ANTICANCER RESEARCH *41*: 113-122 (2021)
doi:10.21873/anticanres.14756

Effect of Hypoxia on Proliferation and the Expression of the Genes HIF-1α and JMJD1A in Head and Neck Squamous Cell Carcinoma Cell Lines

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Abstract. *Background/Aim: The aim of the study was to investigate the effects of hypoxia on proliferation and the expression of HIF-1α (hypoxia-inducible factor 1 alpha) and JMJD1A (jumonji domain 1A) in head and neck squamous cell carcinoma (HNSCC). Materials and Methods: FaDu and HLaC78 cells were incubated for 1-24 h in hypoxia and normoxia. Cell proliferation, mRNA and protein levels of HIF-1α and JMJD1A were quantified by counting, PCR and western blot. Results: Hypoxia led to a constant decrease in cell proliferation. Short hypoxia resulted in an increase in HIF-1α mRNA levels. This effect was reversed after longer incubation. The western blot for HIF-1α showed a maximum accumulation after 3-6 h of hypoxia. In FaDu cells, the concentration of JMJD1A reached a peak after 6 h and decreased thereafter, whereas in HLaC78 cells, it presented a second peak after 48 h. Conclusion: The transcription factors HIF-1α and JMJDA1 were confirmed as relevant hypoxia-dependent regulators of carcinogenesis in HNSCC.*

Oxygen homeostasis is crucial for the survival of mammalian cells. Oxidative phosphorylation provides the required energy supply as adenosine triphosphate. Hypoxia is intolerable for most of the cells (1). It plays an important role in the pathology of different diseases including ischemic stroke (2). Atherosclerosis, thrombosis or an embolism, result in decreased perfusion and impairment of the affected brain tissue (3). However, a heterogeneous distribution in oxygen concentration is crucial for the progression of many malignant tumors. Hypoxic intratumoral areas are caused by an inadequate oxygen supply towards the rapidly proliferating tumor cells (4). In comparison to the healthy tissue of the neck, untreated head and neck squamous cell carcinomas (HNSCC) are clearly less oxygenated in certain areas (5).

The transcription factor hypoxia-inducible factor (HIF) is an important stimulant of malignant progression of tumors and physiological and pathological adaptations (6). HIF is a heterodimer with an oxygen-dependent α subunit and a constitutively expressed β subunit. There are three known subunits 1α, 2α and 3α, while the latter is not ubiquitously expressed and underexplored (7). HIF-1α has a molecular weight of 120 kDa and a half-life of only a few minutes in normoxia. It is stabilized during continuous hypoxia and can accumulate in the nucleus (8). HIF-1β has a molecular weight of 91-94 kDa and is constitutively expressed in the nucleus. Stable HIF-1α accumulates in the cytosol and translocates to the nucleus where it dimerizes with HIF-1β and can bind the hypoxia response element (HRE) of target genes (9).

Furthermore, immunohistochemistry shows an upregulation of HIF in primary and metastatic tumors (10, 11). Etiologically, low oxygenation leads to necrosis in certain tumor areas, which are diffusely distributed and surrounded by normoxic areas. Moreover, in HNSCC, a clearly lower oxygenation of 15 mmHg could be shown compared to healthy tissue with 44 mmHg (5). Other causes for an upregulation of HIF in tumors could be the loss of function of tumor suppressor genes like *p53* or von Hippel-Lindau *via* a decreased degradation of HIF-1α. Clonal selection of cancer cells with an increased HIF-1α activity then leads to the malignant progression of the tumor (12). Tumor hypoxia interferes with the limited success of tumor therapies, by the decreased levels of chemotherapeutics and the intrinsic radiation resistance of hypoxic areas (13). A candidate to overcome the challenges of hypoxia in chemotherapy is tirapazamine, which releases cytotoxic radicals in hypoxia (14). In combination with cisplatin and radiotherapy, tirapazamine achieved a better tumor control

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Key Words: Head and neck squamous cell carcinoma (HNSCC), hypoxia, FaDu, HLaC78, HIF-1α, JMJD1A, proliferation.

in stage III or IV squamous cell carcinoma of the head and neck (15). For an ideal result, the pre-therapeutic measurement of tumor oxygenation seems to be of clinical relevance. Exogenic markers like pimonidazole and 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide (EF5) seemed to be promising. Evans and colleagues showed a high heterogeneity of binding in HNSCC specimens for EF5 (16). Since a pre-therapeutic application of such markers is not practical, studies are focused on the identification of endogenous markers. Janssen and colleagues investigated immunohistochemical parameters of tumor oxygenation and perfusion in HNSCC. They concluded that HIF-1α might not be suitable as a marker for chronic hypoxia, since its expression did not correlate well with pimonidazole staining (17).

