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Lack of Correlation between Expression of HIF-1α Protein and Oxygenation Status in Identical Tissue Areas of Squamous Cell Carcinomas of the Uterine Cervix

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ABSTRACT

Hypoxia inducible factor-1α (HIF-1α) has been proposed as a candidate endogenous marker of tumor hypoxia and as a molecular mediator of hypoxia-driven malignant progression and acquired treatment resistance. In this study, HIF-1α expression in 68 biopsies of oxygenation measurement tracks from squamous cell carcinomas of the uterine cervix of 38 patients was assessed. Expression of HIF-1α was commonly found to increase as a function of distance from microvessels, at the center of tumor cell aggregations, and in the vicinity of necrotic areas. However, there was no correlation of HIF-1α expression with median oxygen tension (oxygen partial pressure; pO_2) and hypoxic fractions (hypoxic fraction < 2.5 mm Hg, hypoxic fraction < 5 mm Hg). The results indicate that HIF-1α should not be used as an endogenous marker of tumor hypoxia in locally advanced squamous cell carcinomas of the uterine cervix. Additionally, no significant prognostic impact of HIF-1α expression was found in this group of patients.

INTRODUCTION

The microenvironment of malignant tumors is characterized by areas of poor oxygenation, a trait that is a consequence of a chaotic vascular architecture, an inadequate microvascular function, enlarged diffusion distances, and tumor-associated anemia (1). The results of various experimental studies suggest that tumor hypoxia plays an important role in the development of typical features of malignancy such as invasiveness, metastatic potential, and acquired resistance to treatment (2). In line with this is the finding that tumors of different entities exhibiting severe hypoxia show unfavorable prognosis when compared with their less hypoxic counterparts (2). This prognostic impact has also been shown for patients treated primarily with surgery (3), indicating that hypoxia-induced malignant progression adds to the problem of a reduced efficacy of sparsely ionizing radiation. The "gold standard" for clinical assessment of the tumor oxygenation status remains at present the Eppendorf histography system, which involves the direct measurement of oxygen tension (oxygen partial pressure; pO_2) using needle electrodes. However, limitations of this technique are its invasiveness and lack of universal applicability, because tumors undergoing investigation need to be accessed without substantial added risk for the patient or disproportionate effort on the part of the clinician. For these reasons, Eppendorf histography pO_2 measurements are not feasible in many tumor entities, and, thus, substitute endogenous or exogenous hypoxia markers would be of great interest in the clinical setting.

Hypoxia-inducible factor 1α (HIF-1α), one subunit of the dimeric basic helix-loop-helix/PER-ARNT-SIM transcription factor HIF-1, has been shown to be tightly regulated with regard to oxygen availability under physiological conditions. HIF-1α protein accumulates in hypoxic cells within minutes and becomes undetectable after reoxygenation within a similar time period (4). In hypoxia, this rapid adaptation is accomplished by a mechanism involving the interruption of the continuous proteasomal degradation of HIF-1α that takes place under normoxic conditions (5, 6). HIF-1α has, therefore, been considered as a candidate endogenous molecular hypoxia marker. HIF-1α protein abundance, however, is known not to be exclusively regulated by oxygen levels. Rather, a wide range of growth factors, cytokines, and oncogenes can also exert a biologically relevant influence on HIF-1α activation (*e.g.*, see ref. 7). In view of the fact that a deregulation of the systems described above is inherent in malignant cells, the relative importance of the oxygen-related and oxygen-independent components of HIF-1α regulation remains to be elucidated.

In addition to its putative role as an endogenous hypoxia marker, HIF-1α has been proposed as a pivotal mediator of hypoxia-driven molecular changes leading to tumor progression. The spectrum of genes transactivated by HIF-1α is indeed known to be involved in metabolic adaptation, cell survival and proliferation, angiogenesis, control of apoptosis, capability of tissue invasion, and metastasis (8). Whether overexpression of HIF-1α and subsequent proteome changes are positive or negative factors for tumor cell growth is a question that is still being discussed controversially. In the present study, protein expression of HIF-1α in tumor microareas of biopsies, taken from needle electrode tracks subsequent to pO_2 measurements, was assessed to elucidate the role of HIF-1α as an endogenous hypoxia marker. Additionally, the role of HIF-1α as a marker of prognosis in 34 (of 38) patients with long-term follow-up was evaluated.

