RESEARCH ARTICLE

Up-regulation of NICE-3 as a Novel EDC Gene Could Contribute to Human Hepatocellular Carcinoma

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Abstract

The epidermal differentiation complex (EDC) contains a large number of gene products which are crucial for the maturation of the human epidermis and can contribute to skin diseases, even carcinogenesis. It is generally accepted that activation of oncogenes and/or inactivation of tumor suppressor genes play pivotal roles in the process of carcinogenesis. Here, NICE-3, a novel EDC gene, was found to be up-regulated in human hepatocellular carcinoma (HCC) by quantitative real-time RT-PCR. Furthermore, overexpression of exogenous NICE-3 by recombinant plasmids could significantly promote cell proliferation, colony formation and soft agar colony formation in Focus and WRL-68 HCC cell lines. Reversely, NICE-3 silencing by RNA interference could markedly inhibit these malignant phenotypes in YY-8103 and MHCC-97H cells. Moreover, cell cycle analysis of MHCC-97H transfected with siRNA by flow cytometry showed that NICE-3 knockdown may inhibit cell growth via arrest in G0/G1 phase and hindering entry of cells into S phase. All data of our findings indicate that NICE-3 may contribute to human hepatocellular carcinoma by promoting cell proliferation.

Keywords: Hepatocellular carcinoma - NICE-3 - C1orf43 - NS5ATP4 - EDC - cell proliferation

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Introduction

According to liver cancer incidence and mortality worldwide in 2008 investigated by the International Agency for Research on Cancer: Liver cancer is the fifth most common cancer in worldwide men (523,000 newly increased cases in 2008, accounting for 7.9% of all cancers) and the seventh in women (226,000 newly increased cases, 6.5% of all cancers); The overwhelming majority of liver cancer occurs in less developed regions, such as China (401,000 newly increased cases in 2008 accounting for 53.5 %); In addition, for it's high fatality , liver cancer becomes the third most common cause of death from cancer all over the world. Hepatocellular carcinoma (HCC) is the most common pathology type of liver cancer. Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) was deemed to the main etiological factor for it's development and progression (Perz et al., 2006). But up to now, the underlying molecular mechanisms of hepatocarcinogenesis are still unclear.

It is generally known that activation of oncogenes or inactivation of tumor suppressor genes is crucial to carcinogenesis. For the past few years, our laboratory had found many up-regulated or down-regulated genes in HCC via transcriptomic study. Some of them are potential oncogenes such as DLK1, E2F8, C9orf100 and so on (Huang et al., 2007; Deng et al., 2010; Wang et al., 2012). Other are potential tumor suppressor genes such as SCARA5, ARHI, SFRP1 etc (Huang et al., 2007; 2009; 2010). Among them, NICE-3, a novel gene, was detected significantly up-regulated in HCC. Via database searches on NCBI and GeneCards, we realize that NICE-3 is also known as C1orf43(chromosome 1 open reading frame 43), HSPC012, NS5ATP4, S863-3, MGC111001, DKFZp586G1722 and is expressed in many tissues.

NICE-3 is a novel member of the epidermal differentiation complex (EDC) and is mapped on 1q21. This gene has three alternatively spliced cDNA sequences and their nucleotide sequences are selfsame except for lacking one or two alternate in-frame exons. Moreover, NICE-3 may have the signature of N-4 cytosine-specific DNA methylases. The EDC contains a great amount of genes which are crucial for the maturation of the human epidermis and contribute to skin diseases, even carcinogenesis (Ingo et al., 2001; Moubayed et al., 2007; Marenholz et al., 2011). Our previous research revealed that HSPC012 as one of NICE-3 transcript variants was detected in $CD34^+$ hematopoietic stem/progenitor

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cells(HSPCs) (Zhang et al., 2000). It is worth mentioning that NICE-3 could be transactivated by hepatitis C virus(HCV) non-structural protein 5A(NS5A) and named NS5ATP4 (Yang et al., 2003). NS5A is a potent transcriptional activator and may contribute to HCV-related HCC by interacting with β-catenin, Bax, p53, PKR and some other mechanisms (Kato et al., 1997; Keng-Hsin et al., 2002; Chung et al., 2003; Giménez-Barcons et al., 2005; Andrew et al., 2010).

