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HuR knockdown changes the oncogenic potential of oral cancer cells

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Abstract

HuR binds to AU-rich element (ARE) containing mRNA to protect them from rapid degradation. Here, we show that knockdown of HuR changes the oncogenic properties of oral cancer cells. Oral squamous cell carcinoma cell lines, HSC-3 and Ca9.22, which express HuR protein and cytoplasmic ARE-mRNA more abundantly than normal cells, were subjected to HuR knockdown. In the HuR-knockdown cancer cells, the cytoplasmic expression of *c-fos*, *c-myc*, and *COX-2* mRNAs was inhibited compared to those in cells that had been transfected with a control siRNA, and the half-lives of these mRNAs were shorter than those of their counterparts in the control cells. HuR-knockdown cells failed to make colonies in soft-agar, suggesting that the cells had lost their ability for anchorage-independent cell growth. Additionally the motile and invasive activities of the cells decreased remarkably by HuR knockdown. Furthermore, the expression of cell cycle-related proteins, such as cyclin A, cyclin B1, cyclin D1, and CDK1, was reduced in the HuR-knockdown cancer cells, and HuR bound to *cdk1* mRNA in order to stabilize it. These findings suggest that HuR knockdown changes the features of oral cancer

cells, at least in part, by affecting their cell cycle, and shows potential as an effective therapeutic approach.

Introduction

Control of the decay of mRNA is considered as one of the important mechanisms of the gene expression system. AU-rich elements (ARE) are commonly present in the 3'-untranslated regions (3'-UTR) of mRNA encoded in so-called early response genes (ERG) such as many proto-oncogenes, growth factors, and cytokines, which are also important for cell growth and proliferation (1, 2). Multiple copies of the typical sequence AUUUA exist in ARE and they target ARE-mRNA for rapid degradation (1, 3). A number of proteins are known to interact with the ARE and modulate either stabilization or destabilization of ARE-mRNA (1-5). One such protein is HuR, which is a member of the embryonic lethal abnormal vision (ELAV) family of RNA-binding proteins (6). It is a 36-kDa polypeptide and contains three RNA recognition motifs (RRM): the first two have been implicated in ARE recognition regions located in 3'-UTR; whereas, the third has been suggested to bind to the poly (A) tail of target mRNA (7). Although HuR is localized predominantly in the nucleus, it can shuttle between the nucleus and cytoplasm, and the exact mechanism by which translocation of HuR is regulated is complex.

It has been shown that many kinds of kinases, such as MAPK-activated protein kinase 2 (MK2) (8), protein kinase C (PKC) (9, 10) and Cdk1 (11, 12) are involved in the phosphorylation of HuR protein and either its import or export between the nucleus and the cytoplasm.

On the other hand, HuR has been shown to associate with pp32, APRIL, and SET (13), which belongs to a family of acidic phosphoproteins.

Under certain conditions such as heat shock or serum stimulation, the pp32-HuR complex is utilized for transportation of ARE-mRNA from the nucleus to the cytoplasm in a CRM1-dependent manner (13, 14).

HuR has been implicated in several biological events such as carcinogenesis, cell proliferation, differentiation, and the response to immune stimuli including inflammation (15). Usually, HuR is exported to the cytoplasm of cancer cells, and cytoplasmic HuR expression has been implicated in the malignancy of several types of carcinomas, such as colon cancer, and has been postulated to contribute to the cancerous malignant phenotype (15, 16).

We have found that HuR and ARE-mRNA is expressed abundantly in the cytoplasm of the cells transformed by adenovirus E4orf6 oncoprotein in a

manner independent of CRM1 (17), and the levels of HuR and ARE-mRNA in the cytoplasm of oral cancer cells are also elevated in a different manner from that of normal cells (18). In the present study, we carried out knockdown of HuR expressed in oral cancer cells to observe the changes in the oncogenic properties of the cancer cells. In the HuR-knockdown cancer cells, the cytoplasmic expression and stabilization of ARE-mRNA were inhibited compared to those of the ARE-mRNA in cells that had been transfected with control siRNA. HuR knockdown cells lost the activities as cancer, such as an anchorage-independent cell growth and a motile/invasive phenotype. These findings suggest that HuR-knockdown has the potential to change the characteristics of oral cancer cells.

Materials and Methods

Cells and cell fractionation

Human oral cancer cells, HSC-3 (a human tongue squamous cell carcinoma cell line) and Ca9.22 (a human gingival squamous cell carcinoma cell line), and human oral normal cells, HGF (a human gingival fibroblast cell line) were cultured at 37°C a 5% CO₂ atmosphere in DMEM containing 10% fetal bovine serum with penicillin/streptomycin (SIGMA). The cells were separated into cytoplasmic and nuclear fractions as described (17). To estimate the accuracy of cell fractionation, cytoplasmic (β -tubulin) and nuclear (hnRNPA1) proteins were detected by Western blotting.

HuR knockdown

HuR siRNA (5'-UUA CCA GUU UCA AUG GUC ATT-3') and control siRNA (Silencer Negative Control #1 siRNA; Invitrogen) were introduced into each cell using Lipofectamin RNAiMAX (Invitrogen) according to the manufacturer's instructions. HuR protein was estimated by Western blotting at 24, 48, and 72 hours and 2 and 3 weeks after the transfection.

Western blot analysis

Western blot analysis was performed as described previously (33). The antibodies used were specific to HuR, hnRPNA1 (Santa Cruz), β -actin (SIGMA), β -tubulin (Upstate), cyclin A, cyclinB1, cyclinD1, and Cdk1 (BD Biosciences). The secondary antibody was horseradish peroxidase-conjugated IgG (Jackson ImmunoResearch laboratories).

Quantitative real-time RT-PCR

Total cellular RNA was isolated using TRI reagent (SIGMA) according to the manufacturer's protocol. Selected 2-5 μ g total RNA, 3 μ g nuclear or cytoplasmic RNA samples were reverse-transcribed using ReverTra Ace (TOYOBO).

Opti-con2 systems and the DyNAmo HS SYBR green qPCR kit (MJ research) were used for the quantitative RT-PCR as described previously (17). cDNA was amplified using the following primers: for *c-fos*: 5'-ccaacctgctgaaggagaag-3', 5'-gctgctgatgctcttgacag-3'; for *c-myc*: 5'-ctcctggcaaaagggtcagag-3', 5'-tcgggtgttgctgatctgtc-3'; for *COX-2*: 5'-tgagcatctacggtttgctg-3', 5'-tgcttgctctggaacaactgc-3'; for *cdk1*:

5'-ttcaggatgtgcttatgcagga-3', 5'-agtgacaaaacacaatcccctgtag-3' and for *GAPDH*: 5'-atcctgggctacactgagca-3', 5'-tgctgtagccaaattcggtg-3'. *GAPDH* was used for normalization.

To evaluate the half-life of total ARE-mRNA, the cells were treated with 5µg/ml actinomycin D (CALBIOCHEM) for 30, 60, or 90 minutes. Total cellular RNA of each cell was subjected to quantitative real-time RT-PCR.