MATERIALS AND METHODS

Patients. All of the patients in this study were part of a prospective clinical trial for the evaluation of the significance of tumor oxygenation in primary, locally advanced carcinomas of the uterine cervix that commenced at the Department of Obstetrics and Gynecology, University of Mainz Medical School in June 1989. The study design was approved by the local Medical Ethics Committee, with patients giving informed written consent before being enrolled. All 38 patients with squamous cell carcinomas from the former study, for whom one or two tumor biopsy specimens of the oxygen measurement tracks were available, were included in the present study. Patients of this subgroup had been recruited between August 1991 and April 1997. Table 1 shows relevant patient and tumor characteristics at the time of the pretreatment pO_2 measurements.

For correlations involving survival, only patients treated with curative intent were included ($n = 34$). In 24 of these, the primary therapy was surgical. Abdominal radical hysterectomy and pelvic/periaortic lymph node dissection was the standard surgical procedure. In 4 (of 24) patients, supralevator exenteration had to be performed instead of radical hysterectomy. The remaining 10 cases were treated with primary radiotherapy, administered as combined teletherapy and brachytherapy. External beam irradiation was applied with 10 MV photons produced by a linear accelerator at the Department of Radiology/Radiooncology. For brachytherapy, a high-dose-rate ^{192}Ir afterloading machine at the Department of Obstetrics and Gynecology was used (for details see ref. 3).

Adjuvant therapies varied during the course of the study. Until 1992, a series of patients received "induction chemotherapy" with cisplatin, vincristine, and bleomycin followed by definitive radiation. During that time, surgically treated patients with parametrial and nodal involvement underwent adjuvant chemotherapy with carboplatin and ifosfamide. From 1993 on,

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Table 1 *Patient and tumor characteristics at the time of pretherapeutic pO_2 measurements*

	No. of patients	Median	Range
FIGO stage			
IB	4		
IIA	1		
IIB	21		
IIIA	0		
IIIB	8 (6)		
IVA	2 (0)		
IVB	2		
Grade			
1	4		
2	17 (15)		
3	11 (10)		
ND	6 (5)		
pT stage			
pT_{1b}	1		
pT_{2a}	2		
pT_{2b}	18		
pT_{3b}	2		
ND	1		
NA	14 (10)		
pN stage			
N_0	9		
N_1	13		
N_X	1		
ND	1		
NA	14 (10)		
Largest tumor diameter, mm		60 (55)	30–150 (30–80)
Menopausal status			
Pre	19 (18)		
Post	19 (16)		
Patient age, yr			
<55	21 (20)	53 (52)	26–73
≥55	17 (14)		(26–73)
Hemoglobin concentration, g/dL			
<12	14 (11)	12.7 (12.8)	9.3–16.0
≥12	24 (23)		

NOTE. Numbers in parentheses indicate deviations for the subgroup of patients treated with curative intent (survival correlations).

Abbreviations: FIGO, International Federation of Gynecologists and Obstetricians; pT stage, pathologic tumor stage; pN stage, pathologic node stage; ND, not documented; NA, not applicable, no surgical treatment (radiation only).

chemotherapy was given concomitantly with radiation using cisplatin or carboplatin instead of the sequential mode, and adjuvant treatment for surgical risk factors was changed to external-beam radiation of the pelvis with 50 Gy using the four-field box technique at 2-Gy/day fractions. Median follow-up time was 28 months (SD, 26 months), ranging from 4 to 85 months.

Tumor Oxygen Tension Measurements. Tumor pO_2 was measured pretherapeutically with the computerized Eppendorf histography system (Eppendorf, Hamburg, Germany), using a protocol that has been described in detail previously (9). Briefly, pO_2 readings were performed in the conscious patient along linear tracks, first in the subcutaneous fat of the mons pubis followed by cervical measurements at the 12 and 6 o'clock sites in macroscopically vital tumor tissue. Within the tumor tissue, up to 35 pO_2 measurements were made along each electrode track (70 readings in total) starting at a tissue depth of 5 mm. The individual pO_2 measurement points were situated 0.7 mm apart, resulting in an overall measurement track length of ~25 mm. Immediately after pO_2 measurements, needle core biopsies (obtained using Biopty; Radioplast, Uppsala, Sweden) of ~2 mm in diameter and 20 mm in length were taken from those tumor areas from which pO_2 readings had been obtained. Both the pO_2 readings and the needle core biopsies were performed without general anesthesia in all of the patients. Intravaginal temperature, arterial blood pressure, heart rate, hemoglobin concentration, hematocrit, and arterial oxyhemoglobin saturation were monitored at the time when pO_2 readings were taken. The pretherapeutic pO_2 measurements were usually performed 1–5 days before oncological treatment.

