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Nuclear Hormone Receptor NR4A2 Is Involved in Cell Transformation and Apoptosis

Ning Ke,[1] Gisela Claassen,[1] De-Hua Yu,[1] Aaron Albers,[1] Wufang Fan,[1] Philip Tan,[1] Mirta Grifman,[1] Xiuyuan Hu,[1] Kristin DeFife,[1] Vivian Nguy,[1] Bernd Meyhack,[2] Arndt Brachat,[2] Flossie Wong-Staal,[1] and Qi-Xiang Li[1]

[1]Immusol, Inc., San Diego, California; and [2]Novartis Institutes for BioMedical Research, Basel, Switzerland

ABSTRACT

HeLaHF cells are transformation revertants of cervical cancer HeLa cells and have lost anchorage-independent growth potential and tumorigenicity. Activation of tumor suppressor(s) was implicated previously in this transformation reversion. In this study, expression profiling analysis was carried out to identify potential oncogenes that are down-regulated in HeLaHF cells. We found that all three members of the NR4A1/Nur77/NGFIB orphan nuclear hormone receptor subfamily (NR4A1, NR4A2, and NR4A3) were down-regulated in the HeLaHF revertant. Small interfering RNA-mediated down-regulation of NR4A2 in HeLa cells, either transiently or stably, resulted in reduced anchorage-independent growth that was largely attributable to increased anoikis. Furthermore, down-regulation of NR4A2 as well as NR4A1 promoted intrinsic apoptosis. These phenotypes were also observed in several other experimental cancer cells, suggesting the observed apoptosis suppression is a more general property of NR4A2 and NR4A1. These phenotypes also suggest that the Nur77/NGFIB subfamily of orphan receptors exhibit certain oncogenic functionalities with regards to cell proliferation and apoptosis and could therefore be evaluated as potential cancer therapeutic targets.

INTRODUCTION

NR4A2 (Nurr1, RNR-1, TONOR) is a transcription factor belonging to the superfamily of steroid nuclear hormone receptors. NR4A2 is a member of the Nur77 orphan receptor subfamily that includes two other members, NR4A1 (Nur77, TR3, and NGFI-B) and NR4A3 (Minor, Nor-1; refs. 1 and 2). All three members bind to the DNA sequence AAAGGTCA (NBRE, a Nur 77-binding response element, or a NGFI-B–binding response element) or AAAT(G/A)(C/T)CA (NurRE, a Nur77-response element) to activate target gene expression (3–6). NR4A1 and NR4A2 can also heterodimerize with retinoic x receptor and activate transcription through a DR-5 element in a 9-*cis*-retinoic acid-dependent manner (7–9). Nur77 family proteins are involved in a wide variety of biological processes and are immediate early genes induced by serum, growth factors, and receptor engagement (10). NR4A1 and NR4A3 have also been shown to be involved in apoptosis pathways; NR4A1 was reported to play either an anti-apoptotic or proapoptotic function depending on context (3, 11–14), whereas NR4A3 was reported to play a partly redundant functional role with NR4A1 in inducing T-cell apoptosis (3). However, the role of NR4A2 in apoptosis and transformation has not been reported.

HeLa is a widely used experimental cell line of cervical cancer origin that exhibits transformed phenotypes, including anchorage-independent growth *in vitro* and tumorigenesis *in vivo*. HeLaHF is a revertant variant isolated from HeLa cells after exposure to mutagen ethane methyl sulfonate (15) and shows a "nontransformed" phenotype: flat and nonrefractile morphology, loss of soft-agar growth, and tumor growth potential. Activation of tumor suppressor genes in HeLaHF has been suggested, including p53, PPAN, IGFBP3, and DKK-1 (16–18).[3] In this study, we examined whether the down-regulation of potential oncogenes may be involved in HeLaHF reversion. We discovered that all three Nur77 orphan nuclear hormone receptors (NR4A1, 2, 3) were significantly down-regulated in the nontransformed HeLaHF revertants, as compared with parental HeLa cells, suggesting possible oncogenic properties for these proteins. Experiments with small interfering (si)RNA-mediated gene silencing showed for the first time that NR4A2 behaves as a pro-survival gene in HeLa cells, as down-regulation induced apoptosis and reduced the anchorage-independent growth of cells. The results were also reproduced in several other cancer cell lines of different origins, suggesting a general pro-survival role for NR4A2. We also observed the anti-apoptotic role for NR4A1, consistent with the previous reports (13, 14). Therefore, the Nur77/NGFI-B family of nuclear hormone receptors seems to have similar pro-survival effects at least in certain cancer cells and could potentially serve as anticancer therapeutic targets.

