Expression of hypoxia-inducible factor-1α and vascular density in mammary adenomas and adenocarcinomas in bitches

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Abstract

Background: The study aimed at examining hypoxia-inducible factor (HIF)-1α expression in adenocarcinomas and adenomas in bitches in regard to tumour malignancy grade, proliferation, apoptosis and vascularisation. Therefore, paraffin sections of 15 adenomas and 64 adenocarcinomas sampled from 79 dogs aged 6 to 16 years were analysed.

Results: A significantly higher HIF-1α expression was noted in adenocarcinomas in comparison to adenomas (P < 0.0004). Moreover, HIF-1α expression in adenocarcinomas correlated positively with tumour malignancy grade (r = 0.59, P < 0.05), Ki-67 antigen expression (r = 0.43; P < 0.0005), TUNEL-positive cells (r = 0.62, P < 0.0001) and tumour vascularity measured by quantification of vessels characterized by the expression of von Willebrand Factor (r = 0.57, P < 0.05).

Conclusion: Results of this study indicate a similar biological role of HIF-1α in dogs and in humans, which may confirm suitability of the animal model in investigations on progression of tumours in humans.

Keywords: HIF-1, Adenocarcinoma, Adenoma, Mammary gland, Dog

Background

Neoplastic cells and cells of malignant tumours at their preliminary stage of development are supplied, in particular, with oxygen and metabolic products via diffusion. This secures conditions for their growth and attainment of a tumour diameter not around 2 mm. Subsequent growth of the tumour exceeding this size requires additional supply provided by blood vessels. Therefore, hypoxia develops within neoplastic tissue and tumour cells begin to manifest an increased demand for glucose and an accelerated glycolysis, in such conditions securing the principal source of ATP [1]. Glycolysis was shown to progress very efficiently in tumours growing in hypoxic conditions since they express hypoxia-inducible factor (HIF-1). This factor is responsible for an increased expression of several proteins, including glycolytic enzymes such as hexokinase-1 and -3, phosphofructokinase L, aldolase A and C, phosphoglycerate kinase-1, enolase-1, lactate dehydrogenase and the so-called glucose transporters GLUT-1 and GLUT-3 [1]. Intensity of glucose uptake by tumour cells was found to manifest positive correlation with their aggressiveness [1]. Moreover, HIF-1 stimulates tumour growth by activation of the VEGF gene transcription, which codes for vascular endothelial growth factor, the principal inducer of angiogenesis. In the absence of neovascularization tumour growth would be inhibited or even the tumour would show regression. Anti-neoplastic therapy takes advantage of this phenomenon by inhibiting angiogenesis in a tumour e.g. using monoclonal VEGF-specific antibodies [1,2]. It should be added that HIF-1 stimulates also transcription of IGF2 gene, coding for insulin-like growth factor 2 (IGF2), which facilitates survival of tumour cells also in an environment with diminished oxygen content [1,3].

HIF-1 is a heterodimer, consisting of HIF-1α and HIF-1β subunits. The HIF-1β subunit undergoes a constitutive expression while the expression of HIF-1α is low in most cells in normoxia conditions. Inhibition of HIF-1α expression results from activity of oxygen-dependent hydroxylases which enzymatically modify HIF-1α chain enabling its binding with von Hippel-Lindau tumour suppressor protein (VHL) [4]. In turn, VHL acts as a recognition factor for ubiquitin-protein ligase E3, which directs HIF-1α to degradation in proteasomes [5,6]. In normoxia conditions, half-life of HIF-1α protein is very short but it

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becomes markedly elongated in hypoxia [7]. Stimulation of HIF-1α synthesis utilizes the signalling pathway leading to a tyrosine kinase receptor, such as HER2 (Human Epidermal Growth Factor Receptor 2), with mediation of phosphatidylinositol-3-kinase (PI3K), serine/threonine kinases (AKT) and mammalian target for rapamycin (mTOR) [8]. The signalling pathway is inhibited by PTEN protein (phosphatase and tensin homologue deleted on chromosome ten), which dephosphorylates the product of PI3K reaction [1]. Therefore, HIF-1 may be regarded as a factor, which allows the cells to adapt to low tissue levels of oxygen.

