Microregional Expression of Glucose Transporter-1 and Oxygenation Status: Lack of Correlation in Locally Advanced Cervical Cancers

Arnulf Mayer,1 Michael Höckel,2 Alexander Wree,1 and Peter Vaupel1

Abstract Purpose: Glucose transporter-1 (GLUT-1), a target gene of hypoxia-inducible factor-1, has been considered a candidate endogenous marker of tumor hypoxia. Expression of GLUT-1 may also serve as an indicator for the induction of the transcriptional response to hypoxia, which has been linked to enhanced proliferation, resistance to therapy, and metastatic propagation of cancer cells. Overexpression of GLUT-1 has been shown to correlate with poor prognosis in several tumor entities, among them cancers of the uterine cervix. The validity of these hypotheses is investigated.

Experimental Design: The expression of GLUT-1 was assessed in 80 biopsies of Eppendorf oxygenation measurement tracks from locally advanced cervical cancers in 47 patients using immunohistochemistry.

Results: No correlation was found between the expression of GLUT-1 and oxygenation variables (median $p_{O_2}$, HF 2.5 and HF 5). Expression of GLUT-1 was found greater in larger tumors ($P = 0.0001$) and to exhibit a linear increase with Fédération Internationale de Gynécologie et d’Obstétrique stage ($P = 0.002$). Overall survival ($P = 0.004$) and recurrence-free survival ($P = 0.007$) were significantly shorter for patients with expression of GLUT-1. In the subgroup of patients treated with surgery, this effect on prognosis was not independent when $pT$ stage or $pN$ stage were included in a multivariate Cox proportional hazards model.

Conclusions: The suitability of GLUT-1 as an endogenous marker of tumor hypoxia seems questionable. The association with prognosis may partially depend on confounding factors.

Under physiologic conditions, glucose transporter-1 (GLUT-1) is expressed most strongly in erythrocytes and blood-brain or blood-nerve barriers (1–3). Overexpression of GLUT-1 is found in many types of solid malignancies (1), among them cancers of the uterine cervix (4). The present study examined the possibility of associating the expression of GLUT-1 with 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

Materials and Methods

Patients. All patients in this study were enrolled in 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

Authors’ Affiliations: 1Institute of Physiology and Pathophysiology, University of Mainz and 2Department of Obstetrics and Gynecology, University of Leipzig, Germany

Received 11/17/04; revised 1/4/05; accepted 1/11/05.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Arnulf Mayer, Institute of Physiology and Pathophysiology, University of Mainz, Duesbergweg 6, 55128 Mainz, Germany. Phone: 49-6131-392-5203; Fax: 49-6131-392-5774; E-mail: arnmayer@uni-mainz.de.

© 2005 American Association for Cancer Research.
Tissue, up to 35 o'clock sites in macroscopically vital tumor tissue. Within the tumor the mons pubis followed by cervical measurements at the 12 and 6 done in the conscious patient along linear tracks, first in the s.c. fat of the pretherapeutic system (Eppendorf, Hamburg, Germany), using a protocol that has been described in detail previously (25). Briefly, \( pO_2 \) readings were done in the conscious patient along linear tracks, first in the s.c. 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 following \( pO_2 \) 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 where \( pO_2 \) readings had been obtained. Both the \( pO_2 \) readings and the needle core biopsies were done without general anesthesia in all 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 done 1 to 5 days before oncological treatment. After histologic examination of the biopsy specimens, \( pO_2 \) measurements in necrotic tissue areas were excluded from analysis.

**Assessment of GLUT-1 expression.** A semiquantitative scoring system was used to assess the degree of GLUT-1 expression in entire biopsy sections: score 0, no staining or only very few positive cells ("absent"); score 1, <10% positive ("weak"); score 2, 11% to 50% positive ("moderate"); score 3, >50% positive ("strong"). Each specimen was scored by two independent observers (A.M. and A.W.). Discordant cases were reevaluated and discussed using a conference microscope.