In situ hybridization

In situ hybridization was modified as described previously (17). The cells were fixed in cold 4% formaldehyde and permeabilized with cold 0.5% Triton X-100. In situ hybridization was performed overnight at 37°C with 50µl of a mixture containing 6.25µg tRNA, 25µg of sperm DNA, 2×SSC, 0.2% BSA, 1mM vanadyl ribonucleoside complexes, 50% formamide, 10% dextran sulfate, and 30ng of 3' digoxigenin-labeled antisense deoxyoligonucleotide probe for *c-fos*, *c-myc*, and *COX-2* mRNA. The coverslips were washed three times with 2×SSC at 37°C and then again with 1×SSC at room temperature. Then, the coverslips were incubated in 4% formaldehyde for 15 min at room temperature and washed

3 times with PBS. The coverslips were incubated for 60 min at room temperature with 1:50 dilution of anti- digoxigenin fluorescein Fab fragments (Roche) with 0.2% Triton X-100/PBS containing 1% BSA and were then washed twice in 0.2% Triton X-100/PBS and twice in PBS. The probe was complementary to nucleotides 288 to 328 of *c-fos*, 1693 to 1719 of *c-myc*, and 1293 to 1336 of *COX-2*. Nuclei were stained using 4', 6' diamidino, 2-phenylindole (DAPI).

Soft-agar colony formation assay

The anchorage-independent growth of HuR-knockdown cells was estimated by a soft-agar colony formation assay as described (34). Single-cell suspensions of 3×10^4 cells were plated per 60-mm culture dish in 3ml of DMEM containing 10% FBS and 0.36% agar on a layer of 5ml of the same medium containing 0.75% agar. Three weeks after plating, the colonies were stained with 0.04% Crystal violet-2% Ethanol in PBS. Photographs of the stained colonies were taken.

RNP immunoprecipitation (RIP) assay

RIP assay was performed as described previously (17). Ca9.22 cells were treated with PBS containing 1% formaldehyde and the lysate was immunoprecipitated with mouse IgG (BD Biosciences) or anti-HuR antibody. The pellets and supernatants were incubated at 70°C for 45 min to reverse the cross-links, the isolated RNA was subjected to reverse transcription, and PCR amplification for *cdk1*, *c-myc* and *GAPDH* was performed using the primers as described above.

Wound healing assay

Ca9.22 cells, 48h after HuR siRNA or control siRNA transfection, were serum-starved for 12h in DMEM containing 0.5% FBS. These cells were wounded with a 200µl pipette tip, washed with PBS and incubated in DMEM containing 0.5% FBS. Migration wounded cells evaluated after 0, 12, 24, 36h with an inverted OLYMPUS CKX41 microscope.

In vitro invasion assays

BD Biocoat Matrigel Invasion Chambers (BD Biosciences) were used in invasion assays as described (35). Ca9.22 cells, 48h after HuR siRNA or control siRNA transfection, were serum-starved for 12h in DMEM

containing 0.5% FBS. Then these cells (1.0×10^5 cells) were suspended in 0.5% FBS medium and added to the upper chamber. The lower chamber was filled with DMEM containing 20% FBS. After 24h incubation, cells were fixed in 10% formaldehyde solution (Wako Pure Chemical) for 20 min at R.T. followed by, stained with Mayer's Hematoxylin (Muto Pure Chemicals) for 30min. Invaded cell were counted by microscope at $\times 100$ magnification.

Results

HuR expression and its knockdown in oral cancer cells

The amounts of HuR protein in HSC-3 (human tongue squamous cell carcinoma), Ca9.22 (human gingival squamous cell carcinoma), and HGF (human gingival fibroblasts) were estimated by Western blotting using β -actin as a control. As expected, the expression of HuR protein was about 3 fold higher in the oral cancer cells compared to that in normal cells (Fig. 1A), indicating that oral cancer cells express HuR at a high level.

In order to examine the effect of HuR in oral cancer cells, we produced HuR-knockdown oral cancer cells. Although, we attempted to produce stable HuR knockdown cells using pSUPER, which directs the synthesis of small interfering RNAs (siRNA) in mammalian cells (19), no cells survived. Next, siRNA for HuR was introduced into HSC-3 and Ca9.22 cells, and the expression of HuR in these cells was examined. The siRNA was very effective, since the HuR siRNA-transfected cells showed a 70~80% reduction in HuR expression compared to that of the control random siRNA-transfected cells (Fig. 1B). Furthermore, the effect of the RNAi continued for more than 96 hours. As the RNAi treatment was most

effective from 48-72 hours after the transfection, these cells were used for the following experiments to examine the biological features of HuR-knockdown cells.

HuR knockdown inhibits the cytoplasmic expression and stabilization of ARE-mRNA in oral cancer cells

To confirm the cytoplasmic expression of ARE-mRNA from HuR-knockdown cancer cells, the quantities of *c-fos*, *c-myc* and *COX-2* mRNAs in the cytoplasm of the cells were estimated using quantitative real-time RT-PCR and the formula $\text{cytoplasm/nuclear} + \text{cytoplasm}$ to reflect the quantity of each mRNA in whole-cell. As shown in Fig. 2, the amount of cytoplasmic ARE-mRNAs in HuR-knockdown cells was low level compared to that in the control siRNA transfected cells. Furthermore, the down-regulation of each mRNA in normal HGF cells was slight compared to cancer cells. This result indicates that HuR knockdown down-regulates the amount of ARE-mRNA expressed in the cytoplasm.

The cytoplasmic accumulation of ARE-mRNA in the HuR-knockdown

cells was confirmed using the *in situ* hybridization technique. In HSC-3 and Ca9.22 cells transfected with control siRNA, *c-fos*, *c-myc*, and *COX-2* mRNAs were dispersed in all cells (Fig. 3). On the other hand, the localization of these ARE-mRNAs was limited to the nucleus or the peripheral region of the nucleus in HuR-knockdown HSC-3 and Ca9.22 cells (Fig. 3). Taken together, these results suggest the possibility that the export of ARE-mRNA was inhibited in the HuR-knockdown cancer cells.

As HuR is involved in protecting ARE-mRNA from degradation in the cytoplasm, we examined the half-life ($t^{1/2}$) of ARE-mRNA in HuR-knockdown cells. The quantities of *c-myc* and *COX-2* mRNAs in HSC-3 and Ca9.22 cells were measured by quantitative real-time RT-PCR, 0, 30, 60, and 90 minutes after actinomycin D (an inhibitor of RNA polymerase II) treatment, and the half-lives of these mRNAs were calculated (Fig. 4). In HuR-knockdown HSC-3 cells, the half-lives of *c-myc* and *COX-2* mRNAs were 27.8 and 20.01 minutes, on the other hand, they were 46.3 and 88.9 minutes in the cells transfected with control siRNA. These data indicate that HuR knockdown shortened the half-life of the ARE-mRNA. Additionally, the same effect was observed in

HuR-knockdown Ca9.22 cells. The half-lives of *c-myc* and *COX-2* mRNAs in HuR-knockdown cells (22.8 and 28.0 minutes respectively) were shorter than those of the control cells (38.9 and 385 minutes respectively). These results indicate that HuR knockdown inhibits the stabilization of ARE-mRNA in oral cancer cells.