A research reported that NICE-3 was overexpressed in Fibrolamellar carcinomas (FLC), a rare type of HCC (Rajesh et al., 2007). However, it is still unclear whether NICE-3 is up-regulated in the typical HCC and the up-regulation of NICE-3 could contribute to the hepatocarcinogenesis. In this study, we found that NICE-3 was markedly up-regulated in HCC tissue specimens compared with correspondingly noncancerous tissues by quantitative real-time RT-PCR. What's more, cell biological results of overexpression of exogenous NICE-3 by recombinant plasmids and knockdown of endogenous NICE-3 by RNA interference, further demonstrated that up-regulation of NICE-3 could contribute to hepatocarcinogenesis. Consequently, NICE-3 may be a candidate oncogene of HCC.

Materials and Methods

Tissue specimens

All 40 paired HCC tissue specimens were obtained from the patients who were diagnosed HCC and accepted hepatectomies in the department of hepatobiliary surgery of Wuxi Municipal People's Hospital. HCC specimens and adjacent non-cancerous tissues (2cm away from the edge of cancer regions) were immediately frozen in liquid nitrogen after resection. Before operation we signed informed consents and this research program was authorized by the ethics committee of Wuxi Municipal People' s hospital and the Chinese National Human Genome Center.

Normal liver RNA , Liver cancer cell lines and cell culture

Fetal liver and adult liver RNA (Clontech) were used for this research to reflect the expression of NICE-3. Fifteen liver tumor-derived cell lines were used in this study: MHCC-97H, MHCC-L,YY-8103,WRL-68, Focus,PLC/PRF/5,QGY-7703,Sk-hep1,HepG2,HepG2.2.15,Hep3B,Huh-7,SNU398,MHCC-LM6, and MHCC-LM3. All of these cell lines were cultured in a 37 °C and 5% CO_2-humidified incubator and fed on the following media: Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS) (Gibco) and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) (Gibco).

Extraction of RNA

RNA was extracted using TRIzol solution(Invitrogen) from HCC Tissue specimens and Liver cancer cell lines according to the manufacturer's instructions. And RNAse-free DNase I was used to remove DNA contamination. Both RNA concentration and quality were measured by absorbance at 260nm and OD260/OD280 ratio using a DNA/Protein Analyzer (DU 530; Beckman).

Semiquantitative RT–PCR and quantitative real-time PCR

Reverse transcription (RT) was accomplished in a 20μl reaction with 2μg RNA via M-MLV reverse transcriptase kit (Promega). To amplify a 292-bp PCR product for NICE-3, we used the following primers: Forward, 5'-CCGCTATGGGACAGGGGTCT-3'; Reverse, 5'-GGGAGTCCTTCAGCTCCGT-3'. For β-actin, we design the following primers: forward, 5'-AGAGCCTCGCCTTTGCCGATCC-3'; reverse: 5'-CTGGGCCTCGTCGCCCACATA-3'. Each Semiquantitative RT–PCR run 28 cycles, and the products were identified by 2% agarose gel electrophoresis staining with ethidium bromide. β-actin served as the internal reference. Furthermore, the relative mRNA level of NICE-3 in HCC specimens and Liver cancer cell lines was measured by quantitative real-time RT-PCR, utilizing the TaKaRa PCR Thermal Cycler Dice Detection System and SYBR green dye (TaKaRa), according to the manufacturer's instructions.A housekeeping gene, β-actin, was served as an endogenous control. All the experiments were repeated independently at least 3 times to guarantee the repeatability and reproducibility of the results. The method of comparative analysis was specified in our previous research (Huang et al., 2010).

Construction of recombinant plasmids

First of all,we designed a pair of primer with double restriction enzyme cutting sites (EcoRI-Forward primer: 5'-GCCGAATTCACC ATGGCGTCCGGCAGTAACT-3', BamHI-Reverse primer:5'-GACGGATCC CAGAGTACTCTCCAATGTGTTATAGTTATCC-3') to amplify the full-length cDNA of NICE-3 (GeneBank Accession Number: NM_001098616) from human hepatocellular cDNA library. Then, used restriction enzymes EcoRI and BamHI to cut the PCR product and expression vector pcDNA3.1 (Invitrogen). Lastly, we can get NICE-3 Flag-tagged overexpression vector by ligation reaction. In addition, the accurate sequence of NICE-3 open reading frame was proved by DNA sequencing.