HIF-inducible genes, which influence cellular mechanisms like angiogenesis, energy metabolism, proliferation, and apoptosis could be of interest as endogenous markers for hypoxia (18). One of the more than hundred HIF-inducible genes is the Jumonji domain-containing protein 1A (JMJD1A) (19). It was isolated in 1991 from testicular tissue and named testis-specific gene A (20). Later, the synonyms lysine-specific demethylase 3A (KDM3A) and JMJD1A were used. The gene consists of 25 exons and expresses its eponymic protein with a molecular weight of 147 kDa, which is located mostly in the nucleus and partly in the cytoplasm (21). It is characterized by its Jumonji C domain, which induces morphological changes in embryogenesis (22). JMJD1A's oxygen-dependent and HIF-mediated expression could be shown both *in vitro* and *in vivo* (23). Its demethylase function can regulate the transcription of further genes, which amplify HIF effects and thus promotes the proliferation and adaption of tumors towards their hypoxic microenvironment (24).

To date, the effects of hypoxia on HIF and JMJD1A, especially in HNSCC, remain unclear. Our aim was to explore the expression of the genes *HIF-1α* and *JMJD1A* and the tumor cell proliferation in HNSCC under hypoxia. A better understanding of the underlying mechanisms could help to improve strategies for cancer treatment in the future.

Materials and Methods

Tumor cell incubation. The HNSCC cell lines FaDu and HLaC78 were obtained by ATCC (*via* LGC Standards, Wesel, Germany)(25, 26). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS and 1% penicillin/streptomycin (Sigma-Aldrich, Schnelldorf, Germany), and cultured at 37˚C with 5% CO_2 in culture flasks. Every other day the medium was replaced. After reaching 70-80% confluence, cells were trypsinized with 0.25% trypsin (Gibco Invitrogen, Karlsruhe, Germany), washed with phosphate-buffered saline (PBS) (Roche Diagnostics GmbH, Mannheim, Germany) and seeded in new culture flasks or treatment wells. Experiments were performed using cells in the exponential growth phase.

Tumor cell proliferation. Tumor cells were first seeded on culture plates and incubated for 24 h at 37˚C, 21% O_2 and 5% CO_2. Thereafter, 6 plates (N_0, N_1, N_3, N_6, N_{24} and N_{48}) were incubated in normoxia, 5 plates were incubated in hypoxia at 37˚C with 5% CO_2 and 1% O_2 (H_1, H_3, H_6, H_{24} and H_{48}) for 1, 3, 6, 24 and 48 h, respectively. The supernatant was removed, cells were trypsinized with 0.25% trypsin, DMEM with supplements was added, and cells were counted electronically (Casy® Technologies, Innovatis AG, Reutlingen, Germany).

Quantitative Real-Time PCR. Harvesting of cells for Real-time polymerase chain reaction (rt-PCR) was performed on ice to prevent degradation of *HIF-1α* mRNA. After removing the supernatant, 5ml PBS were added and cells were scratched, resuspended and centrifuged for 5 min at 4˚C and 1500 rpm. RNA was extracted using a RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically (Eppendorf AG, Hamburg, Germany). Synthesis of cDNA was made from 50 ng of RNA using SuperScript VILO Mastermix (Thermo Fisher Scientific, Waltham, MA, USA) at 42˚C. Two microliters of template cDNA were added to a final volume of 20 μl of reaction mixture consisting of 10 μl TaqMan Gene Expression Master Mix, 1 μl of the according TaqMan Gene Expression Assays (assay ID for *HIF-1α* Hs00153153_m1 and for *KDM3A*/*JMJD1A* Hs00218331_m1, both Thermo Fisher Scientific) and 7 μl RNAse free water. PCR cycle parameters included a first step at 50˚C for 2 min, enzymatic activation for 10 min at 95˚C followed by 40 cycles involving denaturation at 95˚C for 15 sec and amplification at 60˚C for 1 min. Relative gene expression levels of *HIF-1α* and *KDM3A*/*JMJD1A* were quantified with the fluorescent TaqMan® technology. GAPDH (Hs02758991_g1, Thermo Fisher Scientific) was used as an endogenous control to normalize the amount of sample RNA. Each sample was measured in triplicate and the comparative Ct method was used for relative quantification of gene expression as described (27). Data were expressed as the mean of three independent experiments.

Western blot. Harvesting of cells for western blot was performed on ice to prevent degradation of HIF-1α protein. After removing the supernatant, Laemmli buffer (100 mM Tris-HCl pH 6.8, 20% glycerol, 4% sodium dodecyl sulfate, 1% 2-mercaptoethanol and 0.002% Bromphenol blue) was added for homogenization. Cell lysates were heated at 95˚C for 10 min and centrifuged after cooling down. Supernatants were used for western blot analysis. 25 μg of total protein was electrophoresed and transferred to a nitrocellulose membrane. After washing in TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0,1% Tween 20) for 5 min membranes were blocked for 60 min with blocking buffer (5% nonfat dry milk, TBST). Afterwards, membranes were washed in TBST three times for 5 min and incubated with the primary antibody (pAb) at 4˚C overnight at the following dilutions: anti-β-actin pAb 1:5000 (Cat. No. 610958, BD Biosciences, Franklin Lakes, NJ, USA), anti-HIF-1α pAb 1:1000 (Cat. No. AF6746, Bio-Techne, Minneapolis, MN, USA) and anti-JMJD1A pAb 1:500 (Cat. No. MA5-15739, Thermo Fisher Scientific). After three washing steps with TBST membranes were incubated for 1 h with horse radish peroxidase-conjugated anti-mouse (Cat. No. 31437, Thermo Fisher Scientific) or anti-sheep (Cat. No. HAF016, Bio-Techne) IgG at a dilution of 1:10000 or 1:1000, respectively and were finally developed using the SuperSignal West Femto Maximum Sensitivity Substrate Enhanced

Chemiluminescence (Thermo Fisher Scientific). The signals were then transferred to an X-ray film. Protein expression was quantified by densitometry (ImageJ software, NIH, Bethesda, MD, USA) from scanned western blots normalized to the actin signal.