HuR knockdown attenuated the ability of the cells to make colonies in soft-agar

To investigate their ability for anchorage-independent growth, one of the most reliable markers of malignant transformation, the HuR-knockdown cancer cells were subjected to soft-agar colony formation assay. Under our experimental conditions, the HSC-3 cells transfected with control siRNA formed a considerable number of colonies after three weeks of incubation (Fig. 5). In contrast, the HuR-knockdown HSC-3 cells formed very few colonies, even after three weeks incubation. Furthermore, the colony size of the HuR-knockdown HSC-3 cells was smaller than that of the control cells (Fig. 5). These results suggest that HuR plays an important role in anchorage-independent cancer growth.

Expression of cell cycle-related proteins in HuR-knockdown cancer cells.

In a previous report, reduction of HuR expression in untransformed human diploid fibroblasts by the expression of an antisense transcript complementary to HuR mRNA resulted in cell senescence and a reduction in stability of cell cycle (or senescence-related) gene products such as cyclin A and cyclin B1 (20). To examine the effect of HuR-knockdown in oral cancer cells, we confirmed the expression of these cell cycle-related gene products, as well as the expression of cyclin D1 and CDK1. The expression of these proteins was obviously decreased in the HuR-knockdown HSC-3 and Ca9.22 cells (Fig. 6). We confirmed the senescent phenotype of HuR-knockdown cancer cells by senescence-associated β -gal activity. No such activity was seen in the HuR-knockdown oral cancer cells under our experimental conditions (data not shown). The results indicate that HuR-knockdown down-regulates cell cycle related proteins in oral cancer cells.

Since CDK1 is thought as the only essential key regulator of cell cycle (21), we further examined the relation between CDK1 and HuR.

Although *cdk1* mRNA is not in ARED (AU-rich element-containing mRNA database; <http://brp.kfshrc.edu.sa/ARED>) (22), we attempted to search the interaction between HuR and *cdk1* mRNA by using a RNP immunoprecipitation (RIP) assay. In Ca9.22 cells, *cdk1* mRNA was coprecipitated with HuR as in the case that *c-myc* mRNA was subjected to the same RIP analysis as a positive control. On the other hands, HuR has never been associated with *GAPDH* mRNA (Fig. 7A). In order to confirm the stabilization of *cdk1* mRNA, we examine the accumulation and half-life of the mRNA. The accumulated *cdk1* mRNA was reduced and the half-life of the mRNA has been changed from 3.49 h to 1.69 h by HuR knockdown (Fig. 7B and C). These data show that HuR has potential to bind to the *cdk1* mRNA and stabilize the mRNA in oral cancer cells.

HuR knockdown changes the motile and invasive phenotype of oral cancer cells.

To explore the feature of HuR-knockdown oral cancer cells further, the motile and invasive activity of the cells were examined. For this purpose, we performed wound-healing assays and the wound closure was examined

at 12, 24 and 36 h following the treatment. Ca9.22 cells transfected with control siRNA migrated into the wound area by 24 h to an extent that the wound edges were undistinguishable (Fig. 8A). However, the HuR-knockdown Ca9.22 cells displayed significantly slower wound closure even at 36 h after the scratch, remaining 40% of the wound area (Fig. 8A and B).

In order to examine invasive activity, we performed an invasion assay. Invasion chambers with matrigel-coated membranes were used to investigate the invasive properties of HuR-knockdown oral cancer cells. The average invasion rate of HuR-knockdown Ca9.22 cells decreased significantly after 24 h compared to that of control siRNA transfected Ca9.22 cells (Fig. 8C). These data indicate that HuR plays a major role for a motile and invasive activities at least in oral cancer cells.

Discussion

In this report, we demonstrated that HuR knockdown has the potential to change the futures of oral cancer cells. The knockdown decreased the cytoplasmic expression of ARE-mRNA, such as *c-fos*, *c-myc* and *COX-2* mRNA, and it also shortened the half-lives of those ARE-mRNAs, suggesting that HuR-knockdown affects the subcellular distribution and stabilization of ARE-mRNA. The ability of the cancer cells such as a growth in an anchorage-independent manner and motile and invasive activities declined after HuR knockdown. Furthermore, the expression of cell cycle-related proteins, such as cyclin A, cyclin B1, cyclin D, and CDK1, was reduced in the HuR-knockdown cells, and HuR was able to interact with *cdk1* mRNA to stabilize it. These findings indicate that HuR knockdown reduces the oncogenic activity of oral cancer, at least in part, by arresting the cell cycle.

Several studies have reported that the HuR protein is expressed abundantly in cancer cells, such as colon, tongue, ovary, breast, salivary gland, larynx, and prostate cancer, compared to the expression of HuR in normal cells (16, 18, 23-28). However, in the case of cells transformed by

the adenovirus E4orf6, the amount of HuR was not changed by the expression of the viral oncoprotein, although the subcellular distribution of the protein was significantly altered (17). Although, no evidence showing that a virus oncoprotein other than adenovirus E4orf6 changes the behavior of HuR has been reported, there might be a difference in HuR export between cancers induced by a virus and other cancers.

The tumorigenicity of HuR-knockdown cancer cells was estimated by an experiment in which HuR-knockdown colon cancer (RKO) cells developed tumors in nude mouse significantly more slowly than in control siRNA transfected cells (16). Under our experimental conditions, HuR-knockdown oral cancer cells failed to produce colonies in soft-agar; whereas, the control siRNA transfected cells made a lot of colonies (Fig. 5). Furthermore, HuR-knockdown reduced the motile and invasive phenotype of oral cancer cells (Fig. 8). Our results strongly suggest that HuR knockdown is able to change the characteristics of oral cancer cells, providing additional evidence for the importance of HuR in cancer malignancy.

HuR knockdown reduced the expression of cell cycle or

senescence-related proteins like cyclin A, cyclin B1, cyclin D1, and CDK1. Since *cyclin A*, *cyclin B1*, and *cyclin D1* mRNA have been shown as HuR-regulated mRNA (20, 29, 30), and the level of encoded proteins were reduced in the cells that had been transfected with an anti-sense RNA or siRNA for HuR, the results in the present study are very reasonable. As mentioned above, HuR-knockdown cells could not induce the senescent phenotype, even when normal diploid cells induced senescence under the same experimental conditions (20). We consider that the cell-cycle arrest, but not senescence, might have been caused in these cells. We suppose that this is the reason why we were unable to produce stable HuR-knockdown cells using pSUPER as mentioned above.