RNA interference

Small interference RNA (siRNA) specifically silencing NICE-3, were chemically synthesized (GenePharma). Our research involved two siRNA and their sequences were as follows: siRNA-522(5'-CCUUGCAGAUGAUGAUGCUAGACdTdA-3', 5'-UAGUCUAGCAUCAUCAUCUGCAAdGdG-3'); siRNA-936 (5'-GCACUUCCUUGAAUUGAAGAGC UdTdT-3', 5'-AAAGCUCUUCAAUUCAAGGAAG UdGdC-3'). We synthesized a pair of irrelevant nucleotides not targeting any annotated human genes to use as a negative control named siRNA-NC, their sequence as follows: (5'-UUCUCCGAACGUGUCACGUdTdT-3', 5'-ACGUGACACGUUCGGAGAAdTdT-3'). Moreover, to chronically silence NICE-3, we constructed the RNAi plasmid vectors named respectively pSUPER-shNC(negative control), pSUPER-sh522, pSUPER-sh936. Firstly, synthesized the DNA oligonucleotides for

forming short hairpin RNA (shRNA); The sequences for these shRNAs were described as follows: shRNA-NC, forward, 5'-GATCCCCTTCTCCGAACGTGTCACGT TTCAAGAGAACGTGACACGTTCGGAGAATTTTT GGAAA-3';reverse, 5'-AGCTTTTCCAAAAATTCTC CGAACGTGTCACGTTCTCTTGAAACGTGACAC GTTCGGAGAAGGG-3';shRNA-522, forward, 5'-GA TCCCCCCTTGCAGATGATGATGCTAGACTATTCA AGAGATAGTCTAGCATCATCATCTGCAAGGTTT TTGGAAA-3';reverse,5'-AGCTTTTCCAAAAACCT TGCAGATGATGATGCTAGACTATCTCTTGAATAG-TCTAGCATCATCATCTGCAAGGGGG-3'; shRNA-936, forward, 5'-GATCCCCGCACTTCCTTGAATTGAAGA GCTTTTTCAAGAGAAAAGCTCTTCAATTCAAGGA AGTGCTTTTTGGAAA-3',reverse,5'-AGCTTTTCCAA AAAGCACTTCCTTGAATTGAAGAGCTTTTCTCTT GAAAAAGCTCTTCAATTCAAGGAAGTGCGGG-3'. Then, inserted them into the expression vector pSUPER (OligoEngine). Finally, the accurate sequences of shRNA were proved by DNA sequencing.

Cell transfection

For transfection, cells were plated in 6-wells plates at 30%–50% confluence(siRNA transfection) or 80%-90% confluence (plasmid transfection). All cell transfections were implemented by Lipofectamine2000 (Invitrogen) according to the protocols recommended by the manufacturer.

Stable cell lines construction

HCC cells transfected with pcDNA3.1 , pcDNA3.1-NICE-3 and pSUPER (shRNA-NC, shRNA-522, shRNA-936) plasmids were cultured in the medium added G418 (Life Technologies Inc) for 2 weeks.These G418 resistant cells were used as our stable cell Lines.

Cell proliferation

Approximately, 3000-5000 HCC cells per well were seeded in 96-well plates and then cultured for 5 to 7 days. Meanwhile, Cell viability was measured by absorbance at 450 nm with the use of Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) according to the manufacturer's instructions. All of the experiments were independently repeated at least three times.

Colony formation assay

To assay the effect of NICE-3 on colony formation, 3-5×10^3 stable cells(pSUPER-shNC, pSUPER-sh522, pSUPER-sh936, Vector and NICE-3) were seeded on 10-cm culture dish. Next, these cells were cultured in the appropriate medium added G418 (Life Technologies Inc) at a final concentration of 0.6 to 1 mg/mL for 2-3 weeks until forming visible clones. Finally, these cell colonies were stained with coomassie brilliant blue, photographed and counted.

