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Study the Expression of Neogene PGBD3 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 gives what is called neogenes that may play an important role in the human genetic instability. One of these Neogene is piggyBac 3 (PGBD3) which is associated with human Cockayne syndrome and Premature ovarian failure.

The aim of this work is to study the expression of Neogene PGBD3 in colorectal cancer cell lines.

The protein expression of PGBD3 gene have been studied by western blot method 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.

The result of this study showed that the protein expression of PGBD3 gene in all 12 colorectal cancer cell lines were obtained with variable degree of expression which was not seen in healthy colon tissue.

From the results of this work, it can be concluded that PGBD3 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, PGBD3, microsatellite instable, microsatellite stable, colorectal cancer cell lines.*

دراسة التمثيل الجيني للنيوجين PGBD3 في الخلايا السرطانية البشرية للقولون والمستقيم

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الخلاصة:

عملية التدجين الجزيئي التي وقعت في ترانسبوزونات الحامض النووي والتي أنتجت مايسمى بالنيوجينات التي من الممكن أن تلعب دوراً مهماً في عدم الاستقرار الجيني البشري. PGBD3 هو أحد هذه النيوجينات (عائلة من البروتينات) ويرتبط دوره مع متلازمة كوكائيين التي تحصل عند الانسان وفشل المبيض المبكر.

الهدف من هذه الدراسة هو دراسة التمثيل الجيني للنيوجين PGBD3 في الخلايا السرطانية البشرية للقولون والمستقيم.

تم دراسة التمثيل الجيني للبروتين PGBD3 بطريقة Western blot في إثنى عشر نوعاً من الخلايا السرطانية البشرية للقولون والمستقيم (HCT116, SW48, LOVO, DLD1) والتي تعتبر غير مستقرة جينيا على مستوى نيوكيلوتيدات الحامض و(SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463) والتي تعتبر مستقرة جينيا على مستوى نيوكيلوتيدات الحامض النووي وفي عينة من نسيج القولون المأخوذ من شخص غير مصاب بسرطان القولون في هذه الدراسة.

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من خلال هذه الدراسة لقد تم الحصول على تمثيل جيني للبروتين PGBD3 في كل من الاثنى عشر نوعاً من الخلايا السرطانية للقولون والمستقيم بدرجات متفاوتة من هذا التمثيل الجيني مع ظهور عدة أسوية في هذة الخلايا السرطانية والتي لم تظهر في نسيج القولون الغير مصاب بالسرطان.

نستنتج من هذه الدراسة أن البروتين PGBD3 قد يكون له دور في سرطان القولون وسرطان المستقيم أما في الشروع، الترويج أو في تطور وتقدم (تفاقم) السرطان والتي تحتاج الى المزيد من البحوث والتاكيد على هذا الدور.

الكلمات المفتاحية: *ترانسبوزونات الحامض النووي، عملية التدجين ،النيوجين، PGBD3، غير مستقرة جينيا على مستوى نيوكيلوتيدات الحامض النووي، مستقرة جينياً على مستوى نيوكيلوتيدات الحامض النووي، الخلايا السرطانية البشرية للقولون والمستقيم.*

Introduction:

The loss of genomic stability can be considered as a driving force for the development of colorectal cancer[1]. In colorectal cancer, at least 3 distinct pathways of genomic instability have been described, that includes Chromosome instability (CIN), Microsatellite instable (MSI), CpG island methylator phenotype (CIMP)[2].

For instance, at least 47% of the human genome is made up of transposable elements derived sequences, and 43 of the 47 human genes derived from DNA transposons[3] that have been domesticated by a process of molecular domestication resulting in the initiation of novel genes (neogenes) that encode proteins[4,5,6,7], play an important role in the human genetic instability[8].

The most 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[6,7].

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[4].

The piggyBac3 (PGBD3) neogene which was selected for studying its expression, contain a DNA binding domain and catalytic domain[9]. PGBD3 is associated with human Cockayne syndrome and Premature ovarian failure[10,11].

In the present study the model retained for the study of the expression of PGBD3 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 specific antibodies[12], that allow the study of the expression and the analysis of neogenic recombinase corresponding to our PGBD3 neogene derived from DNA transposon.

The aim of this study is to show the protein expression of PGBD3 in these colorectal cancer cell lines with two phenotypes MSI, MSS; and to reveal if truly this Neogene PGBD3 have a role in the genetic instability and in turn a role in the process of initiation, promotion or progression of cancer.

