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ORIGINAL ARTICLE

Expression of Hypoxia-Associated Protein HIF-1α in Follicular Thyroid Cancer is Associated with Distant Metastasis

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Abstract Follicular thyroid carcinomas (FTCs) are the second most common malignant neoplasia of the thyroid and in general its prognosis is quite favorable. However, the occurrence of metastases or non-responsiveness to radioiodine therapy worsens the prognosis considerably. We evaluated immunohistochemically the expression of hypoxia-associated proteins by hypoxia-induced factor 1α (HIF-1α), the stroma-remodeling marker Tenascin C, as well as markers for the epithelial-mesenchymal transition (EMT), namely E-cadherin and slug in a series of 59 sporadic FTCs. In addition, various clinicopathologic parameters were assessed like TNM-staging, age, tumor size as well as tumor characteristics like desmoplasia, necrosis, and calcification. Overexpression of HIF-1α was seen in 29 of 59 tumors (49.2%) including 21 (35.6%) FTC with strong expression of tumor cell groups. HIF-1α correlated significantly with metastasis ($p < 0.001$; Mann-Whitney U test), degree of desmoplasia ($p = 0.042$, Kruskal-Wallis test), tenascin C expression ($p = 0.042$, Kruskal-Wallis test), calcification ($p < 0.025$, Kruskal–Wallis test), necrosis ($p = 0.002$), age ($p = 0.011$, Kruskal-Wallis test) and tumor stage UICC ($p = 0.022$, Kruskal-Wallis test). Furthermore, metastasis was associated with the degree of desmoplasia ($p = 0.014$; Fisher's exact test), calcification ($p = 0.008$, Fisher's exact test), necrosis ($p = 0.042$, Fisher's exact test), tumor size ($p = 0.015$, Mann-Whitney U test), and age ($p = 0.001$, Mann-Whitney U test). In a Cox proportional hazards model, only metastasis remained as an independent risk factor for overall survival (hazard rate: 10.2 [95% CI, 02.19 to 47.26]; $p = 0.003$). Our data suggest that HIF-1α plays a critical role in the remodeling of the extracellular matrix as well as metastasizing process of follicular thyroid carcinoma and targeting hypoxia-associated and -regulated proteins may be considered as potential targets for personalized medicine.

Keywords HIF-1α · Hypoxia-associated proteins · Tenascin · Follicular thyroid cancer · Desmoplastic stroma reaction · Metastasis

Introduction

Growth of tumors requires an increased intratumoral blood supply. This is triggered by tumor hypoxia, which promotes angiogenic mediators and induces HIF-1α, the universal mediator of the cellular adaptation to hypoxic conditions. [1, 2] In many tumors (e.g. pancreatic cancer, breast cancer, cervical carcinoma) the over-expression of HIF-1α has been associated with worse prognosis, selection of a more aggressive phenotype, metastatic spread and resistance to radiation and chemotherapies. [3–7] So far, this is the largest cohort study that investigated the expression of HIF-1α in primary FTC.

Malignant tumors are arranged by stromal elements and cancer cells, which may alter their stroma through induction of myofibroblast differentiation and govern the desmoplastic stroma reaction. Also, stromal cells or cancer-associated

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fibroblasts (CAFs) are able to modify the phenotype, invasiveness, and metastatic capacity of carcinomas, typically promoting the progression. [8]

Desmoplastic stromal reaction, defined as the presence of newly formed connective tissue between tumor cells is composed of fibroblasts and myofibroblasts, expressing α-smooth muscle actin, fibroblast activation protein and extracellular matrix proteins such as tenascin C. An altered stroma has been suggested to be an important player in the development of the invasion process. [9, 10]

The progression of cancer involves an increased cell motility, cell invasion and migration. Epithelial cells lose their polarity and acquire a mesenchymal phenotype, which is known as epithelial-mesenchymal transition (EMT). This transition is a major facilitator of tumor metastasis. Repression of epithelial-specific proteins in the tumor cells is a crucial step of EMT. A main molecular feature of this process is the downregulation of E-cadherin, which serves as hallmark of EMT and is essentially controlled by EMT-mediated proteins such as slug. Slug plays vital roles in the development of motile and invasive manner of cancer cells via EMT progression. [11–15]

Follicular thyroid carcinoma (FTC) 10-year survival rates are about 80%. However, the occurrence of metastases worsens prognosis considerably. The tumor specific mortality of FTC is primarily caused by advanced metastatic spread. If patients don't respond to radioiodine therapy 5-year survival rates decrease to only 15%. Yet the reason why tumors of the same entity display differences in their biological behavior and aggressiveness still has not been sufficiently resolved. [16–18]

In the present study we aimed to investigate the expression of hypoxia-induced factor 1 alpha (HIF-1α) in follicular thyroid carcinoma (FTC) with and without distant metastasis in relation to desmoplasia, the expression of tenascin C, E-cadherin, slug, metastatic potential and histomorphological parameters.