Immunohistochemistry. HIF-1α protein expression was assessed in 68 biopsy specimens taken from the tumor pO_2 measurement tracks obtained directly after pO_2 measurement in 38 patients. Two biopsies, corresponding to the 6 and 12 o'clock positions of the tumor center were available for each of 30 patients, and one biopsy was available for each of the remaining 8 cases. All of the material was fixed in formalin before being embedded in paraffin. Histological slides were prepared from the paraffin blocks and dried overnight at 37°C. On the next day, specimens were dewaxed in two changes of fresh xylene and were rehydrated in a descending alcohol series. Retrieval of antigenic binding sites was performed by heating specimens in 10 mM citrate buffer (pH 6.0) in a microwave oven for 17 min. The immunodetection of HIF-1α was carried out using the DAKO catalyzed signal amplification system in accordance with the manufacturer's instructions, except that all incubation steps were additionally temperature-controlled. Anti-HIF-1α clone H1α67 (Abcam, Cambridge, United Kingdom) was used as the primary antibody at a concentration of ~1 μg/ml in PBS (1:3000 dilution) for 90 min at 37°C. Negative control specimens were incubated with identical concentrations of immunoglobulins of the same isotype (IgG2b, DakoCytomation) under the same conditions. Slides were counterstained with Mayer's hematoxylin, dehydrated in an ascending alcohol series, and covered with a coverslip using Eukitt mounting medium (Riedel-de Haen, Seelze, Germany).

Assessment of HIF-1α Protein Expression. Quantification of HIF-1α protein expression was performed using a computer-assisted image-analysis technique. For each biopsy, one to six digital images (representing >90% of the total area of the specimen) were acquired at 10× magnification using a microscope-based image acquisition system consisting of a Zeiss Axiotron (Zeiss, Oberkochen, Germany) microscope equipped with a NIKON D-100 camera connected to a standard Windows-based personal computer. Digitized images were transferred to the personal computer, were color-enhanced and brightness-adjusted using Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA), and then were analyzed using the software package OPTIMAS (Version 6.2; Media Cybernetics Inc., Silver Spring, MD). To estimate the fraction of the positive tumor cell nuclear area, we applied the following procedure: one or multiple "regions of interest" consisting entirely of tumor cells were marked by freehand using the computer mouse. Then, color thresholds for blue (unstained, HIF-1α-negative) and brown (HIF-1α-positive) nuclear areas were set. Repeated visual control ensured that only nuclear staining and only the appropriate color was highlighted. The fraction of the total nuclear area positive for HIF-1α was calculated using Microsoft Excel and termed the "positive nuclear fraction." This process was repeated up to three times per image. For correlation with clinical data, the mean value of individual specimens was calculated in cases in which two biopsies were available.

Statistical Analysis. All of the statistical tests were performed using the SPSS software package (Version 11.5; SPSS Inc., Chicago, IL). The significance level was set at $\alpha = 5\%$ for all comparisons. Linear correlations between two parameters were described by Spearman's rank correlation coefficient (rho). Two-sided Mann-Whitney *U* tests and Kruskal-Wallis tests were used for comparison of categorized variables. Survival estimates were calculated using the Kaplan-Meier method, and differences between groups were assessed with log-rank statistics.

RESULTS

Expression Patterns of HIF-1α. Nuclear expression of HIF-1α in tumor cells increased as a function of distance away from the stroma and/or vessels, in the center of tumor cell masses and around areas of necrosis. Expression was assessed by estimating the 3,3′-diaminobenzidine (DAB)-positive nuclear fraction. Positive nuclear fraction of HIF-1α-stained tumor cells ranged from 0.2 to 98.6%, with a median value of 23.7% (SD, 27%). A strong correlation was found between positive nuclear fraction values from biopsies taken from the 6 and 12 o'clock positions from individual patients (Spearman-rho = 0.477, $P = 0.008$). In the tumor stroma, which included endothelial cells, HIF-1α was also expressed to a varying degree. This expression was, however, not quantified. Cytoplasmic staining in the tumor cells was evident in the majority of cases, as has been seen in other studies (10).