It is noteworthy that the expression of CDK1 was reduced by HuR knockdown. Recently, Cdk1 has been shown to be the only essential cell cycle Cdk, because, in the absence of interphase Cdk, it can execute all the processes that are required to drive cell division (21, 31), and it is also important for the import of HuR to the nucleus due to its phosphorylation (11). In G2/M phase, HuR is phosphorylated at residue 202 by CDK1, leading to an increased interaction with 14-3-3 in the nucleus, while

unphosphorylated form of HuR exists in the cytoplasm with its target mRNA (11, 32). We show here that HuR can bind to *cdk1* mRNA and stabilize it presumably by exporting the mRNA (Fig. 7). Therefore, HuR treated in our experiments might be an unphosphorylated HuR at residue 202. HuR will be phosphorylated by a lot of CDK1 translated from the stabilized *cdk1* mRNA. Because phosphorylated HuR will become impossible to stabilize *cdk1* mRNA by entering the nucleus, the feedback loop might exist in the HuR phosphorylation and the CDK1 synthesis.

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Disclosure of Potential Conflicts of interest

No potential conflicts of interest were disclosed.

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Legends

FIGURE 1. HuR expression in oral cancer cells. **A.** Western blot analysis of HuR in oral cancer (HSC-3 and Ca9.22) and normal oral (HGF) cells. The quantitative assessment of HuR protein in each cell is shown as a histogram. Shown are fold changes in HuR level as measured by densitometry. **B.** HuR or control (Ctr) siRNA were introduced into HSC-3 and Ca9.22 cells, and the amounts of HuR and β -actin were estimated at the indicated time by Western blot analysis.

FIGURE 2. The cytoplasmic expression of ARE-mRNA in the cells transfected with siRNA for HuR. Western blot analysis of the nuclear (N), cytoplasmic (C), and total cell (TC) fractions of HSC-3 and Ca9.22 cells using the antibodies against β -tubulin and hnRNPA1 (*top*). The relative amount of *c-fos*, *c-myc*, and *COX-2* mRNAs expressed in the cytoplasm of HSC-3, Ca9.22 and HGF cells transfected with HuR siRNA or control (Ctr) siRNA was measured by quantitative real-time RT-PCR (*bottom*). Each quantity was calculated by the formula $C/N+C$. Data are mean $\pm$ SD of three independent experiments.

FIGURE 3. The distribution of ARE-mRNA in HuR-knockdown cells. HSC-3 (*top*) and Ca9.22 (*bottom*) cells transfected with HuR or control (Ctr) siRNA were subjected to *in situ* hybridization using digoxigenin-labeled antisense oligonucleotide probes complementary to *c-fos*, *c-myc*, and *COX-2* mRNAs and rhodamine-conjugated antidigoxigenin antibody. DAPI-stained nuclei are shown.

FIGURE 4. The stabilization of ARE-mRNA in HuR-knockdown cells. HSC-3 and Ca9.22 cells were treated with actinomycin D (Act.D), and the amount of each ARE-mRNA was estimated at the indicated time by quantitative real-time RT-PCR. The value of $t^{1/2}$ indicates the half-life (minutes) of each mRNA. Data are mean $\pm$ SD of three independent experiments.

FIGURE 5. Effect of HuR knockdown on anchorage-independent growth of oral cancer cells. HSC-3 cells transfected with HuR or control (Ctr) siRNA were subjected to a soft-agar colony formation assay. Photographs

of the stained colonies were taken three weeks after the transfection (*top*). The effect of HuR knockdown was estimated by Western blot analysis (*bottom*).

FIGURE 6. The changes in the protein levels of cell cycle related proteins induced by HuR knockdown. HSC-3 and Ca9.22 cells were transfected with HuR or control (Ctr) siRNA, and the expression of cell cycle related proteins (cyclinA, cyclinB1, cyclinD1 and CDK1) was estimated by Western blotting 48 h after the transfection.

FIGURE 7. The interaction of HuR with *cdk1* mRNA and its stabilization in oral cancer cells. **A.** *cdk1* mRNA associated with HuR was isolated by RNP immunoprecipitation analysis using Ca9.22 cells. Mouse IgG was used as a control of the antibody. S, supernatant; P, pellet of immunoprecipitation. **B.** The relative amount of *cdk1* mRNA expressed in Ca9.22 cells transfected with HuR siRNA or control (Ctr) siRNA was measured by quantitative real-time RT-PCR 48h after the transfection. Data are mean $\pm$ SD of five independent experiments. **C.** Ca9.22 cells

were treated with actinomycin D (Act.D), and the amount of *cdk1* mRNA was estimated at the indicated time by quantitative real-time RT-PCR. The value of $t^{1/2}$ indicates the half-life (hours) of the mRNA. Data are mean $\pm$ SD of three independent experiments.

FIGURE 8. HuR knockdown affects the motility and invasion activity of oral cancer cells. **A.** Phase images of Ca9.22 cells transfected with HuR siRNA or control (Ctr) siRNA at 0h, 12h, 24h and 36h after the wounding. **B.** Quantification of cell motility analysed by the wound healing assay. Data are mean $\pm$ SD of three independent experiments. **C.** Serum-starved Ca9.22 cells transfected with HuR siRNA or control (Ctr) siRNA employed in Biocoat Matrigel Invasion chamber. After 24h, cells on the lower side of membrane were fixed, stained and counted. Data are mean $\pm$ SD of three independent analysis.

