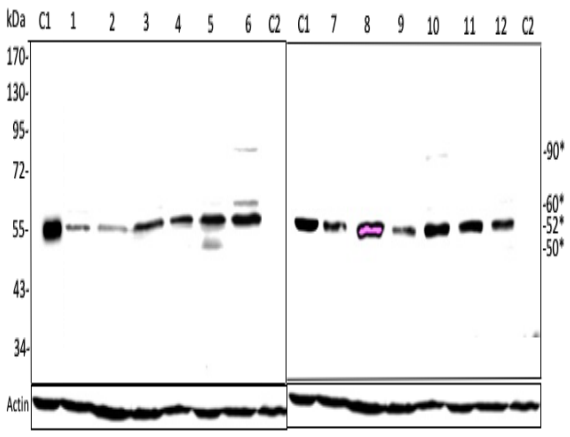

Fig.1. Western blot analyses of *TIGD3*. Lanes 1 to 12 correspond to protein extracts from the human colorectal cancer cell lineages. C1 correspond to protein extracts from HeLa transfected with pVAX-TIGD3. C2 corresponds to an extract of human healthy gut. * indicates the 90, 60 and 50 kDa isoforms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

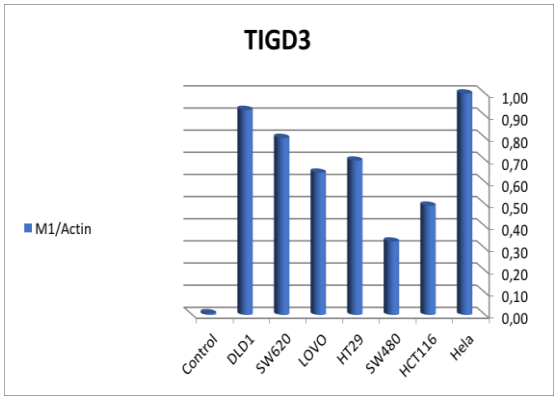

Fig.2. Percentage of TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3,HCT116, SW480, HT29, LOVO, SW620, DLD1 and Control(an extract of human healthy gut)) respectively.

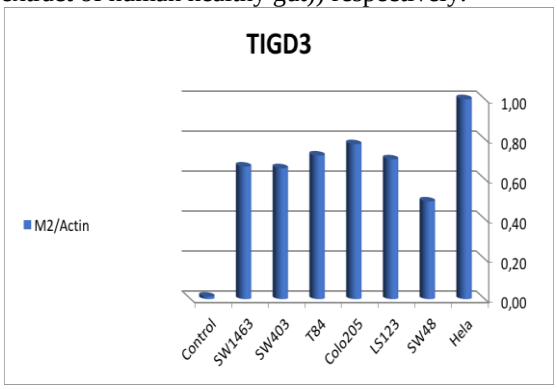

Fig.3. Percentage of TIGD3 expression (Isoform 52 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-TIGD3, SW48, LS123, COLO205, T84, SW403, SW1463 and Control (an extract of human healthy gut)) respectively.

4. Discussion

Our results concerning the protein expression of TIGD3 gene in these colorectal cancer cell lines were realised for the first time, which represents an expression of a domesticated DNA Neogene on such cell lines. No previous bibliography achieves like this study especially because of the unavailability of such specific antibody directed against this type of domesticated DNA Neogene. [11] Even thought the commercial antibodies which are available and are used to test the protein expression of TIGD3 gene in these cell lines have no capability to show any of these results. [11] Also there is no available data show the exact molecular weight of TIGD3 protein by western blot method in previous bibliography, but according to our previous work which revealed the molecular weight of this protein by western blot that equal to 52 kDa. [12] Concerning the multiple isoforms of protein expression of TIGD3 gene which have been shown in our study by western blot in different cell lines were not previously shown and may be explained by the possibility of post translational modification of this protein. The results which are obtained with the antibody; anti TIGD3 that we were produced in collaboration with In Cell Art, therefore provide new information on gene expression TIGD3. [11]

For twelve cell lines of colorectal cancer studied, the number of isoforms expressed in different cell lines were variable. No cell line shows the four isoforms of TIGD3 (90, 60,52 and 50 kDa). Only two lines (DLD1 and T84) expressing the 3 isoforms. The two isoforms (60 and 52 kDa) were observed only in five cell lines (HCT116, SW620, DLD1, SW1463 and T84). The isoform (50 kDa) was observed only in SW620 cell line. Finally, the isoform of 52 kDa was commonly expressed in all studied colorectal cancer lines. To note that the expression of this isoform was higher in cell lines (SW620, DLD1, LOVO, HT29, LS123, Colo205, T84, SW403, SW1463) which were emerged from either metastatic or advance grade colorectal cancer except for LS123 cell line which emerged from non advancing grade (Dukes B). For all these four isoforms detected in our result no expression was found in the C2 (protein extract from healthy gut tissue; figure 1).

All these findings may propose a relationship between the level of expression of this gene and the stage of cancer progression, where highly expressed in cell lines with advancing stage of cancer or metastasis. This may be assumed by either the gene has a role in the progression of cancer or the highly progressed cancer express more this gene, this will need further study and confirmation.

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 liaison between the gene expression and MSS status of the cell line may suggest either the stability at the level of nucleotide (MSS) has a positive effect on the level of TIGD3 gene expression or this gene may have a role in the stability of these nucleotide inside the nucleus of these cell lines. On the other hand, the possible inverse relation between nucleotide instability (MSI) and the level of gene expression may increase the possible theory that the nucleotide stability has a positive effect on gene expression. Unfortunately, there is no available data in the bibliography which tried to show this possible connection between the MSS status at the level of nucleotide and TIGD3 gene expression. For approving this possible link, we need further research and work on this gene.

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 may reveal a relationship between the stability at the level of nucleotide and expressing different isoforms of this gene. In other words, the status of cell line whether MSS or MSI may express different isoforms for the same gene and the appearance of these four isoforms for TIGD3 in these cell lines may be due to their nucleotide stability or instability.

The chromosomal region 11q13.1 where TIGD3 gene is located is not deleted in the cell lines studied SW48, DLD1, HT29, SW480, SW620, LS123, T84, LOVO et SW403. [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.

From the results we were obtained in colorectal cancer cell lines studied, alterations of TP53 and KRAS genes do not appear to be associated with the characteristics of the expression of the TIGD3 gene. These results should be confirmed in a larger series of colorectal cancers. To our knowledge, no studies have been reported on the study of gene expression TIGD3 in these human cancer cell lines.

5. Conclusion

The presence of protein expression for TIGD3 gene in all colorectal cancer cell lines with higher expression in cell lines emerged from advancing or metastatic stage; and absent in healthy tissue may indicate a strong relationship between cancer evolution or progression with gene expression which may be in turn translated to a possible role for this gene in colorectal cancer.

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