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REGULAR ARTICLE

Parkin modulates expression of HIF-1α and HIF-3α during hypoxia in gliobastoma-derived cell lines in vitro

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Abstract Mutation of the Parkin gene causes an autosomal recessive juvenile-onset form of Parkinson's disease. However, recently, it has been also linked to a wide variety of malignancies, including glioblastoma multiforme (GBM). In this pathology, Parkin exhibits a tumor suppressor role by mitigating the proliferation rate in both in vitro and in vivo models. However, Parkin involvement in the hypoxic process has not as yet been investigated. GBM is the most common and aggressive primary brain tumor in adults and is characterized by hypoxic areas. The low oxygen supply causes the expression of hypoxia-inducible factors (HIFs) leading to an accumulation of pro-angiogenic factors and tumoral invasiveness. We assess the relationship between Parkin and two HIFs expressed during hypoxic conditions, namely HIF-1α and HIF-3α. Our data show that Parkin is downregulated under hypoxia and that it interferes with HIF expression based on cellular oxygen tension. These results suggest a role for the involvement of Parkin in GBM, although further studies will be needed to understand the mechanism by which it modulates HIF-1α and HIF-3α expression.

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Introduction

Parkin (PARK2) is one of the largest human genes. Various mutations of this gene have been identified in a familial form of Parkinson's disease known as autosomal recessive juvenile Parkinsonism (ARJP; Quinn et al. 1987). The first isolated PARK2 transcript was cloned by Kitada et al. (1998) but now, GenBank lists 26 human PARK2 transcripts corresponding to 21 different alternative splicings (La Cognata et al. 2014). The Parkin gene encodes a RING-between-RING-type E3 ubiquitin ligase, which plays a role in the ubiquitin proteasome system by recruiting E2 components and facilitating the transfer of ubiquitin to damaged or misfolded target substrates (Imai et al. 2000, 2001; Shimura et al. 2000). Parkin displays wide neuroprotective activity in cellular and animal models: it is involved in mitophagy by maintaining a healthy population of mitochondria, it reduces reactive oxygen substrates (ROS) by increasing the glutathione level in cells and it increases proteasomal degradation of toxic substrates (Tan et al. 2005; Yang et al. 2006; Henn et al. 2007; Fett et al. 2010; Bouman et al. 2011; Trempe and Fon 2013; Winklhofer 2014). Mutations of the Parkin gene have not only been identified in ARJP but have also been demonstrated in a variety of human cancers, including glioblastoma multiforme (GBM; Veeriah et al. 2010). Glioblastoma is the most frequently occurring malignant primary brain tumor (Wen and Kesari 1999). Its histopathological characteristics display areas of hypoxia that trigger angiogenesis. The physiological milieu promotes proliferation and tumoral invasion. The primary transcriptional response of glioblastoma cells to hypoxia is mainly mediated by diverse hypoxia inducible factors

(HIFs). These molecules are constituted by heterodimeric complexes that include an oxygen labile α-subunit and a stable β-subunit (ARNT; Semenza 1999a, 1999b; Maynard and Ohh 2004). The α-subunit is stable under hypoxic conditions but is rapidly degraded under normoxic conditions. In normoxia, HIF-α is mostly cytoplasmic but in hypoxia, it accumulates in the nuclear compartment (Kallio et al. 1998). Here, it forms a heterodimer with its constitutively expressed nuclear-binding partner ARNT (Semenza 1999a, 1999b).

Another proposal is that the translocation of HIF-α into the nucleolus represents a regulatory mechanism by which the activities of several cell-cycle-regulatory proteins are inhibited (Visintin and Amon 2000). In the nucleus, HIF-1 induces the transcription of numerous downstream target genes via their hypoxia response elements (HREs; Semenza 2010). Humans have three HIF-α genes: HIF-1α, HIF-2α and HIF-3α. Among these, HIF-1α appears to be a key regulator that modulates the cellular response to hypoxia. HIF-1α activation in malignant gliomas contributes to intense angiogenesis characterizing these tumors (Zagzag et al. 2000; Kaur et al. 2005). On the other hand, this factor represents an adaptive response for maintaining cell viability under prolonged hypoxia. Indeed, Zhang et al. (2008) demonstrated that HIF-1α-null cells generate more ROS from increased ATP production by losing the ability for mitophagy and then inducing cellular damage and death. HIF-1α is negatively regulated by HIF-3α, a member of the HIF-α family found in mammals and identified by Gu et al. (1998). In normoxia, HIF-3α competes with HIF-1α to form the heterodimer complex with ARNT and consequently, it secures the inhibition of HIF-1α activity, which, in turn, modulates angiogenesis. In accord with this evidence, the suppression of HIF-3α expression under normal cellular oxygen tension leads to an accumulation of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), which is one of the main regulators of vascularization. Previously, Parkin has been demonstrated to display tumor suppressor activity in cancer. Its expression mitigates the proliferation rate of glioma cells, both in vitro and in vivo and it reduces levels of VEGF receptor 2 (VEGFR2), promoting the suppression of tumoral angiogenesis, in particular in GBM (Yeo et al. 2012; Xu et al. 2013). Therefore, in this work, we hypothesized that the tumor suppressor effect of Parkin is exerted through a modulation of hypoxic processes predominantly present in the tumor core. To this end, we evaluated the expression of Parkin during hypoxia induced by the hypoxia-mimetic agent deferoxamine and its effect on the modulation of HIF-1α and HIF-3α by silencing its expression. Our results suggest that Parkin is downregulated under hypoxia and has an important role in this process by regulating HIF expression. Further studies are needed to elucidate the mechanism by which Parkin modulates HIF-1α and HIF-3α expression.

Materials and methods

Cell lines

This study was performed on human glioblastoma cells U87-MG (ATCCC no. HTB-14) and T98G (ATCCC no. CRL-1690) obtained from the American Type Culture Collection (ATCC, Rockville, Md., USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100-μg/ml streptomycin (Lonza, Italy) and maintained under normoxic conditions at 37 °C in a humidified atmosphere of 5 % CO_2 and 21 % O_2. Cells grown in hypoxic conditions were exposed for 24 h to 100 μM deferoxamine mesylate salt (Sigma-Aldrich, Steinheim, Germany; Fujimura et al. 2013).