Methods

Case Selection

Formalin-fixed and paraffin-embedded specimens of 59 patients with FTC that underwent surgical treatment between 2000 and 2012 at the General Hospital of the City of Vienna, Austria, were included into this retrospective study. The study was conducted following the rules of ICH-Guideline for Good Clinical Practice and the ethical principles for medical research according to the declaration of Helsinki. The use of human material for the analysis was approved by the local ethical committee (Ethikkommission, MUW, vienna, vote number: 230/2010). Well-documented follow up was available of all individuals. Disease free survival was calculated from time of primary surgery until first evidence of progression of disease. Survivals until end of observation period or losses to follow up were considered as censored observations.

One staged total thyroidectomy was carried out in 43 patients, in 16 cases two staged thyroidectomy was performed. The tumor area in the organ was sectioned in slices of approximately 3–5 mm, formalin-fixed and embedded in paraffin. In all cases, at least ten tumorblocks with capsular regions or the entire tumor capsule were embedded. Sections of each block were cut at 3 μm and used for haematoxylin and eosin (H&E) staining. All FTCs were classified according to the WHO criteria outlined in 2004 and staged according to the UICC classification outlined in 2009. [19, 20]

Morphology

Desmoplastic stroma reaction (desmoplasia) was defined as the presence of a newly formed fibrotic (collagenous) stroma surrounding the invasive epithelial tumor cells. The tumor capsule was not regarded as desmoplastic stromal reaction per se. Desmoplasia was graded as follows: negative, −; little, + (<10% of tumor tissue); moderate, ++ (<50% of tumor tissue); and strong, +++ (>50% of tumor tissue). Infiltration patterns (vascular and capsular penetration of entire thickness of capsule, extrathyroidal extension), calcification, and necrosis, were assessed. Widely invasive subtype was defined as showing 4 or more vascular invasions, minimal invasive subtype as less than 4 vascular invasions. [21, 22] Preexisting thyroid gland was evaluated for nodular goiter disease and lymphocytic thyroiditis. In addition, concomitant neoplastic diseases were recorded.

Immunohistochemistry

Sections of a representative tumor block were cut at 3 μm. Immunostaining against HIF-1α (mouse monoclonal, clone 54/HIFα, dilution 1:10; BD Transduction Laboratories, NJ, USA), extracellular matrix protein tenascin C (mouse monoclonal, dilution 1:100; Novocastra, Newcastle, UK), E-Cadherin (mouse monoclonal, clone NCH-38, dilution 1:50; Novocastra, Newcastle, UK) and slug (mouse, clone 1A6, dilution 1:150; Novus Biologicals, Littleton, CO, USA) were performed using an automated immunostainer (Ventana Medical Systems, Benchmark Ultra Tucson, AZ, USA). For HIF-1α staining, antigen retrieval was performed by boiling the slides in citrate buffer (pH 6 for 92 and 76 min) and using a commercially available amplification kit (Ventana Medical Systems).

Negative controls included substitution of primary antibodies by non-specific, isotype matched antibodies or omission of the primary antibody. Sections were counterstained with haematoxylin.

The expression of proteins was evaluated independently by two investigators (OK and KA). To obtain concordant results in cases with discrepancy between the two observers, the slides were discussed and re-evaluated on a multiheaded microscope. Evaluating HIF-1α only nuclear reactivity in tumor cells was counted; a tumor was scored positive if any tumor nuclei were stained differentiating single nuclear staining (+) and groups of nuclear staining (++). Stromal staining of tenascin C expression was graded semi-quantitatively as follows: negative, no stromal staining (−); little, staining in <10% of stroma (+); moderate, staining in ≥10 and ≤50% (++); and strong, >50% of stroma (+++). Membranous staining for E-Cadherin was semi-quantitatively scored by use of a four-tier scale: 0, 0–5% positive cancer cells; 1, 6–33%; 2, 34–66%; and 3, 67–100%. Cytoplasmic staining for slug was interpreted based on the intensity as negative, moderate (1+) and strong (2+).

Statistical Analysis

Fisher's exact test, the Mann-Whitney *U* test and the Kruskal-Wallis test were used as appropriate. Multivariate analysis of survival was performed using the Cox proportional hazard model in a backward manner including HIF-1α, tenascin C, age, desmoplasia, necrosis, histological grade and stage (T, N, M) was carried out. A *p*-value of <0.05 was considered to be statistically significant. SPSS 22 (IBM, Armonk, NY) was used for all calculations.