Our study aimed at demonstration of HIF-1α protein expression and determination of its intensity in the most frequently manifested malignant and benign mammary tumours of epithelial origin (adenocarcinomas and adenomas) in bitches. Moreover, an attempt was made to correlate the obtained results with expression levels of the Ki-67 proliferation antigen and with blood vessel density of the tumours.

Methods
The research we performed was approved and financed by the National Science Center of Poland. As this research was performed on archival material routinely collected during surgical-treatment procedures and no additional harm was done to the animals due to the experiments, we did not require an additional ethics approval for our research. All the experiments were performed on disposable material which were not utilized for future scientific experiments. Only paraffin-embedded tissues were used for the study.

Tissue material and immunohistochemistry (IHC)
Material for the study was sampled during surgery in 79 female dogs of various breeds, aged 6 to 16 years. The tumours were verified by histopathological examination of the HE sections and represented adenomas (15 cases) and adenocarcinomas (64 cases).

Formalin-fixed, paraffin-embedded tissue was freshly cut (4 μm). The sections were mounted on Superfrost Plus slides (Menzel Gläser, Braunschweig Germany) and subsequently deparaffinised by boiling in Antigen Retrieval Solution (High pH = 9 for HIF-1α, Low pH = 6 for Ki-67; DakoCytomation, Glostrup, Denmark) using PT Link Rinse Station (DakoCytomation). Then, the sections were incubated (20 min; room temperature, RT) in Link48 automated staining platform (DakoCytomation) utilizing murine primary monoclonal antibodies diluted in the Background Reducing Antibody Diluent (DakoCytomation) and directed against HIF-1α (1:600; Novus Biologicals, Littleton, USA), von Willbrand Factor (vWF; 1:800; DakoCytomation) or Ki-67 (ready-to-use, DakoCytomation). The visualization of the studied antigens was performed using EnVision FLEX (DakoCytomation), according to the manufacturer’s instructions. All the sections were counterstained with Meyer’s hematoxylin. In all the cases, controls were included, in which specific antibody was substituted by the Primary Negative Control (DakoCytomation).

Apoptosis detection was performed utilizing the ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, USA). Paraffin sections were dewaxed in xylene, rehydrated in alcohol and rinsed in distilled water and 1xPBS, pH 7.4. Then, the sections were incubated in Proteinase K (DakoCytomation) for 5 min in RT and rinsed in 1xPBS. Endogenous peroxidase was blocked by 5 min incubation in 3% H2O2/1xPBS. Subsequently, the sections were incubated with Equilibration Buffer for 10 min in RT, with subsequent incubation with TdT Enzyme and Reaction Buffer at 37°C for 1 h. The reaction was stopped after 10 min incubation in the Stop Buffer and rinsed in 1xPBS. Then, anti-dioxygenin peroxidase-conjugated antibodies were applied for 30 min at RT. Following that, the sections were incubated for 10 min with diaminobenzidine (DAB; DakoCytomation) to visualize the TUNEL-positive cell nuclei. Finally, the sections were counterstained with Mayer’s hematoxylin and, after dehydration in alcohols, mounted in SUB-X Mounting Medium (both DakoCytomation).

Quantification of IHC reactions
Microphotographs of all the studied tumours were subjected to computer-assisted image analysis via a computer coupled to an Olympus BX53 optical microscope (Olympus, Japan). The set had the potential to record images and to perform their digital analysis. The measurements took advantage of Cell® software (Olympus Soft Imaging Solution GmbH, Germany).