Moreover,to illustrate the effect of NICE-3 on anchorage-independent growth, the soft agar colony formation was used. Briefly, 4×10^3 stable cells were mixed with 0.5% top agar and seeded on 24-well plates paved 1% base agar. Then these cells were cultured for three weeks. Lastly the cell colonies in soft agar were photographed and counted by a microscope. All of the experiments were independently repeated at least three times.

Western blot analysis

The entire process was accomplished according to the manufacturer's instructions (Santa Cruz Biotechnology). The main steps were introduced briefly as follows: Firstly, total proteins were extracted from cultured cells by using the lysis buffer [25 mmol/L Tris (pH 6.8), 1% SDS, 5 mmol/L EDTA, protease inhibitor cocktail (Sigma)]; These extracts were separated by 12% SDS-PAGE and were then transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with blocking solution (5% nonfat milk and 0.1% Tween-20 in PBS) for 2 hours at room temperature, the membrane was incubated with Anti-Flag antibody (Sigma) and anti-β-actin antibody (Santa Cruz Biotechnology) overnight at 4 °C. Next day,incubation with the secondary antibody at room temperature for 2 hours was in the same way. Finally, the Odyssey Infared Imaging System (Li-COR) was used to record the electrophoretic bands.

Immunofluorescence assay

The stable cells transfected with NICE-3 Flag-tagged overexpression vector were fixed with 4% paraformalclehyde on ice for 30 minutes, then were blocked with 5% horse serum at room temperature for 2 hours. With that, the cells were incubated with mouse anti-Flag antibody(Sigma) overnight at 4 °C. Next day, incubation with Alexa Fluor 488-coupled (green) anti-mouse IgG antibody (Molecular Probes Inc) at 4 °C for 2 hours was followed in the same way. After washing, the nuclei of cells were stained with 4,6-diamidino-2-phenylindole(DAPI). Lastly, the images of immunofluorescence were photographed by a Zeiss confocal microscopy.

Cell cycle analysis and cell synchronization

Flow cytometry was utilized to analyze the effect of NICE-3 on cell cycle. Firstly, HCC cells were cultured in the medium lacking serum for 24 hours for cell cycle synchronization. Next day, these cells were transfected with siRNA-NC, siRNA-522, siRNA-936 respectively and were then harvested at different time points.To analyse the DNA content, the cells were fixed in 70% ethanol, and dealed with RNaseA (10 mg/mL) for half an hour and stained with propidium iodide (10 μg/mL) for 5 minutes.

Statistical analysis

All the data on this research were analysed by student's t-test using GraphPad Prism 5 software. P values, less than 0.05, were considered statistically significant.

Results

NICE-3 expression in HCC is obviously elevated

Our laboratory found many up-regulated or down-regulated genes in HCC using cDNA microarray (data not shown). In these genes, a novel EDC gene named NICE-3 attracted our attention because of it's obvious up-regulation. To further verify this result, the

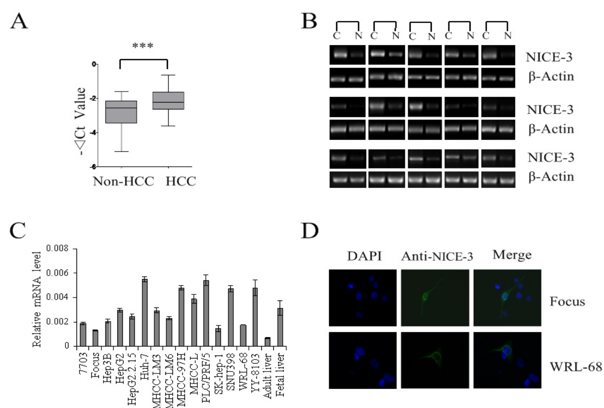

Figure 1. Expression Pattern of NICE-3 in HCC Specimens, Cell Lines and Livers. (A) The mRNA level of NICE-3 in 40 HCC specimens and corresponding Non-HCC livers was measured by quantitative real-time RT-PCR, where β-actin was employed as an internal control. The line within each box represents the median –△Ct value; the upper and lower edges of each box represent the 75th and 25th percentile, respectively; the upper and lower bars indicate the highest and lowest values detected, respectively. P value was calculated by Student's t test. *** indicates P<0.001. (B) Representative semi-quantitative RT–PCR results of 15 pairs of HCC (C) and corresponding Non-HCC livers (N). β-actin was employed as an internal control. (C) The transcript level of NICE-3 was detected in HCC cell lines, fetal livers and adult human livers by real-time quantitative RT-PCR, where β-actin was used as an internal reference. (D) Immunofluorescence assay showed that exogenous NICE-3 protein mainly gathers around the karyotheca in Focus and WRL-68 cells. The nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI)