Results

Characterization of Patients

The mean age of the patients was 59 ± 32 years, 46 (78%) were females, 13 (22%) males, median tumor size was 30 mm (range 9 mm – 100 mm). Eleven (18.8%) of the 59 patients had distant metastases in the lung or bones, 8 of them (synchronic) at the time of primary surgery and 3 developed later (metachronic). The median follow-up period was 53.2 months (mean 55.0 months, range 2–122 months). During the follow-up period, 5 patients (8.5%) developed recurrent disease and 2 patients (3.4%) died of FTCs (Table 1).

Tumor Morphology

2 FTCs were classified as pT1a, 20 as pT1b, 23 as pT2 and 14 as pT3 tumors including 11 tumors with infiltration beyond the thyroid capsule, according to the UICC classification system outlined in 2009. Overall multifocality was seen in 3 tumors. The mean tumor size was 33,7 mm (median: 30,0 mm; min: 9 mm; max: 100 mm).

Regarding the morphological characteristics, a desmoplastic stroma reaction was present in 54 cases (91.5%), including 22, 28 and 4 cases with little, moderate and strong desmoplasia, respectively. Capsular invasion was seen in 44 cases (74.6%) and vascular invasion in 43 cases (72.9%). All tumors showed either capsular invasion or a vascular invasion or both together in 28 cases (47.5%). Widely invasion was counted in 13 cases (22%) and minimal invasion in 46 cases (78%). Small necrotic foci were detected in 8 cases (13.6%), calcification in 22 cases (37.3%). Preexisting thyroid tissue showed nodular goiter in 33 cases (55.9%). A lymphocytic thyroiditis (non-neoplastic inflammation) was seen in 18 cases (30.5%). Concomitant carcinomas such as microcarcinoma of papillary thyroid carcinoma (micro PTC; tumor size ≤1 cm) were seen in 7 cases including 1 case with an additional medullary thyroid carcinoma (Table 1).

HIF-1α, Tenascin C, E-Cadherin and Slug

Only nuclear immunoreactions against HIF-1α were observed. Expression of HIF-1α was seen in 29 (49.2%) FTCs. The positive cases included 21 cases with groups of nuclei stained and 8 cases with single nuclei stainings. Overall, a distinct focal pattern was seen in all cases and in most cases was associated with desmoplastic stromal reaction.

Tenascin C expression was seen in the extracellular matrix of the tumor stroma and in vascular smooth muscle cells. Tumor cell staining was not present. Absence of stromal tenascin C expression was observed only in 1 case, little staining in 8 cases (13.5%), moderate staining in 32 cases (54.2%) and strong staining in 18 cases (30.5%), respectively (Fig. 1).

Absence of membranous expression of E-cadherin was observed in 12 (23.5%) cases. In 18 (35.3%) cases only focal absence of membranous staining was observed. Complete (preserved) membranous E-cadherin staining was seen in 21 (41.2%) cases.

Nuclear immunoreactions against slug were seen in 36 (62.1%) FTCs. In most cases, immunoreactivity was seen focally. Strong immunoreaction was seen in 32 (55.2%), whereas moderate expression was seen in only 4 (6.9%) cases. No immunoreactivity was seen in 22 (37.9%) of the cases.

Correlation of HIF1 α, Distant Metastases and Clinicopathologic Parameters

All cases with metachronal distant metastasis showed HIF expression. HIF-1α correlated significantly with distant metastasis ($p < 0.001$; Mann-Whitney *U* test), with the degree of desmoplasia ($p = 0.042$; Kruskal–Wallis), with tenascin C expression ($p = 0.042$; Kruskal–Wallis), calcification ($p < 0.025$, Mann-Whitney *U* test), necrosis ($p = 0.002$; Mann-Whitney *U* test); age ($p = 0.011$; Kruskal–Wallis) and tumor stage (UICC) ($p = 0.022$; Kruskal–Wallis), but not with

Table 1 Correlation of clinicopathological/morphological parameters and HIF-1α expression

