Research Article

Study the expression of Neogene NAIF1 that derived from DNA transposons in colorectal cancer cell lines

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Abstract

Background: The domesticated genes (Neogenes) in humans which arisen from the process of molecular domestication that occurred on DNA transposons may play an important role in the human genetic instability, and have evolved to have endogenous functions other than genomic transposition. One of these Neogene is nuclear apoptosis-inducing factor 1 (NAIF1) which inducing apoptosis in various human cancers.

Aim: Study the expression of Neogene NAIF1 in colorectal cancer cell lines.

Method: By western blot method we study the protein expression of NAIF1 gene in 12 colorectal cancer cell lines (HCT116, SW48, LOVO, DLD1) that are microsatellite instable MSI and (SW480, SW620, HT29, LS123, COLO205, T84, SW403, SW1463) that are microsatellite stable MSS and in healthy tissue of colon as a control in this study.

Results: Protein expression of NAIF1 gene in all these 12 colorectal cancer cell lines with variable degree of expression and was seen strongly expressed in healthy colon tissue.

Conclusion: NAIF1 protein expression inversely related with more advanced stage or grade of colorectal cancer in these cell lines (in other words, NAIF1 protein expression increase in colorectal cancer with high degree of differentiation).

Key words: DNA transposons, Domestication, Neogene, NAIF1, microsatellite instable, microsatellite stable, colorectal cancer cell lines.

INTRODUCTION

Transposable elements (TEs) are pieces of nucleic acid that encode the inherent ability to mobilize from one genomic location to another. This ability to “jump” is mediated by element-encoded proteins such as DNA transposase or reverse transcriptase.[1,2,3]

TEs are historically subdivided into two major classes defined by their mobilization intermediate. Class I TEs, also known as retrotransposons, encompass elements that move via a “copy-and-paste” mechanism involving an RNA intermediate,[4, 5] while Class II TEs, referred to as DNA transposons, represent TEs that mobilize by a “cut-and-paste” mechanism. DNA transposons are currently thought to be transpositionally inactive in most mammals with bats being the exception;[6,7] however, several genes in the human genome are derived from DNA transposons.[3]

TEs can contribute to the developmental and adaptive regulation of gene expression and are a major source of genetic variation that drives genome evolution.[8] In humans and other mammals, transposable elements comprise about half of the nuclear genome.[9] The majority of primate-specific sequences that regulate gene expression are derived from transposons,[10] and transposons are a major source of structural genetic variation in human populations. [11]

TEs are also considered evolutionary precursors of many genes in mammalian genomes.[12] Of particular concern are the deleterious effects of TEs exerted by their transposition that may result in potential insertions and deletions within the coding sequences that may disrupt gene expression, as well as damaging

recombination events.[13,14] TEs can disrupt genes, alter their transcription or serve as ground for recombination, and have been implicated in diseases such as cancer and diabetes.[15,16]

DNA transposons that have been domesticated by a process of molecular domestication resulting in the initiation of novel genes (neogenes) that encode proteins.[17,18,19,20] These proteins play an important role in the human genetic instability.[21] Also these domesticated genes in humans have evolved to have endogenous functions other than genomic transposition per se. For example, human RAG1 (Recombination Activating Gene) is a domesticated Transib (Family subclass of DNA transposons) transposase that has retained its active transposase domain, and can transpose ITR (inverted terminal repeat) -containing transposons in vitro, but catalyzes somatic recombination of immunoglobulin and T-cell receptor genes in lymphocytes across signal sequences that might be derived from related transposons.[22,23] Human SETMAR (SET+HSmar) is a Mariner-derived transposase with a divergent DDN (Catalytic triad) transposase domain that has retained its endonuclease activity and functions in double-strand DNA repair by non-homologous end joining.[24] The human genome encodes over 40 other genes derived from DNA transposases,[9,25] including THAP9 (Thanatos-Associated protein 9) that was recently found to mobilize transposons in human cells with as of yet unknown function.[26]

One of these neogenes is Nuclear apoptosis-inducing factor 1 (NAIF1), which derived from the DNA transposon by molecular domestication. NAIF1 is a nuclear protein that contains a Myb-like domain at its N-terminal region.