Short hairpin RNA transfection of U87MG and T98G cell lines

Cells were transfected with short hairpin RNA against Parkin (Parkin shRNA Plasmid (h); Santa Cruz Biotechnology), according to the protocol provided by the manufacturer. Briefly, a total of 5×10^4 cells were seeded into each well of a 6-well tissue plate in antibiotic-free normal growth medium supplemented with FBS. The next day, when cells were 50–70 % confluent, the cells were transfected with Parkin shRNA by using the plasmid transfection reagent for 7 h. Thereafter, normal growth medium containing the double concentration of both serum and antibiotics was added for an additional 18–24 h. Approximately 48 h after transfection, cells were treated with 0.5 μg/ml puromycin for positive selection. A group of cells was transfected with shRNA Plasmid-A as a negative control (Control shRNA).

Western blot analysis

To determine the expression levels of HIF-1α, HIF-3α and Parkin proteins, Western blot analysis was performed as previously described by Maugeri et al. (2015). Briefly, proteins were extracted with a buffer containing 20 mM TRIS (pH 7.4), 2 mM EDTA, 0.5 mM EGTA, 50 mM mercaptoethanol, 0.32 mM sucrose and a protease inhibitor cocktail (Roche Diagnostics) by using a Teflon-glass homogenizer and were then sonicated twice for 20 s by using an ultrasonic probe, followed by centrifugation at 10,000*g* for 10 min at 4 °C. The concentration of the protein was determined by the Quant-iT Protein Assay Kit (Invitrogen). Each sample containing about 28 μg protein homogenate was diluted in 2× Laemmli buffer (Biorad, Carlsbad, Calif., USA), heated at 70 °C for 10 min. Proteins were separated on Biorad Criterion XT BIS-TRIS 4–15 % and then electrotransferred onto a nitrocellulose membrane (Biorad). Blots

were blocked by using the Odyssey Blocking Buffer (LI-COR Biosciences) and probed with appropriate antibodies: rabbit anti-Park2 polyclonal antibody (cat. no. PAB1105, Abnova, 1:500), mouse anti-HIF-1alpha (cat. no. NB100-105, Novus Biologicals, 1:500), rabbit anti-HIF-3alpha (H-170; cat. no. sc-28707, Santa Cruz Biotechnology; 1:200) and rabbit anti-β-tubulin (cat. no. sc-9104, Santa Cruz Biotechnology; 1:500). The secondary antibodies, namely goat antirabbit IRDye 800CW(cat. no. 926-32211, Li-Cor Biosciences) or goat antimouse IRDye 680CW (cat. no. 926-68020D, Li-Cor Biosciences) were used at dilutions of 1:20,000 and 1:30,000, respectively. Blots were scanned with the Odyssey Infrared Imaging System (Odyssey), which is highly sensitive for signals obtained both from low and from high abundance proteins detected by chemiluminescence analysis. To quantify and translate these results into values for use in statistical analysis, we performed a densitometric analysis of signals on blots by using ImageJ software (NIH, Bethesda, Md., USA; available at http://rsb.info.nih.gov/ij/index.html). The percentage of the total density of each band was normalized to the density of the relative loading control, which, in our analysis, was represented by β-tubulin. The combinatorial use of these two methods allowed us statistically to estimate the expression level of each protein detected on the blots.

Immunolocalization

To determine the cellular distribution of HIF-1α and HIF-3α proteins, immunofluorescence analysis was performed on U87MG and T98G cells as previously described by Scuderi et al. (2015). Both cell lines cultured on glass cover slips were fixed in 4 % paraformaldehyde in phosphate-buffered saline (PBS; 15 min at room temperature), permeabilized with 0.2 % Triton X-100, blocked with 0.1 % BSA in PBS and then probed with anti-HIF-1alpha (1:50) and anti-HIF-3alpha (1:50) antibody. Signals were revealed with Alexa Fluor 488 goat anti-rabbit or Alexa Fluor 488 goat anti-mouse, respectively, for 1.5 h at room temperature (shielded from light). DNA was counterstained with 4,6-diamidino-2-phenylindole (DAPI; cat. no 940110, Vector Laboratories). After a series of washes in PBS and double-distilled water, the fixed cells were cover-slipped with Vectashield mounting medium (Vector Laboratories, Burlingame, Calif., USA). Immunolocalization was analyzed by confocal laser scanning microscopy (Zeiss LSM700). Green and blue signals were detected with laser light at 488 nm/10 mW and 405 nm/5 mW, respectively and by using the objective "PLAN-APOCHROMAT" 63×/1,40 OIL DIC M27. Each scan was individually digitalized by a high sensitivity photomultiplier