MATERIALS AND METHODS

Affymetrix GeneChip Expression Analysis. Total cell RNA was isolated with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and further purified with RNeasy columns (Qiagen, Valencia, CA). Two independent RNA isolations were used for each cell line. Ten micrograms of RNA were used for each sample to generate cDNA with the SuperScript Choice System (Invitrogen), and the cDNA was *in vitro* transcribed to generate biotinylated cRNA (BioArray HighYield RNA Transcript Labeling kit, Affymetrix, Santa Clara, CA). The cRNA was fragmented and hybridized to U133A probe arrays during a 16 hour incubation at 45°C. The probe arrays were washed and stained with streptavidin phycoerythrin in a GeneChip Fluidics Station 400 (Affymetrix). Finally, the probe arrays were scanned with a G2500A GeneArray scanner (Affymetrix).

The data were analyzed with Microarray Suite 5.0 and Data Mining Tool 2.0 (Affymetrix) to determine probe sets with different expression levels between HeLa and HF cells. Samples were queried with software algorithms to determine qualitative information, such as increased or decreased expression, present or absent expression, and quantitative information, such as fold change and signal intensity. Microarray Suite was also used to determine relative expression of the genes in different cell lines ($n = 3$ for these cell lines).

Cells. HeLa is a cervical carcinoma cell line, and HeLaHF is a HeLa revertant with loss-of-transformation phenotype (15). DLD-1 and HCT116 are human colon carcinoma cell lines. PC3 is a prostate cancer cell line, and M14 is a melanoma cell line. HeLa and HF were cultured in DMEM 1× (10% fetal bovine serum, 2 mmol/L L-glutamine, 1× non-essential amino acids, and 1% sodium pyruvate (Invitrogen), whereas HCT116, DLD1 and M14 were cultured in RPMI 1640 1× media (10% fetal bovine serum, 2 mmol/L L-glutamine, 1× non-essential amino acids, and 1% sodium pyruvate; Invitrogen) in a humidified incubator with 5.0% CO_2 at 37.0°C.

Measurement of Cell Growth. Anchorage-dependent and anchorage-independent growth in 96-well, as well as anchorage-independent colony formation in a 10-cm plate were described in detail previously (19). Cell growth in 96-well plates was scored by alamarBlue staining (Biosource International, Camarillo, CA) and colony formation in 10-cm plates was determined by Qcount (Spiral Technology, Norwood, MA).

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Requests for reprints: Qi-Xiang Li, Immusol, Inc., 10790 Roselle Street, San Diego, CA 92121. Phone: 858-824-1100, ext. 101; Fax: 858-824-1112. E-mail: li@immusol.com.

[3] D. H. Yu *et al.*, unpublished data.

siRNA Transfection and Retroviral Vector Transduction of siRNA Expression Vector. The preparation and transient transfection of *in vitro* transcribed siRNAs were described in detail previously (19). The siRNA target sequences are as follows: *NR4A2*-A (5′-TCGCTGTCAGTACTGCCGAtt-3′), *NR4A2*-B (5′-GAGAGTGGAAGAACTGCAAtt-3′), control (CNTL; 5′-GCGCGCTTTGTAGGATTCG-3′), *NR4A1* (5′-GGAAGTTGTCCGAACAGAC-3′).

Construction, preparation, and transduction of lentiviral vectors expressing siRNAs have also been described before (19). HeLa, PC3, HCT116, and DLD1 cells stably expressing siRNAs were then generated by transduction and selection in media containing desired concentrations of puromycin. A desired number of cells were then seeded into 96-well liquid, 96-well soft agar, or 10-cm plates to assay for soft agar growth. For transient transduction experiments, transduction was conducted in 96-well plates, and apoptosis was assayed 4 days later with the DNA fragmentation-based ELISA assay (cell death detection ELISA Plus, Roche Applied Science, Indianapolis, IN), which measures the amount of fragmented and solubilized nucleosomal DNA that is a hallmark of apoptosis.

Assay for Anoikis. To measure anoikis, cells were either kept attached, or detached by trypsinization and resuspension in methycellulose in a 12-well plate for 18 hours to mimic the detachment conditions that promote anoikis. Apoposis was quantitated with DNA fragmentation-based ELISA kit according to manufacturer's instructions (Roche Applied Science).