Microscope examination allowed determination of the malignancy grade of the adenocarcinomas. The grade was established using the scale of Bloom-Richardson in modification of Elston and Ellis [9]. The evaluation method of the malignancy grade included three parameters scored in the scale from 0 to 3 points: formation of tubules (evident, moderate, slight), polymorphism of cell nuclei (slight, moderate, marked), number of mitotic figures per 10 microscopic fields at the magnification of ×400 (0–7, 8–16, ≥17). The sum of the points provided potential to distinguish three malignancy grades (G) among the tumours: 0–5 pts. – G1, 6–7 pts. – G2, 8–9 pts. – G3.

Expression of HIF-1α was appraised using the modified semi-quantitative immunoreactive score (IRS) scale according to Remmele (Table 1) [10]. The method takes into account both proportion of positively stained cells and intensity of the colour reaction, while the final score is the product of the parameters, with values ranging from 0 to 12 points (no reaction = 0 points (−); weak reaction = 1–2
points (+), moderate reaction = 3–4 points (++), intense reaction = 6–12 points (+++)).

Microvessel density (MVD) of vWF-positive vessels was quantified under × 200 magnification in five intratumoral areas of the lesion and the final score was determined as a mean of the five quantified areas.

The Ki-67 antigen expression and TUNEL stained sections were scored under × 400 magnification in five areas, in which number of positive cells presenting brown reaction colour were counted. The final score represented the percentage of positive tumour cells to all tumour cells in the examined sections.

**Statistical analysis**
The results were subjected to statistical analysis using Prism 5.0 (GraphPad, La Jolla, USA) software, employing Mann–Whitney test and Spearman’s correlation analysis.
In all the analyses, results were considered statistically significant when $P < 0.05$.

**Results**

Expression of HIF-1α protein was demonstrated both in mammary adenomas and adenocarcinomas in bitches (Figures 1A, 1B). In all the cases with positive reaction (90% adenocarcinomas and in 86% adenomas) a nuclear-cytoplasmic HIF-1α expression was noted. Moreover, in the two groups of tumours evident differences were seen in intensity of the protein expression. In adenocarcinomas, over 39% tumours manifested HIF-1α expression evaluated at +, over 26% expression evaluated at ++ and 25% at ++++. In adenomas, 86% of the examined tumours manifested expression of the protein which, however, did not exceed the + intensity. It should be noted that over 9% of adenocarcinomas and over 13% of adenomas manifested no HIF-1α expression. Statistical analysis using Mann-Whitney test demonstrated a significantly higher HIF-1α expression in adenocarcinomas than in adenomas ($P = 0.0004$) (Figure 2A).

The relationship between distribution of HIF-1α expression intensity and malignancy grade was also of interest. In G1 adenocarcinomas almost 28% of the tumours manifested no HIF-1α expression, 61% of them manifested the expression at + level and 11% at ++ level. In cases of G2 adenocarcinomas, 3% of the tumours manifested no HIF-1α expression, over 37% showed + expression, over 37% of them demonstrated ++ expression and almost 22% ++++ expression. G3 adenocarcinomas exhibited HIF-1α expression at + level in over 14% of cases, at ++ level in over 28% of cases and at +++ level in over 57% of cases. It should be added that only in the group of G3
adenocarcinomas over 50% of the tumours manifested high expression of the protein (++) while no such strong HIF-1α expression could be noted in G1 or G2 tumours.

Spearman's correlation test demonstrated a pronounced positive correlation between HIF-1α expression and tumour malignancy grade \( (r = 0.59; P < 0.05) \). Mann–Whitney test revealed significant differences in HIF-1α expression between particular malignancy grades of adenocarcinomas \( (G1 \text{ vs. } G2 \quad P = 0.251; G1 \text{ vs. } G3 \quad P = 0.098; G2 \text{ vs. } G3 \quad P = 0.586) \) – Figure 2B.

