Study the expression of PGBD3 Neogene, which derived from DNA transposons in Leukaemia cell lines

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دراسة التمثيل الجيني للبروتين PGBD3 في خلايا سرطان الدم

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الخلاصة: ان عملية التدجين الجزيئي التي حدثت في بعض من ترانسبوزونات الحامض النووي والتي ادت الى انتاج جينات جديدة مسماة بالنيوجينات. النيوجينات والتي من الممكن أن تلعب دورا مهما في عدم الاستقرار الجيني البشري. واحدة من هذه النيوجينات هو PGBD3 والذي يؤدي دوره مرتبط بمتلازمة كوكايين التي تحصل عند الانسان وفشل المبيض المبكر.

الهدف من هذه الدراسة هو دراسة التمثيل الجيني للبروتين PGBD3 في خلايا سرطان الدم.

تم دراسة التمثيل الجيني للبروتين PGBD3 بطريقة "western blot" في سبعة انواع من خلايا سرطان الدم (HL60، NB4 ، KG1 ، KG1a ML2 THP1 U937), وفي عينة من نسيج الدم الماخوذة من شخص غير مصاب بسرطان الدم في هده الدراسة.

من خلال هذه الدراسة لقد تم الحصول على تمثيل جيني للبروتين PGBD3 في كل من السبعة انواع من خلايا سرطان الدم بدرجات متفاوتة من هذا التمثيل الجيني والتي لم تظهر في نسيج الدم الغير مصاب بالسرطان.

نستنتج من هذه الدراسة إن البروتين PGBD3 قد يكون له دور في سرطان الدم اما في الشروع، الترويج او في تطور وتقدم (تفاقم) السرطان والتي تحتاج إلى المزيد من البحوث والتأكيد على هدا الدور.

الكلمات المفتاحية: ترانسبوزونات الحامض النووي، عملية التدجين، النيوجين، PGBD3، غير مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي، مستقرة جينيا على مستوى نيوكليوتيدات الحامض النووي، خلايا سرطان الدم.

Abstract: The process of molecular domestication that occurs in some DNA transposons which leads to the production of genes called neogenes. These neogenes 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.

Aim: To study the expression of PGBD3 Neogene in leukaemia cell lines.

Method: By western blot method we study the protein expression of PGBD3 gene in seven leukaemia cell lines (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) and in healthy tissue of blood (blood sample taken from healthy person) as a control in this study.

Results: We obtained a protein expression of PGBD3 gene in all seven leukaemia cell lines with

variable degree of expression which was not seen in healthy blood tissue.

Conclusion: PGBD3 gene may have a role in blood cancer either in initiation, promotion or progression, which needs further research and confirmation.

Keywords: DNA transposons, Domestication, Neogene**,** PGBD3**,** microsatellite instable, microsatellite stable, and leukaemia cell lines.

Introduction

Transposable elements (TEs) are distinct DNA segments that are capable to move from one locus to another within genomes of host cells using a cut-and-paste mechanism [1,2]. Their extended distribution among all major branches of life, their diversity, and intrinsic biological features have made TEs a noticeable source of genetic innovations during species evolution [3,4]. Moreover, transposons may be useful genomic tools for transgenesis, insertional mutagenesis and DNA delivery vehicles in gene therapy [5-9].

TEs are able to induce various genetic alterations upon insertion as a consequence of the transposition process (insertions, excisions, duplications or translocations in the site of integration). For example, DNA transposons can inactivate or alter the expression of genes by insertion within introns, exons or regulatory regions [10-14]. In addition, TEs can assist in the reorganization of a genome by the mobilization of non-transposon DNA [15-17] or by acting as recombination substrates. This recombination would occur by homology between two sequences of a transposon located in the same or different chromosomes, which could be the origin for several types of chromosome alterations [18]. Indeed, TEs can contribute in the loss of genomic DNA by internal deletions [19] or other mechanisms [20,21].

