The p14ARF Tumor Suppressor Protein Facilitates Nucleolar Sequestration of Hypoxia-inducible Factor-1α (HIF-1α) and Inhibits HIF-1-mediated Transcription*

Oncogenic alterations can influence tumor cell survival partly by affecting the activity of the hypoxia-inducible factor-1 (HIF-1) transcription factor. The α subunit of HIF-1 was found to be frequently overexpressed in advanced tumors, which was proposed to help the adaptation of tumor cells to hypoxia. Here we show that an important tumor suppressor protein, p14ARF (alternative reading frame product of the INK4A locus) can directly inhibit the transcriptional activity of HIF-1 by sequestering its α subunit into the nucleolus. The interaction requires neither p53 nor HDM2. This is one of the first reports that describe the interaction of p14ARF with a protein besides HDM2, which may define a p53-independent tumor suppressor activity for p14ARF.

Recently, it has become increasingly clear that hypoxia plays an important role in the development of solid tumors. Elevated expression of the hypoxia-responsive proangiogenic factors and glycolytic enzymes contributes to the manifestation of the “lethal cancer phenotype” (1). The heterodimeric HIF-1α transcription factor is the major regulator of many genes that are activated under low oxygen concentration. It has been reported that HIF-1α is the hypoxia-responsive component of the dimer, while HIF-1β is expressed constitutively (2). HIF-1α is accumulated in hypoxic cells, which is mainly due to its increased resistance to proteasomal degradation (3, 4). Additionally, an increase of the transactivation potential of HIF-1α, which may be related to its phosphorylation status, has been reported (5).

Recent studies have revealed that mutation of oncogenes and tumor suppressor genes can also result in changes in the expression and/or activity of HIF-1α (6–10). The most frequently mutated tumor suppressor protein, p53, can also negatively affect the activity of HIF-1. According to an earlier report, the ability of p53 to inhibit HIF-1 is based on a competition between the two transcription factors for the p300/CBP transcriptional co-activator (11). A recent study suggests that p53 can also affect the stability of HIF-1α through the regulation of the HDM2 proto-oncogene (10). The expression of HDM2 is activated by p53, which in turn inhibits p53, creating a negative feedback loop that is mainly responsible for the low p53 activity in normal nonstressed cells (12). HDM2 is an E3 ubiquitin ligase that inactivates p53 primarily by accelerating its degradation through the ubiquitin proteasome pathway (13). HDM2 can also form a ternary complex with HIF-1α and p53 (10). It has been suggested that in this complex HDM2 preferentially promotes the ubiquitination and subsequent degradation of HIF-1α. This model may explain the observed shorter half-life of HIF-1α in cells expressing wild-type p53 compared with those in which p53 activity is compromised.

The p14ARF tumor suppressor protein (p19ARF in mouse) has recently been recognized as an important negative regulator of HDM2 (14). p14ARF can strongly bind HDM2 and facilitates its translocation from the nucleus to the nucleolus (15, 16). The nucleolar sequestration is suggested to prevent HDM2 from promoting the degradation of p53, resulting in elevated p53 activity. In this study, we examined whether the inhibition of HDM2 by p14ARF can also have a positive effect on HIF-1 activity. Surprisingly, we found that HIF-1, unlike p53, was strongly inhibited by p14ARF. The inhibition is at least partially p53-independent and strongly correlates with the ability of p14ARF to bind HIF-1α. The interaction does not require HDM2 or p53 and induces nucleolar relocalization of HIF-1α. Although several reports have suggested that the p14ARF/p19ARF tumor suppressor may have p53-independent functions (17, 18), only a very recent study identified several members of the E2F family of transcription factors as binding partners for p19ARF (19). Our data extend these findings by showing that the human p14ARF protein is capable of interacting with the α subunit of the HIF-1 transcription factor, which may also contribute to some of the p53-independent tumor suppressor activities of the protein.