Correlation of Nuclear HIF-1α Expression in Tumor Cells with Oxygenation Data. No correlation between HIF-1α expression ($n = 68$) and the oxygenation parameters mean pO_2, median pO_2 (Fig. 1), and hypoxic fractions (hypoxic fraction <2.5 mm Hg and hypoxic

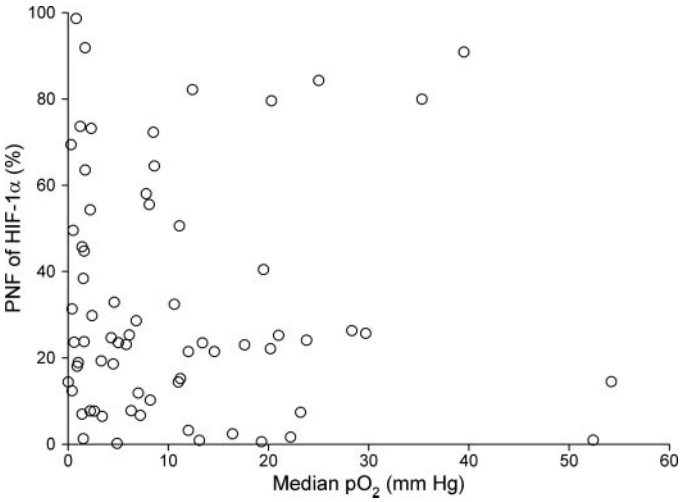

Fig. 1. Positive nuclear fraction (*PNF*) of HIF-1α as a function of median pO_2 values in identical tissue areas of locally advanced squamous cell carcinomas of the uterine cervix. *Each data point*, one oxygen microsensor track.

fraction <5 mm Hg) was found. Severely hypoxic tumors exhibited a wide range of HIF-1α expression (see Fig. 2). The same was true for "well-oxygenated" tumors that partially showed a pronounced expression of HIF-1α (see Fig. 3).

Correlation of HIF-1α with Clinical and Pathohistological Parameters. There was no significant correlation between HIF-1α expression (pooled positive nuclear fraction data) and clinical or pathohistological parameters, International Federation of Gynecologists and Obstetricians (FIGO) stage, maximum clinical diameter, maximum histological diameter, patient age, menopausal status, hemoglobin concentration, tumor-node-metastasis stage, and grading.

Correlation of Nuclear Expression of HIF-1α with Survival. Using the median as a cutoff point, pooled positive nuclear fraction data from 6 and 12 o'clock positions were divided into two groups (weak *versus* strong expression). Although no significant differences were found, weak trends were evident for longer overall ($P = 0.11$) and recurrence-free survival ($P = 0.19$) for the group with strong expression of HIF-1α. Subgroup analyses were not performed because of the small number of cases. In this cohort of patients, the median pO_2 also had no prognostic influence on either overall survival ($P = 0.11$) or recurrence-free survival ($P = 0.08$).