NAIF1 is an apoptotic pathway gene that induces apoptosis in various human cancers. In gastric cancer, NAIF1 inhibits gastric cancer migration and invasion by targeting MAPK or caspase-3.[27,28] In non-small-cell lung cancer, NAIF1 interacts with miR-24 to inhibit cancer growth.[29] In human prostate cancer, NAIF1 was suggested to be a potential biomarker,[30] yet its exact expression pattern or functional role remains elusive.

The relationship between NAIF1 and inhibition the progression of cancer by inducing apoptosis by two hypothesis according to Luo et al; 1st: NAIF1 may interact with some DNA binding protein, like histone, to help to change the configuration of DNA and then regulate some gene expression sequentially, inducing apoptosis, 2nd: NAIF1 plays an important role in control of the expression of some pivotal anti-cancer or apoptotsis-related genes in physiological level.

These findings may indicate that it is very likely that NAIF1 may predominantly act as a tumor-suppressive gene, possibly through the activation of apoptotic pathways, in various types of cancers.[31] The exact mechanism of NAIF1 inducing apoptosis and the physiological role need further exploration.

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 epithelial colorectal cancerous cell lines, using the protein extracted from these cancer cell lines and by the antibodies synthesized by Arnaoty et al,[32] that allow the study of the expression and the analysis of neogenic recombinase corresponding to our NAIF1 neogene derived from DNA transposon.

The aim of this study is to show the protein expression of NAIF1 in these colorectal cancer cell lines with two phenotypes microsatellite instable, microsatellite stable (MSI, MSS); and to reveal its role in inducing apoptosis in human cancer cell lines and in turn its role in inhibition of proliferation, migration and invasion of cancer.

PATIENTS AND METHODS

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 NAIF1. 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.

Cell lines proteins extraction and Dosing

Whole protein from all cell lines were extracted with using lyses buffer (SDS 20%, NaCl 100mM, beta mercapto ethanol 10mM, Protease inhibitor). Heating at 65°C for 5 minutes then breaking the DNA by ultrasound wave for 20 seconds and centrifuging the in 15,000 rpm at 20°C for 10 minutes. 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 and white blood cells 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 InCellArt, 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 30 min.

RESULTS

Expression of the protein NAIF1 in colorectal cancer cell lines

The study of the protein expression of the gene NAIF1 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 NAIF1 (35 kDa a molecular weight equal to that of the *NAIF1* transposase) (figure.1). This unique isoform 35 kDa was expressed in all these 12 colorectal cancer cell lines in variable degree or pattern.

We note that it was lightly 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 highly 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).

At the same time, we note a very high and strong expression of this gene 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, in other words relation between gene expression and degree of tissue differentiation; which was highly expressed in normal gut tissue and slightly expressed in metastatic cancerous tissue (figure 1,2,3).

The expression of NAIF1 gene in other cell lines was seen in between these two extremes (metastatic and early stage) as in HCT116, HT29, LOVO, DLD1, SW48 (figure 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 microsatellite stable (MSS) genetic status.

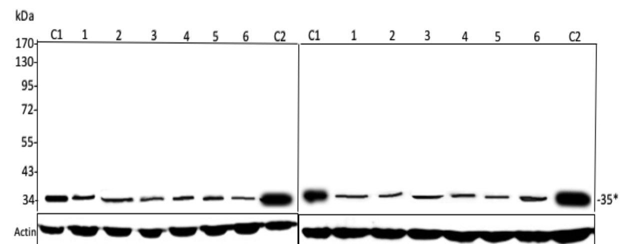

Figure 1: Western blot analyses of protein extracts from colorectal cancer cell lineages with antisera directed against the *NAIF1*. Lanes 1 to 12 correspond to protein extracts from the human colorectal cancer cell lineages HCT116, SW480, HT29, LOVO, SW620, DLD1, SW48, LS123, COLO205, T84, SW403, and SW1463 respectively. C1 correspond to protein extracts from HeLa transfected with pVAX-NAIF1. C2 corresponds to an extract of human healthy gut. * indicates the 35 kDa isoforms of NAIF1; *, indicates a 35 kDa isoform with a molecular weight equal to that of the *NAIF1* 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. The isoforms shown in black have not so far been described in databases.