EXPERIMENTAL PROCEDURES
Cell Culture—U-2OS cells were grown in McCoy’s 5A medium supplemented with 15% fetal bovine serum. The Hep3B and HT1080 cell lines were maintained in a-minimum essential medium supplemented with 10 or 15% fetal bovine serum, respectively. The p53−/−, MDM2−/− mouse embryonic fibroblasts were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. All of the media and the fetal bovine serum were purchased from Life Technologies, Inc.

Transfection, Transient Transfection Assay, and Cell Cycle Analysis—Mouse embryonic fibroblasts were transfected with Cytofectene (Bio-Rad), while the rest of the cell lines were transfected with Fugene 6 (Roche Biochemicals) following the manufacturer’s instructions. For transient transfection assays, cells were seeded in 24-well microtiter plates 1 day before the transfection. Generally, 500 ng of DNA/well was used including 25 ng of reporter plasmid and 125 ng of expression construct. The total amount of DNA was brought up to 500 ng using the “empty” expression vector. Endogenous HIF-1α protein was induced in cells by the addition of 125 μM of desferrioxamine for 16–20 h. Hypoxia treatments were performed in a hypoxic chamber (<0.2% O2; Sheldon...
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Corp.). β-galactosidase and firefly luciferase activities were measured using chemiluminescent β-galactosidase and luciferase assay kits from Roche Biochemicals. All of the transfections were performed in triplicate. For Western blot analysis, cells were plated in 100-mm dishes, and the transfected cells were harvested and fixed in 0.25% paraformaldehyde for 5 min followed by a 5-min postfixation in 70% ethanol. Before analysis, the cells were treated with RNaseA (100 μg/ml) and stained with propidium iodide (50 μg/ml). The cell cycle profiles were analyzed on a FACScalibur flow cyrometer (Becton Dickinson).

Plasmids—The p14ARF cDNA was isolated by reverse transcription-PCR using poly(A)+ RNA prepared from Hep3B cells. The first strand cDNA was synthesized using a kit from Roche Biochemicals. After the cDNA synthesis, the p14ARF cDNA was amplified by PCR using the following primers: 5'-GGCCGCAGCTCATGTTGCAGGTG-3' and 5'-COGATTTCGCCAGCCAGCAG-3'. The p14ARF cDNA was cloned into pcDNA1/Amp (Invitrogen), and its sequence was confirmed by sequencing. The FLAG-tagged full-length and deleted versions of p14ARF were constructed in the same expression vector by PCR using appropriate primers. A pNiV vector (Invitrogen) containing the full-length HIF-1α cDNA was obtained from N. Denko (Stanford University). The cDNA was cut out from the plasmid with KpnI and NcoI restriction enzymes and cloned into pCEP4 (Invitrogen). A FLAG-tag was fused to the C-terminal end of the cDNA by PCR. The hypoxia-responsive reporter plasmid was constructed by inserting four tandem copies of a 54-base pair double-stranded oligonucleotide corresponding to the human vascular endothelial growth factor HRE into the Nhel site of pGL2 promoter (Promega). In the resulting plasmid, the luciferase gene was replaced with a 5.7-kilobase pair HindIII-BamHI fragment of pCH110 (Amersham Pharmacia Biotech) containing the bacterial β-galactosidase gene. Finally, to reduce the background activity of the reporter, the 0.4-kilobase pair BglII-HindIII fragment containing the minimal SV40 promoter was removed from the plasmid. To construct a p53-responsive reporter plasmid, 10 copies of a double-stranded oligonucleotide containing a consensus p53 binding site (5'-GGCGCAGCTCATGTTGCAGGTG-3') were inserted into the SmaI site of the pGL2 promoter vector in a tandem manner. To obtain the HIF-1α-EF1 fusion constructs, different segments of the HIF-1α coding region were amplified by PCR and cloned into the pCruHA expression vectors (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The EF1G open reading frame was amplified by PCR using pEGFP-1 (CLONTECH) as a template and inserted into the above-described plasmid in frame with the HIF-1α fragments. The HIF-1α-DaRed-1 fusion gene was constructed by inserting the HIF-1α open reading frame as a 2.6-kilobase pair KpnI-HpaI fragment into the pDsRed1-N1 expression vector (CLONTECH). To obtain a frame fusion, pDsRed1-N1 was digested with BamHI and KpnI restriction enzymes, and primer to the ligation the BamHI end was repaired with the Klenow fragment of Escherichia coli DNA polymerase I. The plasmid sequences were verified by sequencing.