RESULTS

Nur77/NGFIB Subfamily Nuclear Hormone Receptors NR4A1, NR4A2, and NR4A3 Are Down-Regulated in Nontransformed HeLaHF Revertant Cells. Cellular transformation is often the result of activation of oncogenes or inactivation of tumor suppressor genes, whereas transformation reversion could result from activation of tumor suppressors or inactivation of oncogenes. Frequently, activation and inactivation of gene functions can be reflected in changes of gene expression. Global gene expression profiling such as DNA chip analysis is the most powerful approach in revealing changes in gene expression. We used the Affymetrix GeneChip DNA microarray (U138 A-chip) to analyze the HeLa and HeLaHF cell pair to identify genes that are significantly altered in their relative expression levels. The expression of hundreds of genes is affected in the HeLa/HF system, with changes up to 250-fold. We have focused on genes that are down-regulated in HeLaHF cells as possible oncogenes, which can be evaluated as potential drug targets. Among the down-regulated genes, we identified three nuclear hormone receptors belonging to the *Nur77/NGFIB* subfamily of orphan nuclear hormone receptors: *NR4A1* (accession, NM_002135 and Affychip probe_set, 202340_s_at, 8.2-fold down-regulated), *NR4A2* (accession, NM_006186, and Affychip probe set, 204622_x_at, 8.5-fold down-regulated; Fig. 1) and *NR4A3* (accession, NM_006981, and Affychip probe_set: 207978_x_at _at, 7.7-fold down-regulated). These down-regulations were further confirmed by real-time PCR. The *NR4A2* and *NR4A1* Affymatrix expression data, along with the confirmation real-time reverse transcription (RT)-PCR data are shown in Fig. 1. These observations suggest potential oncogenic properties for these proteins and prompted us to investigate whether these three receptor genes are involved in the HeLaHF reversion phenotype.

siRNA-Mediated Down-Regulation of NR4A2 Reduces Anchorage-Independent Growth in HeLa and Other Cancer Cell Lines. Anchorage-independent growth is a hallmark of cell transformation, and soft agar colony formation assay is the most commonly used *in vitro* assay to assess anchorage-independent growth. Soft agar growth is also the distinguishing phenotype between parental HeLa and revertant HF cells *in vitro* (15, 17, 19). To examine the potential role of the NR4A2 in the differential phenotypes of HeLa and HF cells, two siRNAs against the gene were used to reduce the gene expression in HeLa cells, and the soft agar growth was then assessed. Initially, a 1-week 96-well soft agar assay that we described recently was used (19). The short duration (7 days) of this assay format allows for functional validation of genes with transient transfection procedures. The results showed that NR4A2 siRNAs reduced NR4A2 mRNA as well as soft agar growth in HeLa cells (Fig. 2*A* and *B*). The correlation between the anchorage-independent growth reduction and the down-regulation of the NR4A2 mRNA implied the phenotype was caused by mRNA down-regulation. This strong cause-effect correlation suggests that NR4A2 functions as a putative "oncogene" in HeLa cell transformation.

Next we tested whether the transient effect could be repeated in the traditional soft agar assay with cells stably expressing NR4A2 siRNA. We previously described a hairpin siRNA expressing lentiviral vector (pHIV-7-puro) and showed that it can be effectively used to deliver siRNA into different cells to mediate gene silencing (19). Lentiviral vectors expressing the hairpin form of either NR4A2 siRNA B or the control siRNA were generated. The vectors were stably transduced into HeLa cells, and an approximately 50% reduction in NR4A2 gene expression (Fig. 2*D*) was observed in cells expressing NR4A2 siRNA as compared with cells with the control siRNA. Although this stable knock-down of NR4A2 mRNA in HeLa seemed to have little effect on cell morphology and growth in liquid culture (data not shown), reduction in soft-agar growth was observed as measured by the 96-well soft agar assay (Fig. 2*C*). To determine whether the overall reduced soft agar growth observed in 96-well format was because of slower proliferation or reduced cloning efficiency, we conducted a standard soft agar growth experiment with traditional soft agar colony formation assay, and a similar reduction was also observed (Fig. 2*C*), suggesting the overall reduction in anchorage-independent growth is largely because of the reduction in cloning efficiency. These results confirmed the observation of the transient transfection experiments.