	FTC Total n (%)	HIF-1α (+) n (%)	HIF-1α (−) n (%)	*p* values
Tenascin C	59 (100%)	29 (49.2%)	30 (50.8%)	$p = 0.042$
no stromal staining (−)	1(1.7%)	0 (0%)	1(1.7%)	
little, staining in < 10% of stroma (+)	8(13.5%)	0 (0%)	8(13.5%)	
moderate, staining in ≥ 10 and ≤ 50% (++)	32(54.2%)	16(27.1%)	16(27.1%)	
strong, >50% of stroma (+++)	18(30.5%)	11(18.6%)	7(11.9%)	
Desmoplastic stroma reaction	59 (100%)			$p = 0.042$
positiv	54(91.5%)	29(49.5%)	25(42.4%)	
little	22(37.3%)	10(16.9%)	12(20.3%)	
moderate	28(47.4%)	15(25.4%)	13(22%)	
strong	4(6.8%)	4(6.8%)	0 (0%)	
T stage (according to UICC 2009)	59 (100%)			$p = 0.022$
I	20 (33.9%)	9(15.3%)	11(18.6%)	
II	18 (30.5%)	6(10.2%)	12(20.3%)	
III	10 (16.9%)	3(5.0%)	7(11.9%)	
IVa	0 (0%)	0 (0%)	0 (0%)	
IVb	0 (0%)	0 (0%)	0 (0%)	
IVc	11 (18.6%)	11(18.6%)	0 (0%)	
Capsular invasion	44(74.6%)	23(39.0%)	21(35.6%)	$p = 0.027$
Vascular invasion	43(72.9%)	22(37.3%)	21(35.6%)	n.s.
Capsular and vascular invasion	28(47.5)	16(27.2%)	12(20.3%)	n.s.
minimal invasive	46(78%)	21(35.6%)	38(64.4%)	n.s.
widely invasive	13(22%)	6(10.2%)	7(11.9%)	n.s.
Distant metastasis (lung and bones)	11(18.6%)	11(18.6%)	0 (0%)	$p < 0.001$
Multifocality	3(5.1%)	2(3.4%)	1(1.7%)	n.s.
Lymph node metastasis	2(3.4%)	2(3.4%)	0 (0%)	n.s.
Calcification	22(37.3%)	15(25.4%)	7(11.9%)	$p < 0.025$
Necrosis	8(13.6%)	8(13.6%)	0 (0%)	$p = 0.002$
Non-neoplastic inflammation	18(30.5%)	6(10.2%)	12(20.3%)	n.s.
oncocytic type	26(44.1%)	10(16.9%)	16(27.1%)	n.s.
Goiter	33(55.9%)	17(28.8%)	16(27.1%)	n.s.
Tumour diameter (mean)	33.7 mm	36.0 mm	31.7 mm	n.s.
Recurrence	5(8.5%)	5(8.5%)	0 (0%)	n.s.
Mean age (years)	54.3y (24-78y)	55.1y (35-77y)	54.1y (24-78y)	$p = 0.011$
Female	46 (78%)	21(45%)	25(42.4%)	n.s.
Male	13 (22%)	8(13.6%)	5(8.5%)	n.s.

the downregulation of E-cadherin ($p = 0.913$, Mann-Whitney *U* test), expression of slug ($p = 0.973$, Mann-Whitney *U* test). Furthermore, metastasis was associated with the degree of desmoplasia ($p = 0.014$; Kruskal–Wallis), with calcification ($p = 0.008$; Fisher's exact test), necrosis ($p = 0.042$; Fisher's exact test), tumor size ($p = 0.015$; Mann-Whitney *U* test), and age ($p = 0.001$; Mann-Whitney *U* test), but not with the down-regulation of E-cadherin ($p = 0.135$, Mann-Whitney *U* test), expression of slug ($p = 0.317$, Mann-Whitney *U* test). In a Cox proportional hazards model, metastasis remained as an independent risk factor for overall survival (hazard rate: 10.2 [95% CI, 02.19 to 47.26]; $p = 0.003$) (Table 1).

Discussion

In this study, we investigated the expression of HIF-1α and correlation to clinicopathologic data in FTC. So far, this is the largest cohort study that investigated the expression of HIF-1α in primary FTC, it has been reported before in only one