Western Blot Analysis and Immunoprecipitation—Cells were lysed in a lysis buffer containing 10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 400 mM NaCl, 10% glycerol, 0.5% Nonidet P-40, 5 mM NaF, 1 mM diethiothreitol, 0.5 mM NaVO4. The lysis buffer was supplemented with 1 mM phenylmethylsulfonyl fluoride and Complete protease inhibitor mix (Roche Biochemicals). After sonication, the extracts were centrifuged for 20 min at 4 °C at 14,000 rpm. The supernatants were transferred into clean tubes, and the protein concentration was determined with the D, Protein Assay Kit (Bio-Rad). The proteins were resolved by 4–20% polyacrylamide gels (Bio-Rad) and transferred to Hybond nitrocellulose membrane (Amersham Pharmacia Biotech). The transfer and the subsequent processing of the membrane were performed according to the manufacturer’s instructions. For detection, the ECL system was used from Amersham Pharmacia Biotech. Immunoprecipitation was carried out from 300–500 μg of cell extract. The volume of the cell extract was brought up to 250 μl with lysis buffer. Next, 250 μl of dilution buffer (same as the lysis buffer with the extracts and incubated overnight at 4 °C on a shaking platform. The following morning, 20 μl of protein A-Sepharose slurry was added to the tubes, and the incubation was continued for 2–4 h at 4 °C. The Sepharose beads were washed in three consecutive steps with the following buffers: 1) NET-gel buffer (35 containing 500 mM NaCl; 2) NET-gel buffer supplemented with 0.1% SDS; and 3) 10 mM Tris-HCl, pH 7.6, 0.1% Nonidet P-40. The washed Sepharose beads were resuspended in 20 μl of 2× sample buffer (35), boiled for 5 min, and analyzed by Western blotting as described above. In this study, the following antibodies were used: monoclonal anti-FLAG M2 antibody (Sigma), polyclonal anti-p53 antibody (Santa Cruz Biotechnology), monoclonal anti-HIF-1α antibody clone 1H6 (Novus Biologicals), polyclonal anti-p14ARF antibody FL-132 (Santa Cruz Biotechnology), polyclonal anti-p14ARF antibody (Abcam), polyclonal anti-GFP antibody FL (Santa Cruz Biotechnology), monoclonal anti-p21WAF1/CIP1 antibody (Transduction Laboratories), monoclonal anti-p53 antibody PAb1801 (NeoMarkers), and monoclonal anti-ubiquitin antibody MS-3 (Santa Cruz Biotechnology).

Indirect Immunofluorescence and Fluorescent Microscopy—Cells growing on coverslips were transfected with the appropriate expression vectors. Twenty-four to thirty-six hours after transfection, the cells were fixed with 4% paraformaldehyde for 30 min at room temperature. For the anti-nucleolin staining, the cells were fixed with cold methanol for 5 min. To block the nonspecific antibody binding, the coverslips were incubated for 1 h at 37 °C with 0.5% bovine serum albumin dissolved in T-TBS (0.1% Tween 20). The coverslips were successively incubated with the primary and secondary antibodies at 37 °C for 1 h each. The antibodies were diluted in T-TBS. The coverslips were extensively washed with T-TBS and mounted on microscope slides using Vectashield mounting medium (Vector Laboratories). The Cy3- and fluorescein isothiocyanate-conjugated secondary antibodies were purchased from Jackson Laboratories. The slides were examined by an Axioplan Fluorescent Microscope (Zeiss).

