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Nuclear Apoptosis-Inducing Factor1 (NAIF1) expression in Glioma cell lines

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Abstract
The neogenic recombinases could be a source of genetic variety, and they could be involved in the pathways of genetic instability in carcinogenesis. The nuclear apoptosis-inducing factor 1 (NAIF1), which codes the protein NAIF1 that triggers apoptosis in several human malignancies, is one of these neogenic recombinases.
The purpose of this research is to look at the expression of the NAIF1 neogene in seven different glioma cell lines (H4, HS683, 42-MG-BA, A172, U87MG, U118MG, SK-N-MC).
The protein expression of the NAIF1 gene in samples of protein isolated from various glioma cell lines was investigated using the western blot method.
The findings revealed that while NAIF1 protein expression was observed in all cell lines, it was moderately expressed in all primary non-metastatic cell lines and very weakly expressed in one cell line generated from a metastatic site, SK-N-MC.
It can be concluded that NAIF1 protein expression is negatively related to advanced cancer stage or grade, and that it may have a role in suppression of cancer proliferation, migration, and invasion via inducing apoptosis.
Keywords: *DNA transposons, Domestication, Neogene, NAIF1, Glioma cell lines.*

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I. Introduction

The development of novel genes known as "Neogenes" that encode proteins [1-4], which play an essential role in human genetic instability [5], is caused by a process of molecular domestication that occurs in some DNA transposons by the host. One of these neogene is NAIF1, which is a nuclear protein with a Myb-like domain located at its N-terminus.

The human gene encoding nuclear apoptosis inducing factor 1 (NAIF1) located on chromosome 9q34.11, has been found to inhibit the advancement of numerous malignant malignancies. [6-12] NAIF1 has an inhibitory role in the early stages of gastric cancer genesis and has been found to be downregulated or absent in gastric cancer tissues. [7,8] According to Fu Y et al, overexpression of NAIF1 inhibit prostate cancer cell growth and migration, [6]

The goal of this study is to demonstrate the expression of Nuclear apoptosis-inducing factor 1 (NAIF1) in Glioma cell lines with phenotype MSS (microsatellite stable), which has been done previously in human colorectal cancer tissue [9] and human gastric cancer. [7,8]

II. Materials And Methods:

In this study, an in vitro model of human glioma, glioblastoma, neuroglioma cell lines was used to examine the expression of NAIF1 protein using the western blot method. Utilizing the protein derived from these cancer cell lines, as well as antibodies synthesized by Arnaoty et al. [13] that enable the investigation and analysis of neogenic recombinase matching to the NAIF1 neogene produced from DNA transposon.

Cell culture

Seven glioma, glioblastoma, neuroglioma cell lines (H4, HS683, 42MG-BA, A172, U87MG, U118MG, SK-N-MC) were grown in Dulbecco's Modified Eagle's Medium (DMEM) 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% CO2. All cell lines were kindly provided by INSERM U915 /Tours/ France. Because human brain tissue was unavailable as a negative control, blood samples were acquired from healthy individuals after separating just the white blood cells.

Cell lines proteins extraction and Dosing

Cell cultures were lysed in lyses buffer (SDS 20%, NaCl 100mM, BetaMercaptoEthanol 10mM, protease inhibitor), heated at 65°C for 5 minutes, DNA broken by ultrasound wave for 20 seconds, centrifuged at 15,000 rpm for 10 minutes at 20°C, supernatant collected, and isolated protein quantified using a modified Bradford assay.

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. [14]

III. Results:

Expression of NAIF1 in glioma, glioblastoma, neuroglioma **cell lines**

The findings revealed a distinct product of NAIF1 expression in all glioma cell lines (H4, HS683, 42MG-BA, A172, U87MG, U118MG, SK-N-MC) by western blot, equivalent to a molecular weight of 35 kDa [14], which is the same as that of NAIF1 transposase figure 1.