Next, we examined the putative oncogenic function of NR4A2 in other cancer cell lines. Transduction of the NR4A2 siRNA-expressing lentiviral vectors into PC3 (prostate carcinoma) cells, DLD1 (colon carcinoma), and HCT116 (colon carcinoma) cells also caused down-regulated NR4A2 expression and reduced anchorage-independent

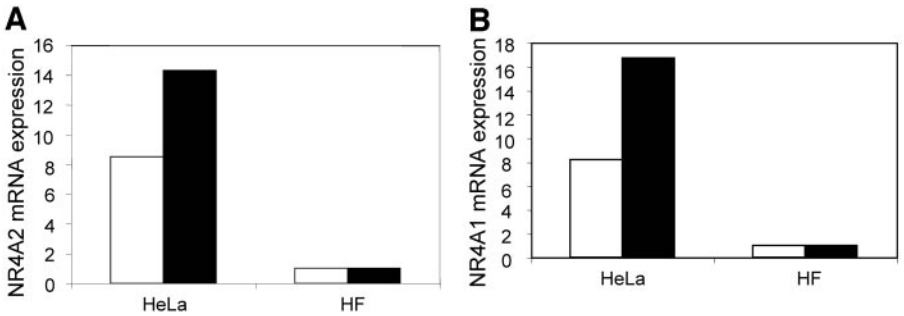

Fig. 1. Relative NR4A2 and NR4A1 expression levels in HeLa compared with HeLaHF cells. Relative levels are normalized to the level in HeLaHF cells and are done either by Affymatrix profiling or real-time RT-PCR (Taqman). RNA samples from sub-confluent cultures were prepared by standard procedures, and affymatrix analysis is described in Materials and Methods. These RNAs were also used to do real-time RT-PCR. The mRNA level is normalized to the internal control ribosomal 18S RNA level. The relative mRNA levels are depicted as arbitrary units. The numbers shown are the average of three independent experiments. □, Affy; ■, Taqman.

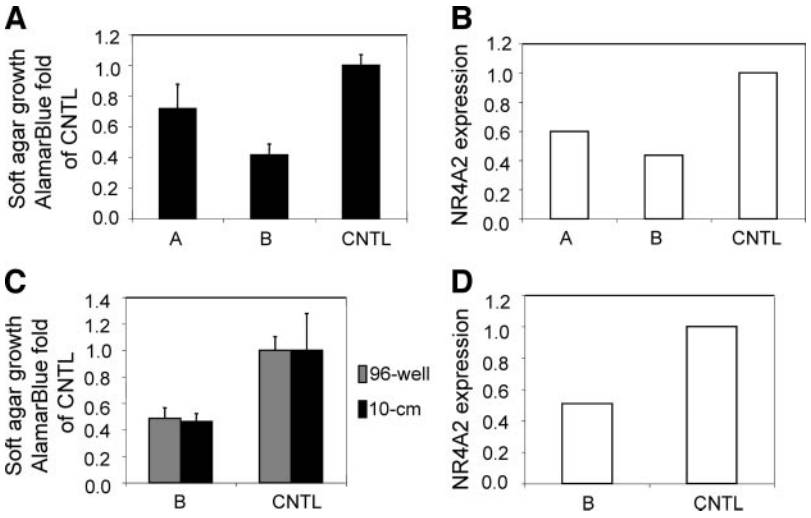

Fig. 2. Soft agar growth in HeLa cells that express NR4A2 siRNAs both transiently and stably. *A*, HeLa cells were transiently transfected with 10 nmol/L *in vitro*-transcribed siRNAs against NR4A2 (siNR4A2-A and siNR4A2-B, labeled *A* and *B* in the figure) and control siRNA with Oligofectamine reagent (Invitrogen). Twenty-four hours after transfection, cells were trypsinized, and 1,000 cells (per well) were seeded into soft agar media and allowed to grow for 7 days before being scored by alamarBlue staining. Each sample was in triplicate. *Error bars* indicate SDs. *B*, HeLa cells from A were also subjected to Taqman analysis as described in the legend to Fig. 1. *C*, HeLa cells containing lentiviral vector stably expressing hairpin siRNA against NR4A2 and a nonspecific control siRNA (CNTL) were obtained by transduction. For 10-cm dish cultures; 5,000 cells were plated in soft agar and allowed to grow for 3 weeks before being scored by Qcount (Spiral Technology); for 96-well cultures, 1,000 cells (per well) of each type were seeded into soft agar and allowed to grow for 1 week before being scored by alamarBlue staining. Each sample was done in triplicate. *Error bars* indicate SDs. *D*, total RNA was prepared from the stable HeLa cells containing either NR4A2 or control siRNA, and RNA was subjected to the real-time RT-PCR (Taqman) analysis to detect NR4A2 mRNA levels. Each sample was in triplicate.

growth (Fig. 3). Thus, NR4A2 seems to promote cellular transformation in multiple cancer cell lines.