FIGURE 1

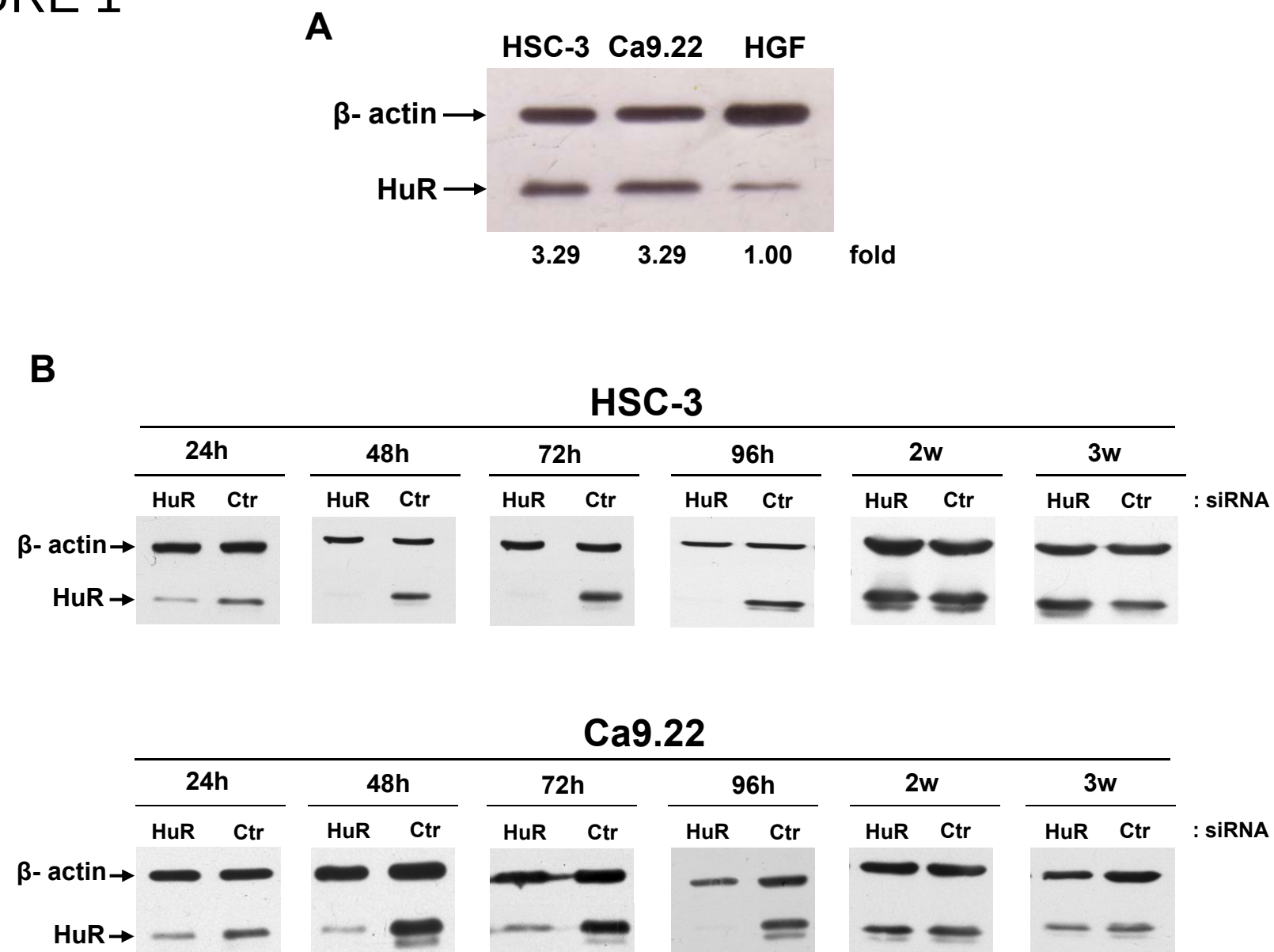


FIGURE 2

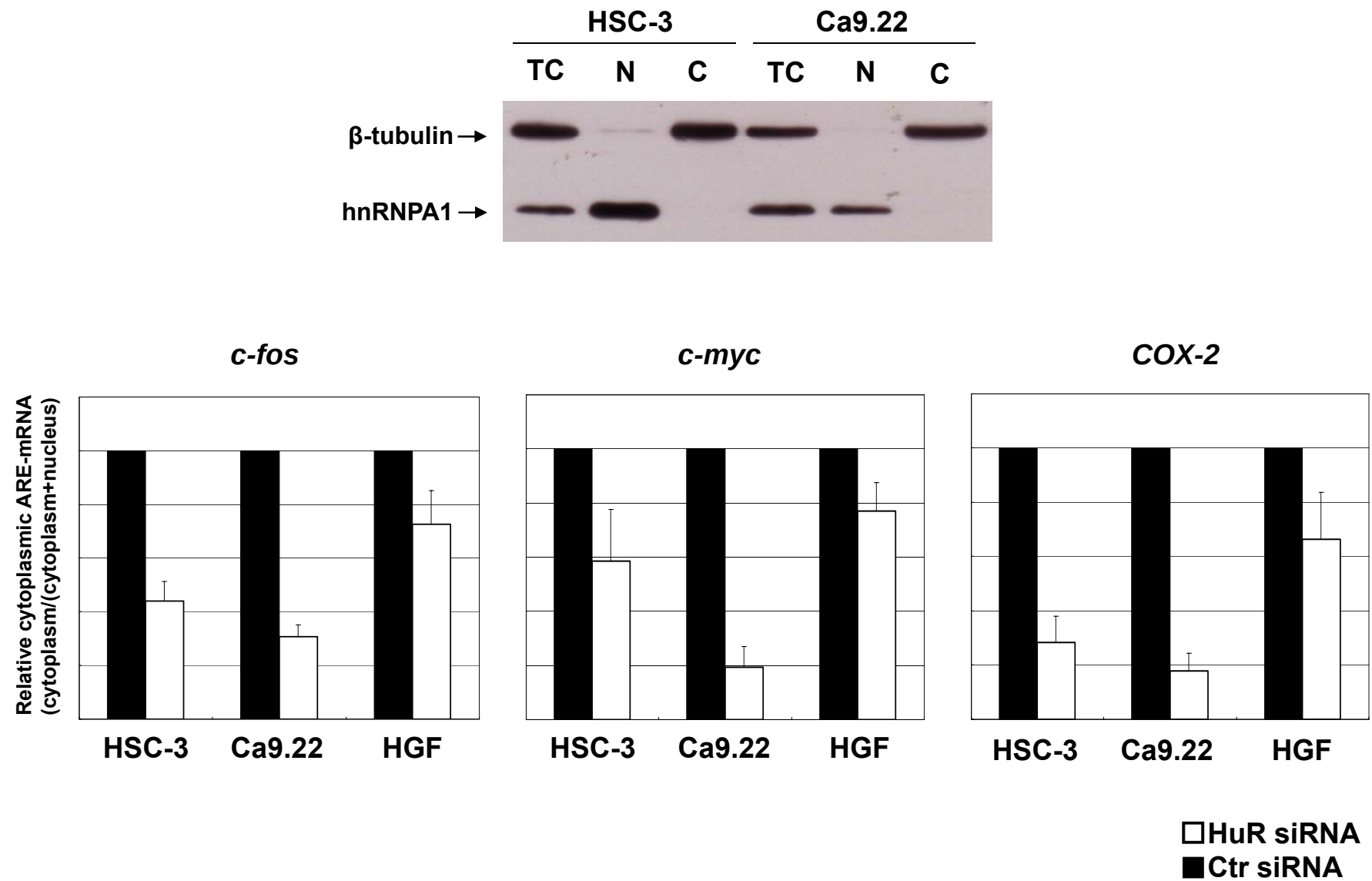


FIGURE 3