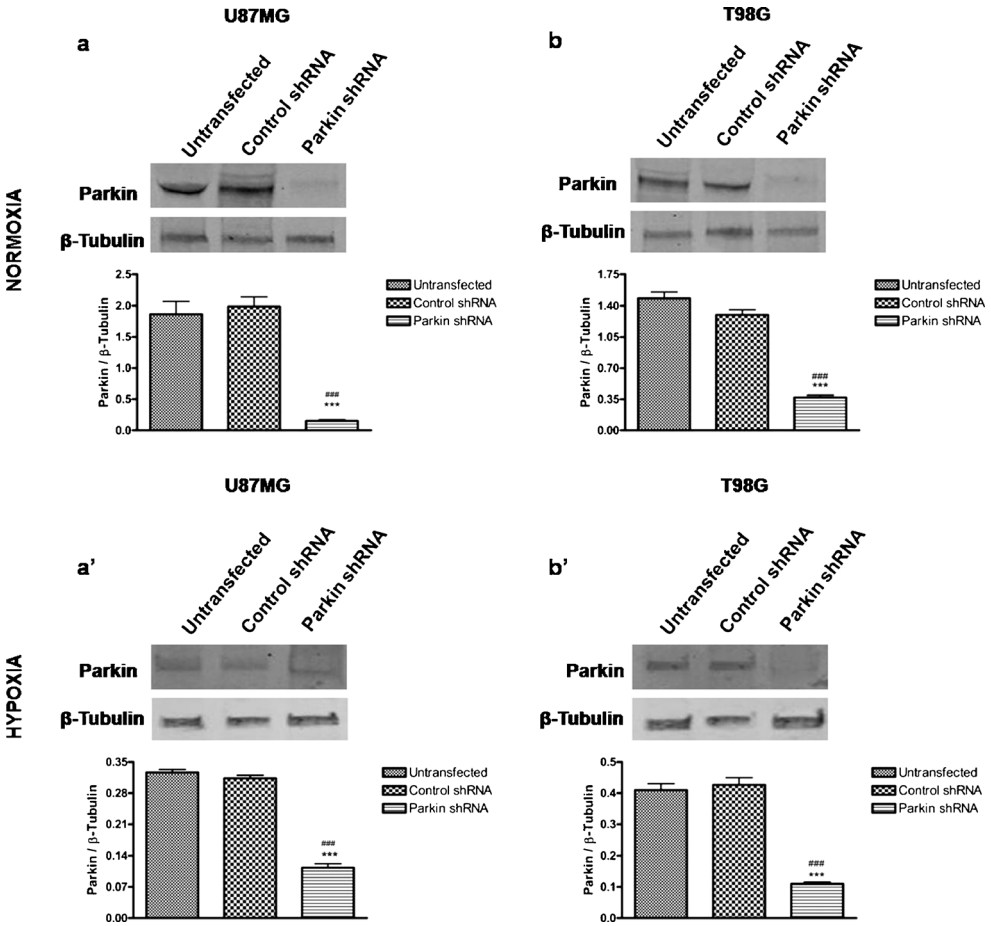

Fig. 1 Parkin silencing in glioblastoma cells. Representative immunoblots of Parkin expression in U87MG (**a**, **a'**) and T98G (**b**, **b'**) cells that were untransfected or transfected with empty vector (*Control shRNA*) or with Parkin shRNA (*Parkin shRNA*). Cells were grown under normoxic (**a**, **b**) or hypoxic condition (**a'**, **b'**) conditions. *Bar graphs* show quantitative analysis of signals obtained by immunoblots resulting from three independent experiments. Relative band densities were quantified by using ImageJ software. Protein levels are expressed as arbitrary units obtained after normalization to β-tubulin, which was used as a loading control. Data are expressed as means ± SEM. ***$P < 0.001$ vs Untransfected; ###$P < 0.001$ vs Control shRNA as determined by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test (*shRNA* short hairpin RNA)

tube by using the following acquisition setup: Gain master: 776; digital offset: −202; digital gain: 1.0. All acquisitions were performed with ZEN-2010 software.

Statistical analysis

Data are represented as means ± SEM. One-way analysis of variance was used to compare differences among groups and statistical significance was assessed by the Tukey–Kramer post hoc test. The level of significance for all statistical tests was $P \leq 0.05$.

Results

Expression of HIF-1α and HIF-3α in Parkin knock down glioma cells during normoxia or hypoxia

To study whether Parkin modulated HIF-1α and HIF-3α expression, we used shRNA transfection to knock down Parkin gene expression. As a negative control, we transfected cells with shRNA Plasmid-A (Control shRNA). Figure 1 shows our results regarding Parkin expression in two distinct cell lines before and after the silencing of this gene, in both normoxic and hypoxic conditions. Parkin protein was expressed both in untransfected and in control shRNA groups during normoxia. In hypoxia, its expression was lower compared with that in normoxia. However, Parkin expression was significantly reduced in cells transfected with shRNA Parkin construct (Fig. 1a, a', b, b').

Then, we assessed the involvement of Parkin in the hypoxic process by analyzing HIF-1α and HIF-3α expression following its silencing. As shown in Fig. 2, under normoxia, HIF-1α was weakly expressed in both cell lines, whereas HIF-3α expression was increased. The silencing of Parkin reversed the expression pattern of both proteins. Indeed, in both U87MG and T98G cells, the knock down of Parkin significantly increased HIF-1α and decreased HIF-3α expression as compared with the control (Fig. 2a, c, $^{###}P < 0.001$ vs Control shRNA; Fig. 2b, d, $^{###}P < 0.001$ vs Control shRNA). As previously demonstrated, hypoxia induced an increase of both HIF-1α and HIF-3α expression. Similar to normoxic condition, HIF-3α levels were significantly reduced in cells transfected with shRNA Parkin constructs (Fig. 3b, d, $^{###}P < 0.001$ vs Control shRNA). In contrast to the results observed in normoxia, the loss of Parkin significantly reduced