Statistical analysis. All data were transferred to standard spreadsheets and analyzed by statistical analysis (GraphPad Prism 6.07 Software, La Jolla, California, USA). Since we assessed the effects of multiple factors (different degrees of oxygenation for different incubation times), 2-way ANOVA was performed to evaluate statistical significance. As a correction for multiple testing, Tukey's multiple comparison test was performed. Differences were considered statistically significant when the *p*-value was <0.05, and significant differences are indicated by an asterisk.

Results

Tumor cell viability and proliferation

Viability. The viability of FaDu and HLaC78 cells was stable between 85 and 90 percent in hypoxia and normoxia for at least 24 h. After 48 h in hypoxia viability of HLaC78 decreased (N_{48}: 84.76±7.21%; H_{48}: 72.32±8.38%) more distinctly than that of FaDu cells (N_{48}: 89.99±2.12%; H_{48}: 84.30±1.11%). Differences were not significant (FaDu: p=0.86 for different incubation times and p=0.50 for different levels of oxygenation; HLaC78: p=0.10 for different incubation times and p=0.12 for different levels of oxygenation, Figure 1).

Proliferation. FaDu cell proliferation was low in the first h under both in normoxia and hypoxia. After 24 h, proliferation increased more intensely in normoxia (198±19%) than in hypoxia (160±15%). After 48 h, differences in proliferation increased to 366±76% in normoxia and 230±71% in hypoxia. Differences were statistically significant for an incubation of 48 h in normoxia (p<0.0001 for different incubation times and p=0.11 for different levels of oxygenation by 2-way ANOVA, with $p(N_0/N_{48})$=0.0002 and $p(N_{24}/N_{48})$=0.04 after correction for multiple testing). HLaC78 cell proliferation showed a higher increase after 24 h to 257±120% in normoxia and only 138±61% in hypoxia. After 48 h, the proliferation benefit in normoxia increased to 347±122% compared to 125±30% in hypoxia. Differences were not significant (p=0.12 for different incubation times and p=0.08 for different levels of oxygenation, Figure 2).

Real-time PCR

HIF-1α mRNA. For FaDu cells in hypoxia, *HIF-1α* mRNA levels increased after 1 h (H_1:111±9%) and continuously decreased to about a fourth after 48 h (H_{48}: 28±15%). For FaDu cells in normoxia, *HIF-1α* mRNA levels were stable between 88 and 109%. Differences were statistically significant (p=0.0008 for different incubation times and p=0.0006 for different levels of oxygenation by 2-way ANOVA, with $p(H_0/H_{24})$=0.02, $p(H_0/H_{48})$=0.005 and $p(N_{48}/H_{48})$=0.001 after correction for multiple testing).

For HLaC78 cell in hypoxia, *HIF-1α* mRNA levels continuously decreased even more to about a fifth after 48 h (H_{48}:18±1%). For HLaC78 cells in normoxia, *HIF-1α* mRNA levels alternated between a decrease after 24 h (N_{24}: 79±25%) and an increase after 48 h (N_{48}: 199±61%). Differences were statistically significant (p=0.20 for different incubation times and p=0.001 for different levels of oxygenation by 2-way ANOVA, with $p(N_{24}/N_{48})$=0.049 and $p(N_{48}/H_{48})$=0.0007 after correction for multiple testing, Figure 3).

JMJD1A mRNA. For FaDu cells in hypoxia, *JMJD1A* mRNA levels nearly doubled after 3 h (H_3:184±39%), further increased after 6 h (H_6: 361±47%) and slightly decreased after 24 and 48 h (H_{24}: 332±61%; H_{48}: 273±59%). For FaDu cells in normoxia, *JMJD1A* mRNA levels were stable between 88 and 108% until 24 h and increased to 154±17% after 48 h. Differences were statistically significant (p=0.0004 for different incubation times and p<0.0001 for different levels of oxygenation by 2-way ANOVA, with $p(N_{24}/H_{24})$=0.001, $p(H_0/H_6)$=0.0002, $p(H_0/H_{24})$=0.001 and $p(H_0/H_{48})$=0.023 after correction for multiple testing).

