Chapter 31
Tumor Oxygenation: An Appraisal of Past and Present Concepts and a Look into the Future

Arisztid G. B. Kovách Lecture

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Abstract Since 1970, the multifactorial pathogenesis of the deficient and heterogeneous oxygenation of transplanted murine tumors and of human cancers (including parameters determining oxygen delivery, e.g., blood flow, diffusion geometry, oxygen transport capacity of the blood) has been investigated in vivo. Hypoxia and/or anoxia was quantitatively assessed and characterized using microtechniques and special preclinical tumor models. Hypoxia subtypes were identified, and critical supply conditions were theoretically analyzed. In the 1980s, first experiments on humans were carried out in cancers of the rectum and of the oral cavity. In the 1990s, the clinical investigations were carried out on cancers of the breast and of the uterine cervix, clearly showing that hypoxia is a hallmark of locally advanced human tumors. In multivariate analysis, hypoxia was found to be an independent, adverse prognostic factor for patient survival due to hypoxia-driven malignant progression and hypoxia-associated resistance to anticancer therapy.

31.1 Introduction

During the directorship of Professor Gerhard Thews, research at the Institute of Physiology, University of Mainz, traditionally focussed on oxygen transport in blood, lung, brain, and heart. Joining his research team in 1970 as a postdoctoral research fellow, I was asked to "investigate the oxygen transport and respiratory gas exchange in other clinically relevant tissues and organs to expand the scope of research of the Institute" (G. Thews). After a careful and time-consuming literature

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search, I finally decided to study oxygen transport to the spleen (murine, rabbit, human) and to solid, malignant tumors, since reliable oxygenation data for these tissues were not available at that time, especially in terms of translation of the preclinical data to the clinical setting. In the following, chronology-oriented chapters are presented, and selected data obtained over the last 40 years are described, clearly showing the progress in relevant information.

The oxygenation status and data on the respiratory gas exchange of the spleen have been described earlier [1–3].

31.2 The Past

Since 1970, in vivo investigations have been carried out on isotransplanted rat tumors after the development and implementation of a "tissue-isolated" tumor model in the rat kidney involving a single artery feeding the tumor and a single vein draining the tumor, thus enabling the measurement of total blood flow and of the biologically relevant arteriovenous concentration differences of the substrates and catabolites of interest [4–6]. Key results using this tumor preparation are as follows (for details, see [4–12]): (a) tumor blood flow (TBF) and oxygen availability exhibit pronounced intra-tumor and inter-tumor heterogeneities; (b) tumor oxygenation is distinctly poorer than in normal tissue and shows similar heterogeneities to those found for TBF; (c) increasing oxygen availability through increasing TBF, arterial oxygen content, and hemoglobin concentration (cHb) can increase oxygen uptake and can improve tissue oxygenation; (d) oxygen availability is the major determinant of the oxygen consumption rate of cancers in situ; (e) oxygen consumption rate of cancers in situ is thus a function of TBF and arterial oxygen content; (f) weight-related TBF and tissue oxygenation generally decrease with increasing tumor size (not necessarily applicable to the clinical setting, see below); and (g) contrary to conventional belief, there is no evidence for a general mitochondrial dysfunction, speaking against a principal role of the Warburg effect in its original concept [6, 13].

Modulation of the tumor oxygenation status has been described as a result of therapeutic measures (irradiation [14], localized hyperthermia [15–19], photodynamic therapy [20], normobaric and hyperbaric hyperoxia [6, 21, 22], improvement of perfusion [23, 24], and correction of anemia using erythropoietin [25]).

Between 1977 and 1985, HbO_2 saturation of single red blood cells (RBCs) in tumor microvessels was registered in experimental rat tumors [26, 27], in cancers of the oral cavity and of the rectum, and in primary and metastatic bone tumors [28–30]. In accordance with the studies on experimental murine tumors, the oxygenation status in human cancers was poorer than in the normal tissue, exhibited pronounced intra-tumor and inter-tumor heterogeneities, and was positively correlated with the vascular density. In contrast to the experimental situation, the O_2 status in human tumors showed no size dependency.

In 1985, investigations were started to assess the oxygen status of orthotopically xenografted human breast cancers in immune-deficient rnu/rnu rats. In order to allow

measurements of TBF and the relevant arteriovenous concentration differences, a novel "tissue-isolated tumor" model was implemented [31, 32]. Experiments using different tumor histologies showed that – comparable to experimental murine tumors – the oxygen consumption and the median tissue pO_2 both were a function of TBF and the oxygen availability, respectively [33]. Theoretical analysis of the oxygen supply conditions in these xenografted human tumors led to the conclusion that oxygen seems to be the limiting substrate for unlimited proliferation and glucose for tumor cell survival [34, 35].