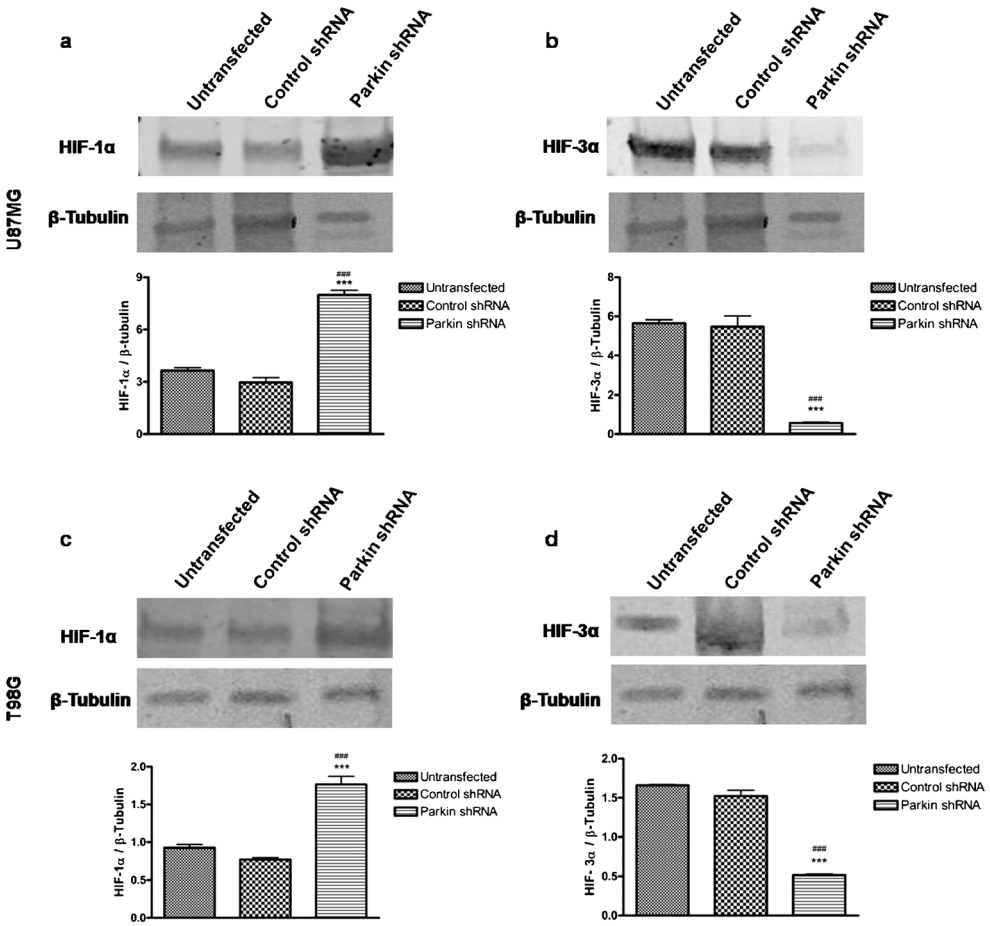

Fig. 2 Effect of Parkin knock down on HIF-1α and HIF-3α expression in glioblastoma cells during normoxia. Representative immunoblot of HIF1α and HIF-3α expression in U87MG (**a**, **b**) and T98G (**c**, **d**) cells that were untransfected or transfected with empty vector (Control shRNA) or with Parkin shRNA (Parkin shRNA) during normoxia. *Bar graphs* show quantitative analysis of signals obtained by immunoblots resulting from three independent experiments. Relative band densities were quantified by using ImageJ software. Protein levels are expressed as arbitrary units obtained after normalization to β-tubulin, which was used as a loading control. Data represent means ± SEM. ***$P < 0.001$ vs Untransfected; $^{###}P < 0.001$ vs Control shRNA, as determined by one-way ANOVA followed by the Tukey post hoc test

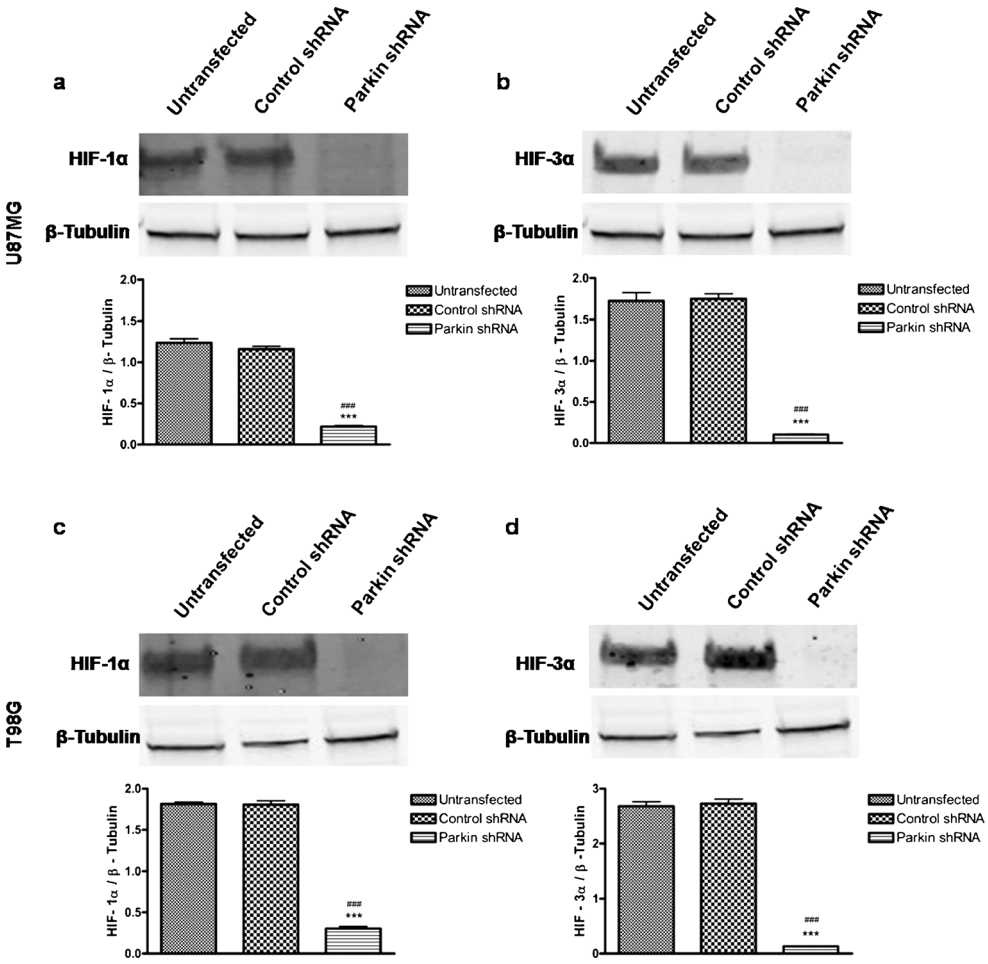

Fig. 3 Effect of Parkin knock down on HIF-1 α and HIF-3α expression in glioblastoma cells during hypoxia. Representative immunoblot of HIF1α and HIF-3α expression in U87MG (**a**, **b**) and T98G (**c**, **d**) cells that were untransfected or transfected with empty vector (Control shRNA) or with Parkin shRNA (Parkin shRNA) during hypoxia. *Bar graphs* show quantitative analysis of signals obtained by immunoblots resulting from three independent experiments. Relative band densities were quantified by using ImageJ software. Protein levels are expressed as arbitrary units obtained after normalization to β-tubulin, which was used as a loading control. Data represent means ± SEM. ***$P < 0.001$ vs Untransfected; $^{###}P < 0.001$ vs Control shRNA, as determined by one-way ANOVA followed by the Tukey post hoc test