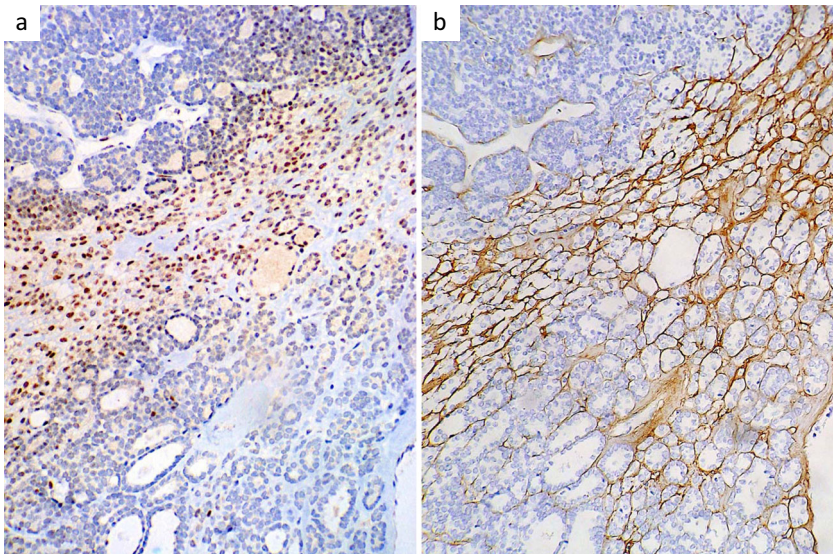

Fig. 1 Hypoxia-inducible factor 1α (HIF1α) and tenascin C in a follicular thyroid carcinoma. **a** Focal unequivocal nuclear HIF-1α immunoreactivity in an area with desmoplastic stroma reaction (original magnification, ×200). **b** Corresponding area shows a strong tenascin C expression in the extracellular matrix of the desmoplastic tumour stroma (original magnification, ×200)

functional study with a low case number without examination of metastasis, Burrows et al. also observed a variable intensity of staining. [23] In our study, nuclear expression of HIF-1α was present in every second FTC (49.2%). The pattern of the expression was focally accentuated, most likely due to focal hypoxic conditions.

Hypoxia inducible factor-1α expression was significantly associated with metastasis, and tumor stage (UICC). Furthermore, HIF-1α expression strongly correlated with desmoplastic stromal reaction, necrosis and tenascin C expression. These data suggest an important role of HIF-1α and its downstream proteins in the remodeling of the tumor stroma and in the process of angioinvasion and development of metastases. Since we found HIF 1a upregulation, tenascin C as well as desmoplastic reaction only focally intratumoral heterogeneity seems to play a role in the metastatic progression of this tumor type. Our data confirm the association between metastasis and lower prognosis on disease-free survival, age and tumor size. [18, 24]

HIF-1α was also shown to be present in papillary thyroid carcinoma and medullary thyroid carcinomas. [25–27] There, expression of HIF-1α was significantly associated with the presence of lymph node metastases and with stroma remodeling, too, stressing an important role in the invasive behavior of differentiated thyroid gland tumors. In contrast to papillary and also medullary thyroid carcinoma FTC primarily metastasize into distant organs, thus although a different metastatic procedure HIF-1α seem to play an important role in this kind of metastasis as well. HIF1a expression was not only seen in synchronal metastasis, but in all cases with metachronal distant metastasis. In other carcinomas, like breast, ovarian cervix, colorectal cancer and oligodendroglioma, the association of distant metastasis or worse prognosis and expression of hypoxia inducible factors is well known. [3, 7, 28–30]

The complexity of known HIF-1α target genes can be divided in a first program that responses to hypoxia through a switch from oxidative phosphorylation to anaerobic glycolysis and a second homeostatic program that increases oxygen levels through vasodilatation (iNOS) and vascular permeability (VEGF), and long-term through induction of neoangiogenesis and erythropoiesis (VEGF). [31–33] Hypoxia may promote neoangiogenesis via growth factors secreted by tumor cells and lead to hypoxia-induced metastases. [34, 35]

HIF-1α plays a key role in the hypoxia-induced transcription of several proteins. Under normal oxygen levels it binds to VHL protein and is rapidly degraded within the cytoplasm. Under hypoxic conditions, HIF-1α is stabilized and translocated into the nucleus. After heterodimerization with the hypoxia-inducible beta subunit, it acts as a transcription factor leading to transcription for genes that facilitate metabolic adaptation to hypoxia, particularly increasing cell proliferation and survival. Gene expression profiling identified several hundred direct HIF-1α targets on a genome-wide scale. HIF-1α also indirectly regulates gene expression by transactivating genes encoding microRNAs and chromatin modifying enzymes. [36] HIF-1α plays a key role in many critical aspects of cancer biology including angiogenesis, stem cell maintenance, metabolic reprogramming, autocrine growth factor signaling, epithelial-mesenchymal transition, invasion, metastasis, and resistance to radiation therapy and chemotherapy. [37, 38, 42].

The association of HIF-1α and EMT has been well described in the literature. In cell culture and knockout mice model, overexpression of HIF-1α induced FTC cells to undergo EMT and downregulated the epithelial marker E-cadherin. [39, 40] Hypoxia is an important factor that activates HIF signaling within tumors, and down- stream target gene expression. HIF-1α activates the expression and activity of several EMT-inducing factors including SNAIL, SLUG, TWIST and ZEB1

and inhibits the expression of E-cadherin. Activation of cancer-associated fibroblasts (CAF) and its target gene CAIX leads to EMT-inducing conditions for tumor cells. [11, 13–15, 41, 42] In our study, we could show expression of slug and loss of membranous E-cadherin expression in the majority of the FTCs. However, we could not show any significant correlation of HIF-1α or the occurrence of metastasis with slug or E-cadherin expression suggesting that other pathways/proteins might be involved in cancer progression of FTC.

