

## The proteomic expression of Neogene nuclear apoptosis-inducing factor 1(NAIF1) in Leukaemia cell lines

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### Abstract

The process of molecular domestication which had been occurred in some DNA transposons (2nd class of transposable elements) led to the formation of novel genes, which are called "Neogenes". These neogenes may play an important role in the human genetic instability, human diseases and cancer. One of these Neogene is nuclear apoptosis-inducing factor 1(NAIF1) which inducing apoptosis in various human cancers.

The aim of this work is to study the expression of NAIF1 Neogene in leukaemia cell lines.

The protein expression of NAIF1 gene has been studied by western blot method in seven leukaemia cell lines (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) and in a healthy tissue of blood as a control.

The result of this study showed that the protein expression of NAIF1 in leukaemia cell lines were found in variable degree of expression, which was highly expressed in healthy blood tissue.

From the results of this work, it can be concluded that NAIF1 protein may have a role in inhibition of proliferation, migration and invasion of leukaemia by inducing apoptosis, which need further research and confirmation.

**Keywords:** DNA transposons, Domestication, Neogene, NAIF1, microsatellite instable, microsatellite stable, and leukaemia cell lines.

### الخلاصة:

ان عملية التدجين الجيني التي حدثت في بعض من ترانسبوزونات الحمض النووي والتي أدت الى انتاج جينات جديدة مسماة بالنيوجينات. النيوجينات والتي من الممكن ان تلعب دور مهم في عدم الاستقرار الجيني البشري، الامراض والسرطانات، واحدة من هذه النيوجينات هو NAIF1 (عامل الموت الخلوي المبرمج) والذي يؤدي الى الموت الخلوي المبرمج في عديد من السرطانات البشرية.

الهدف من هذه الدراسة هو دراسة التمثيل الجيني للبروتين NAIF1 في خلايا سرطان الدم.

تم دراسة التمثيل الجيني للبروتين NAIF1 بطريقة "western blot" في سبعة أنواع من خلايا سرطان الدم (KG1a، U937، THP1، ML2، KG1، NB4، HL60) وفي عينة من نسيج الدم المأخوذة من شخص غير مصاب بسرطان الدم في هذه الدراسة.

من خلال هذه الدراسة، لقد تم الحصول على تمثيل جيني للبروتين NAIF1 في كل من السبعة أنواع من خلايا سرطان بدرجات متفاوتة من هذا التمثيل الجيني والتي ظهرت بمستوى عالي جدا في نسيج الدم الغير مصاب بالسرطان.

نستنتج من هذه الدراسة ان البروتين NAIF1 قد يكون له دور في كبت الانتشار والهجرة والغزو السرطاني لسرطان الدم من خلاه دورة في الموت الخلوي المبرمج والتي تحتاج الى المزيد من البحوث والتأكيد على هذا الدور.

### Introduction

Transposable elements are DNA sequences that have capability to change its location from one to another, in the human genome.

Transposable elements comprise approximately 45% of the human genome [1].

Several mechanisms were suggested the activity of transposable elements can positively and negatively affect the human genome; for example, transposable elements

mobilization can induce gene inactivation, alter gene expression or produce illegitimate recombination and a significant role in genome evolution [2].

Transposable elements can disrupt genes, modulate their transcription or serve as ground for recombination, and have been implicated in diseases such as cancer and diabetes [3,4].

Two classes of transposable elements were

found; class I (retrotransposons) and class II (DNA transposons)<sup>[5,6]</sup>. DNA transposons are currently thought to be transpositionally inactive in most mammals with bats being the exception<sup>[7,8]</sup>; however, several genes in the human genome are derived from DNA transposons [1]. By a process of molecular domestication which occur in some DNA transposons by the host lead to the formation of novel genes which are called “Neogenes” that encode proteins<sup>[9,10,11,12]</sup>, that play an important role in genetic instability of human<sup>[13]</sup>.

NAIF1 is a nuclear protein, which contains a Myb-like domain at its N-terminal region. NAIF1 is an apoptotic pathway gene that has a role in inducing apoptosis in various human cancers. In human gastric cancer, NAIF1 inhibits gastric cancer migration and invasion<sup>[14,15]</sup>. In non-small-cell lung cancer, NAIF1 can inhibit cancer growth<sup>[16]</sup>. In human prostate cancer, NAIF1 was suggested to be a potential biomarker<sup>[17]</sup>, yet its exact expression pattern or functional role in human remains elusive especially in cancer.