Using ^{31}P-NMR-spectroscopy studies starting in 1987, correlations between the bioenergetic status, the tissue oxygenation, and the intracellular pH (pH_i) were evaluated. In experimental murine tumors, pH_i was neutral to alkaline whereas the extracellular pH (pH_e) was acidic [36]. Intracellular pH was found to be alkaline as long as the median tissue pO_2 was above 10 mmHg. Below this threshold, pH_i became acidic [37–41] and the gradient between the intracellular pH and extracellular pH flattened.

In 1989 systematic investigations on gynecological patient cancers (cervix, breast, vulva) were initiated. In these patients, the pretherapeutic oxygenation status of primary and recurrent tumors was assessed using the pO_2 histography system [42–46]. Publication of these data had a tremendous impact in defining the role of tumor hypoxia in malignant progression and therapeutic resistance [47–52]. Key findings were as follows: (a) approx. 60 % of pretreatment cervical cancers were hypoxic; (b) cancer oxygenation was distinctly poorer than that of the normal tissues at the site of tumor growth; (c) the extent of hypoxia was independent of clinical size, stage, histology, grade, lymph node status, and various patient demographics; (d) hypoxia was aggravated in anemic patients; (e) hypoxia was less pronounced on transgression of stage IVA cervical cancers into the bladder wall; (f) recurrent tumors were more hypoxic than their primaries; and (g) there was no typical topological distribution of hypoxic areas within tumors (periphery vs. center).

Since 1990, investigations on hypoxia-driven malignant progression followed, based on the observations that in multivariate analysis, hypoxia was found to be a strong, independent, and adverse prognostic factor for overall and disease-free survival in cervical cancer patients [53–58].

In the last 10 years, the recognition of tumor hypoxia as a pivotal factor driving the development of a highly malignant phenotype – in which the HIF system, genetic instability, and clonal selection play a central role – has encouraged attempts to correlate the expression of "endogenous" hypoxia markers (HIFs, GLUT-1, CA IX) with the oxygenation status in identical, non-necrotic tumor microareas. Our results clearly showed that there is no correlation between the protein expression of these markers and pO_2 data measured with O_2 microsensors [59–62]. This supports the hypothesis that the HIF system can be stabilized even under normoxic conditions (e.g., by oncogenic growth factors, certain cytokines, glucose deprivation, acidosis, and gene mutations). From this it can be concluded that HIF-1α and its target genes cannot be considered as strict hypoxia markers, but instead should be considered to be markers of hypoxia-associated malignant progression.

31.3 The Present

Since 2008 our research focus is on the classification and quantification of hypoxia subtypes in xenografted human squamous cell carcinomas of the head and neck. In these experiments, hypoxia subtypes are categorized as follows: (a) continuous (chronic) hypoxia due to diffusion limitations or sustained microvascular flow stop by disturbed Starling forces, (b) intermittent (acute) hypoxia due to temporary obstructions of tumor microvessels or distinct fluctuations of RBC fluxes, and (c) hypoxemic hypoxia due to patient anemia or plasma flow in microvessels only [63]. Using tumor cryosections and (immuno-) fluorescence, detection and quantification of these subtypes showed that chronic hypoxia is the dominating subtype in vital tumor tissue, followed by acute and hypoxemic hypoxia. Analyses using microcirculatory supply units yielded pronounced (tumor size-dependent) intra-tumor heterogeneity and distinct variability between different tumor lines [64–67].

31.4 The Future

The extent of hypoxia subtypes, their respective fractions of total hypoxia, their time frames, and biological and therapeutic consequences will be investigated in the near future. Furthermore, detection and reliable quantification of hypoxia subtypes in the clinical setting are urgently needed, especially for critical judgment of fractionation schedules in radio(chemo-)therapy.