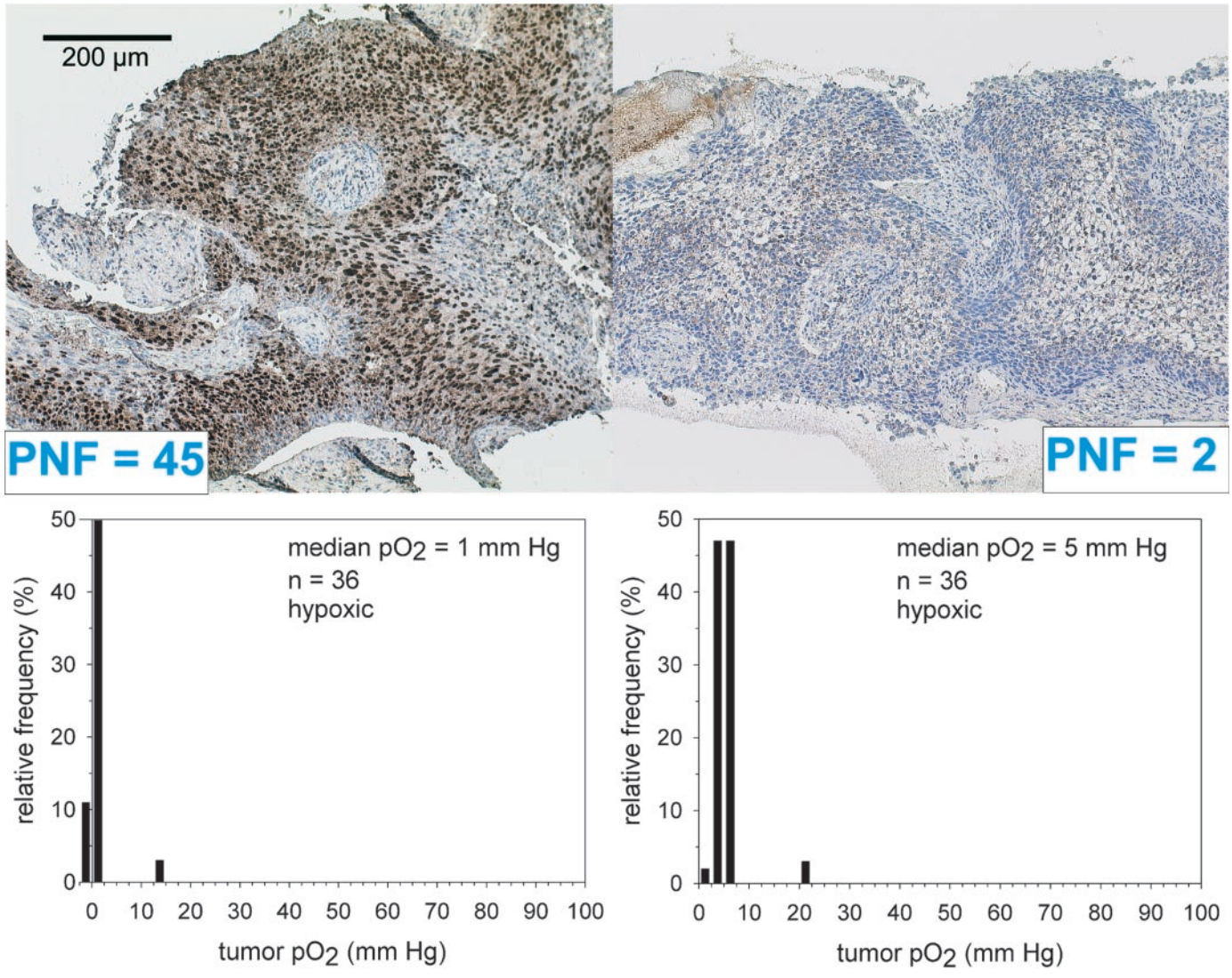

Fig. 2. Expression patterns and positive nuclear fraction (*PNF*) values of HIF-1α (histological photographs, 10× magnification; *scale bar in upper left panel*, 200 µm) with corresponding pO_2 histograms for hypoxic tumors. Examples of high and low expression of HIF-1α are depicted. n = number of pO_2 readings in the respective measurement track.