In our study cohort, the expression of HIF-1α significantly correlated with the presence of tenascin C. Tenascin C is an extracellular matrix protein, expressed in stromal remodeling leading to desmoplastic morphology, which might increase the ability of tumor cells to invade lymphatic vessels. The aberrant expression of Tenascin C is known to promote cell migration, inhibit cell adhesion to fibronectin and induce cancer progression and stromal remodeling in model systems. [10, 43, 44] We could not show any significant correlation with slug or E-cadherin.

The activation of the HIF1 pathway may also contribute to the remodeling of the tumor stroma and the development of lymph node metastases. The correlation of hypoxia-associated proteins with fibrotic foci is also known in other tumor types, e.g. in pancreatic ductal adenocarcinoma and invasive breast carcinoma. When fibrosis is associated with tumor necrosis, it may result from hypoxia effects, too. [45, 46]

HIF-1α is not only activated by hypoxic conditions, but also influenced oncogenic stimuli. Mitogen activated protein kinase/extracellular signal regulated kinase (MAPK/MEK/ERK) kinase has been implicated as a regulator of HIF-1α not only by the phosphorylation of HIF-α but also by increasing its protein synthesis. [37, 47] Since many FTC harbor a mutation in the MAPK/MEK/ERK pathway, e.g. diverse RAS mutations, an oncogenic influence of the HIF 1α expression cannot be excluded. Further molecular studies are needed to elucidate the role of different genome alterations and the activation of the HIF-1α pathway. [9, 19, 48, 49]

Numerous drugs have been developed to inhibit HIF-1α activity on protein synthesis, mRNA levels, transcriptional activity or HIF-1α degradation. Traditional chemotherapy taken together inhibitors of HIF-1α may improve the efficacy of anti-angiogenic agents. [36] Interestingly, data from several mouse models indicate that use of VEGF receptor inhibitors reduced primary tumor growth and vascularization but increased metastasis, probably because impaired angiogenesis led to increased intratumoral hypoxia and increased HIF-1α activity. [50]

Conclusion

We showed that HIF-1α was expressed in almost 50% of FTCs and associated with distant metastasis. HIF-1α also correlated with the presence of tenascin C and desmoplastic stroma remodeling morphology. These data support the concept that hypoxia through HIF-1α activation acts as a hallmarks of cancer progression. Combination therapy targeting hypoxia-regulated proteins may provide new therapeutic options.

Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Desgrosellier JS, Cheresh DA (2010) Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 10:9–22. doi:10.1038/nrc2748
2. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899. doi:10.1016/j.cell.2010.01.025
3. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G (2000) Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 60:4693–4696
4. Kitada T, Seki S, Sakaguchi H, Sawada T, Hirakawa K, Wakasa K (2003) Clinicopathological significance of hypoxia-inducible factor-1alpha expression in human pancreatic carcinoma. Histopathology 43:550–555
5. Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, Hankinson O, Pugh CW, Ratcliffe PJ (1997) Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc Natl Acad Sci USA 94:8104–8109
6. Pugh CW, Gleadle J, Maxwell PH (2001) Hypoxia and oxidative stress in breast cancer. Hypoxia signalling pathways. Breast Cancer Res: BCR 3:313–317
7. Schindl M, Schoppmann SF, Samonigg H, Hausmaninger H, Kwasny W, Gnant M, Jakesz R, Kubista E, Birner P, Oberhuber G, Group ABaCCS (2002) Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin Cancer Res 8:1831–1837
8. Zalatnai A (2006) Molecular aspects of stromal-parenchymal interactions in malignant neoplasms. Curr Mol Med 6:685–693
9. De Wever O, Mareel M (2003) Role of tissue stroma in cancer cell invasion. J Pathol 200:429–447. doi:10.1002/path.1398
10. Yoshida T, Akatsuka T, Imanaka-Yoshida K (2015) Tenascin-C and integrins in cancer. Cell Adhes Migr 9:96–104. doi:10.1080/19336918.2015.1008332
11. Dong W, Qin G, Shen R (2016) Rab11-FIP2 promotes the metastasis of gastric cancer cells. Int J Cancer 138:1680–1688. doi:10.1002/ijc.29899
12. Huang CH, Yang WH, Chang SY, Tai SK, Tzeng CH, Kao JY, Wu KJ, Yang MH (2009) Regulation of membrane-type 4 matrix metalloproteinase by SLUG contributes to hypoxia-mediated metastasis. Neoplasia 11:1371–1382
13. Rankin EB, Giaccia AJ (2016) Hypoxic control of metastasis. Science 352:175–180. doi:10.1126/science.aaf4405