The western blot analysis of protein extracts from glioma, glioblastoma, neuroglioma cell lineages with antisera directed against the NAIF1 is shown in this figure. Lanes 1 to 7 correspond to protein extracts from the human glioma, glioblastoma, neuroglioma cell lineages (H4, HS683, 42MG-BA, A172, U87MG, U118MG, SK-N-MC) respectively. C1 correspond to protein extracts from HeLa transfected with pVAX- NAIF1, C2 corresponds to an extract of human healthy blood tissue. By hybridizing the membranes with a particular monoclonal antibody, the amount of the housekeeping protein actin in each lane was determined. In the left margins, the molecular weights are indicated. The neogenic isoforms' molecular weights are shown in the right margin.

The NAIF1 protein was expressed in varying degrees in all of the glioma, glioblastoma, neuroglioma cell lines tested. This protein was very mildly expressed in SK-N-MC cell line, which is derived from metastatic high-grade tumor. Variable degree of expression was seen in other six-glioma cell lines, which were derived from primary non-metastatic tumor (figure 1 and 2). Figure 2, which represent the percentage of NAIF1 expression (35 kDa) in glioma, glioblastoma, neuroglioma cell lines (C1: HeLa transfected with pVAX-NAIF1, H4, HS683, 42MG-BA, A172, U87MG, U118MG, SK-N-MC, C2: corresponds to an extract of human healthy blood tissue) respectively. These percentages were computed using a multigauge analysis tool that divided the signals from each cell line by the amount of protein actin they contained.

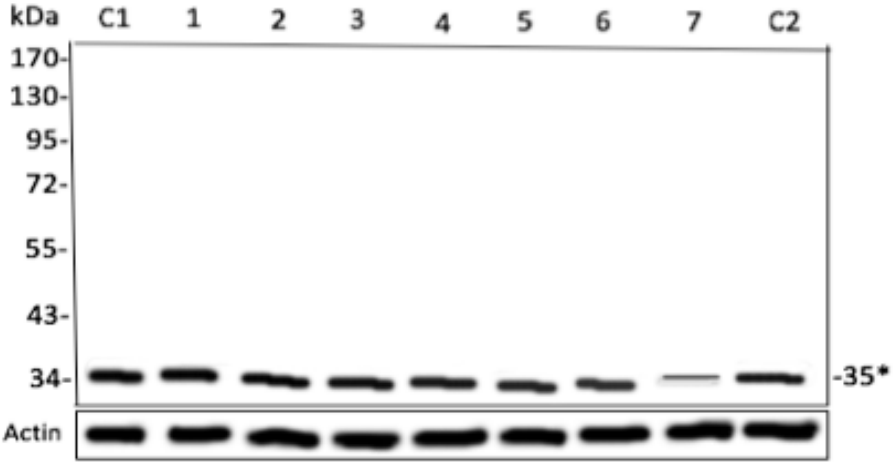

Fig.1. Western blot analyses NAIF1. Lanes 1 to 7 correspond to protein extracts from the human glioma cell lineages (H4, HS683, 42MG-BA, A172, U87MG, U118MG, SK-N-MC) respectively. C1 correspond to protein extracts from HeLa transfected with pVAX- NAIF1. C2 corresponds to an extract of human healthy blood tissue. * indicates the 35 kDa isoforms of NAIF1 transposase.