For HLaC78 cells in hypoxia, *JMJD1A* mRNA levels continuously increased threefold after 6 h (H_1: 145±21%; H_3: 193±57%; H_6: 271±63%) and reached a maximum after 48 h (H_{48}:554±172%). For HLaC78 cells in normoxia, *JMJD1A* mRNA levels alternated more than in FaDu cells with a decrease to about a half after 3 h (N_3: 49±18%) and about a twice after 48 h (N_{48}: 191±34%). Differences were statistically significant (p=0.002 for different incubation times and p=0.001 for different levels of oxygenation by 2-way ANOVA, with $p(N_{48}/H_{48})$=0.012 and $p(H_0/H_{48})$=0.001 after correction for multiple testing, Figure 4).

Western blot

HLaC78. For HLaC78 cells in hypoxia, HIF-1α protein showed a maximum after 6 h and decreased after longer incubation times. In normoxia, there were only weak signals for HIF-1α after 6 and 24 h. JMJD1A protein showed signals in all conditions. While it decreased in normoxia to a minimum after 48 h, and was mostly constant in hypoxia (Figure 5).

FaDu. For FaDu cells in normoxia, HIF-1α protein showed a slight increase of the weak signal, while in hypoxia it reached a maximum after 3 and 6 h and decreased after 24 and 48 h. JMJD1A protein increased in hypoxia to a maximum after 48 h. In normoxia, there was a steadily weak signal (Figure 6).

Discussion

The role of hypoxia has been described for many malignant tumors. Vaupel and colleagues showed that 50-60% of locally progressed tumors show hypoxic or anoxic

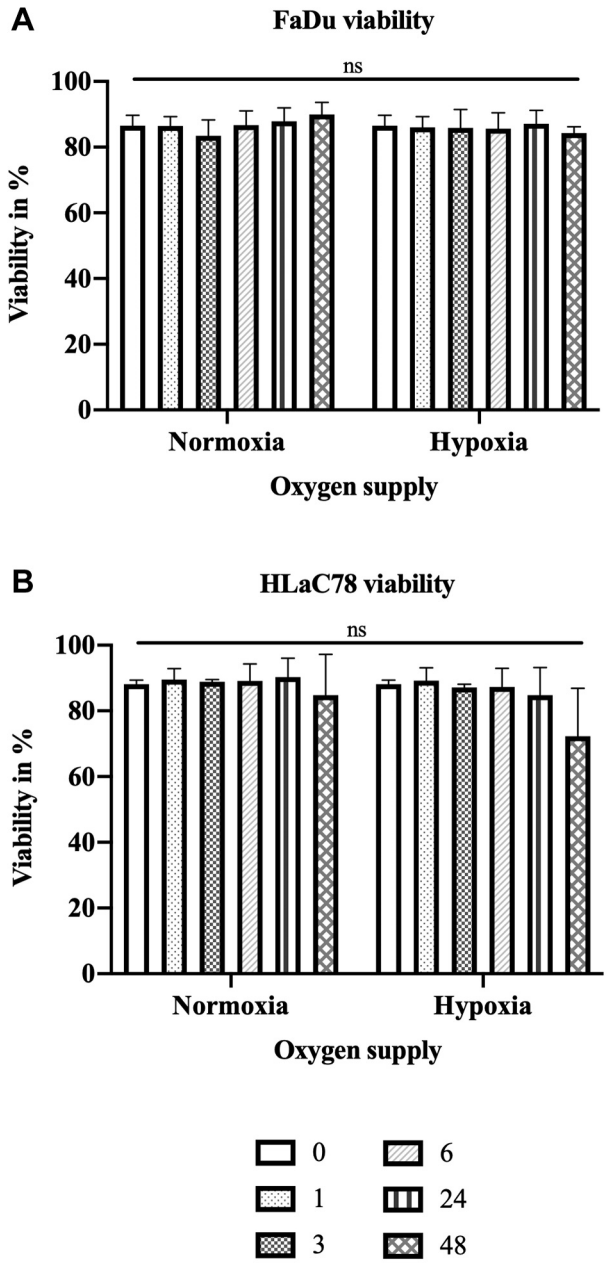

Figure 1. *Tumor cell viability in normoxia and hypoxia. The viability of (A) FaDu and (B) HLaC78 cells ranged between 85 and 90% in hypoxia and normoxia for at least 24 h. After 48 h in hypoxia, viability of HLaC78 cells decreased more distinctly compared to FaDu cells. The experiments were independently performed three times, and the lines on the graph show the arithmetic mean with the standard deviation. Differences were not statistically significant (ns: nonsignificant).*