RESULTS

p53-independent Inhibition of HIF-1 Activity by the p14ARF Tumor Suppressor—To monitor HIF-1 activity in cells, a reporter plasmid was constructed by inserting four tandem copies of the 54-base pair hypoxia response element from the human vascular endothelial growth factor gene promoter upstream of the bacterial β-galactosidase gene. The reporter construct was highly responsive to hypoxia and HIF-1α overexpression. In addition, the plasmid could also be induced by CoCl2 and desferrioxamine treatments, which were both shown to be able to cause the stabilization of the HIF-1α protein similarly to hypoxia (2). Forced expression of the p14ARF tumor suppressor protein drastically inhibited the HIF-1-dependent activity of the reporter in the human osteosarcoma cell line U-2OS, without affecting the desferrioxamine-induced expression of HIF-1α (Figs. 2a and 3b). A similar inhibition was exhibited by p14ARF in experiments where the HIF-1-responsive reporter plasmid was activated by either hypoxia (Fig. 1a) or forced expression of HIF-1α (Fig. 2b). As expected, p14ARF expression elevated both the protein level of p53 (Fig. 2a) and its transcriptional activity as judged by the increased expression of the p53 target gene, p21WAF1/CIP1 (Fig. 2a) and the elevated activity of a reporter plasmid containing concatamerized p53 binding sites in front of the firefly luciferase gene (Fig. 2b). This observation raised the possibility that the decreased HIF-1 activity was due to the activation of p53. To prevent p53 activation, p53-specific E3 ubiquitin ligases, human papilloma virus (HPV) E6 or HDM2, were co-expressed with p14ARF in U-2OS cells. Although both proteins could efficiently prevent the activation of p53, they were unable to restore HIF-1 activity. Importantly, forced expression of neither HPV E6 nor HDM2 alone had any significant effect on HIF-1 activity (Fig. 2b). These data combined with the observation that p14ARF could also strongly inhibit the transcriptional activity of HIF-1 in the p53 null Hep3B cell line suggest that the inhibition is at least partially p53-independent (Fig. 1b). HIF-1 activity in the presence of p14ARF was 3–10% of control in Hep3B cells, while it was less than 1% in U-2OS cells, indicating that, in agreement with previous reports, p53 could also contribute to the inhibition of HIF-1 (11).

To identify the inhibitory domain within p14ARF, deletion
mutants of the ARF cDNA were constructed, and their ability to inhibit the transcriptional activity of HIF-1 was tested in transient transfection assays. The N-terminal 64-aa fragment of p14ARF inhibited HIF-1 to a similar extent as the full-length protein, while the C-terminal 68-aa region did not exhibit significant inhibitory activity (Fig. 1b). Western blot analysis indicated that the different polypeptides were expressed at comparable levels (Fig. 2a).

It has been reported before that forced expression of p14ARF can arrest U-2OS cells at both G1 and G2 phases (20). To rule out the possibility that the inhibition of HIF-1 activity was due to a nonspecific effect of the cell cycle arrest, cell cycle profiles
of U-2OS cells transfected either with the full-length or the deleted p14<sup>ARF</sup> expression constructs were analyzed by fluorescence-activated cell sorting. As expected, full-length p14<sup>ARF</sup> induced G1 and G2 arrest; however, the cell cycle distribution of cells expressing either the N-terminal or the C-terminal fragment of p14<sup>ARF</sup> did not change significantly compared with control cells that were transfected with the “empty” expression vector (Fig. 1c). Since the N-terminal fragment of p14<sup>ARF</sup> inhibited HIF-1 almost as efficiently as the full-length protein, it is unlikely that the inhibition is a result of cell cycle arrest. Additionally, since the forced expression of p14<sup>ARF</sup> did not considerably affect the activities of reporter constructs driven by either the SV40 early promoter or the cytomegalovirus early promoter, we can rule out the possibility that the p14<sup>ARF</sup>-induced reduction of HIF-1 activity is a result of a nonspecific inhibition of the general transcription apparatus (Fig. 1d).