Materials and Methods:

Cell lines culture

Twelve colorectal cancer cell lines and six leukaemia cell lines were included in this study; colorectal cancer cell lines are:(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 PGBD3. Culture conditions for all at 37◦C in a

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humidified 5% CO2. All of these 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 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. For the samples of healthy gut tissue were extracted according to the protocol provided by our laboratories.

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.

Results:

Expression of the protein PGBD3 in colorectal cancer cell lines

The study of the protein expression of the gene PGBD3 in the 12 colorectal cancer cell lines which have different molecular characteristics (MSI, MSS) by western blot method highlighted unique product of expression of this gene corresponding to PGBD3 (67.5 kDa a molecular weight equal to that of the *PGBD3* transposase) (figure.1). This figure represents a western blot analyses of protein extracts from colorectal cancer cell lineages with antisera directed against the *PGBD3*. Lanes 1 to 12 correspond to protein extracts from the human colorectal cancer cell lineages HCT116, SW480, HT29, LOVO, SW620, DLD1, SW48, LS123, COLO205, T84, SW403, and SW1463 respectively. 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. The isoforms shown in black have not so far been described in databases. This unique isoform 67.5 kDa was expressed in all these 12 colorectal cancer cell lines in variable degree or pattern.

The result showed that it was strongly expressed in cell lines which emerged from advanced Dukes C, grade IV or metastatic stage colorectal cancer (SW620, COLO205, T84, SW1463, SW403). Also it was little expressed in cell lines which emerged from early or primary stage colorectal cancer (SW480 and LS123 both of them emerged from colorectal cancer Dukes B).

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At the same time, the result revealed an absence of this gene expression in sample taken from healthy gut tissue, these findings may suggest a relationship between level of gene expression and grade or stage of this cancer which was highly expressed in metastatic and absent in non cancerous tissue (figures 1, 2 and 3). Figure 2 which represents the percentage of PGBD3 expression (67.5 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-PGBD3, SW620, HT29, SW1463, HCT116, LOVO, SW48, and Control (an extract of human healthy gut)) respectively. These percentages were calculated by programme of multigauge analyses for the signals taken from each cell line divided on their contents or amount of protein actin. Figure-3 is the similar to figure-2 (percentage of PGBD3 expression 67.5 kDa) but in the other colorectal cancer cell lines SW480, LS123, DLD1, SW403, COLO205, T84.

The expression of PGBD3 gene in other cell lines was seen in between these two extremes (metastatic and early stage) as in HCT116, HT29, LOVO, DLD1, SW48 (figures 1,2,3). Although the difference in expression of this gene between these two categories of cell lines according to their emergence either metastatic or early stage but all these cell lines (SW620, COLO205, T84, SW1463, SW403, SW480 and LS123) were of MSS (microsatellite stable) genetic status.

The possible relationship between cancer stage and gene expression need further research and work on this gene to approve his possible role in colorectal cancer. Unfortunately, no data available concerns this gene expression and its role in cancer.

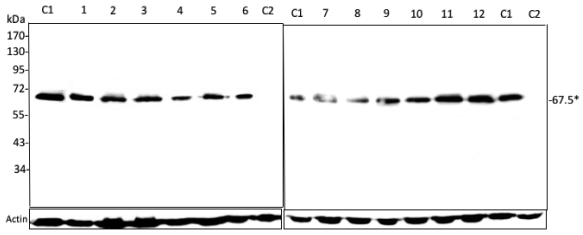

Figure-1: Western blot analyses PGBD3. C1 correspond to protein extracts from HeLa transfected with pVAX-PGBD3. C2 corresponds to an extract of human healthy gut.
* indicates the 67.5 kDa isoforms of PGBD3 transposase.

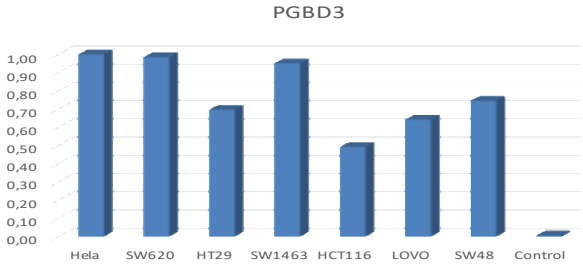

Figure-2: Percentage of PGBD3 expression (67.5 kDa) in different colorectal cancer cell lines.