NR4A2 Down-Regulation Elevates Cell Anoikis Levels. Anoikis is apoptosis caused by cell detachment from a substratum and is one of the key factors preventing nontransformed cells from proliferating and forming colonies in a soft agar matrix. We recently observed significantly elevated anoikis in HeLaHF as compared with HeLa,[4] which may contribute to the reversion reflected in the abolished soft agar growth potential. We investigated whether NR4A2 siRNA-mediated reduction in anchorage-independency is because of increase in anoikis. HeLa and HCT116 cells were transduced with the pHIV-7-puro/NR4A2-siRNA or control siRNA vectors and plated in both standard tissue culture medium (attached) and in methylcellulose semi-solid media (detached). We then did an ELISA-based terminal deoxynucleotidyltransferase-mediated nick end labeling assay to quantify the extent of DNA fragmentation, nucleosomal fragmentation being a hallmark of apoptosis. We observed significantly increased apoptosis in the methylcellulose semi-solid cultures for HeLa or HCT116 cells with NR4A2 siRNA as compared with cells containing control siRNA vectors (Fig. 4), whereas there was no detectable difference in apoptosis in liquid culture (data not shown). These results suggest that the reduction in soft-agar cloning efficiency for cells expressing NR4A2 siRNA was, at least in part, because of an increase in cell anoikis.

Down-Regulation of NR4A2 and NR4A1 Induces Cell Apoptosis. NR4A2 down-regulation enhances apoptosis after cell detachment, suggesting its involvement in suppressing apoptosis pathways. To assess this further, we examined whether the down-regulation of NR4A2 would induce intrinsic apoptosis regardless of cell attachment. Because we did not observe apoptosis induction for cells stably expressing NR4A2 siRNA treatment, transient transduction followed by apoptosis assays were conducted. Cells were analyzed for apoptosis 5 days after transduction with lentiviral vector against NR4A2, and the results showed significant increases of apoptosis levels for HeLa and M14 cells transduced with the NR4A2 siRNA vectors as compared with the control vector (Fig. 5). This observation confirmed the role of NR4A2 in intrinsic apoptosis pathways. The apparent discrepancy between the normal liquid culture growth of stable cells expressing siNR4A2 and transient apoptotic induction by NR4A2 siRNA expression could be explained by several possibilities. First, differences in the extent of down-regulation may exist among cell populations, causing cells with more pronounced down-regulation to apoptose and be removed during antibiotic selection. Cells with less down-regulation may be stably selected but were sensitive to apoptotic stimuli, such as cell detachment. Second, compensatory mechanisms resulting from altered expression of other genes (either from the same family members or other genes) in some cells may play a role in preventing them from apoptosis during culture, although this assumption is purely speculative and remains to be shown.

The involvement of NR4A1 in apoptosis seems to be context-dependent: either proapoptotic or antiapoptotic depending on the experimental model (11–14). Our expression profiling suggested its potential role in transformation. Therefore, we also tested whether NR4A1 siRNA-mediated silencing would lead to apoptosis. Transient transduction/apoptosis experiments were conducted with lentiviral vector against NR4A1, similar to that of NR4A2 experiment. The results also showed a significant increase in apoptosis after NR4A1 gene inactivation (Fig. 5). In separate experiments, we also observed an increase in TRAIL-induced apoptosis after NR4A1 siRNA knockdown.[5] Thus, our results indicate that NR4A1 is involved in both intrinsic and extrinsic apoptosis pathways.

DISCUSSION

We have recently developed a one-week 96-well soft agar assay for oncogene identification and validation (19). Because of the short duration of the assay, transient transfection can be used to introduce siRNAs to determine transformation phenotype, which allows parallel testing of multiple genes and of multiple siRNAs etc. This study is the first demonstration using the 96-well soft agar assay to identify a novel gene with transformation properties by siRNA transient transfection. In addition, the assay may also be useful for identifying potential transformation-suppressing compounds that would otherwise have been missed if traditional cytotoxic assays had been used.