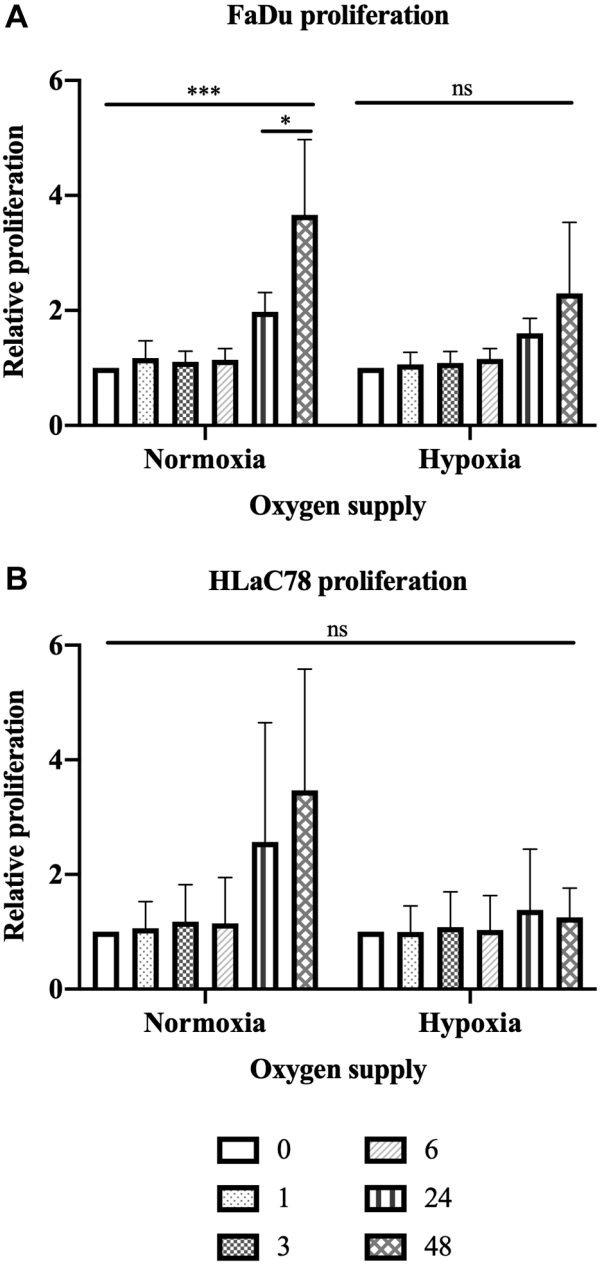

Figure 2. *Tumor cell proliferation in normoxia and hypoxia. Proliferation of (A) FaDu and (B) HLaC78 cells was low in the first 6 h in normoxia and hypoxia. After 24 and 48 h, proliferation increased more intensely in normoxia than in hypoxia for both tumor cells. The experiments were independently performed three times, and the lines on the graph show the arithmetic mean with the standard deviation. Differences in (A) were statistically significant (ns: nonsignificant, *p<0.05, **p<0.01, ***p<0.001).*

areas (28). Brizel and colleagues showed that the oxygenation of the primary tumor is a prognostic factor in HNSCC (29). They determined the oxygenation of primary tumors pre-therapeutically and chose a partial pressure of oxygen of 10 mmHg as threshold for hypoxia. The disease-free survival after one year was 78% for patients with

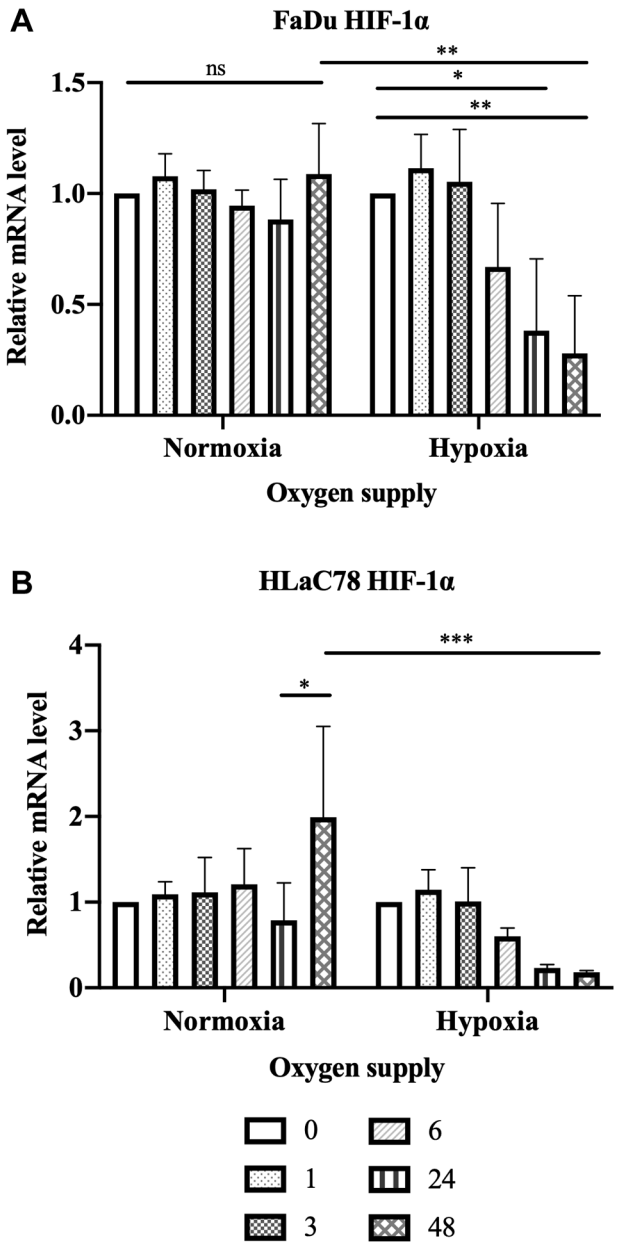

Figure 3. *HIF-1α gene expression in normoxia and hypoxia. (A) In FaDu cells, HIF-1α mRNA slightly increased after 1 h and continuously decreased to about 25 percent after 48 h in hypoxia, while it was stable in normoxia. (B) In HLaC78 cells, HIF-1α mRNA decreased to about 20 percent after 48 h in hypoxia, while it strongly alternated in normoxia. The experiments were independently performed three times, and the lines on the graph show the arithmetic mean with the standard deviation. Differences were statistically significant (ns: nonsignificant, *p<0.05, **p<0.01, ***p<0.001).*