Tumour cell proliferation was determined by assessing the expression of the Ki-67 antigen (Figure 1C). Similarly, positive correlations were disclosed between tumour's malignancy grade and expression of Ki-67 proliferation antigen \( (r = 0.61; P < 0.0001) \), and between expressions of HIF-1α and Ki-67 \( (r = 0.43; P < 0.0005) \) – Figure 3A.

Studies on intensity of apoptosis using the TUNEL approach (Figure 1D) demonstrated that level of apoptosis in canine mammary adenocarcinomas manifested positive correlation with expression of HIF-1α protein and the correlation demonstrated a high level \( (r = 0.62; P < 0.0001) \) – Figure 3B.

An extremely important and interesting aspect of the study was the examination of a correlation between HIF-1α protein expression and vWF MVD (Figures 1E, 1F). Both in adenomas and in adenocarcinomas the correlation proved to be pronounced and positive. Its level was slightly higher in malignant tumours (adenocarcinomas: \( r = 0.57; P < 0.05 \) – Figure 3C) than in benign tumours (adenomas \( r = 0.52; P < 0.05 \) ). Mann–Whitney test demonstrated significant differences in vWF MVD between individual malignancy grades \( (G1 \text{ vs. } G2 \quad P = 0.251; G1 \text{ vs. } G3 \quad P = 0.098; G2 \text{ vs. } G3 \quad P = 0.586) \) – Figure 4.

Discussion

Immunohistochemical studies on human tumours demonstrated pronounced expression of HIF-1α in many common tumours, which probably represents a consequence of both hypoxia within the tumour and various genetic disturbances in neoplastic cells [11]. Moreover, HIF-1α expression was shown to manifest positive correlation with expression of VEGF and with density of microvessels in most of tumours of central nervous system [12], ovarian cancer [13], ductal mammary carcinoma [14], colon adenocarcinoma [15], endometrial adenocarcinoma [16], ductal pancreatic adenocarcinoma [17], small-cell pulmonary carcinoma [18]. The relationship seems to be of significance since not only elevated HIF-1α expression is linked to increased VEGF levels, but absence of HIF-1α expression in several cases results in a decreased VEGF level and, thus, an inhibited process of neoangiogenesis. The latter observation has been corroborated in studies on embryonal stem cells of mice devoid of HIF-1α expression, in which VEGF mRNA level was markedly decreased and could not be induced by hypoxia. Similarly in our study we have demonstrated a pronounced positive correlation between expression of HIF-1α and density of microvessels both in a malignant (adenocarcinoma) and benign (adenoma) tumours, even if in the latter case the correlation has shown a slightly lower level. However, it should be added that some reports documented absence of a significant correlation between expressions of mRNA for HIF-1α and VEGF in hypophyseal adenomas [19]. Their authors suggested that in such tumours VEGF expression may exhibit no pronounced dependence on expression of HIF-1α.

A very important element of HIF-1α biological activity is the induction of protein synthesis involved, i.a., in development of metastases, including vimentin, fibronectin, metalloproteinase 2, cathepsin D, urokinase-type plasminogen activator receptor (uPAR). Moreover, HIF-1α protein can induce a decrease in E-cadherin expression, the protein important for cell adhesion [20]. In studies on in vitro invasiveness of human pulmonary adenocarcinoma cells, Shyu et al. [21] demonstrated that in conditions of normoxia, HIF-1α manifested expression in cells presenting high...
invasiveness (CL1-5) while no expression of the factor was noted in cells of a low invasiveness (CL1). In parallel, the authors found that an increased HIF-1α expression in cells of human pulmonary carcinoma was linked to an increased invasiveness, probably due to an increased expression of uPAR receptor and metalloproteinase 1 and 2 (MMP1 and MMP2). In our study we have analogously found that HIF-1α expression has manifested positive correlation with certain prognostically unfavourable traits of the tumours, i.e., with malignancy grade and proliferative potential, reflected in expression of the Ki-67 antigen.