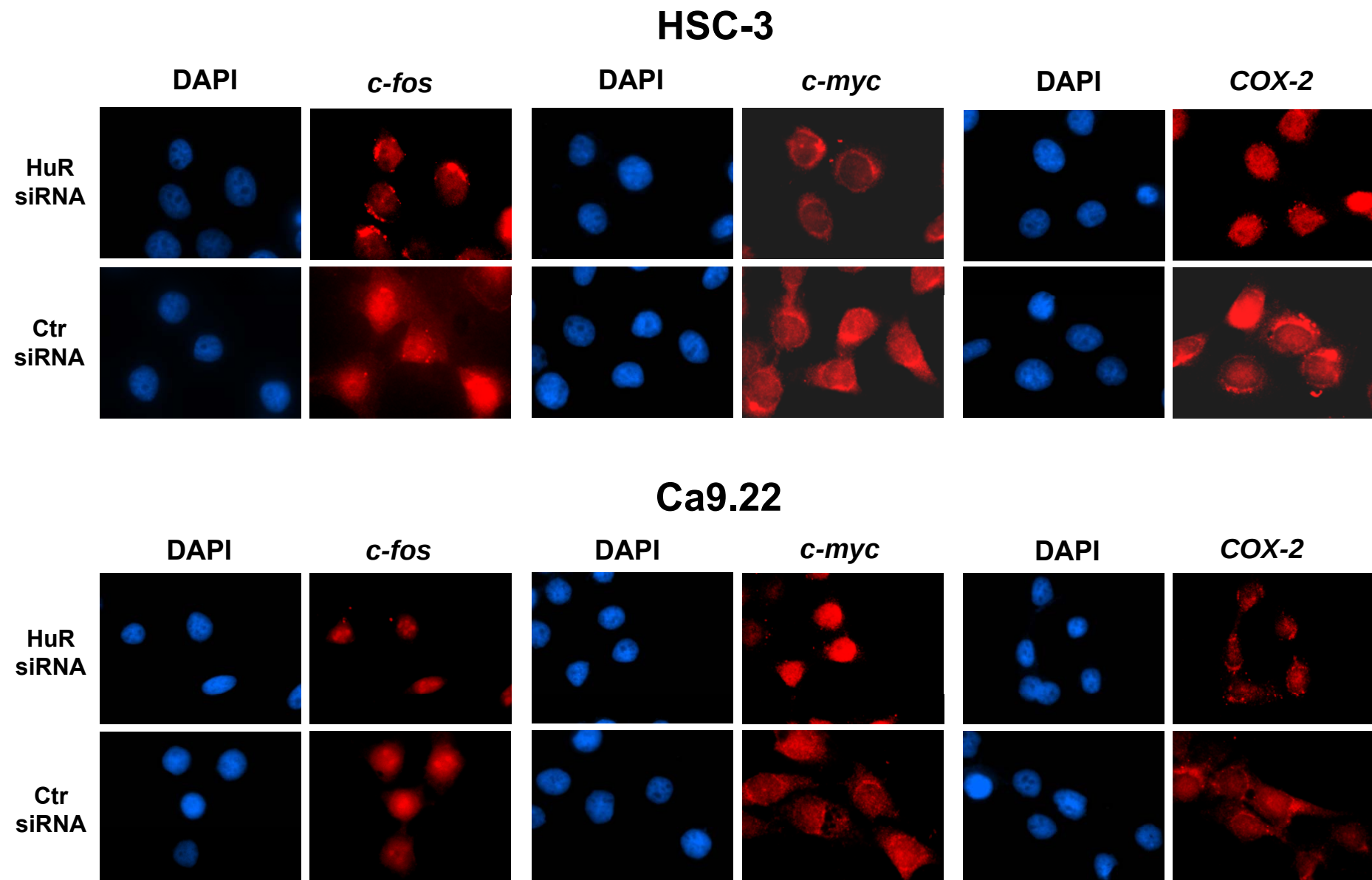


FIGURE 4

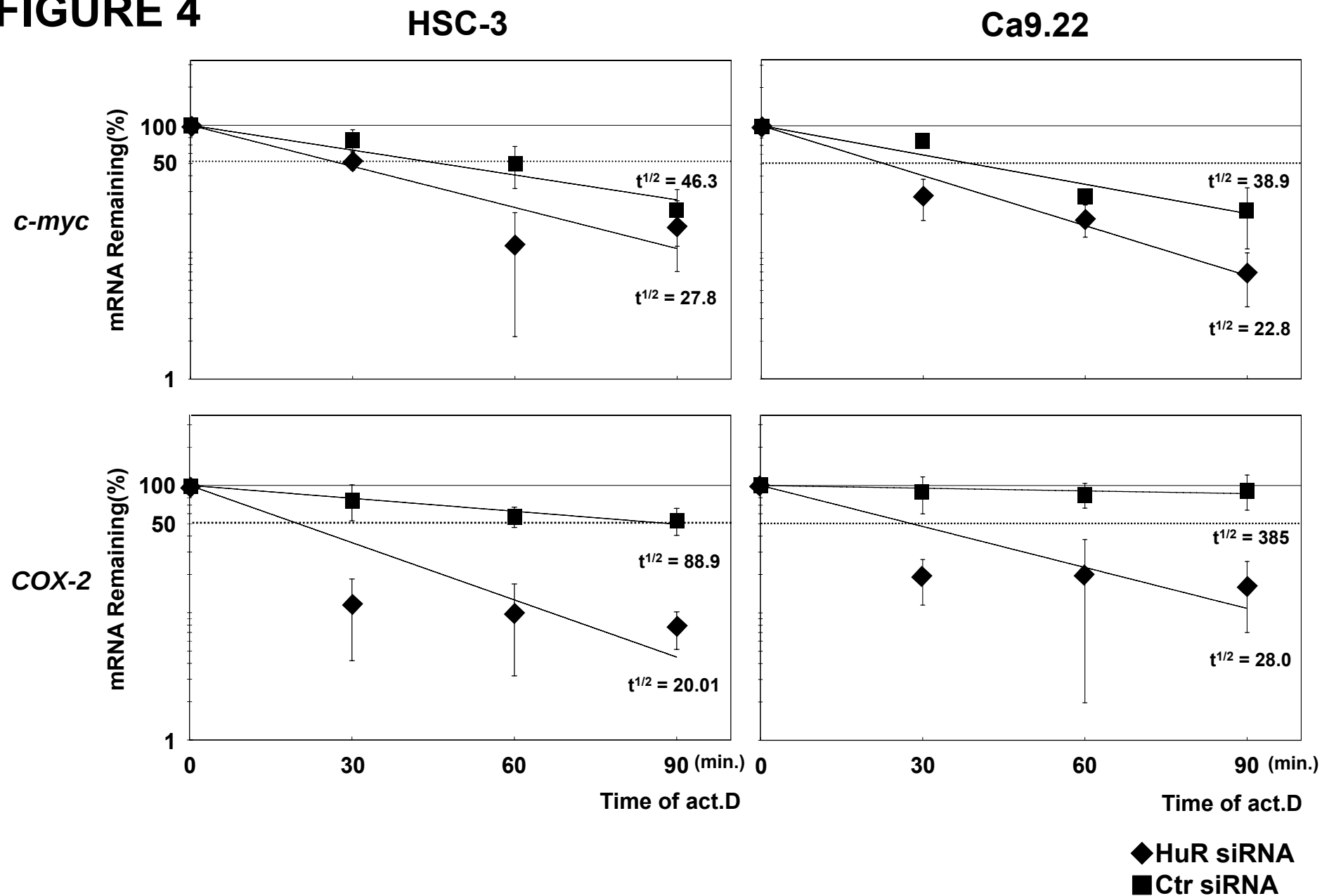


FIGURE 5

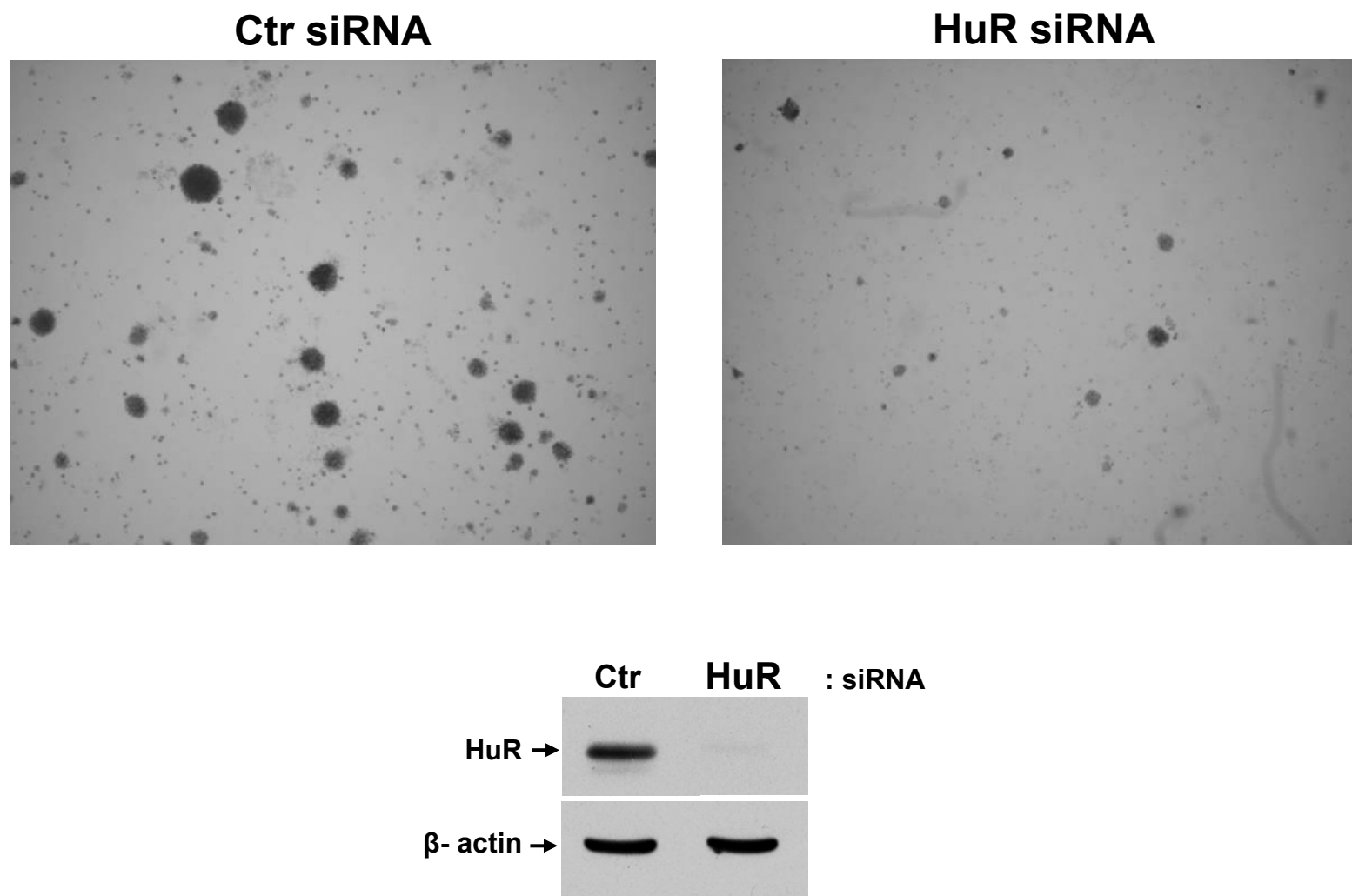