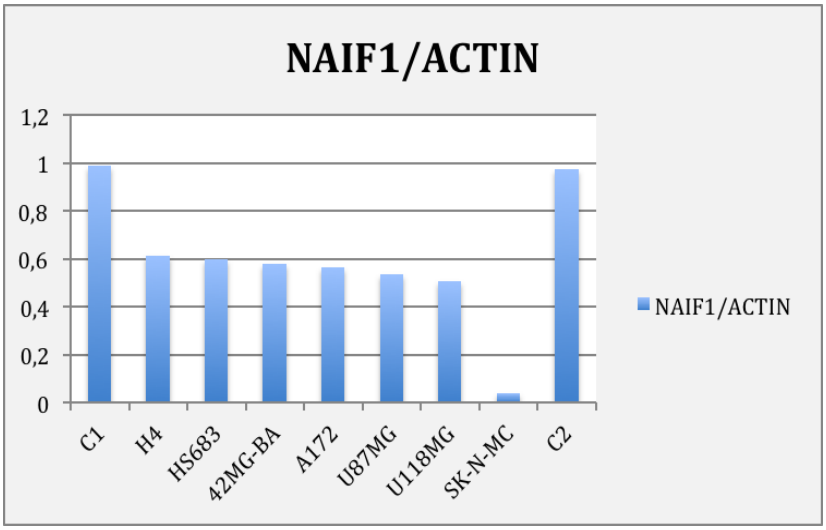

Fig.2. Percentage of NAIF1 expression (35 kDa) in glioma cell lines.

IV. Discussion:

The proteomic expression of the NAIF1 gene has been studied extensively. [6-12] Previous research into the expression of the NAIF1 gene revealed that NAIF1 is overexpressed in cancer tissue and cancer cell lines. [7,8,12] We previously looked at the expression of this Neogene (NAIF1) in human colorectal cancer cell lines and tissues, and the results of western blotting showed that NAIF1 was expressed significantly in normal healthy gut tissue, but only moderately or barely in high-grade cancer cell lines and tissues. [9] Our findings in this investigation were similar or virtually identical to those in the previous study. The low level of expression in the Glioma SK-N-MC cell line, which is derived from metastatic high-grade tumor collected from disseminated malignancy in the skull bone. High levels of expression for this gene in healthy blood tissue and previously in healthy colorectal tissue [9]; this observation could be explained by an inverse association between gene expression and tumor stage or grade, and hence a putative involvement for this gene in cancer regression or suppression. These findings corroborate Luo's findings in tissues, demonstrating that NAIF1 protein is extensively expressed in normal human stomach tissue and down regulated or absent in gastric cancer tissue. [7] This inverse link between gene expression and cancer stage or grade will need to be investigated further. However, nothing is known about the link between NAIF1 and cancer initiation and progression. These data suggest that NAIF1 is most likely to serve as a tumor suppressor gene in diverse malignancies, probably via activating apoptotic pathways. [11] The specific method by which NAIF1 induces apoptosis, as well as its physiological relevance, need to be investigated further. All of the cell lines investigated were MSS (microsatellite stable) genetically [15], but their emergence was either metastatic (SK-N-MC) or primary (H4, HS683, 42MG-BA, A172, U87MG, U118MG). We discovered that differences in NAIF1 protein expression are related to the degree of differentiation rather than the genetic status of these cell lines. That is, whether the malignant cell's genetic state is microsatellite stable or microsatellite instable has no effect on the degree of gene expression. In other words, these cell lines' microsatellite stability did not demonstrate the same level of gene expression. Unfortunately, no information in the bibliography attempted to uncover this potential relationship between microsatellite nucleotide stability and NAIF1 gene expression. More research is needed to confirm the association. The NAIF1 gene is located on chromosome 9q34.11[13], which has not shown any mutations (deletion, translocation, substitution,...) in the cell lines studied, which could indicate that they do express this gene, but to varying degrees depending on whether they are metastatic or primary, or in other words, "cancer stage and differentiation."

V. Conclusion:

The presence of NAIF1 protein in all Glioma cell lines, with reduced expression in cell line that have progressed or metastatic stages, and higher expression in healthy tissue, could imply a strong link between gene expression and cancer suppression or regression. As a result, NAIF1 may have therapeutic promise in the treatment of cancer and could lead to novel anti-cancer drug development strategies.

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Ethical Clearance: Samples were taken from the INSERM UMR 1087, Nantes /France, no samples were taken from patients to have their consents to participation in this type of research.

Conflict of Interest: There is no conflict.

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