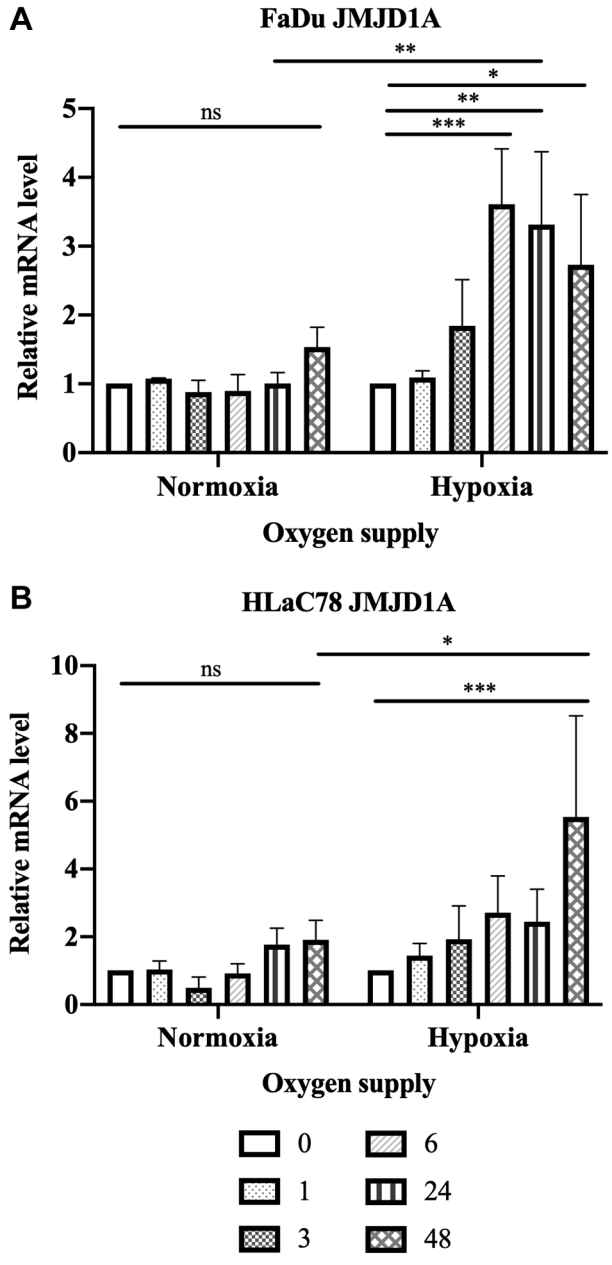

Figure 4. *JMJD1A gene expression in normoxia and hypoxia. (A) In FaDu cells, JMJD1A mRNA increased until 6 h in hypoxia and decreased afterwards, while it was stable in normoxia. (B) In HLaC78 cells, JMJD1A mRNA continuously increased in hypoxia, while it strongly alternated in normoxia. The experiments were independently performed three times, and the lines on the graph show the arithmetic mean with the standard deviation. Differences were statistically significant (ns: nonsignificant, *p<0.05, **p<0.01, ***p<0.001).*

tumors, which had an oxygenation of more than 10 mmHg in contrast to 22% for an oxygenation of less than 10 mmHg. Especially, if radiotherapy is planned, tumor oxygenation plays a relevant role. Nordsmark and colleagues showed in 31 patients that the local tumor control probability after two years was significantly higher