Previously, it has been reported that the MDM2 oncoprotein can physically interact with both p14<sup>ARF</sup> and HIF-1α. To examine the possibility that the HIF-1α-p14<sup>ARF</sup> interaction is mediated by MDM2, immunoprecipitations were carried out from cell extracts prepared from MDM2<sup>−/−</sup>, p53<sup>−/−</sup> mouse embryonic fibroblasts that were transiently transfected with p14<sup>ARF</sup> and HIF-1α expression vectors. As shown in Fig. 3d, HIF-1α was efficiently co-precipitated with p14<sup>ARF</sup>, indicating...
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FIG. 3. HIF-1α and p14ARF can form a stable complex in vivo. U-2OS cells were transfected with the indicated expression vectors (a). The expression of the proteins was verified by Western blotting (20 μg of extract/well) (extract controls, top two panels). Immunoprecipitations (IP) were carried out from 300 μg of total cell extracts, and the precipitated proteins were analyzed by Western blotting (WB). The antibodies used are indicated beside the panels. Overexpressed p14ARF can bind to the desferrioxamine (DFO)-induced endogenous HIF-1α protein in U-2OS cells (b). The arrowhead points to the position of the co-precipitated HIF-1α, while the asterisk indicates a nonspecific band. The endogenous p14ARF and HIF-1α proteins were analyzed in HT1080 cells. HIF-1α expression was induced in HT1080 cells with desferrioxamine treatment. Immunoprecipitations were performed from 500 μg of total cell extracts either with the irrelevant anti-HA.11 antibody (negative control) or an anti-p14ARF antibody. HIF-1α can interact with p14ARF in the absence of MDM2 and p53 proteins (d). MDM2−/−, p53−/− mouse embryonic fibroblasts were transfected with the indicated expression vectors, and immunoprecipitations were carried out from 300 μg of total cell extracts.

that the interaction between the two proteins did not require the presence of either MDM2 or p53.

p14ARF Facilitates the Translocation of HIF-1α to the Nucleolus—Since it was described before that the nucleolar p14ARF/p19ARF protein was capable of sequestering HDM2/MDM2 into the nucleolus, we examined whether p14ARF could change the subcellular localization of HIF-1α in a similar manner. The localization of HIF-1α was followed by fusing the DsRed1 fluorescent protein in frame to the HIF-1α cDNA. When the HIF-1α-DsRed1 fusion protein was expressed alone in U-2OS cells, it was localized to the nucleus but was mostly excluded from the nucleoli. Co-expression of p14ARF induced nucleolar accumulation of the fusion protein (Fig. 4a). This result was also confirmed in experiments where the localization of overexpressed HIF-1α was followed by indirect immunofluorescence. The identity of the nuclear structures occupied by the p14ARF-HIF-1α complexes was verified by staining with anti-nucleolin antibodies (Fig. 4b).

To determine which region of HIF-1α is responsible for the p14ARF-induced nucleolar relocalization, different fragments of HIF-1α were fused to EGFP. The fusion proteins were expressed in U-2OS cells either alone or in combination with p14ARF. As seen in Fig. 5, co-expression of p14ARF could only change the subcellular localization of the fusion protein that contained the N-terminal 199-aa segment of HIF-1α (1–199HIF-1α-EGFP). In the absence of p14ARF, this protein exhibited a slight nuclear accumulation; however, co-expression of p14ARF induced its almost complete relocalization to the nucleoli. We could also show that the N-terminal 64-aa fragment of p14ARF was necessary and sufficient to induce the nucleolar accumulation of 1–199HIF-1α-EGFP. On the contrary, the C-terminal 68-aa fragment of p14ARF was unable to do so despite the fact that this polypeptide could also localize to the nucleoli (although less efficiently than the full-length protein) (Fig. 6).