14. Serrano-Gomez SJ, Maziveyi M, Alahari SK (2016) Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Mol Cancer 15:18
15. Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression nature reviews. Cancer 2:442–454. doi:10.1038/nrc822
16. Chow SM, Law SC, Mendenhall WM, Au SK, Yau S, Yuen KT, Law CC, Lau WH (2002) Follicular thyroid carcinoma: prognostic factors and the role of radioiodine. Cancer 95:488–498. doi:10.1002/cncr.10683
17. Ito Y, Hirokawa M, Masuoka H, Yabuta T, Fukushima M, Kihara M, Higashiyama T, Takamura Y, Kobayashi K, Miya A, Miyauchi A (2013) Distant metastasis at diagnosis and large tumor size are significant prognostic factors of widely invasive follicular thyroid carcinoma. Endocr J 60:829–833
18. Ito Y, Miyauchi A, Tomoda C, Hirokawa M, Kobayashi K, Miya A (2013) Prognostic significance of patient age in minimally and widely invasive follicular thyroid carcinoma: investigation of three age groups. Endocr J 61:265–271
19. DeLellis RA (2006) Pathology and genetics of thyroid carcinoma J Surg Oncol 94:662–669. doi:10.1002/jso.20700
20. Sobin L, Gospodarowicz M, Wittekind C (2009) TNM classification of malignant tumours. Wiley-Blackwell, New York
21. Ghossein RA, Hiltzik DH, Carlson DL, Patel S, Shaha A, Shah JP, Tuttle RM, Singh B (2006) Prognostic factors of recurrence in encapsulated Hurthle cell carcinoma of the thyroid gland: a clinicopathologic study of 50 cases. Cancer 106:1669–1676. doi:10.1002/cncr.21825
22. Lang W, Choritz H, Hundeshagen H (1986) Risk factors in follicular thyroid carcinomas. A retrospective follow-up study covering a 14-year period with emphasis on morphological findings. Am J Surg Pathol 10:246–255
23. Burrows N, Resch J, Cowen RL, von Wasielewski R, Hoang-Vu C, West CM, Williams KJ, Brabant G (2010) Expression of hypoxia-inducible factor 1 alpha in thyroid carcinomas. Endocr Relat Cancer 17:61–72. doi:10.1677/ERC-08-0251
24. Ito Y, Miyauchi A, Tomoda C, Hirokawa M, Kobayashi K, Miya A (2014) Prognostic significance of patient age in minimally and widely invasive follicular thyroid carcinoma: investigation of three age groups. Endocr J 61:265–271
25. Koperek O, Akin E, Asari R, Niederle B, Neuhold N (2013) Expression of hypoxia-inducible factor 1 alpha in papillary thyroid carcinoma is associated with desmoplastic stromal reaction and lymph node metastasis. Virchows Archiv : Int J Pathol 463:795–802. doi:10.1007/s00428-013-1484-3
26. Koperek O, Bergner O, Pichlhöfer B, Oberndorfer F, Hainfellner JA, Kaserer K, Horvat R, Harris AL, Niederle B, Birner P (2011) Expression of hypoxia-associated proteins in sporadic medullary thyroid cancer is associated with desmoplastic stroma reaction and lymph node metastasis and may indicate somatic mutations in the VHL gene. J Pathol 225:63–72
27. Semenza G (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 33:207–214
28. Birner P, Gatterbauer B, Oberhuber G, Schindl M, Rössler K, Prodinger A, Budka H, Hainfellner JA (2001) Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer 92:165–171
29. Cleven AHG, van Engeland M, Wouters BG, de Bruïne AP (2007) Stromal expression of hypoxia regulated proteins is an adverse prognostic factor in colorectal carcinomas. Cell Oncol 29:229–240
30. Schoppmann SF, Fenzl A, Schindl M, Bachleitner-Hofmann T, Nagy K, Gnant M, Horvat R, Jakesz R, Birner P (2006) Hypoxia inducible factor-1alpha correlates with VEGF-C expression and lymphangiogenesis in breast cancer. Breast Cancer Res Treat 99:135–141
31. Bertout JA, Patel SA, Simon MC (2008) The impact of O2 availability on human cancer. Nat Rev Cancer 8:967–975. doi:10.1038/nrc2540
32. Dery M, Michaud M, Richard D (2005) Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. Int J Biochem Cell Biol 37:535–540