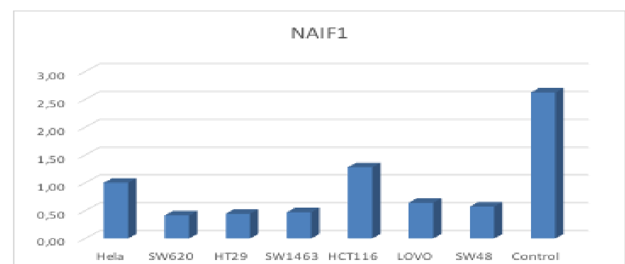

Figure 2: Percentage of NAIF1 expression (35 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-NAIF1, SW620, HT29, SW1463, HCT116, LOVO, SW48, and Control (an extract of human healthy gut)) respectively. These percentages were calculated by programme of Multigauge analyses for the signals taken from each

cell line divided on their contents or amount of protein actin.

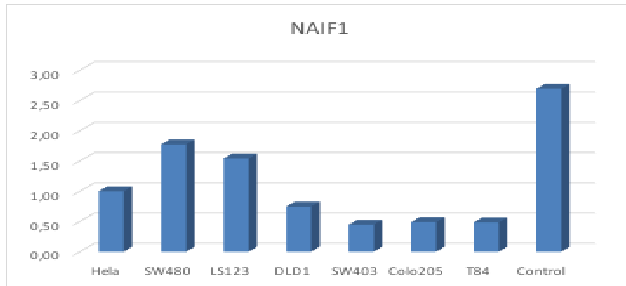

Figure 3: Percentage of NAIF1 expression (35 kDa) in different colorectal cancer cell lines (HeLa transfected with pVAX-NAIF1, SW480, LS123, DLD1, SW403, COLO205, T84 and Control (an extract of human healthy gut)) respectively. These percentages were calculated by programme of Multigauge analyses for the signals taken from each cell line divided on their contents or amount of protein actin.

DISCUSSION

Several studies have been performed to describe the NAIF1 gene; however, little is known about its proteomic profile NAIF1 is overexpressed in cancer tissue or cancer cell lines.[27,28,33] Results from western blotting demonstrated that NAIF1 was minimally expressed in metastatic colorectal cancer cell lines, more in cancer cell lines originated from early stage colorectal cancer and highly expressed in normal gut tissue (figures 1,2,3).

For twelve cell lines of colorectal cancer studied, NAIF1 gene expression was variable. To note that the expression of this gene is lower in cell lines (SW620, COLO205, T84, SW1463, SW403) which were emerged from either metastatic or advance grade colorectal cancer, whereas more 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,[17] confirming that there is a possible relationship between NAIF1 gene expression and stage or grade of colorectal cancer (degree of differentiation), as we found low level of expression seen in SW620 cell line and the inverse with SW480.

Interestingly, our results also show a very strong expression of NAIF1 gene in healthy gut tissue, this may confirm the possible relationship between NAIF1 gene expression and stage or grade of colorectal cancer (degree of differentiation). This may be assumed by either the gene has a role in inhibition the progression of cancer by inducing apoptosis or the highly progressed cancer express this gene little. Further investigation will be required to clarify this relationship. However, little information exists on the relationship between NAIF1 and cancer genesis and progress. The data presented herein is analogous to 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.[28]

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 microsatellite stable (MSS) genetic status. This finding may indicate that the cell genetic instability at the level of nucleotide microsatellite instable (MSI) (as represented by the widespread insertion or deletion of simple repeat nucleotides) not affect the level of NAIF1 gene expression or the gene NAIF1 not affect the molecular status of these cells. This may be due to the fact that colorectal cancers arise through a multistep carcinogenic process in which genetic and epigenetic alterations accumulate in a sequential manner.[34, 35]

Also the absence of difference in NAIF1 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 tumors and MSS tumors.[36] 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 NAIF1 gene expression. For approving this possible relationship, we need further research and work on this gene.

Conclusion

The presence of protein expression of NAIF1 gene in all colorectal cancer cell lines with lower expression in cell lines emerged from advanced or metastatic stage. Higher expression in healthy tissue may indicates a strong relationship between cancer inhibition or regression with this gene expression. Therefore, NAIF1 may have a role in the diagnosis and treatment of colorectal cancer and may provide new clues for developing anti-cancer drugs.

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