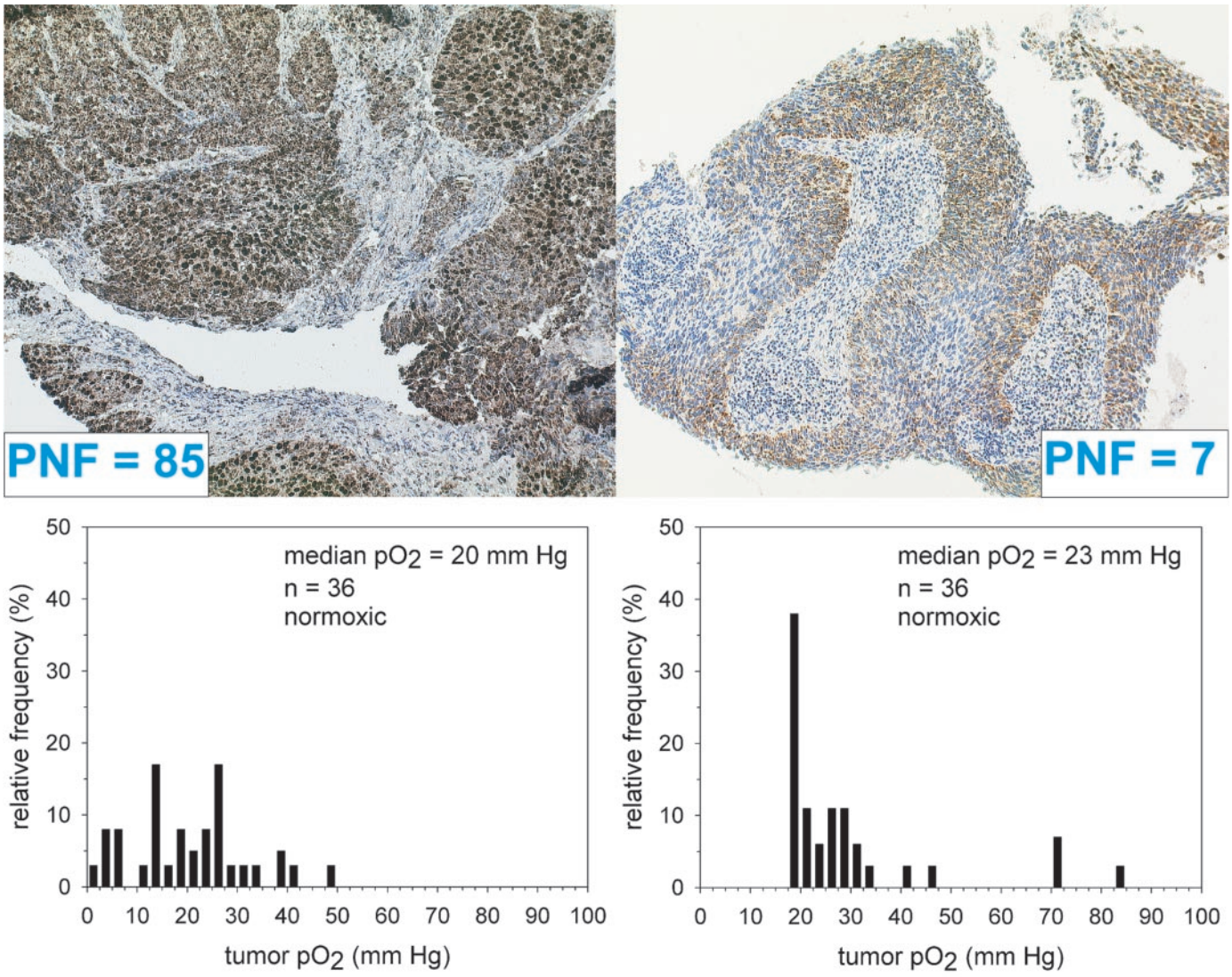

Fig. 3. Expression patterns and positive nuclear fraction (*PNF*) values of HIF-1α with corresponding pO_2 histograms for normoxic tumors. Examples of high and low expression of HIF-1α are depicted. For further details see legend of Fig. 2.

DISCUSSION

In the present study, the expression of HIF-1α protein, as assessed by immunohistochemistry in biopsy specimens of squamous cell carcinomas of the uterine cervix, did not correlate with tumor oxygenation in the same tissue areas. HIF-1α showed a characteristic nuclear expression pattern, rising toward the center of tumor cell aggregations, with increasing distance from the stromal compartment (containing microvessels) and at the margin of necrotic areas (see Figs. 2 and 3). This expression pattern of HIF-1α is as might theoretically be expected if its biological function is considered; it is also in accordance with patterns already reported in the literature (11–13). However, contrary to our study, Haugland *et al.* (11) found greater expression of HIF-1α in cervical cancers with larger fractions of pO_2 measurements below 5 mm Hg. The highest expression of HIF-1α in their study was ~11%, whereas it was close to 100% in our study. Two lines of explanation may account for this discrepancy. Firstly, there are significant methodological differences between the two studies. Whereas Haugland *et al.* (11) also used computer-based image analysis for the interpretation of immunolabeling, they did not analyze HIF-1α expression specifically in tumor cells, in contrast to the present study. This may partially explain the much lower percentage of positive cells in their study, because expression of HIF-1α in the tumor stroma is usually much lower than within the tumor cells. Haugland *et al.* (11) also did not directly assess the fraction of positive nuclei but rather the percentage of positively staining pixels over the total tumor area and then applied a correction factor for nuclear size. The latter procedure may yield inaccurate results because the staining intensity may still show some variation with nuclear size, *e.g.*, the antigen expression in cells with larger nuclei may be overestimated. Secondly, we chose to restrict our study to squamous histology because preliminary analyses of our data had shown evidence for a strong correlation of HIF-1α expression with hypoxic fractions for non-squamous cell histology in our patient cohort (expression of HIF-1α was also assessed in 23 non-squamous cell carcinoma biopsies, data not shown). However, the number of biopsies of non-squamous cell carcinomas was too low to allow for a definitive statement, and inclusion of all histological subtypes in one study would have resulted in an overall weak and possibly misleading statistical correlation (the significance of which would have depended entirely on the non-squamous cell carcinomas). Because the percentage of tumors with non-squamous histology in the study of Haugland *et al.* (11) was relatively high (≈27%), it would have been interesting

to know whether the correlation of HIF-1α expression and hypoxic fractions would still be present when only cases of squamous histology were assessed. In line with our results, Janssen *et al.* (13), who assessed squamous cell carcinomas of the head and neck, could not find a correlation between the expression of HIF-1α and the extrinsic hypoxia marker pimonidazole.