HIF-1α expression during hypoxia (Fig. 3a, c, $^{###}P < 0.001$ vs Control shRNA). We hypothesized that cells required Parkin for HIF-1α expression during hypoxia because its loss significantly attenuated the expression of this factor (Fig. 3a, c, $^{###}P < 0.001$ vs Control shRNA).

Immunolocalization of HIF-1α and HIF-3α in Parkin knock down glioma cells during normoxia or hypoxia

We detected, by immunofluorescence analysis, the cellular distribution of HIF-1α and HIF-3α under normoxia or hypoxia following the knock down of Parkin gene expression in U87MG and T98G cells (Figs. 4, 5, 6, 7).

Under normal oxygen tension, we observed a weak cytoplasmic immunoreactivity of HIF-1α (Figs. 4b–e, 6b–e), whereas HIF-3α was highly expressed in the cytoplasm and nucleus (Figs. 4k–n, 6k–n) of both untransfected and control shRNA cells. In contrast, Parkin silencing interfered with the nuclear distribution of the two factors by enhancing HIF-1α expression (Figs. 4h, 6h) and reducing HIF-3α expression (Figs. 4q, 6q).

During hypoxia, both HIFs were highly expressed in both the cytoplasm and nucleus of control groups. However, their immunoreactivity was reduced in Parkin shRNA cells (Figs. 5h, 7q). In particular, in T98G cells that had been Parkin-silenced, HIF-3α was exclusively immunolocalized in nucleoli (Fig. 7q).

Discussion

The tumor suppressor effect of Parkin in cancer has been widely investigated in both in vitro and in vivo animal models (Yeo et al. 2012). Many studies have demonstrated that the restoration of Parkin expression in diverse Parkin-deficient cancer cells, including GBM, results in a marked decrease in their proliferation rate (Veeriah et al. 2010; Tay et al. 2010; Fujiwara et al. 2008). Furthermore, Parkin-null mice have been observed to exhibit a tendency to develop carcinoma. However, no data exist with regard to the role of Parkin in cells exposed to hypoxia, which affects some internal core regions of tumors during their growth. In gliomas, low oxygen

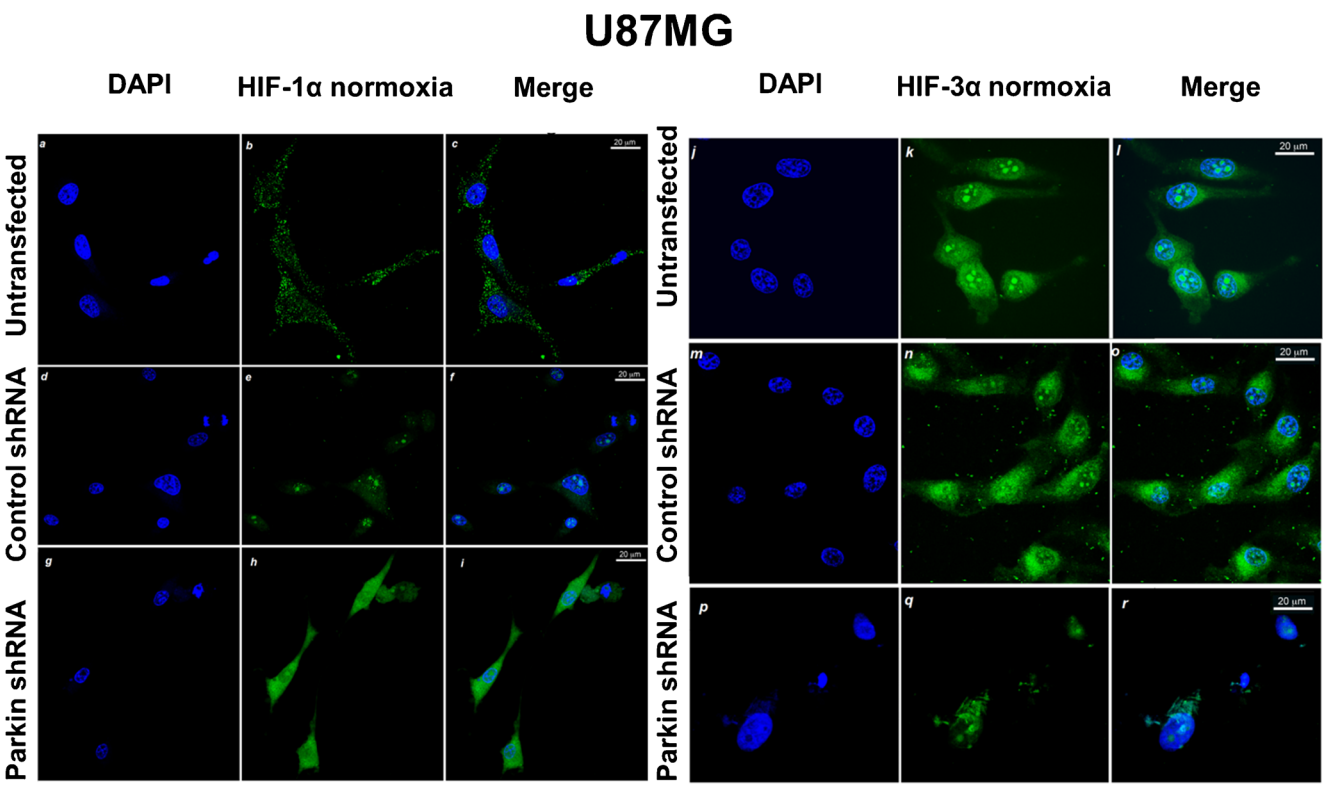

Fig. 4 Immunolocalization of HIF-1α and HIF-3α following Parkin knock down in U87MG cells during normoxia. Representative photomicrographs showing HIF-1α (**b**, **e**, **h**) and HIF-3α (**k**, **n**, **q**) expression (*green*) in U87MG cells grown in normoxic condition. Nuclei were stained (*blue*) with DAPI (**a**, **d**, **g**, **j**, **m**, **p**). Merged images (**c**, **f**, **i**, **l**, **o**, **r**). Photomicrographs are representative results of fields taken randomly from slides and scanned by confocal laser scanning microscopy (CLSM; Zeiss LSM700)