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Appendix A Citation Record of Most-Cited Publications of Vaupel et al. (ISI Web of Science, August 21, 2012)

1705 × Vaupel et al (1989) Cancer Res 49: 6449
1018 × Höckel et al (1996) Cancer Res 56:4509
895 × Höckel & Vaupel (2001) J Natl Cancer Inst 93:268
618 × Vaupel et al (1991) Cancer Res 51:3316

518 × Höckel et al (1993) Radiother Oncol 26:45
320 × Vaupel & Mayer (2007) Cancer Metastasis Rev 26:225
315 × Vaupel et al (1981) Cancer Res 41:2008
289 × Höckel et al (1991) Cancer Res 51:6098
235 × Vaupel (2004) Semin Radiat Oncol 14:198
231 × Vaupel et al (2001) Semin Oncol 28 (suppl 8):29
220 × Höckel et al (1999) Cancer Res 59:4525
206 × Vaupel (2004) Oncologist 9 (suppl 5):10
205 × Vaupel et al (2001) Med Oncol 18:243
205 × Kallinowski et al (1990) Int J Radiat Oncol Biol Phys 19:953
204 × Tatum et al (2006) Int J Radiat Biol 82:699
203 × Höckel et al (1996) Semin Radiat Oncol 6:3
168 × Höckel & Vaupel (2001) Semin Oncol 28 (suppl 8)36
164 × Vaupel & Harrison (2002) Oncologist 9 (suppl 5):4
161 × Kallinowski et al (1989) Cancer Res 49:3759
– 150 × Bicher et al (1980) Radiology 137:523

References

1. Vaupel P, Braunbeck W, Thews G (1973) Respiratory gas exchange and pO_2 distribution in splenic tissue. Adv Exp Med Biol 37A:401–406
2. Vaupel P, Manz R, Müller-Klieser W (1979) Respiratory gas exchange in the rat spleen in situ and intrasplenic oxyhemoglobin saturation. Pflügers Arch 379(1):109–111
3. Vaupel P, Frinak S, Müller-Klieser W, Manz R (1981) The end of a postulate: there are no hostile metabolic conditions within the normal spleen. Bibl Anat 20:403–406
4. Vaupel P, Günther H, Grote J, Aumüller G (1971) Atemgaswechsel und Glucosestoffwechsel von Tumoren (DS- Carcinosarkom) in vivo. Z Ges Exp Med 156(4):283–294
5. Schwarz W, Schulz V, Kersten M, Wörz R, Vaupel P (1971) Durchblutung und Sauerstoffverbrauch gewebsisolierter Impftumoren (DS- Carcinosarkom) in vivo. Z Krebsforsch 75(3):161–173
6. Vaupel P (1974) Atemgaswechsel und Glucosestoffwechsel von Implantationstumoren (DS-Carcinosarkom) in vivo. Funktionsanalyse biolog Systeme 1:1–138
7. Vaupel P (1977) Hypoxia in neoplastic tissue. Microvasc Res 13(3):399–408
8. Manz R, Otte J, Thews G, Vaupel P (1983) Relationship between size and oxygenation status of malignant tumors. Adv Exp Med Biol 159:391–398
9. Thews G, Vaupel P (1976) O_2 supply conditions in tumor tissue in vivo. Adv Exp Med Biol 75:537–548
10. Vaupel P, Thews G (1974) PO_2 distribution in tumor tissue of DS-carcinosarcoma. Oncology 30(6):475–484
11. Vaupel P, Frinak S, Bicher HI (1981) Heterogeneous oxygen partial pressure and pH distribution in C3H mouse mammary adenocarcinoma. Cancer Res 41(5):2008–2013
12. Vaupel P, Müller-Klieser W (1986) Cell line and growth site as relevant parameters governing tumor tissue oxygenation. Adv Exp Med Biol 200:633–643
13. Vaupel P, Mayer A (2012) Availability, not respiratory capacity governs oxygen consumption of solid tumors. Int J Biochem Cell Biol 44(9):1477–1481
14. Vaupel P, Frinak S, O'Hara M (1984) Reoxygenation of malignant mammary tumors after a single large dose of irradiation. Adv Exp Med Biol 180:773–782