Shin et al. [18] found that levels of HIF-1α and VEGF mRNA expression were higher in tissues of an early or advanced colon carcinoma than in colon adenoma. They suggested that MMP-2, HIF-1α and VEGF might represent useful parameters for detection of early carcinogenesis and progression of colon carcinoma. In turn, Osada et al. [13] showed that HIF-1α expression in cell nucleus and HIF-2α in cytoplasm of neoplastic cells was linked to an unfavourable prognosis in women with ovarian carcinoma. The authors found also that HIF-1α expression in cell nucleus represented an independent prognostic factor in women with such a cancer. Similar results were obtained by Daponte et al. [22], who noted that survival of females with serous ovarian carcinoma, the cells of which demonstrated strong HIF-1α expression, was significantly abbreviated as compared to patients in whom neoplastic cells showed low or no HIF-1α expression. A pronounced expression of HIF-1α is linked to an increased risk of death at early stages of various types of malignant tumours, including carcinomas of uterine cervix, esophageal, mammary carcinoma and cerebral oligodendroglioma [12,23-25]. Moreover, reports are available that, augmented expression of HIF-1α in epithelial and mesenchymal tumours utilizing hypoxia inducible factor 1 (HIF-1) and blood vessel density in bitches of the Polish Ministry of Science and Higher Education. The authors thank Ms Aleksandra Jethon from the Department of Histology and Embryology, Wroclaw Medical University, for her technical assistance in performing immunohistochemical reactions.

Conclusions
High expression of HIF-1α in neoplastic cells, expressing their adjustment to hypoxia conditions in the tumour, in the course of why some tumours may provide a useful marker of canine tumour aggressiveness based on observed positive correlations with the tumour malignancy grade and the Ki-67 antigen expression. The HIF-1α expression in mammary adenomas and adenocarcinomas of bitches may indicate that the biological role of the protein is similar in tumours of canines and humans. This may confirm suitability of using the animal model in studies on progression of tumours in the man.

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

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References
1. Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 2002, 8:52–67.
2. Braghieri M, Sabbaga J, Hoff M: Bevacizumbab: overview of the literature. Expert Rev Anticancer Ther 2012, 12:567–580.
3. Savai R, Schermüly RT, Voswinckel R, Renigunta A, Reichmann B, Eul B, Grimminger F, Seeger W, Rose F, Hanze J: HIF-Talpha attenuates tumor growth in spite of augmented vascularization in an A549 adenocarcinoma mouse model. Int J Oncol 2005, 27:393–400.
4. Epstein AC, Geadlick JM, McNeill LA, Hewitson KS, O’Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhandha A, Tian YM, Masson N, Hamilton DL, Jaakola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ: C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001, 107:53–54.
5. Ivan M, Kondo K, Yang H, Kim W, Vailano J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr: HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001, 292:464–468.
6. Jaakola P, Mole DR, Tian YM, Wilson MI, Gelbert J, Gaskell SJ, Kriegshiem A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ: Targeting of HIF-α to the von Hippel–Lindau ubiquitylation complex by O2-regulated prolyl hydroxylase. Science 2001, 292:468–472.
7. Huang LE, Arany Z, Livingston DM, Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its a subunit. J Biol Chem 1996, 271:32253–32259.
8. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL: HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1α (HIF-1 α) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001, 21:4095–4104.
9. Elston CW, Ellis IO: Pathological prognostic factors in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991, 19:403–410.
10. Remmele W, Stegner HE: Vorschlag zur einheitlichen Definition eines immunoreaktiven Score (IRS) fur den immunhistochemischen Oestrogenrezeptor-Nachweis (ER-ICA) im Mammakarzinomgewebe. Pathologie 1987, 8:138–140.

11. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL: The expression and distribution of the hypoxia-inducible transcription factors HIF-1a and HIF-2a in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000, 157:411–421.