All three members of the Nur77/NGFIB orphan nuclear receptor subfamily were expressed at significantly lower levels in the nontransformed revertant HeLaHF cells as compared with transformed HeLa parental cells. Down-regulation of NR4A2 mediated by multiple siRNAs reduced soft agar growth of HeLa cells and several other cancer cell lines, which could be partially explained by an increase in anoikis. Furthermore, transient down-regulation of NR4A2 induces

[4] D. H. Yu *et al.*, unpublished observation.

[5] P. Tan *et al*, unpublished observations.

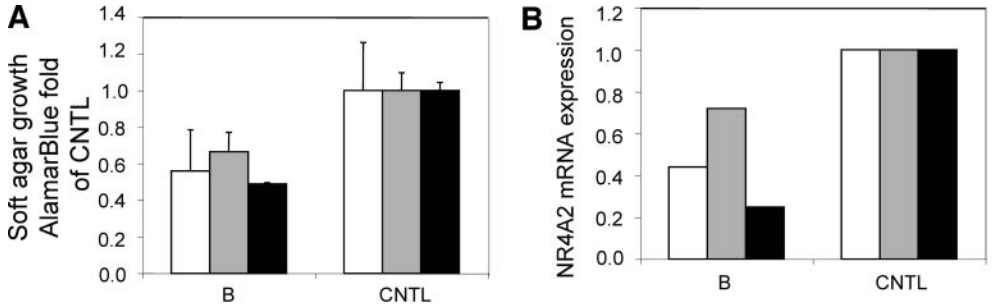

Fig. 3. Soft agar growth in other cells (DLD1, HCT116, and PC3) that stably express NR4A2 siRNAs. *A*, DLD1, HCT116, or PC3 cells containing lentiviral vector stably expressing hairpin siRNA against NR4A2 and a nonspecific control siRNA (CNTL) were obtained by transduction. One thousand cells (per well) of each type were seeded into soft agar and allowed to grow for 1 week before being scored by alamarBlue staining. Each sample was done in triplicate. *Error bars* indicate SDs. *D*, total RNA was prepared from the stable cells containing either NR4A2 or control siRNA, and RNA was subjected to the real-time RT-PCR (Taqman) analysis to detect NR4A2 mRNA levels. Each sample was in triplicate. □, HCT116; ▒, DLD1; ■, PC3.

cell apoptosis. These observations suggest the involvement of NR4A2 in cell apoptosis pathways.

The involvement of NR4A2 in cyto-protective functions in the central nervous system has been implicated in previous studies. NR4A2 is expressed exclusively in brain tissue, unlike NR4A1 and NR4A3. NR4A2 knockout mice were impaired in development and maintenance of midbrain dopaminergic neurons, and they die shortly after birth (1, 20–22). Heterozygous mice (NR4A2±) seemed healthy, but were more susceptible to the action of neurotoxin (23). NR4A2 has also been linked to Parkinson's disease, as mutations in the NR4A2 gene were found recently in certain patients (24). Cyto-protective function of NR4A2 was also shown in an overexpression study, because overexpressing NR4A2 increased the resistance of neural stem cells to neurotoxins 6-hydroxy-dopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (25). Together, these findings point to a cyto-protective function for NR4A2 in the central nervous system. Our study using siRNA-mediated down-regulation showed that NR4A2 was involved in anchorage-independent growth and survival in various tumor cell lines. This is the first report describing "cyto-protective-like" function in cancer cells for NR4A2 outside of the neuronal system via antiapoptosis mechanisms. Although little is known regarding the mechanism of NR4A2-mediated antiapoptosis, gene expression profiling could be used to explore the downstream pathways affected by NR4A2.

NR4A1 is expressed in adrenal gland and thymus (9, 26). It was previously reported to be proapoptotic in thymocytes and some cancer cell lines including LnCap (11, 12), but antiapoptotic in ceramide-induced apoptosis and tumor necrosis factor-induced apoptosis in other cell systems (13, 14). This study reported that siRNA against NR4A1 caused apoptosis in a few cancer cell lines tested, and sensitized cells to trail-induced apoptosis, thus supporting the NR4A1 involvement in inhibiting intrinsic and extrinsic apoptotic pathways.