### Table 1. Patient and tumor characteristics at the time of pretherapeutic oxygen tension measurements

<table>
<thead>
<tr>
<th>No. patients</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I B</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>9 (6)</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>2 (0)</td>
<td></td>
</tr>
<tr>
<td>IVB</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7 (6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19 (17)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 (13)</td>
<td></td>
</tr>
<tr>
<td>ND</td>
<td>7 (6)</td>
<td></td>
</tr>
<tr>
<td>pT stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1b</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>pT2a</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>pT2b</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>pT3b</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>17 (12)</td>
<td></td>
</tr>
<tr>
<td>pN stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>NX</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>17 (12)</td>
<td></td>
</tr>
<tr>
<td>Largest tumor diameter (mm)</td>
<td>60 (50)</td>
<td>0-150 (0-80)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>24 (23)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>23 (19)</td>
<td></td>
</tr>
<tr>
<td>Patient age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>27 (26)</td>
<td>52</td>
</tr>
<tr>
<td>≥55</td>
<td>20 (16)</td>
<td>26-80</td>
</tr>
<tr>
<td>Hemoglobin concentration (g/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 g/dL</td>
<td>13 (11)</td>
<td>12.8 (12.9)</td>
</tr>
<tr>
<td>≥12 g/dL</td>
<td>33 (31)</td>
<td></td>
</tr>
<tr>
<td>ND</td>
<td>1 (0)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Numbers in brackets indicate deviations for the subgroup of patients treated with curative intent (survival correlations). Abbreviations: ND, not documented; NA, not applicable, no surgical treatment (radiation only).
significance level was set at $\alpha = 5\%$ for all comparisons. Linear correlations between two variables 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. The Cox proportional hazards model was used for the multivariate analysis of the effect of individual factors on survival.

Results

GLUT-1 expression. GLUT-1 expression exhibited a characteristic pattern, with staining intensity increasing as a function of distance from the vascularized tumor stroma (Fig. 1), being particularly strong in the viable cell layers immediately adjacent to necrotic areas. Erythrocytes and perineural tissue invariably stained positive. Variation of erythrocyte staining intensity was...
very low, indicating a negligible batch to batch variation in overall GLUT-1 immunoreactivity. No positive staining was seen in the vascular tumor stroma. GLUT-1 expression was present in 59 of 80 biopsies (~74%). Of these, GLUT-1 expression was weak in 37 cases (~63%), moderate in 18 cases (~30%), and strong in four cases (~7%).

**GLUT-1 expression, clinical, and pathohistologic data.** GLUT-1 expression increased linearly with Fédération Internationale de Gynécologie et d’Obstétrique (FIGO) stage ($r = 0.35, P = 0.002$; see Fig. 2). Significantly higher expression of GLUT-1 was also found in larger tumors ($r = 0.42, P = 0.0001$) and in tumors with a higher pT stage ($r = 0.34, P = 0.015$). GLUT-1 expression showed no correlation with histologic grading, pN stage, patient age, parity, and pretherapeutic hemoglobin level.

**GLUT-1 expression and oxygenation status.** The Kruskal-Wallis test showed no differences in median $pO_2$, hypoxic fraction $\leq 2.5$ mm Hg (HF 2.5) and hypoxic fraction $\leq 5$ mm Hg (HF 5) between the four GLUT-1 expression scores. There were also no statistically significant differences in GLUT-1 expression between individual categories (e.g., absent expression versus strong expression; Fig. 3). A subgroup analysis of squamous cell carcinomas only ($n = 60$) also showed no differences in any of the oxygenation variables between the four classes of intensity of GLUT-1 expression. A weak trend ($r = 0.34; P = 0.14, n = 20$) was seen for higher values of HF 5 in non–squamous cell histology cases with higher GLUT-1 expression. As Fig. 3 shows, some severely hypoxic tumors had weak to absent expression of GLUT-1 and normoxic tumors repeatedly showed moderate to strong GLUT-1 expression.

**GLUT-1 expression and survival.** Univariate Kaplan-Meier survival analysis showed significantly improved overall ($P = 0.004$) and recurrence-free ($P = 0.007$) survival for patients whose tumors entirely lacked (both biopsies negative in cases where two biopsies were available) expression of GLUT-1 (see Fig. 4). In addition, correlations with poorer overall and recurrence-free survival, respectively, were found for the presence of lymph node metastasis ($P = 0.0002$ and $P = 0.0001$), higher pT stage ($P = 0.02$ and $P = 0.0367$) and higher FIGO stage ($P = 0.0067$ and $P = 0.0248$). When either pT stage or pN stage were included in a multivariate Cox proportional hazards analysis (only applicable in cases treated with surgery), there was no significant independent influence of GLUT-1 expression on prognosis. Only pN stage remained a significant prognostic factor for overall ($P = 0.015$) and recurrence-free ($P = 0.007$) survival.