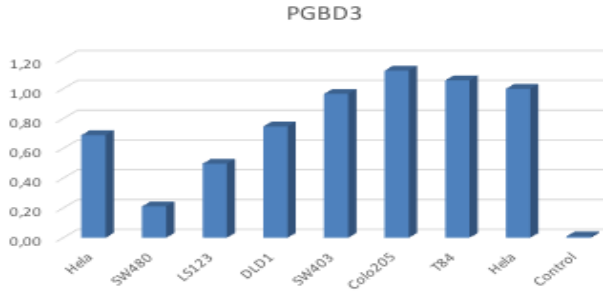

Figure-3: Percentage of PGBD3 expression (67.5 kDa) in different colorectal cancer cell lines.

Discussion:

The results concerning the protein expression of PGBD3 gene in these colorectal cancer and leukaemia 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 on cancerous cell lines. Even thought the commercial antibodies which are used to test the protein expression

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of PGBD3 gene in these cell lines have no capability to show any of these results[12]. There is an available data show the exact molecular weight of PGBD3 protein equal to 68 KDa by western blot performed with the use of anti-PGBD3 antibody (Abnova PAB21786) in human heart tissue, COV434 cells, adult ovary tissue and granulosa cells showed that PGBD3 protein was expressed in human heart tissue, ovary but not in granulosa cells[11]**.** According to our previous and recent work which revealed the molecular weight of this protein by western blot that equal to 67.5 kDa[12] and this finding was in coherence with PGBD3 protein expression obtained by Qin *et al,*[11].

These results which were obtained with the antibody; anti PGBD3 that we were produced in collaboration with In Cell Art, therefore provide new information on gene expression PGBD3[12].

For twelve cell lines of colorectal cancer studied, PGBD3 gene expression was variable. To note that the expression of this gene was higher in cell lines (SW620, COLO205, T84, SW1463, SW403) which were emerged from either metastatic or advance grade colorectal cancer, and little expression was seen in cell lines (SW480 and LS123) which emerged from non advancing grade or early stage colorectal cancer (Dukes B). If we take the two cell lines SW480 and SW620 originally were derived from the same patient, respectively from a primary tumor and metastasis, and they share several translocations[13], confir-ming that there is a possible relation-ship between PGBD3 gene expres-sion and stage or grade of colorectal cancer, as we found high level of expression seen in SW620 cell line and the inverse with SW480.

Interestingly, our results also show absence of PGBD3 gene expression in healthy gut tissue, this may confirm the possible relationship between PGBD3 gene expression and stage or grade of colorectal cancer. 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, further investigation will be required to clarify this possible relationship. Although the difference in expression of this gene between these two categories of cell lines according to their emergence either metastatic (SW620, COLO205, T84, SW1463, SW403) or early stage (SW480 and LS123) but all these cell lines were of MSS (microsatellite stable) genetic status. This finding may indicate that the cell genetic instability at the level of nucleotide MSI (microsatellite instable as represented by the widespread insertion or deletion of simple repeat nucleotides) not affect the level of PGBD3 gene expression. This may be due to the fact in which colorectal cancers arise through a multistep carcinogenic process in which genetic and epigenetic alterations accumulate in a sequential manner[14,15].

Also the absence of difference in PGBD3 gene expression between the two categories colorectal cancer whether MSS or MSI may be due to the level of MSI is low (MSI L) in which there is no distinctive difference between (MSI L) and (MSS) and the difference between MSI-L and MSS is merely quantitative and that it is unlikely that there are qualitatively different genetic pathways to MSI-L and MSS tumours[16].

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 PGBD3 gene expression. For approving this possible link, we need further research and work on this gene.

The chromosomal region 10q11 where PGBD3 gene is located is not deleted in the cell lines studied SW48, DLD1, HT29, SW480, SW620, LS123, T84, LOVO and SW403[13, 17-22]. In addition, the data observed in colorectal cancer series indicate that chromosomal deletions type LOH (Loss of heterozygoty) are less common on the 10q

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chromosome arm than the other arm[23,24]. Therefore, the expression of a PGBD3 gene is not related to allelic loss on chromosome 10q.

From the results we were obtained in colorectal cancer cell lines studied, alterations of TP53 and KRAS genes whether mutated or wild type not appear to be associated with the level of expression of the PGBD3 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 PGBD3 in these human cancer cell lines.

Conclusion:

The presence of protein expression for PGBD3 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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