tension is associated with tissue necrosis highlighted by the presence of pseudopalisading cells (Brat et al. 2004). On the other hand, in the same region, the hypoxic process triggers angiogenesis leading to microvascular proliferation representing a mechanism for the survival of healthy cells, whereas in cancer, this event is associated with increased invasiveness. In accord with its tumor suppressor function, we demonstrated that, during hypoxia, the expression of Parkin is reduced. This condition is also associated with increased expression levels of both HIF-1α and HIF-3α as analyzed in this model.

Under normal oxygen tension, in both control groups, namely untransfected cells and cells with control shRNA, the expression of HIF-1α is very low, either in U87MG or in T98G cells. Indeed, in accordance with previous data, during this condition, HIF-1α is rapidly degraded (Semenza 2010). On the contrary, the loss of Parkin causes a significant increase of its expression. This suggests that Parkin is a negative regulator of this factor under normal oxygen tension, probably by inducing its proteasome degradation. However, Parkin seems to regulate positively the expression of HIF-3α. Unlike HIF-1α, this factor is highly expressed in the control groups, whereas its expression is absent after Parkin gene knock down. HIF-3α downregulation cancels its inhibitory effect on HIF-1α expression.

In agreement with previous results, we observed that hypoxia induces the expression of these two factors in both cell lines (Semenza 2009; Greer et al. 2012; Heidbreder et al. 2003). Indeed, the response of tumor cells to low oxygen tension is regulated via the stabilization and activation of HIFs (Barker et al. 1996). During hypoxia, low Parkin expression is associated, as predicted, with a concomitant increase of both HIF-1α and HIF-3α. The increase of the latter factor might be a result of its modulatory effect on HIF-1α expression, since it competes for binding with the same β-subunit, namely ARNT.

During hypoxia, Parkin silencing eliminates HIF-3α expression by affirming its positive regulatory role on this factor. However, contrary to predictions, the knock down of Parkin also reduces HIF-1α levels. In this condition, as shown by immunofluorescence analysis, cells are still viable, although they seem to suffer and show restricted nuclei. Therefore, we hypothesize that the hypoxic event might trigger other mechanisms. To investigate more extensively the way that Parkin affects HIF levels during hypoxia, we plan to over-express the protein under this condition. Such an approach should better clarify whether the effect observed is attributable to the relative oxygen tension level or whether it is