The aim of this study is to show the protein expression of Nuclear apoptosis-inducing factor 1 (NAIF1), derived from the DNA transposon by molecular domestication in leukaemia cell lines with phenotype MSS (microsatellite stable) and to examine the role of NAIF1 neogene in the genetic instability and in turn a role in the process of regression or inhibition, of blood cancer as previously done in human colorectal cancer cell lines and in human gastric cancer.

## Materials and Methods:

In the present study the model retained for the study of the expression of NAIF1 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<sup>[18]</sup>, that allow the study of the expression and the analysis of neogenic recombinase corresponding to NAIF1 neogene derived from

DNA transposon.

## Cell 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 NAIF1. All cultures were kept at 37 °C in a humidified 5% CO<sub>2</sub>. 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<sup>[19]</sup>.

## 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 mem-

brane 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<sup>[19]</sup>.

## Results:

### Expression of NAIF1 in leukaemia cell lines

Results highlighted a unique product of expression of NAIF1 in all leukaemia cell lines (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) by western blot, corresponding to 35 kDa molecular weight<sup>[19]</sup> equal to that of the *NAIF1* transposase (figure 1). This figure represents a western blot analyses of protein extracts of leukaemia cell lineages with antisera directed against the *NAIF1*. Lanes 1 to 7 correspond to protein extracts from the human leukaemia cell lineages (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) 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.

All studied leukaemia cell lines showed an

expression of NAIF1 protein in a variable degree of expression. This protein was mildly expressed in U937 cell line (Myelomonocytic histiocytic lymphoma (Diffuse B-cell lymphoma) established from a patient with high grade lymphoma which, disseminated to pleural effusion). Overexpression was seen in (HL60, NB4, KG1a, ML2, THP1) cell lines (acute myeloid leukaemia cells, originated from bone marrow of the NB4 and peripheral blood of HL60, KG1a, ML2, THP1). Moderate expression was seen in KG1 cell line; a cell line of acute myeloid leukaemia (monocytic), originated from bone marrow (figure 1 and 2). Figure 2 which represents the Percentage of NAIF1 expression (35 kDa) in leukaemia cell lines (HeLa transfected with pVAX-NAIF1, HL60, NB4, KG1, KG1a, ML2, THP1, U937, and Control (an extract of human healthy blood sample)) respectively. These percentages were calculated by programme of multigaueanalyses 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 NAIF1 gene was highly expressed in the control (C2), which represented a protein extract from white blood cells of healthy individual.

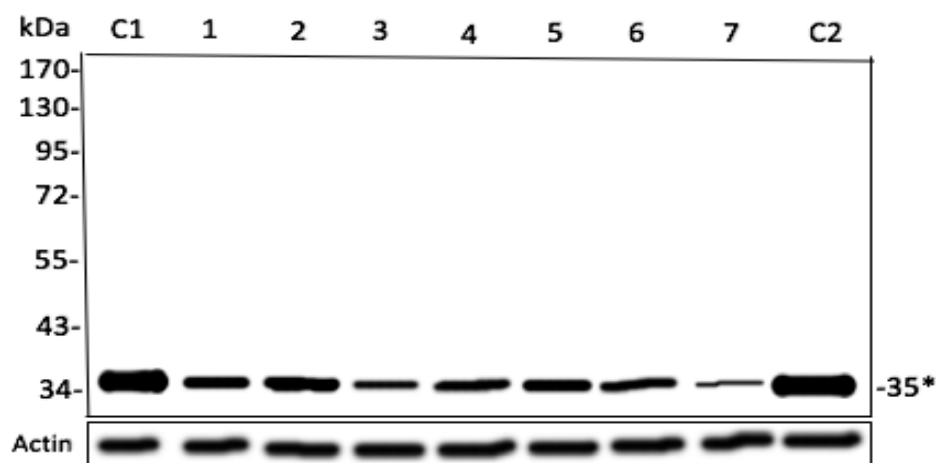
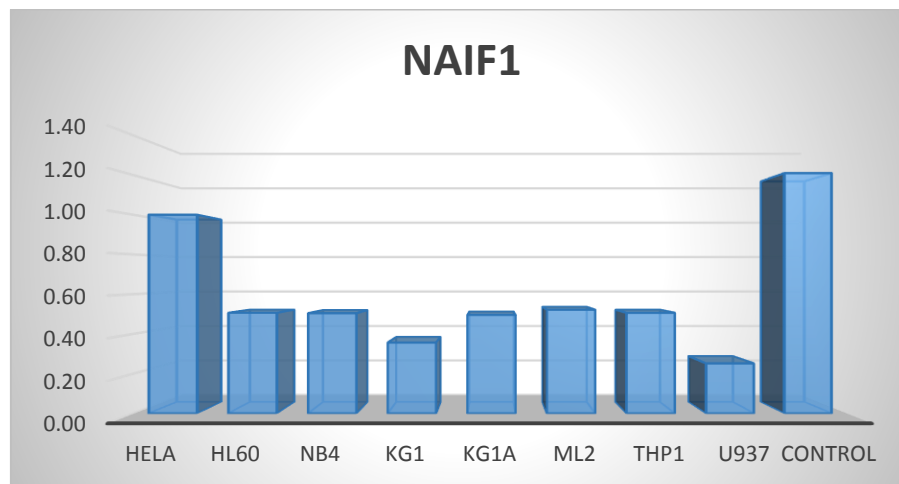