12. Bimer P, Gatterbauer B, Oberhuber G, Schindl M, Rössler K, Prodinger A, Budka H, Hainfellner JA: Expression of hypoxia-inducible factor-1α in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer 2001, 92:165–171.

13. Osada R, Horuchi A, Kikuchi N, Yoshida J, Hayashi A, Ota M, Katsumaya Y, Melillo G, Koniishi I: Expression of hypoxia-inducible factor 1α, hypoxia-inducible factor 2α, and von Hippel–Lindau protein in epithelial ovarian neoplasms and allelic loss of von Hippel-Lindau gene: nuclear expression of hypoxia-inducible factor 1α is an independent prognostic factor in ovarian carcinoma. Hum Pathol 2007, 38:1310–1320.

14. Bos R, Zhang H, Hansahan CF, Mommers EC, Semenza GL, Pinedo HM, Abeloff MD, Simons JW, van Diest PJ, van der Wall E: Levels of hypoxia-inducible factor-1α during breast carcinogenesis. J Natl Cancer Inst 2001, 93:309–314.

15. Jiang CQ, Fan LF, Liu ZS, Qian Q, Xia D, Diao LM, He YM, Ai ZL: Expression levels and significance of hypoxia inducible factor-1 alpha and vascular endothelial growth factor in human colorectal adenocarcinoma. Chin Med J 2004, 117:1541–1546.

16. Ozbudak IH, Karaveli S, Simsek T, Erdogan G, Pestereli E: Neoangiogenesis and expression of hypoxia-inducible factor 1alpha, vascular endothelial growth factor, and glucose transporter-1 in endometrioid type endometrium adenocarcinomas. Gynecol Oncol 2008, 108:603–608.

17. Sun HC, Qiu ZL, Liu J, Sun J, Jiang T, Huang KJ, Yao MH, Huang C: Expression of hypoxia-inducible factor-1 alpha and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. Int J Oncol 2007, 30:1359–1367.

18. Shin JE, Jung SA, Kim SE, Joo YH, Shim KN, Kim TH, Yoo K, Moon IH: Expression of MMP-2, HIF-1alpha and VEGF in colon adenoma and colon cancer. Korean J Gastroenterol 2007, 50:5–18.

19. Kim K, Yoshida D, Teramoto A: Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in pituitary adenomas. Endocr Pathol 2005, 16:115–121.

20. Brahim-Horn MC, Pouysségur J: Harnessing the hypoxia-inducible factor in cancer and ischemic disease. Biochem Pharmacol 2007, 73:450–457.

21. Shyu KG, Hsu FL, Wang MU, Wang BW, Lin S: Hypoxia-inducible factor 1alpha regulates lung adenocarcinoma cell invasion. Exp Cell Res 2007, 313:181–191.

22. Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE, Koukoulis G: Prognostic significance of hypoxia-inducible factor 1 alpha (HIF-1alpha) expression in serous ovarian cancer: an immunohistochemical study. BMC Cancer 2008, 335:1–10.

23. Bimer P, Schindl M, Oberhuber G, Plank C, Breitenecker G, Oberhuber G: Overexpression of hypoxia-inducible factor 1a is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000, 60:4596–4693.

24. Ogawa K, Chiba I, Morioka T, Shimoji H, Tamaki W, Takamura R, Nishimaki T, Yoshimi N, Murayama S: Clinical significance of HIF-1 alpha expression in patients with esophageal cancer treated with concurrent chemoradiotherapy. Anticancer Res 2011, 31:2351–2359.

25. Koutourakis M, Giatromanolaki A, Skarlatos J, Corti L, Blandamura S, Pizzaro M, Gatter KC, Harris AL: Hypoxia inducible factor (HIF-1a and HIF-2a) expression in early esophageal cancer and response to photodynamic therapy and radiotherapy. Cancer Res 2001, 61:1830–1832.

26. Zhong H, De Marzo AM, Laughner E, Linn M, Hiltun DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW: Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. Cancer Res 1999, 59:5830–5835.

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