15. Bicher HI, Hetzel FW, Sandhu TS et al (1980) Effects of hyperthermia on normal and tumor microenvironment. Radiology 137(2):523–530
16. Vaupel P, Frinak S, Müller-Klieser W, Bicher HI (1982) Impact of localized hyperthermia on the cellular microenvironment in solid tumors. Natl Cancer Inst Monogr 61:207–209
17. Vaupel P, Kallinowski F (1987) Physiological effects of hyperthermia. Recent Results Cancer Res 104:71–109
18. Vaupel P, Kallinowski F, Kluge M (1988) Pathophysiology of tumors in hyperthermia. Recent Results Cancer Res 107:65–75
19. Vaupel P, Kelleher DK (2010) Pathophysiological and vascular characteristics of tumors and their importance for hyperthermia: heterogeneity is the key issue. Int J Hyperthermia 26(3):211–223
20. Kelleher DK, Thews O, Scherz A, Salomon Y, Vaupel P (2004) Perfusion, oxygenation status and growth of experimental tumors upon photodynamic therapy with Pd-bacteriopheophorbide. Int J Oncol 24(6):1505–1511
21. Müller-Klieser W, Vaupel P, Manz R (1983) Tumor oxygenation under normobaric and hyperbaric conditions. Br J Radiol 56(668):559–564
22. Thews O, Kelleher DK, Vaupel P (2002) Dynamics of tumor oxygenation and red blood cell flux in response to inspiratory hyperoxia combined with different levels of inspiratory hypercapnia. Radiother Oncol 62(1):77–85
23. Vaupel P, Kelleher DK, Thews O (1998) Modulation of tumor oxygenation. Int J Radiat Oncol Biol Phys 42(4):843–848
24. Jung C, Müller-Klieser W, Vaupel P (1984) Tumor blood flow and O_2 availability during hemodilution. Adv Exp Med Biol 180:281–291
25. Kelleher DK, Matthienssen U, Thews O, Vaupel P (1996) Blood flow, oxygenation, and bioenergetic status of tumors following erythropoietin treatment in normal and anemic rats. Cancer Res 56(20):4728–4734
26. Vaupel P, Grunewald WA, Manz R, Sowa W (1977) Intracapillary HbO_2 saturation in tumor tissue of DS- Carcinosarcomas during normoxia. Adv Exp Med Biol 94:367–375
27. Vaupel P, Manz R, Müller-Klieser W, Grunewald WA (1979) Intracapillary HbO_2 saturation in malignant tumors during normoxia and hyperoxia. Microvasc Res 17(2):181–191
28. Müller-Klieser W, Vaupel P, Manz R, Schmidseder R (1981) Intracapillary oxyhemoglobin saturation in malignant tumors in humans. Int J Radiat Oncol Biol Phys 7(10):1397–1404
29. Wendling P, Manz R, Thews G, Vaupel P (1984) Heterogeneous oxygenation of rectal carcinomas in humans: a critical parameter for preoperative irradiation? Adv Exp Med Biol 180:293–300
30. Strube HD, Vaupel P, Manz R (1983) Inhomogene Oxygenierung bei primär malignen Knochentumoren sowie bei Knochenmetastasen und mögliche Konsequenzen für die Tumortherapie. Z Orthop 121:514
31. Vaupel P, Kallinowski F, Dave S, Gabbert H, Bastert G (1985) Human mammary carcinomas in nude rats–a new approach for investigating oxygen transport and substrate utilization in human tissues. Adv Exp Med Biol 191:737–751
32. Dave S, Kallinowski F, Vaupel P (1985) Blood flow and oxygen supply to human mammary carcinomas transplanted into nude rats. Adv Exp Med Biol 191:753–762
33. Vaupel P, Fortmeyer HP, Runkel S, Kalinowski F (1987) Blood flow, oxygen consumption and tissue oxygenation of human breast cancer xenografts in nude rats. Cancer Res 47(13):3496–3503
34. Vaupel P, Kallinowski F, Groebe K (1988) Evaluation of oxygen diffusion distances in human breast cancer using inherent in vivo data: role of various pathogenetic mechanisms in the development of tumor hypoxia. Adv Exp Med Biol 222:719–726
35. Groebe K, Vaupel P (1988) Evaluation of oxygen diffusion distances in human breast cancer xenografts using tumor-specific in vivo data: role of various mechanisms in the development of tumor hypoxia. Int J Radiat Oncol Biol Phys 15(3):691–697
36. Kallinowski F, Vaupel P (1988) pH distributions in spontaneous and isotransplanted rat tumors. Br J Cancer 58(3):314–321