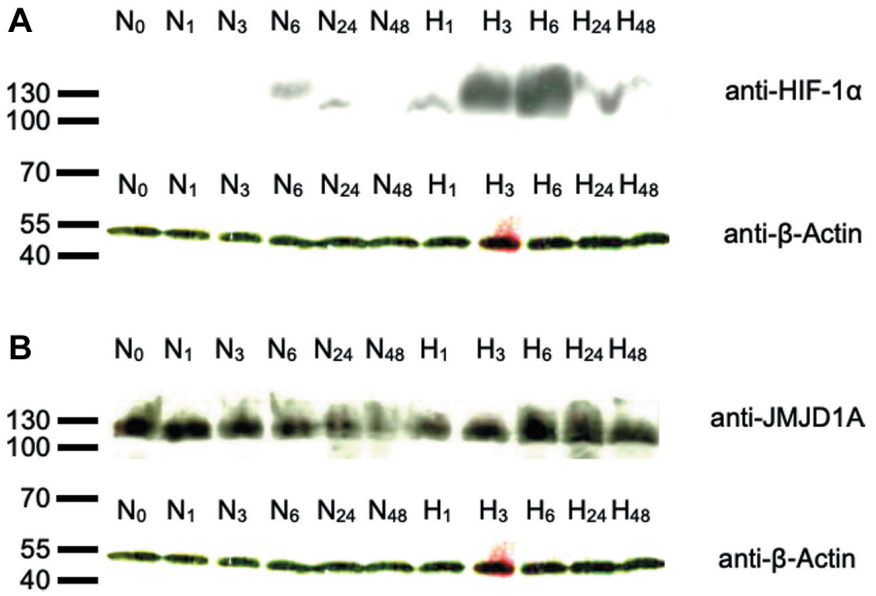

Figure 5. *HIF-1α and JMJD1A protein levels in HLaC78 cells, in normoxia and hypoxia. For HLaC78 cells, (A) HIF-1α protein showed a maximum after 6 h and decreased after longer incubation times in hypoxia. In normoxia, there were only weak signals for HIF-1α after 6 and 24 h. (B) JMJD1A protein showed signals in all conditions. While it decreased in normoxia to a minimum after 48 h, it was mostly constant in hypoxia.*

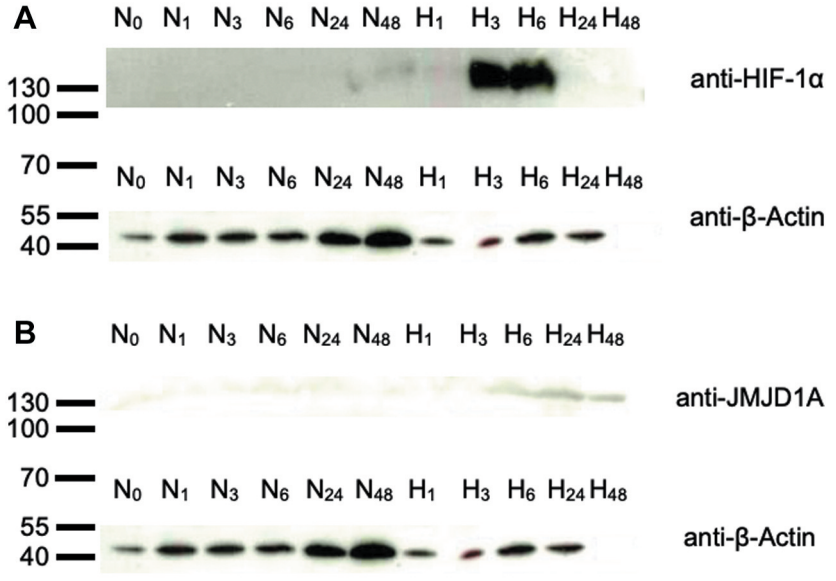

Figure 6. *HIF-1α and JMJD1A protein levels in FaDu cells, in normoxia and hypoxia. For FaDu cells, (A) HIF-1α protein showed a slight increase of the weak signal in normoxia, while in hypoxia it reached a maximum after 3 and 6 h and decreased after 24 and 48 h. (B) JMJD1A protein increased in hypoxia to a maximum after 48 h. In normoxia, there was a steadily weak signal.*

(90%) in normoxic tumors as compared with the hypoxic subgroup (45%) (30). Stadler and colleagues showed in a collective of 59 patients that higher hypoxic tumor subvolume negatively influenced the overall survival of HNSCC patients, regardless of the primary therapy modality (31). Later, an international multi center survey with 397 patients confirmed the impact of tumor hypoxia on the prognosis of HNSCC in a larger collective (32).

A characterization of tumors showed that hypoxia induces a clonal selection, which favors tumor cells that adapt their gene expression best to an aggravated environment. The resulting tumors show a malignant progression to an aggressive phenotype with a low differentiation, increased angiogenesis, invasive growth pattern and high potential of metastatic spread (4). HIF-1 and its subunit α seem to have an important function in this development as they are highly expressed in many malignant tumors like bladder, prostate, colon, lung, gastric, breast, renal, ovarian and pancreatic cancer (10, 11).

However, the aim of this work was to determine the expression levels of HIF-1α in the pharyngolaryngeal tumor cell lines FaDu and HLaC78 as well as their proliferation properties in hypoxia. Both cell lines showed reduced viability and proliferation in hypoxia after 24 and 48 h. Similar results were obtained in different human carcinomas under prolonged hypoxia (33). Immunohistochemical staining in human tumor xenograft systems showed a reduction of tumor cell proliferation in hypoxia. Co-staining with pimonidazole as a hypoxia marker and bromodeoxyuridine as a proliferation marker identified hypoxic and nonproliferative cells in different sites inside the tumor (34). Thus, there is a small number of proliferative cells in hypoxic areas. Also, HNSCC xenografts showed overlapping areas of cells positive for hypoxia and proliferation markers (35).