p14ARF Binds Strongly to the Proline-Serine-Threonine-rich Protein Stability Domain (PSTD) of HIF-1α—The p14ARF binding sites of HIF-1α were mapped by using immunoprecipitation. Fragments of HIF-1α covering the entire protein were fused in frame to EGFP, and the resulting fusion proteins were separately co-expressed with FLAG-tagged p14ARF in Hep3B cells. Immunoprecipitations were performed using anti-FLAG antibodies, and the presence of p14ARF in the precipitated material was analyzed with anti-FLAG antibodies (Fig. 7). p14ARF bound strongly to the 463–652 segment of HIF-1α. This fragment covers almost the entire proline-serine-threonine-rich protein stability domain (PSTD), which was previously suggested to play an important role in the regulation of HIF-1α (4, 21, 22). Weaker binding of p14ARF to the 1–199 and 200–462 segments of HIF-1α could also be seen. These data combined with the results of the cellular localization studies suggest that the N-terminal 199-aa fragment of HIF-1α may...
contain a cryptic nucleolar localization signal, which is activated by p14ARF, resulting in nucleolar sequestration of the polypeptide. Although the PSTD exhibits higher affinity toward p14ARF, it does not translocate efficiently to the nucleoli, probably as a result of the absence of a nucleolar localization signal of its own. A similar mechanism for the p14ARF/p19ARF-induced nucleolar sequestration of HDM2/MDM2 has been proposed recently (16, 23).

DISCUSSION

During the growth of solid tumors, a strong selective pressure is imposed on the tumor cells to undergo both metabolic and genetic alterations, which help their survival under the adverse environmental conditions accompanying the unregulated expansion of the tumor mass. Increasing numbers of observations suggest that mutations in oncogenes and tumor suppressor genes, besides altering the cell cycle and/or apoptotic properties of tumor cells, may also have an impact on their general metabolism (24). The heterodimeric HIF-1 transcription factor appears to play a central role in this adaptation process. HIF-1 was identified as a transcription factor that regulates the expression of many genes involved in the organism’s responses to reduced availability of oxygen under both physiological and pathophysiological conditions. Activated oncogenes (e.g. v-src, Ha-ras, and K-ras) have been reported to be capable of inducing HIF-1 by increasing the stability and/or activity of HIF-1α. On the contrary, overexpression of tumor suppressors (e.g. PTEN (phosphatase and tensin homologue deleted on chromosome 10), von Hippel-Lindau tumor suppressor protein, and p53) has been shown to attenuate the hypoxia-mediated stabilization of this protein. Here we demonstrate that the p14ARF tumor suppressor protein can also affect HIF-1-mediated transcription.

p14ARF/p19ARF is an alternative product of the INK4A locus and is a component of the p53 tumor suppressor pathway (25). Since its expression is induced by several oncogenes (c-myc, E1A, ras, v-abl, E2F-1), it has been suggested that its function is to protect the cells from excessive mitogenic signaling (26). p14ARF/p19ARF can directly bind to HDM2/MDM2, which induces the nucleolar sequestration of the complex. In vitro, p19ARF was also capable of directly inhibiting HDM2’s ability to ubiquitinate p53 (27). According to a recent report, HDM2 can also accelerate the degradation of HIF-1α supposedly by promoting its ubiquitination (10). Based on these findings, one would assume that the inhibition of HDM2 by p14ARF may result in increased activity of the HIF-1 transcription factor. Surprisingly, we found that p14ARF strongly inhibited HIF-1-
mediated transcription, which most likely ensued from its ability to bind HIF-1α. Interaction could be detected between the endogenous proteins, and it did not require the presence of either HDM2 or p53. Importantly, in our experimental system, p14ARF could not decrease the stability of the endogenous HIF-1α protein. These observations suggest that the inhibition of HIF-1 by p14 ARF is mechanistically different from the HDM2-mediated inhibition reported previously. In cellular localization studies, we demonstrated that p14ARF induced the nucleolar sequestration of HIF-1α. Of note, it was reported that in the human prostate cancer cell line, PC-3, the endogenous HIF-1α protein was accumulated in the nucleoli (28). Based on immunocytochemical staining, p14ARF is expressed at high levels in PC-3 cells and predominantly localized to the nucleoli,2 lending further support to the suggestion that the p14ARF-HIF-1α interaction can take place under physiological conditions.