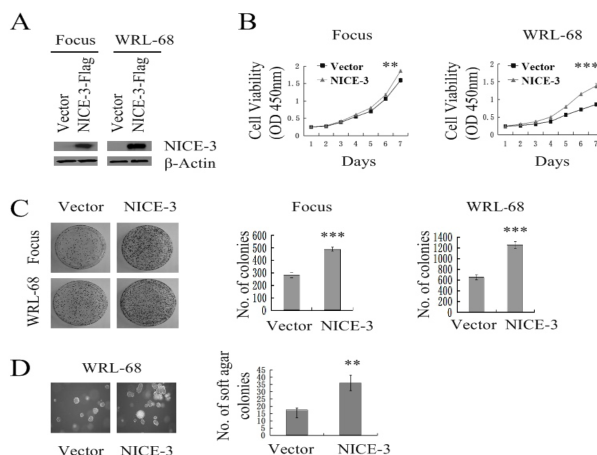

Figure 2. NICE-3 Overexpression Promoted Cell Proliferation, Colony Formation and Soft Agar Colony Formation. (A) The expression of exogenous protein NICE-3 in Focus and WRL-68 cells was testified by Western blot analysis. (B) Ectopic NICE-3 promoted the proliferation of Focus and WRL-68 cells compared with Vector (pcDNA3.1) used as a control. The different symbols represent the corresponding mean values of the experiments in triplicate (mean ± SD). (C and D) NICE-3 overexpression promoted colony formation (Focus and WRL-68) and soft agar colony formation(WRL-68) in comparison with Vector (pcDNA3.1) used as a control. Here we show the representative photos. The histograms represent the numbers of colonies which are the mean values of three independent experiments. All experiments were independently repeated at least three times. **P<0.01, ***P<0.001

expression level of NICE-3 was detected in 40 paired HCC tissue specimens by quantitative real-time RT-PCR and semi-quantitative RT–PCR. Expectedly, NICE-3 was significantly up-regulated in HCC tissue specimens compared to correspondingly adjacent noncancerous tissues (Figure1A, B). Furthermore, we detected the expression pattern of NICE-3 in available HCC cell lines and adult and fetal human livers by quantitative real-time RT-PCR. We found that NICE-3 could express in all these HCC cell lines and livers, especially the relative expression levels in HCC cell lines and fetal human liver were all very high but low in healthy adult liver (Figure 1C).

Exogenous NICE-3 mainly gathers around the cell karyotheca

To illustrate the subcellular distribution of NICE-3, immunofluorescence assay was used to detect where exogenous NICE-3 is in cells of Focus and WRL-68. The fluorescence microscope photographs demonstrate that exogenous NICE-3 is full of the cytoplasm, plasma membrane, what's more, mainly gathers around the karyotheca (Figure 1D). It suggests that NICE-3 may function by interacting with the nucleus in HCC cells.

NICE-3 overexpression promotes cell proliferation, colony formation and soft agar colony formation

We utilized the stable cells Focus and WRL-68 transfected with pcDNA3.1(Vector) and pcDNA3.1-NICE-3(NICE-3) to observe the effect of NICE-3 overexpression on the cellular proliferation, colony formation and soft agar colony formation. The expression of exogenous protein NICE-3 was testified by using Western blot analysis (Figure 2A).

The cell growth curve drawn with absorbance at 450nm and photos of the cell colonies stained with coomassie brilliant blue showed that the stable cells Focus and WRL-68 transfected with pcDNA3.1-NICE-3 (NICE-3) had larger value of cell viability and more colonies than the corresponding stable cells transfected with pcDNA3.1 (Vector) (Figure 2B, C). Moreover, the effect of NICE-3 on anchorage-independent growth was evaluated by WRL-68 soft agar colony formation experiment. Expectedly, the stable cell WRL-68 transfected with pcDNA3.1-NICE-3(NICE-3) had more and larger colonies compared with the stable cell WRL-68 transfected with pcDNA3.1(Vector) (Figure 3D). In a word, it indicated that NICE-3 overexpression enchanced cellular proliferation , colony formation and soft agar colony formation.