Transposable elements have been “domesticated” by the host in process called molecular domestication which give rise to genes called neogenes. These neogenes are able to achieve a specific function in the cell [22].

An example of these neogenes is the piggyBac3 (PGBD3) neogene which was selected for studying his expression, it contains a DNA binding domain and catalytic domain [23]. PGBD3 is associated with human Cockayne syndrome and Premature ovarian failure [24,25].

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 leukaemia cell lines, using the protein extracted from these cancer cell lines and by the antibodies synthesized by Arnaoty et al [26], that allow the study of the expression and the analysis of neogenic recombinase corresponding to PGBD3 neogene derived from DNA transposon.

The aim of this study is to show the protein expression of PGBD3 in leukaemia cell lines with phenotype MSS (microsatellite stable) and to reveal if truly this Neogene PGBD3 has a role in the genetic instability and in turn a role in the process of initiation, promotion or progression of blood cancer as previously done in human colorectal cancer cell lines.

Materials and Methods

Cell lines culture

Seven leukaemia cell lines (HL60, NB4, KG1, KG1a, ML2, THP1 and

U937) were grown in RPMI 1640 medium supplemented with 10% FBS and streptomycin/penicillin 5.5µg/ml.

Hela cell line was also used for achieving transfection with PGBD3. All cultures were kept at 37 ◦C in a humidified 5% CO2. All cell lines were kindly provided by INSERM U915 /Tours/ France. Blood samples were taken from healthy individual after separating only the white blood cells.

Cell lines proteins extraction and Dosing

Cell cultures were lysed using lyses buffer (SDS 20%, NaCl 100mM, BetaMercaptoEthanol 10mM, protease inhibitor), heated at 65°C for 5 minutes, then DNA broken by ultrasound wave for 20 seconds, centrifuged the tube in 15,000 rpm at 20°C for 10 minutes, the supernatant was collected and the isolated protein was quantified by modified Bradford assay. Controls (healthy) white blood cells were isolated according to a standard protocol optimised in our laboratories [23].

Western blot assay

Samples were prepared by boiling the isolated protein (40 µg) of total protein were placed in each well. The samples were 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 our team [24], 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 an available enhanced chemiluminescence kit (Amersham Biosciences) according to the manufacturer's instructions, the membrane was exposed to film for 1 and 30min [23].

Results

Expression of the protein PGBD3 in leukaemia cell lines

The study of the protein expression of the gene PGBD3 in these leukaemia cell lines reveal nearly the same result, which revealed by the TIGD3 gene (previously done). Western blot method highlighted also a unique product of expression of this gene corresponding to 67.5 kDa a molecular weight equal to that of the *PGBD3* transposase (figure 1). This figure represents western blot analyses of protein extracts of leukaemia cell lineages with antisera directed against the *PGBD3*. Lanes 1 to 7 correspond to protein extracts from the human leukaemia cell lineages (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) respectively. Hybridizing the membranes with a specific monoclonal antibody checked the amount of the housekeeping protein, actin, in each lane. Molecular weights are indicated in the left margins. Molecular weights of the neogenic isoforms are indicated in the right margin.

All these leukaemia cell lines studied, shows an expression of PGBD3 gene but in variable degree of expression. This gene was highly expressed in U937 cell line (Myelomonocytic histiocytic lymphoma (Diffuse B-cell lymphoma) originated from a patient with high grade lymphoma which disseminated to pleural effusion). Less

expression was seen in (KG1, ML2) cell lines (cell lines of acute myeloid leukaemia, originated from bone marrow for the KG1 and peripheral blood for ML2). Moderate expression was seen in other four cell lines (HL60, NB4, KG1a and THP1) cell lines of acute myeloid leukaemia, originated from bone marrow for the NB4 and peripheral blood for HL60, KG1a and THP1) (figure 1,2). Figure 2, which represent the Percentage of PGBD3 expression (67.5 kDa) in leukaemia cell lines (HeLa transfected with pVAX-PGBD3, HL60, NB4, KG1, KG1a, ML2, THP1, U937, and Control (an extract of human healthy blood sample)) 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.