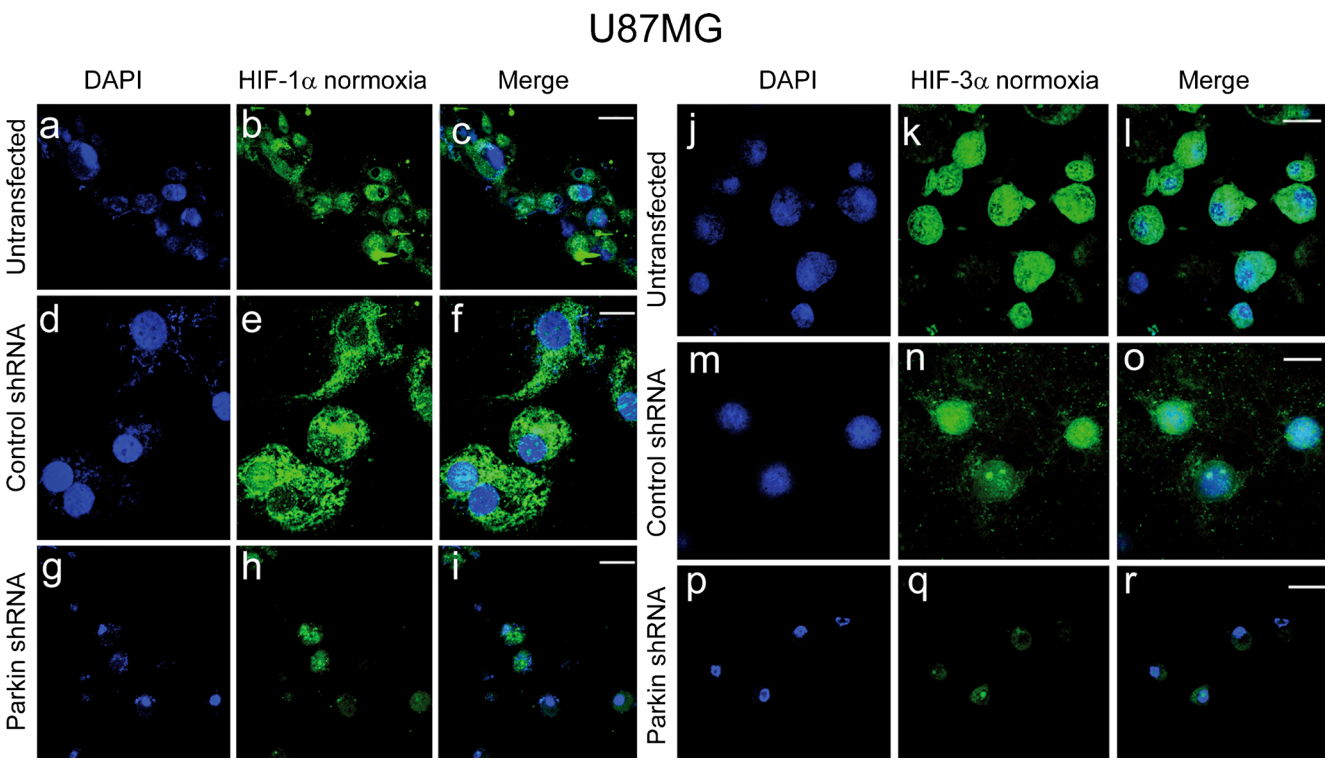

Fig. 5 Immunolocalization of HIF-1α and HIF-3α following Parkin knock down in U87MG cells during hypoxia. Representative photomicrographs showing HIF-1α (**b**, **e**, **h**) and HIF-3α (**k**, **n**, **q**) expression (*green*) in U87MG cells grown in hypoxic condition. Nuclei were stained (*blue*) with DAPI (**a**, **d**, **g**, **j**, **m**, **p**. Merged images (**c**, **f**, **i**, **l**, **o**, **r**). Photomicrographs are representative results of fields taken randomly from slides and scanned by CLSM (Zeiss LSM700)

dependent on the different Parkin levels under hypoxic or normoxic conditions.

Indeed, to date, the mechanisms underlying the regulation by Parkin on HIF-1α and HIF-3α levels under normoxic or hypoxic condition are still unknown. However, Parkin might regulate these factors by interfering with their oxygen-dependent degradation. Parkin, like other ubiquitin ligases such as von Hippel–Lindau protein (pVHL), cooperates to form the ubiquitin-proteasome system (Lee et al. 2005; Dachsel et al. 2005; Corn et al. 2003). Therefore, we hypothesize that Parkin acts similarly to pVHL. Mutation of this latter leads to von Hippel–Lindau syndrome, a rare genetic condition that increases the risk for certain malignancies (Rabinowitz 2013). During normoxia, pVHL, which is considered a tumor suppressor gene, targets HIFs for ubiquitination (Gossage et al. 2015). However, under hypoxic conditions, the HIF-α subunit is not recognized by pVHL and it consequently accumulates and dimerizes with HIF-1β (Ivan and Kaelin 2001). In accordance, the loss of function of pVHL is associated with HIF-α accumulation (Rabinowitz 2013).

Similarly, we assume that Parkin, like pVHL, plays a role as a tumor suppressor gene by mediating HIF-1α proteasomal degradation. The decrease of HIF-1α in malignant gliomas might contribute to the reduction of the intense angiogenesis that is typical of these tumors. However, such an action might also be performed indirectly through the positive modulation of HIF-3α, which competes with HIF-1α for binding to the β subunits. Moreover, previous data have demonstrated that, under normoxia, the suppression of this latter factor leads to an accumulation of pro-angiogenic factors, such as VEGF (Maynard and Ohh 2004).

Under low oxygen tension, cancer cells adapt to hypoxia. In this condition, HIF-1α, a key regulator of angiogenesis, is significantly increased (Semenza 2007). We hypothesize that Parkin enacts its tumor suppressor function through HIF-3α upregulation. This event, in turn, interferes with HIF-1α overexpression and therefore with the tumoral progression mediated by angiogenesis. Although we consider that Parkin acts similarly to pVHL, we cannot rule out that such mechanisms are mediated through an interaction between these genes. However, data regarding an association between Parkin and pVHL have not as yet been reported.

The loss of Parkin interferes with the cellular distribution of these proteins. Its silencing in normoxia induces HIF-1α accumulation in the nucleus, an event that usually occurs only during hypoxic processes. Nuclear translocation of HIF-1α induces the transcription of several genes. In this condition, however, Parkin knock down reduces the HIF-3α immunoreactivity both in the cytoplasm and in the nucleus as compared with controls. This suggests that Parkin positively affects HIF-