Silencing NICE-3 inhibits cell proliferation , colony formation and soft agar colony formation

To reversely describe the effect of NICE-3 on cellular proliferation, colony formation and soft agar colony formation, we synthetized three specific siRNA (si-NC, si-522, si-936) and constructed the corresponding RNAi plasmid vectors (pSUPER-shNC, pSUPER-sh522, pSUPER-sh936). SiRNA silenced gene expression in a short term, while pSUPER-shRNA could silence gene expression all through. As negative controls, siRNA-NC and pSUPER-shNC which couldn't silence any genes expression were used to exclude the influences

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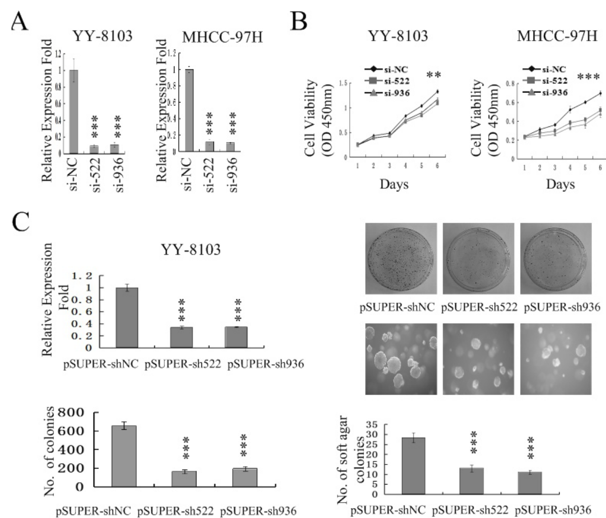

Figure 3. Silencing NICE-3 Inhibited Cell Proliferation, Colony Formation and Soft Agar Colony Formation. (A) The Silencing efficiencies of si-522 and si-936 were assessed by quantitative real-time RT-PCR. (B) Silencing NICE-3 by siRNA (si-522 and si-936) inhibited cell proliferation compared with si-NC which was seen as a control. The different symbols represent the corresponding mean values of the experiments in triplicate (mean ± SD). (C) Both pSUPER-sh522 and pSUPER-sh936 could knockdown NICE-3 significantly. Silencing NICE-3 by pSUPER-sh522 and pSUPER-sh936 could inhibit both colony formation and soft agar colony formation in YY-8103 cell in comparison with pSUPER-shNC used as a control. Here we show the representative photos. The histograms represent the numbers of colonies which are the mean values of three independent experiments. All experiments were independently repeated at least three times. **P<0.01, ***P<0.001

of irrelevant siRNA and pSUPER vector. First of all, the silencing efficiencies of them were assessed via quantitative real-time RT-PCR. Expectably, si-522, si-936, pSUPER-sh522 and pSUPER-sh936 observably knocked down the expression of NICE-3 compared with si-NC or pSUPER-shNC (Figure 3A, C).

YY-8103 and MHCC-97H transfected with si-NC, si-522, si-936 were used for the cell proliferation experiment. The stable cells YY-8103 transfected with pSUPER-shNC, pSUPER-sh522 and pSUPER-sh936 were used to assay colony formation and soft agar colony formation. In the proliferation experiment, the cell viability of YY-8103 and MHCC-97H transfected with si-NC were larger than corresponding ones transfected with si-522 or si-936 (Figure 3B). The stable cells YY-8103 transfected with pSUPER-shNC had more and larger colonies compared with corresponding ones transfected with other plasmids (pSUPER-sh522 and pSUPER-sh936) (Figure 3C). Consequently, the above results showed that silencing NICE-3 can inhibit HCC cells proliferation, colony formation and soft agar colony formation. In a word, all data indicated that NICE-3 should be necessary for HCC cell proliferation and colony formation .