Also we observe that the protein expression of PGBD3 gene was absent in the sample control C2 that correspond to protein extract from white blood cells of healthy individual. This finding may be explained by the same suggestion in case of expression of TIGD3 in leukaemia cell lines and PGBD3 in colorectal cancer cell lines, which was negative in the sample of healthy blood and gut tissue previously.

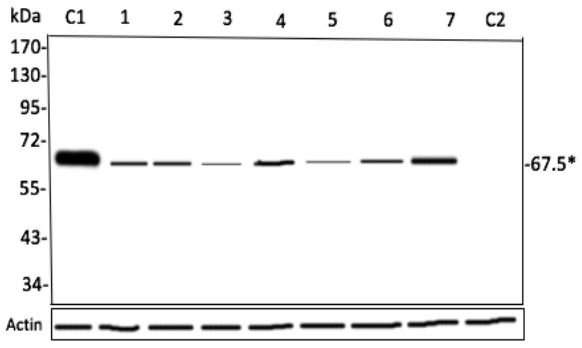

Fig.1. Western blot analyses PGBD3. Lanes 1 to 7 correspond to protein extracts from the human leukaemia cell lineages (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) respectively. C1 correspond to protein extracts from HeLa transfected with pVAX- PGBD3. C2 corresponds to an extract of human healthy blood tissue. * indicates the 67.5 kDa isoforms of PGBD3 transposase.

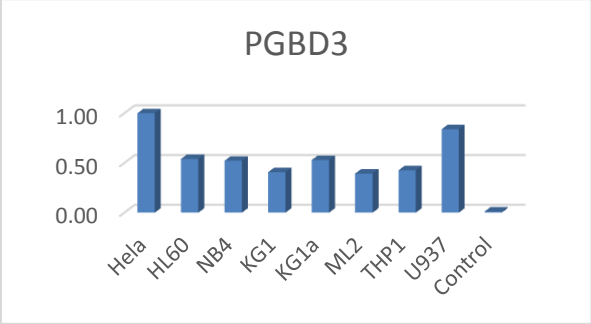

Fig.2. Percentage of PGBD3 expression (67.5 kDa) in leukaemia cell lines.

Discussion

Very little data is available concerning the proteomic profile of PGBD3 neogene in human tissue. Previously we studied the expression of this Neogene in human colorectal cancer cell lines and the results from western blotting demonstrated that PGBD3 was expressed minimally or absent in human healthy gut tissue while the inverse was shown in high grade cancer. Here in this study results showed the same findings. High level of expression in leukaemic cell line which derived from highly metastatic tumor where taken from disseminated cancer in pleural effusion. Little or absent expression of this gene in healthy blood tissue, this finding may be interpretated by a possible relationship between gene expression and stage or grade of tumor and inturn possible role for this gene in this type of 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, this will need further study and confirmation.

Although the difference in expression of this gene between these two

categories of cell lines according to their emergence either metastatic (U937) or primary (HL60, NB4, KG1, KG1a, ML2, THP1) but all these cell lines were of MSS (microsatellite stable) genetic status. That's mean the genetic status of the cell whether MSI or MSS not affect the level of gene expression, in other words the MSS status of these cell lines not given the same level of 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 PGBD3 gene expression. For approving this possible link, we need further research and work on this gene.

The interesting thing is the chromosomal region 10q11 where PGBD3 gene is located is not deleted in all these cell lines studied which may indicate that they really express this gene but in variable degree depending on cancer stage and differentiation.
From the results we obtained in leukaemia cell lines studied, alteration or mutation of TP53 gene doesn't appear to be associated with the expression of the PGBD3 gene.

Conclusion

The presence of protein expression for PGBD3 gene in all leukaemia cell lines with higher expression in cell line 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 in turn be translated to a possible role for this gene in blood cancer.

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