FIGURE 6

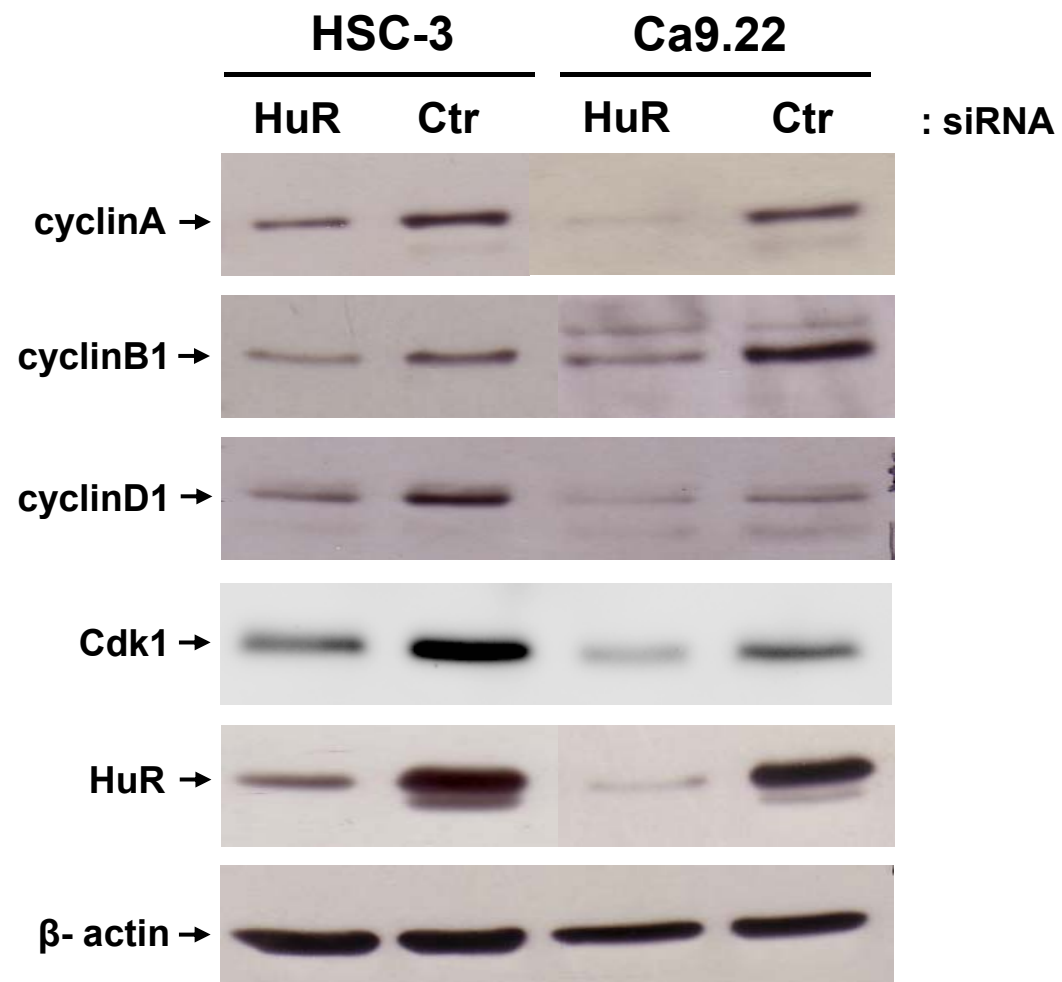
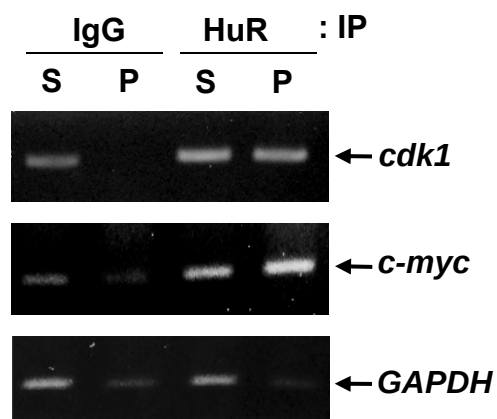
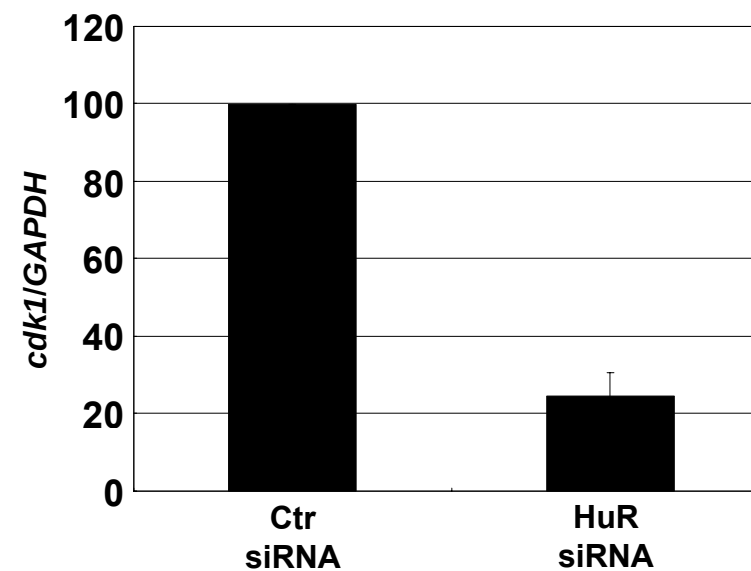