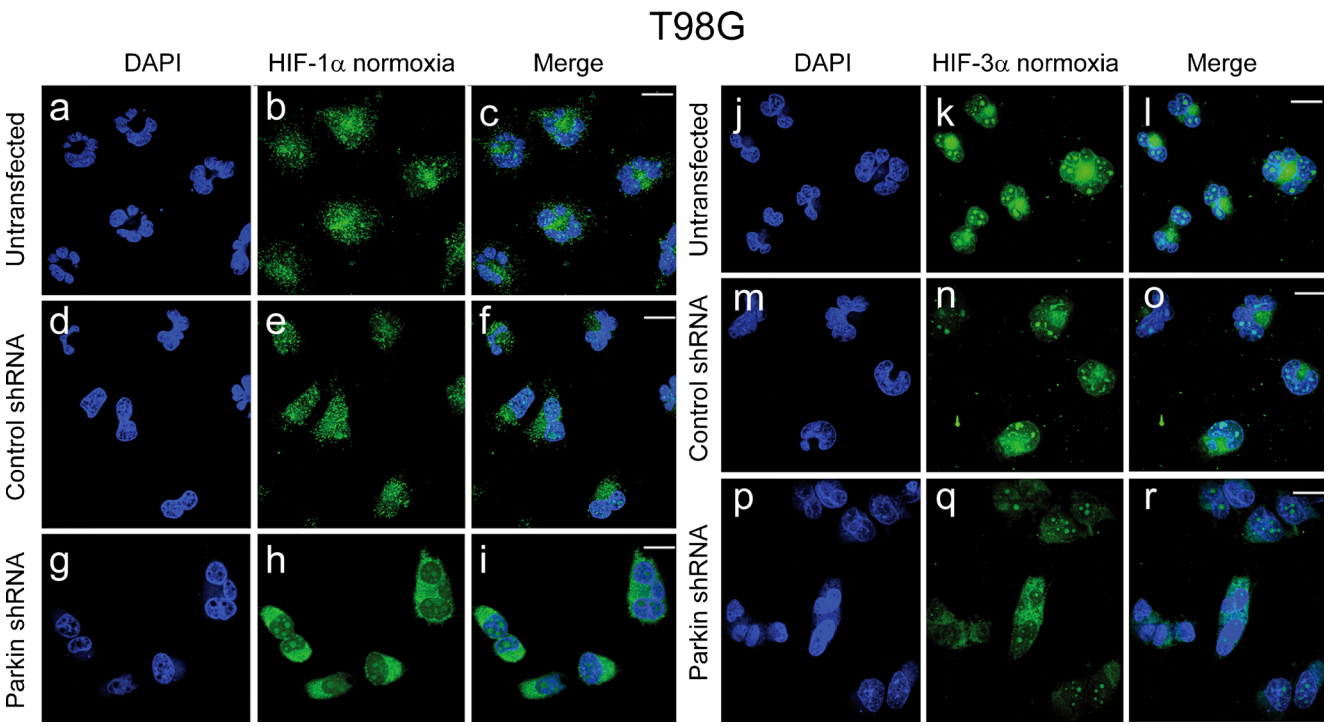

Fig. 6 Immunolocalization of HIF-1α and HIF-3α following Parkin knock down in T98G cells during normoxia. Representative photomicrographs showing HIF-1α (**b**, **e**, **h**) and HIF-3α (**k**, **n**, **q**) expression (*green*) in T98G cells grown in normoxic condition. Nuclei were stained (*blue*) with DAPI (**a**, **d**, **g**, **j**, **m**, **p**). Merged images (**c**, **f**, **i**, **l**, **o**, **r**). Photomicrographs are representative results of fields taken randomly from slides and scanned by CLSM (Zeiss LSM700)

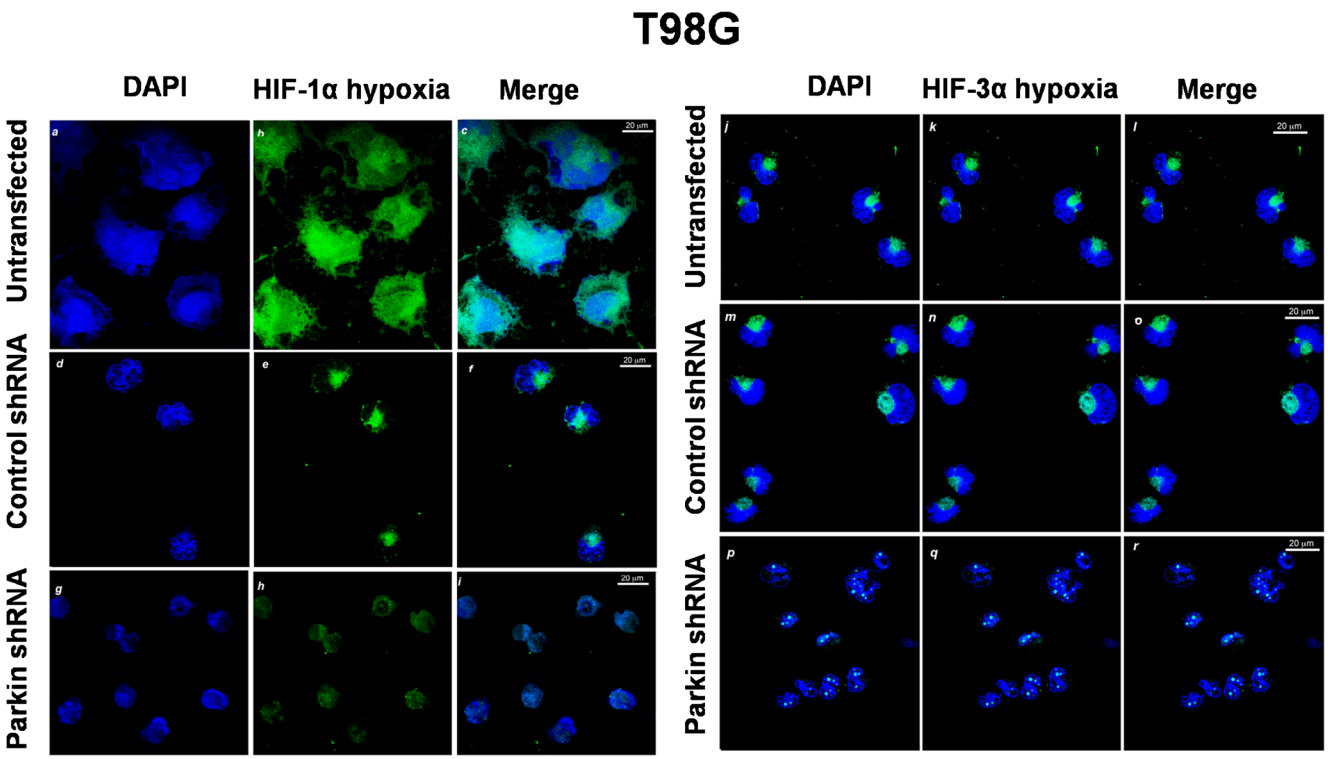

Fig. 7 Immunolocalization of HIF-1α and HIF-3α following Parkin knock down in T98G cells during hypoxia. Representative photomicrographs showing HIF-1α (**b**, **e**, **h**) and HIF-3α (**k**, **n**, **q**) expression (*green*) in T98G cells grown in hypoxic condition. Nuclei were stained (*blue*) with DAPI (**a**, **d**, **g**, **j**, **m**, **p**). Merged images (**c**, **f**, **i**, **l**, **o**, **r**). Photomicrographs are representative results of fields taken randomly from slides and scanned by CLSM (Zeiss LSM700)

3α expression, since its absence interferes with the expression of this factor in both cellular compartments.

During hypoxic conditions, Parkin silencing is associated with reduced immunoreactivity for both factors in the cytoplasmic and nuclear compartments, confirming data obtained by Western blot analysis. Furthermore, in T98G cells that are Parkin-silenced, HIF-3α is exclusively immunolocalized in nucleoli.

In conclusion, these data suggest a regulatory role of the Parkin gene on HIF expression related to cellular oxygen tension. However, further studies are needed to clarify the mechanisms by which Parkin modulates their expression.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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