37. Vaupel P, Okunieff P, Kallinowski F, Neuringer LJ (1989) Correlations between 31P-NMR spectroscopy and tissue O_2 tension measurements in a murine fibrosarcoma. Radiat Res 120(3):477–493
38. Vaupel P, Schaefer C, Okunieff P (1994) Intracellular acidosis in murine fibrosarcomas coincides with ATP depletion, hypoxia and high levels of lactate and total Pi. NMR Biomed 7(3):128–136
39. Vaupel P, Okunieff P, Neuringer LJ (1989) Blood flow, tissue oxygenation, pH distribution, and energy metabolism of murine mammary adenocarcinomas during growth. Adv Exp Med Biol 248:835–845
40. Vaupel P (1996) Is there a critical tissue oxygen tension for bioenergetic status and cellular pH regulation in solid tumors? Experientia 52(5):464–468
41. Vaupel P, Kallinowski F, Okunieff P (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 49(23):6449–6465
42. Vaupel P, Schlenger K, Knoop C, Höckel M (1991) Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurements. Cancer Res 51(12):3316–3322
43. Höckel M, Schlenger K, Knoop C, Vaupel P (1991) Oxygenation of carcinomas of the uterine cervix: evaluation of computerized O_2 tension measurements. Cancer Res 51(22):6098–6102
44. Höckel M, Schlenger K, Höckel S, Aral B, Schäffer U, Vaupel P (1998) Tumor hypoxia in pelvic recurrences of cervical cancer. Int J Cancer 79(4):365–369
45. Vaupel P, Mayer A, Höckel M (2006) Oxygenation status of primary and recurrent squamous cell carcinomas of the vulva. Eur J Gynaecol Oncol 27(2):142–146
46. Vaupel P, Höckel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal 9(8):1221–1235
47. Höckel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic and molecular aspects. J Natl Cancer Inst 93(4):266–276
48. Höckel M, Schlenger K, Mitze M, Schäffer U, Vaupel P (1996) Hypoxia and radiation response in human tumors. Semin Radiat Oncol 6(1):3–9
49. Vaupel P, Kelleher DK, Höckel M (2001) Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin Oncol 28(2 Suppl 8):29–35
50. Vaupel P (2004) Tumor microenvironmental physiology and its implications for radiation oncology. Semin Radiat Oncol 14:198–206
51. Vaupel P, Mayer A, Höckel M (2004) Tumor hypoxia and malignant progression. Methods Enzymol 381:335–354
52. Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 26(2):225–239
53. Höckel M, Knoop C, Schlenger K et al (1993) Intratumoral pO_2 predicts survival in advanced cancer of the uterine cervix. Radiother Oncol 26(1):45–50
54. Höckel M, Schlenger K, Aral B, Mitze M, Schäffer U, Vaupel P (1996) Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 56(19):4509–4515
55. Höckel M, Schlenger K, Höckel S, Vaupel P (1999) Hypoxic cervical cancers with low apoptotic index are highly aggressive. Cancer Res 59(18):4525–4528
56. Vaupel P (2008) Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. Oncologist 13(3):21–26
57. Vaupel P, Harrison L (2004) Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist 9(5):4–9
58. Vaupel P (2004) The role of hypoxia- induced factors in tumor progression. Oncologist 9(5):10–17
59. Mayer A, Wree A, Höckel M, Leo C, Pilch H, Vaupel P (2004) Lack of correlation between expression of HIF-1α protein and oxygenation status in identical tissue areas of squamous cell cancers of the uterine cervix. Cancer Res 64(16):5876–5881
60. Mayer A, Höckel M, Wree A, Vaupel P (2005) Microregional expression of glucose transporter GLUT-1 and oxygenation status: lack of correlation in locally advanced cervical cancers. Clin Cancer Res 11(7):2768–2773

61. Mayer A, Höckel M, Vaupel P (2005) Carbonic anhydrase IX expression and tumor oxygenation status do not correlate at the microregional level in locally advanced cancers of the uterine cervix. Clin Cancer Res 11(20):7220–7225
62. Mayer A, Höckel M, Vaupel P (2006) Endogenous hypoxia markers in locally advanced cancers of the uterine cervix: reality or wishful thinking? Strahlenther Onkol 182(9):501–510
63. Bayer C, Shi K, Astner ST, Maftei CA, Vaupel P (2011) Acute versus chronic hypoxia: why a simplified classification is simply not enough. Int J Radiat Oncol Biol Phys 80(4):965–968
64. Maftei CA, Bayer C, Shi K, Astner ST, Vaupel P (2011) Quantitative assessment of hypoxia subtypes in microcirculatory supply units of malignant tumors using (immuno-) fluorescence techniques. Strahlenther Onkol 187:260–266
65. Maftei AC, Bayer C, Shi K, Astner ST, Vaupel P (2011) Changes in the fraction of total hypoxia and hypoxia subtypes in human squamous cell carcinomas upon fractionated irradiation: evaluation using pattern recognition in microcirculatory supply units. Radiother Oncol 101(1):209–216
66. Maftei AC, Bayer C, Shi K, Vaupel P (2012) Intra- and intertumor heterogeneities in total, and acute hypoxia in xenografted squamous cell carcinomas: detection and quantification using (immuno-) fluorescence techniques. Strahlenther Onkol 188(7):606–615
67. Bayer C, Vaupel P (2012) Acute versus chronic hypoxia in tumors: controversial data concerning time frames and biological consequences. Strahlenther Onkol 188(7):616–627