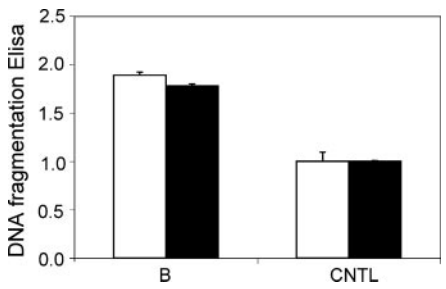

Fig. 4. Role of NR4A2 in anoikis. HeLa and HCT116 cells expressing siRNA against NR4A2 and control were detached and resuspended in methylcellulose for 16 hours. The cell lysates were made, and DNA fragmentation was measured with the cell death detection ELISA Plus kit (Roche Biochemicals). The results shown are the mean values from three independent experiments. □, HeLa; ■, HCT116.

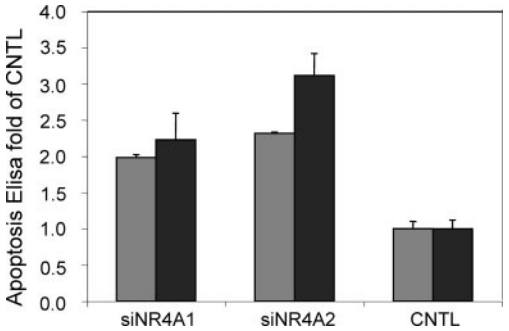

Fig. 5. Role of NR4A1/NR4A2 in apoptosis protection. HeLa and melanoma M14 cells were transduced with hairpin lentiviral constructs expressing either NR4A2 or control siRNAs in 96-well plates. Four days after transduction, the cell lysates were made, and DNA fragmentation was measured with the cell death detection ELISA Plus kit (Roche Biochemicals). The results shown are mean from three independent experiments. ▒, HeLa; ■, M14.

NR4A3 is expressed similarly to NR4A1, and has been implicated in redundancy functions, such as induction of apoptosis in T cells (3). Our preliminary results also show a similar role of NR4A3 knockdown on soft agar growth of HeLa cells.[6] Recent *in vivo* experiments by others indicated that NR4A3 knockout mice were embryonically lethal (27), further suggesting a cyto-protective role for NR4A3. Therefore, all three members of Nurr77 receptor family seem to play pro-survival functions in certain contexts, whereas NR4A1 and NR4A3 also play proapoptotic functions in other contexts. It would be interesting therefore to determine whether NR4A2 may also play proapoptotic function in other circumstances, as shown for NR4A1 and NR4A3.

In separate experiments, we retransformed HeLaHF cells with functionally selected ribozymes. These retransformed cells regained transformation phenotypes such as anchorage-independent growth and tumor formation (17).[7] Interestingly, NR4A1 and NR4A2 were found again to be up-regulated in these retransformed cells and in the xenograft tumors derived from the retransformed cells, as compared with parental nontransformed HeLaHF. These data also further indicate the involvement of the nuclear receptors in cell transformation pathways in HeLa cells and their potential involvement in tumor formation in animals. Although it is unknown whether the oncogenic (or antiapoptotic) properties of NR4A1 and NR4A2 play any impor-

[6] N. Ke *et al.*, unpublished observations.
[7] Q. X. Li *et al.*, unpublished observations.

tant biological role in human cancer development, *in vivo* tumorigenicity experiments using tumor cells in which NR4A1 and NR4A2 are silenced would help to show whether these genes are true oncogenes in a traditional sense.

If these receptors were found to play significant roles in human cancers, they could be further evaluated as cancer therapeutic drug targets based on their pro-survival properties. Like other nuclear receptors, the structure of NR4A1 family proteins includes an activation domain, a DNA-binding domain, and a ligand-binding domain (LBD; 28). Whereas NR4A1, 2 and 3 are highly homologous in the DNA-binding domain (~91–95%) and modestly homologous in the LBD domain (~60%), their activation domain is divergent. At present, the crystal structures for LBD have been published for NR4A2 and the Drosophila ortholog NR4A4 (29, 30). Interestingly, the LBD is similar to the agonist-bound, transcriptionally active LBDs in other nuclear hormone receptors. However, their ligand-binding pocket is tightly packed with side chains from several conserved bulky hydrophobic residues, which would prevent ligand entrance. This structural information may help to guide drug design should these receptors prove to be promising candidate cancer drug targets.

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