The finding that HIF-1α expression can occur in cancer cells in an oxygenation-independent manner is not novel. First of all, it is generally accepted that HIF-1α expression is increased independently of oxygenation status in tumors belonging to the von Hippel-Lindau disease spectrum (*e.g.*, clear-cell renal cell cancer, hemangioblastoma). Here, the underlying mutation of the *VHL* gene causes an "inactivation" of the physiological mechanism of normoxic proteasomal degradation of HIF-1α, leading to constitutively elevated protein levels. Diverse human cancer cell lines have been shown to express heterogeneous levels of HIF-1α at normoxia (14). Normoxic stabilization of HIF-1α protein has been demonstrated to occur through the activation of oncogenes (15), growth factors, and cytokines (7). Additionally, chromosomal amplification of the HIF-1α gene, a characteristic mechanism for the activation of oncogenes, has recently been described for a prostate cancer cell line (16). In this context, it is important to state that, because transcriptional activity of HIF-1α is regulated in a manner independent of protein stability, both may be differentially influenced by some of these processes. Therefore, the possibility that protein expression of HIF-1α may not directly reflect transcriptional activity cannot be ruled out. Because stromal cells are less likely to be influenced by the factors cited above, it would have been interesting to study possible correlations of the oxygenation status and the stromal expression of HIF-1α. This assessment was prevented by a low reproducibility of the positive nuclear fraction-method for the estimation of the HIF-1α expression in this compartment, because the stromal signal is less clearly confined to the nuclei. In addition, there is a higher batch-to-batch variation of signal intensity between individual staining procedures.

We also could not confirm correlations of HIF-1α expression with poor prognosis, which have been reported in recent studies on uterine cervix cancers (10, 17) and in a variety of studies on other tumor entities (12, 18, 19). Regarding prognosis, we thus confirm the results of Haugland *et al.* (11), who found no prognostic impact of HIF-1α expression in uterine cervical cancers. Despite this lack of a significant prognostic correlation, we observed a trend toward longer overall ($P = 0.11$) and recurrence-free survival ($P = 0.19$) in cases with higher expression of HIF-1α (positive nuclear fraction of HIF-1α above the median). Thus far, only one study on squamous cell cancers (of the head and neck) has shown a result similar to this trend found in our study (20). The authors of the cited study attributed the poorer prognosis of cases with lower expression of HIF-1α to the fact that the primary treatment of patients in their cohort was surgical, thus circumventing a major influence of hypoxia-induced radiation resistance. In this setting, intrinsic malignant traits of HIF-1α negative cells, *e.g.*, apoptosis deficiency [Carmeliet *et al.* (21), see below], may dominate the clinical course of the disease. The discrepancies in the results obtained in the studies mentioned may also reflect the fact that because of the diversity of target genes, HIF-1α activation within cancer cells does not result in universally predictable consequences. Only in some instances has HIF-1α been shown to be a positive factor for tumor growth. For example, Ryan *et al.* (22) showed this to be the case in mouse embryonic fibroblasts growing as allografted sarcomas. Downstream effects of HIF-1α induction, such as an influence on the capability for metabolic adaptation through *trans*-activation of enzymes involved in glycolysis, cellular glucose uptake, and pH regulation, as well as the activation of growth factor genes, may account for this finding, although the latter study did not identify a specific mechanism responsible for the observations made. Interestingly, no differences in the degree of vascularization between *HIF-1α*$^{-/-}$ and *HIF-1α*$^{+/+}$ tumors were found in the study, although HIF-1α is capable of inducing angiogenesis via up-regulation of factors such as vascular endothelial growth factor. Other HIF-1α downstream effects, however, have been shown to have adverse consequences for tumor growth and are, thus, more likely to explain the findings presented in this paper. Inactivation of HIF-1α in embryonic stem cells was found to lead to increased proliferation and a reduction of apoptosis under hypoxic conditions (21). Additionally, low expression of HIF-1α was associated with increased clonogenic survival under hypoxia in breast cancer cells (23). For these reasons, a cell type-specific impact of HIF-1α expression seems likely. A preliminary analysis of the impact of HIF-1α expression in the non-squamous cell cancers ($n = 23$; data not shown) showed a significantly poorer outcome for cases with stronger expression of HIF-1α.