p14ARF represses HIF-1 transcriptional activity, most likely by inducing the translocation of HIF-1α into the nucleolus. The nucleolar sequestration may prevent HIF-1α from reaching its targets and/or interacting partners needed for the hypoxia-responsive transcription. Recently, it has been proposed that nucleolar sequestration represents a novel regulatory mechanism, by which the activities of several cell cycle-regulatory proteins are inhibited (29). Our data, combined with a recent observation that the E2F family of transcription factors can also be sequestered into the nucleoli through binding to p19ARF (19), extend this hypothesis and suggest that not only cell cycle regulatory proteins but transcription factors may also be controlled by nucleolar sequestration. It is conceivable that the p14ARF/p19ARF protein plays an important role in this process, probably by acting as an adapter molecule for a nucleolar transport mechanism.

Since the half-life of HIF-1α in normoxia is very short (t1/2 ~5 min), one may hypothesize that the regulation of HIF-1 activity by p14ARF would become important under conditions where HIF-1 is constitutively active. This is the situation in high grade tumors, where the expression of HIF-1α can be detected even in normoxia, partly as a result of the stabilizing effects of the oncogenic mutations discussed above (28, 30). Several of

FIG. 5. p14ARF induces the nucleolar sequestration of the N-terminal 199-aa segment of HIF-1α. EGFP was fused in frame to the N-terminal end of different fragments of HIF-1α. The fusion proteins were expressed separately in U-2OS cells either alone or in combination with p14ARF. Twenty-four hours after transfection, the cells were analyzed by fluorescent microscopy. The left panels show the subcellular distribution of the fusion proteins in the absence of p14ARF, while the panels on the right show the localization of the same proteins in the presence of p14ARF.

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2 K. Fatyol and A. A. Szalay, unpublished data.
Highly vascularized aggressive glioblastoma multiforme, p14ARF was found to be one of the most frequently mutated oncogenes (32). Undoubtedly, one of the reasons for this is that the mutation of p14ARF at least partially inactivates the p53 tumor suppressor pathway. However, since the inactivation of p53 itself would be even more advantageous, we can hypothesize that p14ARF may indeed have p53-independent effects in these cells, one of which may be the direct inhibition of HIF-1 by p14ARF described here.

Inhibition of the HIF-1-regulated transcriptional program has been identified as a promising therapeutic strategy in tumor cells (33). One possible way to achieve this goal is to prevent the interaction between HIF-1α and the p300 transcriptional co-activator. Based on this premise, polypeptides derived from the C-terminal transactivation domain of HIF-1α were used successfully to inhibit HIF-1-mediated transcription (34). This strategy, however, may have inherent limitations. Since the p300 co-activator is required for the activity of a large number of transcription factors, the interference with p300 function may result in unintended side effects. For example, the CH1 domain of p300, which is required for HIF-1α binding, can also interact with p53. Therefore, the blocking of the CH1 domain with the use of competitive polypeptides may also inhibit p53 activity, which is obviously undesirable in tumor cells. Our results presented here suggest an alternative way to selectively inhibit HIF-1 activity. By understanding the details of the HIF-1-p14ARF interaction, one may be able to design polypeptides or small molecules, which can inhibit HIF-1 activity with high specificity.

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