FIGURE 7

A



B



C

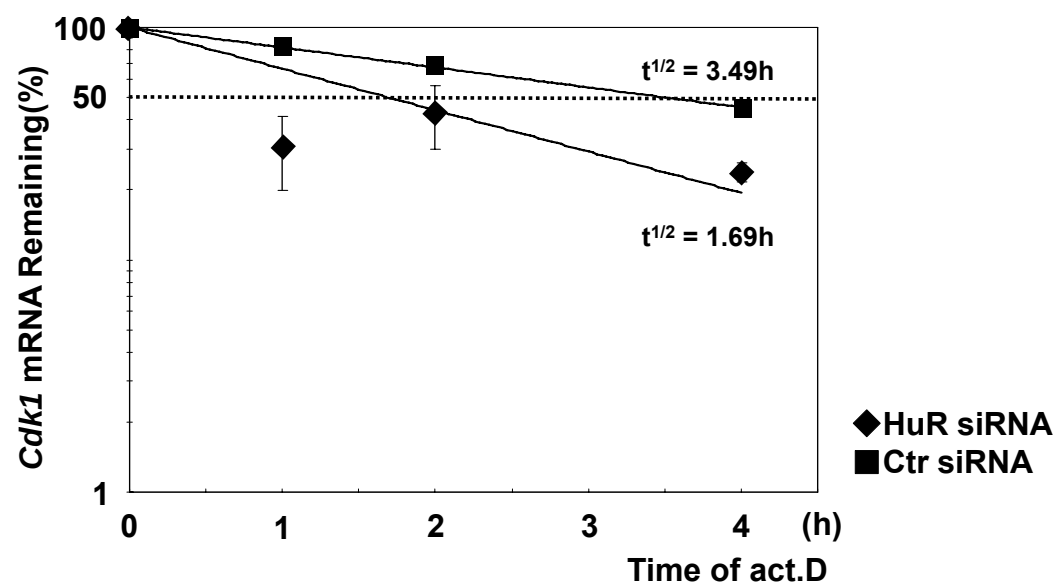
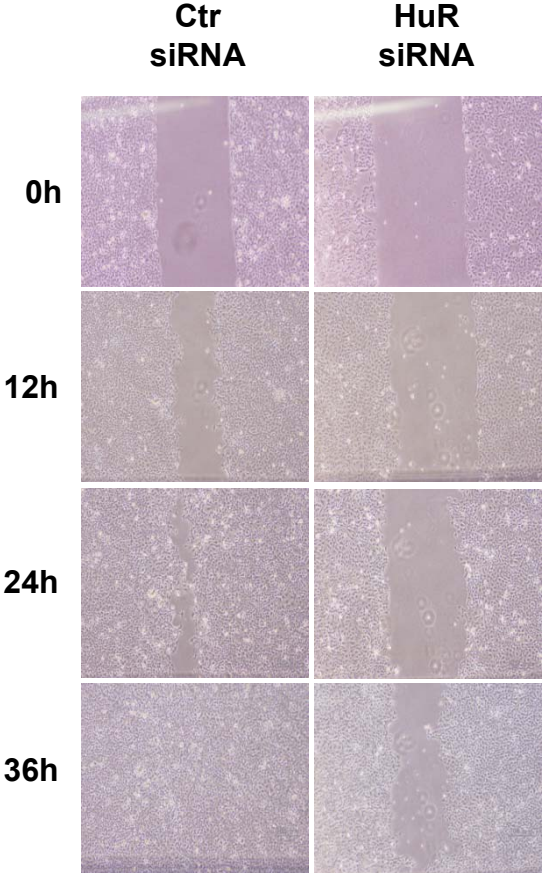
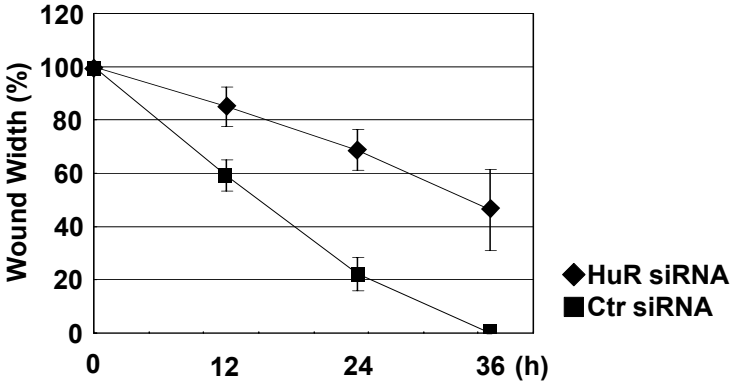


FIGURE 8

A



B



C