It should also be noted that the relatively low patient number and the diversity of treatment modalities in this study may limit the power of the prognostic correlations. Additionally, the relatively small size of the biopsies in combination with the heterogeneity of HIF-1α expression may theoretically have led to a misjudgment of the overall expression in some cases (although the significant correlation of the two biopsy positions suggests otherwise). The analysis of a larger and more homogeneous patient group in a prognostic study might identify HIF-1α as having a significant negative impact. From the data presented here, the suitability of HIF-1α protein expression assessment as an endogenous hypoxia marker in squamous cell carcinomas of the uterine cervix seems questionable. Additional studies are, thus, needed to find other appropriate markers that more reliably and independently reflect the extent of hypoxia in human tumors.

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REFERENCES

1. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 1989;49(23): 6449–65.
2. Höckel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst (Bethesda) 2001;93(4):266–76.
3. Höckel M, Schlenger K, Aral B, Mitze M, Schäffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56(19):4509–15.
4. Jewell UR, Kvietikova I, Scheid A, Bauer C, Wenger RH, Gassmann M. Induction of HIF-1α in response to hypoxia is instantaneous. FASEB J 2001;15(7):1312–4.
5. Maxwell PH, Ratcliffe PJ. Oxygen sensors and angiogenesis. Semin Cell Dev Biol 2002;13(1):29–37.
6. Giaccia A, Siim BG, Johnson RS. HIF-1 as a target for drug development. Nat Rev Drug Discov 2003;2(10):803–11.
7. Bilton RL, Booker GW. The subtle side to hypoxia inducible factor (HIFα) regulation. Eur J Biochem 2003;270(5):791–8.
8. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3(10): b1721–32.
9. Höckel M, Schlenger K, Knoop C, Vaupel P. Oxygenation of carcinomas of the uterine cervix: evaluation by computerized O_2 tension measurements. Cancer Res 1991;51(22):6098–102.
10. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000;60(17):4693–6.
11. Haugland HK, Vukovic V, Pintilie M, *et al.* Expression of hypoxia-inducible factor-1α in cervical carcinomas: correlation with tumor oxygenation. Int J Radiat Oncol Biol Phys 2002;53(4):854–61.
12. Aebersold DM, Burri P, Beer KT, *et al.* Expression of hypoxia-inducible factor-1α: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001;61(7):2911–6.
13. Janssen HL, Haustermans KM, Sprong D, *et al.* HIF-1A, pimonidazole, and iododeoxyuridine to estimate hypoxia and perfusion in human head-and-neck tumors. Int J Radiat Oncol Biol Phys 2002;54(5):1537–49.

14. Zhong H, Mabjeesh N, Willard M, Simons J. Nuclear expression of hypoxia-inducible factor 1α protein is heterogeneous in human malignant cells under normoxic conditions. Cancer Lett 2002;181(2):233–8.
15. Chan DA, Sutphin PD, Denko NC, Giaccia AJ. Role of prolyl hydroxylation in oncogenically stabilized hypoxia-inducible factor-1α. J Biol Chem 2002;277(42): 40112–7.
16. Saramaki OR, Savinainen KJ, Nupponen NN, Bratt O, Visakorpi T. Amplification of hypoxia-inducible factor 1α gene in prostate cancer. Cancer Genet Cytogenet 2001; 128(1):31–4.
17. Burri P, Djonov V, Aebersold DM, *et al.* Significant correlation of hypoxia-inducible factor-1α with treatment outcome in cervical cancer treated with radical radiotherapy. Int J Radiat Oncol Biol Phys 2003;56(2):494–501.
18. Schindl M, Schoppmann SF, Samonigg H, *et al.* Overexpression of hypoxia-inducible factor 1α is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin Cancer Res 2002;8(6):1831–7.
19. Birner P, Gatterbauer B, Oberhuber G, *et al.* Expression of hypoxia-inducible factor-1α in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer (Phila) 2001;92(1):165–71.
20. Beasley NJ, Leek R, Alam M, *et al.* Hypoxia-inducible factors HIF-1α and HIF-2α in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res 2002;62(9):2493–7.
21. Carmeliet P, Dor Y, Herbert JM, *et al.* Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature (Lond) 1998;394(6692): 485–90.
22. Ryan HE, Poloni M, McNulty W, *et al.* Hypoxia-inducible factor-1α is a positive factor in solid tumor growth. Cancer Res 2000;60(15):4010–5.
23. Blancher C, Moore JW, Talks KL, Houlbrook S, Harris AL. Relationship of hypoxia-inducible factor (HIF)-1α and HIF-2α expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines. Cancer Res 2000;60(24):7106–13.