**Discussion**

The primary aim of this study was to evaluate the suitability of GLUT-1 as an endogenous marker of tumor hypoxia. The expression of GLUT-1 was analyzed using immunohistochemistry in biopsy specimens taken from oxygenation measurement tracks done with the Eppendorf microsensor system. The fact that both measurements originate from identical tissue microareas is a novel feature of this study. Using this methodology, no correlation of GLUT-1 expression and oxygenation variables (median $pO_2$, HF 2.5 and HF 5) were found. Several severely hypoxic tissue biopsies showed weak or no expression of GLUT-1, whereas moderate to strong expression was repeatedly found in normoxic specimens. In a recent study, Airley et al. (4) described a weak, albeit statistically significant, correlation of higher GLUT-1 expression in cases with higher values of HF 2.5. This finding may in the first instance be interpreted as being contradictory to our results. On closer examination however, the suitability of GLUT-1 as an endogenous hypoxia marker seems highly questionable from the data of both studies. In agreement with our own results, Airley et al. (4) found no correlation of GLUT-1 expression with HF 5 and the study also does not mention a correlation with the median $pO_2$. Both studies show that GLUT-1 expression may be absent in a significant amount of severely hypoxic tumors and that well-oxygenated tumors may exhibit very strong expression of GLUT-1. Two recent studies compared GLUT-1 (and carbonic anhydrase IX) expression with the accumulation of the “hypoxia-marker” pimonidazole and found a strong spatial colocalization and correlation between the two variables, concluding that both proteins may be regarded as endogenous hypoxia markers (17, 26). This interpretation is problematic.
since pimonidazole, as well as another “extrinsic” hypoxia marker, EF5, have been shown not to correlate with the oxygenation status, as directly measured with microelectrodes (27–30). It also has to be kept in mind that prognostic correlations have thus far only been shown for the “snapshot” picture of hypoxia acquired with the Eppendorf microsensor technique, assessing all pathophysiologically relevant types of hypoxia (acute and chronic), provided measurements in necrotic tissue areas can be excluded as done in our study. Similar to our findings, Hedley et al. (31) in a recent study from recent findings by other groups, the role of GLUT-1 as an endogenous marker of tumor hypoxia is questionable, at least for cancers of the uterine cervix. There is an association of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

A remarkable finding in the present study is the correlation of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

In conclusion, from the data of the present study as well as from recent findings by other groups, the role of GLUT-1 as an endogenous marker of tumor hypoxia is questionable, at least for cancers of the uterine cervix. There is an association of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

A remarkable finding in the present study is the correlation of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

Another important issue is the prognostic effect of GLUT-1 as a marker of the hypoxic response. In univariate analysis, an improved overall and recurrence-free survival in patients with completely absent GLUT-1 expression (i.e., two negative biopsies) was found. Multivariate Cox regression analysis revealed that both correlations were independent of FIGO stage, clinical tumor size, histologic grading, patient age, and pretherapeutic hemoglobin concentration. Inclusion of pT stage or pN stage into the model, however, abrogated the independent prognostic effect of GLUT-1 expression. The dependency of the prognostic relevance of GLUT-1 expression on FIGO stage and N stage has been described for other tumor entities (e.g., breast carcinoma; ref. 23) and colorectal cancer (16, 22). The only study that evaluated the prognostic effect of GLUT-1 expression in cancers of the uterine cervix found a significant correlation with prognosis only for metastasis-free survival. A possible dependency of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

A remarkable finding in the present study is the correlation of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

A remarkable finding in the present study is the correlation of GLUT-1 expression with FIGO stage, T stage, and maximum clinical tumor size. Correlations between GLUT-1 expression and tumor size have also been described by others (14, 39–41) and may have important pathophysiologic implications. Because it is known (24) that tumor oxygenation is independent of this correlation on nodal status could not be analyzed, as only patients treated with radiotherapy were examined (4).

Expression of GLUT-1, much like HIF-1α, is induced by a variety of stimuli besides hypoxia. For GLUT-1, established inducing factors are glucose deprivation (33), oncogenic transformation (e.g., overexpression of c-MYC; ref. 34), inhibition of oxidative phosphorylation (35), angiotensin II (in mesangial cells; ref. 36), and osmotic stress (37, 38). According to our interpretation of the data, induction of HIF-1α and subsequent transactivation of GLUT-1 by hypoxia, although undoubtedly present, cannot be selectively identified due to the heterogeneous occurrence of the other above-mentioned factors in human cancer specimens.

Acknowledgments

We thank Prof. Dr. M.A. Konerding (Department of Anatomy, University of Mainz) for providing the DISKUS image acquisition system, Beate Köhler for excellent technical assistance, and Dr. Debra K. Kelleher for her valuable assistance in preparing this article.
References


Microregional Expression of Glucose Transporter-1 and Oxygenation Status: Lack of Correlation in Locally Advanced Cervical Cancers

Arnulf Mayer, Michael Höckel, Alexander Wree, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/7/2768

Cited articles
This article cites 39 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/7/2768.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/11/7/2768.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.