Figure-1: Western blot analyses NAIF1. Lanes 1 to 7 correspond to protein extracts from the human leukaemia cell lineages (HL60, NB4, KG1, KG1a, ML2, THP1 and U937) respectively. C1 correspond to protein extracts from HeLa transfected with pVAX- NAIF1. C2 corresponds to an extract of human healthy blood tissue. \* indicates the 35 kDa isoforms of NAIF1 transposase.



**Figure-2: Percentage of NAIF1 expression (35 kDa) in leukaemia cell lines.**

## Discussion

Many studies have described the proteomic expression of NAIF1 gene [14,15,16,20,21]. The previous studies were done on NAIF1 gene expression showed that, NAIF1 is overexpressed in cancer tissue or cancer cell lines [14,15,20]. Previously we studied the expression of this Neogene (NAIF1) in human colorectal cancer cell lines and the results from western blotting demonstrated that NAIF1 was expressed strongly in human healthy gut tissue while the inverse (moderate or little expression) was shown in high-grade cancer. Here in this study our results showed similar or nearly the same results. Low level of expression in leukaemic cell line was derived from a highly metastatic tumour, where taken from disseminated cancer in pleural effusion. High level of expression for this gene in healthy blood tissue, this finding may be interpreted by a possible relationship between gene expression and stage or grade of tumour (inversely related) and inturn possible role for this gene in the regression or inhibition in this type of cancer. These results are in coherence Luo's finding in tissues, they demonstrated NAIF1 protein is highly expressed in human normal gastric tissue and down-regulated or lost in gastric cancer tissue [15]. Further investigation will be required to clarify this inverse relationship between gene expression and stage or grade of cancer (degree of differentiation). However, little information exists on the

relationship between NAIF1 and cancer genesis and progress. These findings may indicate that it is very likely that NAIF1 may predominantly act as a tumour-suppressive gene, possibly through the activation of apoptotic pathways, in various types of cancers [21]. The exact mechanism of NAIF1 inducing apoptosis and the physiological role need further exploration.

All studied cell lines were of MSS (microsatellite stable) genetic status [22], but they differ according to their emergence either metastatic (U937) or primary (HL60, NB4, KG1, KG1a, ML2, THP1). Here we revealed that the difference in expression of NAIF1 protein is related to degree of differentiation not to the genetic status of these cell lines. That means the genetic status of the cancerous cell whether microsatellite stable or microsatellite instable not affecting the level of gene expression. In other words, the microsatellite stability in these cell lines did not show the same level of gene expression. Unfortunately, no information available in the bibliography tried to reveal this possible link between the microsatellite stable status at the level of nucleotide and NAIF1 gene expression. To confirm the correlation, further research is required.

NAIF1 gene is located on the chromosomal region 9q34.11 [19]; this region not shown any types of mutation (deletion, translocation, substitution,...) in the studied cell lines which may indicate that they

really express this gene but in variable degree depending on their emergence either metastatic or primary in other words; “cancer stage and differentiation”.

Concerning the TP53 gene status whether altered or mutated not appear to affect the level of NAIF1 gene expression in these leukaemia cell lines studied because all these cell lines show a mutated status of TP53 [22].

### Conclusion:

The presence of NAIF1 protein in all leukaemia cell lines with lower expression in cell line emerged from advanced or metastatic stage; and higher in healthy tissue may indicate a strong relationship between gene expression and cancer inhibition or regression. Therefore, NAIF1 may have therapeutic potential in the treatment of blood cancer and may provide new strategies for developing anti-cancer drugs.

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