This study showed an early increase and subsequently a steady decrease of *HIF-1α* mRNA to 28 and 18 percent for FaDu and HLaC78 cells, respectively, after 48 h of hypoxia. Western blot showed an accumulation of HIF-1α after 3 and 6 h, which declined after longer incubation under hypoxic conditions. Other studies showed an accumulation of HIF-1α in FaDu cells already after 1 h in hypoxia (36). This confirms the rapid stabilization of HIF-1α within a few h and could indicate degradation of HIF-1α after longer incubation. The decline of *HIF-1α* mRNA after more than 3 h could demonstrate a secondary event of a reduced gene expression. One possible explanation is the glucose dependency of HIF-1α, as suggested by Vordermark and colleagues (37). It might be that necessary substrates are depleted after longer hypoxic incubation. Yet, this is in contrast with the increase of HIF-1α mRNA after 48 h in normoxia in this study. An explanation for this contradiction could be that the rapidly proliferating cells consumed other essential nutrients in the culture medium, while the glucose concentration is still sufficient for an accumulation of HIF-1α. The nutrient deficit might induce cellular stress and subsequently trigger an increased expression of HIF-1α. For FaDu cells, however, Vordermark and colleagues showed that other factors of the tumor microenvironment like pH and growth factors do not influence the accumulation of HIF-1α.

This study shows hints for a decrease of HIF-1α after prolonged hypoxia in FaDu and HLaC78 cells. These results have to be confirmed in further studies. One objective is to prevent an intracellular accumulation of HIF-1α in different ways. For example, a gene silence using the antisense technique has been shown to down-regulate HIF-1α expression (38). Furthermore, there are possible pharmacological targets. The international hypoxia conference of 2003 has named two promising drugs: geldanamycin, which can directly inhibit HIF-1α, and topotecan, which inhibits HIF-1α indirectly *via* inhibition of topoisomerase I (39). The anti-cancer drug tirapazamine shows cytotoxic activity in hypoxia (40). This effect has shown therapeutic effects on non-small cell lung carcinoma and nasopharynx carcinoma (14, 41). Another possibility is to use hypoxia in gene therapy. Obligate anaerobes like Clostridia can target hypoxic and necrotic areas and express anticarcinogenic substances (42). Further studies have to expand on those findings and continue characterizing HNSCC in hypoxia.

To evaluate its interaction with HIF-1α in HNSCC, this study also investigated the role of JMJD1A. Its demethylase activity is oxygen-dependent but is only inhibited by hypoxia below 0.2 percent of oxygen concentration while it is fully active in hypoxia of one percent. This oxygen concentration leads to an intracellular accumulation of HIF-1α and activation of its target genes like *JMJD1A* (43). In contrast to other target genes, the activation of *JMJD1A* is specific for HIF-1α while it is not activated by isoforms like HIF-2 (44). This specificity does not seem to be accidental, JMJD1A interacts with HIF-1α by forming a complex and activates its enzymatic function of regulating further target genes in early hypoxia (45).

Compared to HIF-1α, this study showed a higher induction of *JMJD1A* mRNA. In FaDu cells, relative gene expression increased threefold after 6 h and decreased after longer hypoxia. HLaC78 cells showed a second peak with a fivefold JMJD1A expression after 48 h. Western blot analysis revealed nearly constant levels of JMJD1A protein in hypoxia for HLaC78 cells while it accumulated in FaDu over time. Taken together, this indicates that JMJD1A plays a role in early and late stages of hypoxia.

For tumors other than HNSCC, the role of JMJD1A in carcinogenesis has already been investigated. In prostate carcinoma, JMJD1A is over-expressed and enhances the prognostic prostate-specific antigen in hypoxia (46, 47). In hepatocellular carcinoma, JMJD1A is over-expressed and deteriorates the prognosis. Its suppression could reduce hypoxic effects like inhibition of proliferation and enhancement of migration and invasiveness in hepatocellular carcinoma cell lines (48, 49). Similar effects were seen in bladder, lung, uterus and renal cancer (50-53). JMJD1A could be a prognostic marker for colon carcinoma and intensifies this malignancy (54, 55). In contrast, a study of 185 patients with nasopharyngeal carcinoma showed that an underexpression of JMJD1A worsens prognosis compared to

overexpressing tumors (56). More recent studies, however, revealed the protumorigenic characteristics of JMJD1A in solid tumors like hepatocellular carcinoma, cervix carcinoma, rhabdomyosarcoma, glioblastoma and melanoma, as inhibition of JMJD1A promoted the success of anti-angiogenic therapy (57). These results highlight the expected role of JMJD1A and the tumor microenvironment for the malignant progression of different tumors. This study elucidates the role of the oxygen-dependent transcription factor JMJD1A and its interaction with HIF-1α in a hypoxic environment. Future studies will analyze the underlying regulatory mechanisms.

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

CW performed all experiments, analyzed the results and was the primary author of the manuscript. MB, TM and TG contributed to the design of the experiments and the interpretation of the results. SH and AS conceived the study, analyzed the results and were major contributors to the manuscript. NK and RH analyzed the data and were major contributors to the manuscript. All Authors read and approved the final manuscript.

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Received November 14, 2020
Revised November 24, 2020
Accepted November 25, 2020