Silencing NICE-3 caused a arrest in G0/G1 phase and inhibited the entry of S phase in cell cycle

Cell cycle analysis via flow cytometry was used to explore the mechanism of NICE-3 in HCC cells. After cell cycle synchronization, MHCCH-97H was transfected

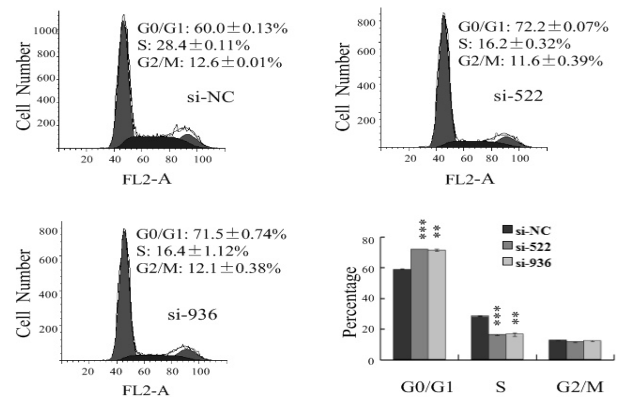

Figure 4. Silencing NICE-3 Caused A Arrest in G0/G1 Phase and Inhibited the Entry of S Phase in Cell Cycle. MHCC-97H cells harvested at the 24th hour after transfection with siRNA were analyzed by flow cytometry. Si-NC was taken as a control. The histograms represent the percentages of each phase in cell cycle which are the mean values of three independent experiments. All experiments were independently repeated at least three times. **P<0.01, ***P<0.001

with si-NC, si-522 and si-936. The Silencing efficiencies of siRNA were confirmed by quantitative real-time RT-PCR. Then, we collected the cells and monitored their cell cycle distribution. Excitingly, NICE-3 knockdown caused a arrest in G0/G1 phase and hindered cells into S phase (Figure 4). So we inferred that NICE-3 could play an important role in the cell cycle of G0/G1 or S phase.

Discussion

NICE-3 is a novel EDC gene and mapped on 1q21 which may contain an oncogene of HCC. The epidermal differentiation complex (EDC) comprised three gene families: cornified envelope precursors, S100 proteins and S100 fused genes and many newly identified EDC genes including NICE-3 (Ingo et al., 2001; Leung et al., 2002). The deregulation of EDC members has been found in various human cancers. For example, S100A7 as a important member of the EDC was associated with poor prognosis in head and neck cancer, and enhances mammary tumorigenesis (Tripathi et al., 2010; Nasser et al., 2012). S100 gene family members could play important roles in bladder cancer and malignant melanoma (Maelandsmo et al., 1997; Ruisheng et al., 2007). Importantly, the overexpression of NICE-3 could reflect the bad malignance and poor prognosis outcome in breast cancer (Martin et al., 2004; Elin et al., 2008). In HCC, only a research reported that NICE-3 was up-regulated in Fibrolamellar carcinomas (FLC), a rare type of HCC (Rajesh et al., 2007). However, it is still unclear whether the up-regulation of NICE-3 is in the typical HCC and could contribute to the hepatocarcinogenesis until now.

In this study, we firstly confirmed that NICE-3 was significantly up-regulated in typical HCC besides FLC by quantitative real-time RT-PCR. Overexpression of exogenous NICE-3 could promote cell proliferation, colony formation and soft agar colony formation of Focus and WRL-68 cells. Whereas, NICE-3 knockdown could inhibit these above malignant phenotypes in YY-8103 and MHCC-97H cells. Furthermore, cell cycle analysis on MHCC-97H transfected with siRNA by flow cytometry

showed that NICE-3 knockdown could cause a arrest of G0/G1 phase and hinder cells into S phase, which inhibited cellular proliferation. All our findings fully manifested that up-regulation of NICE-3 could contribute to HCC's oncogenesis and progression. NICE-3 is also known as NS5ATP4 which was transactivated by HCV NS5A (Yang et al., 2003). This may hew out a brand new mechanism how does HCV NS5A lead to HCC.

In a word, we detected a novel EDC gene NICE-3 which may be a candidate oncogene of HCC for the first time. Furthermore, cell cycle analysis indicated that NICE-3 knockdown may inhibit cell proliferation via causing a arrest in G0/G1 phase and hindering cells into S phase .But the complex molecular mechanisms of NICE-3 contribution to HCC still need our further investigation.

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