33. Erler JT, Giaccia AJ (2006) Lysyl oxidase mediates hypoxic control of metastasis. Cancer Res 66:10238–10241. doi:10.1158/0008-5472.CAN-06-3197
34. Hockel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93:266–276
35. Sullivan R, Graham CH (2007) Hypoxia-driven selection of the metastatic phenotype. Cancer Metastasis Rev 26:319–331. doi:10.1007/s10555-007-9062-2
36. Rapisarda A, Shoemaker RH, Melillo G (2009) Antiangiogenic agents and HIF-1 inhibitors meet at the crossroads. Cell Cycle (Georgetown, Tex) 8:4040–4043
37. Burrows N, Babur M, Resch J, Ridsdale S, Mejin M, Rowling EJ, Brabant G, Williams KJ (2011) GDC-0941 inhibits metastatic characteristics of thyroid carcinomas by targeting both the phosphoinositide-3 kinase (PI3K) and hypoxia-inducible factor-1α (HIF-1α) pathways. J Clin Endocrinol Metabol 96:E1934–E1943. doi:10.4061/2011/762905
38. Manalo D, Rowan A, Lavoie T, Natarajan L, Kelly B, Ye S, Garcia J, Semenza G (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 105:659–669
39. Chen S, Chen JZ, Zhang JQ, Chen HX, Yan ML, Huang L, Tian YF, Chen YL, Wang YD (2016) Hypoxia induces TWIST-activated epithelial-mesenchymal transition and proliferation of pancreatic cancer cells in vitro and in nude mice. Cancer Lett 383:73–84. doi:10.1016/j.canlet.2016.09.027
40. Lan L, Luo Y, Cui D, Shi BY, Deng W, Huo LL, Chen HL, Zhang GY, Deng LL (2013) Epithelial-mesenchymal transition triggers cancer stem cell generation in human thyroid cancer cells. Int J Oncol 43:113–120. doi:10.3892/ijo.2013.1913
41. Giannoni E, Bianchini F, Calorini L, Chiarugi P (2011) Cancer associated fibroblasts exploit reactive oxygen species through a proinflammatory signature leading to epithelial mesenchymal transition and stemness. Antioxid Redox Signal 14:2361–2371
42. Zhu GH, Huang C, Feng ZZ, Lv XH, Qiu ZJ (2013) Hypoxia-induced snail expression through transcriptional regulation by HIF-1α in pancreatic cancer cells. Dig Dis Sci 58:3503–3515
43. Chiquet-Ehrismann R, Tucker RP (2004) Connective tissues: signalling by tenascins. Int J Biochem Cell Biol 36:1085–1089. doi:10.1016/j.biocel.2004.01.007
44. Elvidge GP, Glenny L, Appelhoff RJ, Ratcliffe PJ, Ragoussis J, Gleadle JM (2006) Concordant regulation of Gene expression by hypoxia and 2-Oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1, HIF-2, and other pathways. J Biol Chem 281:15215–15226
45. Colpaert C, Vermeulen P, van Beest P, Goovaerts G, Weyler J, Van Dam P, Dirix L, Van Marck E (2001) Intratumoral hypoxia resulting in the presence of a fibrotic focus is an independent predictor of early distant relapse in lymph node-negative breast cancer patients. Histopathology 39:416–425
46. Couvelard A, O'Toole D, Leek R, Turley H, Sauvanet A, Degott C, Ruszniewski P, Belghiti J, Harris AL, Gatter K, Pezzella F (2005) Expression of hypoxia-inducible factors is correlated with the presence of a fibrotic focus and angiogenesis in pancreatic ductal adenocarcinomas. Histopathology 46:668–676

47. Takacova M, Bullova P, Simko V, Skvarkova L, Poturnajova M, Feketeova L, Babal P, Kivela AJ, Kuopio T, Kopacek J, Pastorek J, Parkkila S, Pastorekova S (2014) Expression pattern of carbonic anhydrase IX in medullary thyroid carcinoma supports a role for RET-mediated activation of the HIF pathway. Am J Pathol 184: 953–965 doi:10.1016/j.ajpath.2014.01.002
48. Faam B, Ghaffari MA, Ghadiri A, Azizi F (2015) Epigenetic modifications in human thyroid cancer. Biomed Reports 3:3–8. doi:10.3892/br.2014.375
49. Nikiforov YE (2011) Molecular analysis of thyroid tumors. Mod Pathol : Off J U S Can Acad Pathol, Inc 24:S34. doi:10.1038/modpathol.2010.167
50. Ebos JML, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS (2009) Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 15: 232–239