Blood Flow, Metabolism, Cellular Microenvironment, and Growth Rate of Human Tumor Xenografts


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ABSTRACT

Better understanding of the micromilieu of human tumors in situ is mandatory for further improvement of diagnostic and therapeutic interventions. Since investigations of untreated tumors of a wide size range are precluded in humans for ethical reasons, size-dependent changes in the pathophysiology of primary and metastatic human tumors were studied using "tissue-isolated" xenografts in nude rats. Tumor types included lung and breast cancers, ovarian and thyroid carcinomas, uterine tumors, and melanomas. A 10-fold variation in weight-adjusted tumor perfusion indicated large variations in angiogenesis which were unrelated to tumor type. Flow values obtained were consistent with data from clinical observations and were comparable to that in isografted rodent tumors. Using actual consumption and supply rates, maximum oxygen and glucose uptake rates were calculated for each tumor type. The capacity to consume oxygen and glucose varied 9-fold and 4-fold, respectively. However, considering actual consumption rates, blood flow was the principal modulator of substrate supply and tumor metabolism in these human tumor xenografts. Consequently, therapeutically relevant parameters of the metabolic micromilieu largely depended on the efficacy of the tumor circulation. Hereby, high metabolic rates concomitant with high flow values coincided with rapid tumor growth. Thus, in order to design the best individualized therapy, flow-related data should supplement histological classification and clinical staging and grading. Further development of relatively noninvasive technologies (magnetic resonance imaging, magnetic resonance spectroscopy, or positron emission tomography) might permit such monitoring.

INTRODUCTION

During malignant transformation, genomic changes occur (1, 2). As a consequence, the enzymology of cancer cells is altered (3, 4), and inherent resistance to radiation and chemotherapy can increase (5, 6). In vitro, gene expression, growth factor response, nutrient supply, cellular proliferation, and treatment sensitivity are interrelated (7–9).

In vivo, growth of solid tumors requires the formation of new blood vessels (10). It has not yet been conclusively shown whether or not oncogenesis and angiogenesis are linked (11). Several angiogenic factors, produced by tumor or immune cells, mediate the process of neovascularization (12).

Considering isografted rodent tumors, it is well established that the efficiency of the new vascular network, and consequently of microcirculation, governs nutritive tumor perfusion (13–15). Within one tumor line, a flow decline per unit of tumor weight during growth can lead to the development of hypoxia, acidosis, and substrate depletion in tumor microareas, which can markedly alter the tumor response to nonsurgical treatment modalities (16–18). Such microareas are heterogeneously distributed within individual tumors due to an anisotropic flow distribution (13–15).

Data on primary or metastatic tumors in patients is inconclusive. Repeated investigation of such tumors at different growth stages is usually impossible due to the need for immediate therapeutic intervention. In order to bridge the gap, we investigated therapeutically relevant pathophysiological properties of various xenografted human tumors.

MATERIALS AND METHODS

Animals and Tumors. Different primary or metastatic human tumors were investigated (6 breast cancers, 1 lung cancer, 2 ovarian carcinomas, 2 uterine tumors, 2 thyroid cancers, 2 malignant melanomas). Tumor tissue was xenografted into athymic mice (NMRI-nu/nu) without prior adaptation to culture conditions. Details of the breeding and maintenance of immunodeficient rodents were described previously (19). After at least four mouse passages, tumors from this "bank" were transplanted into athymic, T-cell-deficient (WAG/Fra-nu/nu) rats. Tissue was grown either s.c. in the flank or as "tissue-isolated" tumor preparations in the groin (implantation technique described in Refs. 20 and 21). In addition, DS-carciinosarcomas in Sprague-Dawley rats were studied in order to allow a comparison of the results obtained with those previously reported on isografted rodent tumors.

Tumor Perfusion and Substrate Turnover. The "tissue-isolated" tumors were supplied by a single artery and drained by one vein and thus permitted the measurement of global tumor blood flow, oxygen consumption, glucose uptake, and lactate release. The techniques used have been described previously (21, 22). In brief, cannulas were inserted into the right jugular vein, the left carotid artery, and the tumor-draining vein after the animals were anesthetized (sodium pentobarbital, Nembutal; Ceva, Paris; 35 mg/kg i.p.). Total tumor blood flow was measured by timed collection of the tumor venous outflow. Arterial and tumor venous blood samples were obtained, and relevant parameters of the respiratory gas exchange were determined (O2 and CO2 tensions, pH, oxyhemoglobin saturation, hematocrit, hemoglobin concentration). From these data, the O2 content of the blood samples was calculated assuming maximal oxygen binding by hemoglobin, oxygen dissociation curves as given by Bork et al. (23) and a constant oxygen solubility coefficient as reported by Zander (24). Glucose and lactate concentrations were determined enzymatically. From these data, the respective uptake or release rates could be calculated taking into account the actual tumor perfusion rate and the relevant concentration differences between arterial and tumor venous blood. Blood loss due to sampling was adequately replaced by fresh donor blood. During the experiments, relevant systemic parameters, such as arterial blood pressure, acid-base status, and rectal temperature, were monitored.

Measurement of Tumor Tissue Oxygenation. pO2 measurements were performed in s.c. tumors with steel-sheathed O2-sensitive needle electrodes (recessed gold-in-glass electrode; outer diameter, 350 μm; diameter of the cathode, 12 μm). The electrode was moved through the tissue in steps of 1 mm. The forward motion was immediately followed by a backward step of 0.3 mm in order to relieve tissue pressure. The local oxygen partial pressures were measured 1 s after the backward motion (KIMO 6650, Sigma pO2, histogram; Eppendorf, Hamburg, Federal Republic of Germany). Several tracks were performed in each tumor recording a total of 60–120 pO2 values. For comparison, pO2 data were also determined in rat liver. Mean arterial blood pressure, rectal temperature, and relevant arterial blood gas parameters were monitored throughout the experiments.

Theoretical Analysis. From all parameters investigated, descriptive statistical parameters were calculated. Mean values and standard errors were calculated. 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. Financial support from the "Gesellschaft der Goenner und Foerderer der Grundlagenforschung des Krebses" (Mainz, FRG) is gratefully acknowledged.

Received 12/21/88; revised 3/13/89; accepted 4/5/89.

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are given in the text if not indicated otherwise. Differences between groups were assessed for statistical significance using the Kruskal-Wallis H test, the t test for paired and unpaired observations, and the Mann-Whitney U test. Perfusion-related changes were statistically evaluated using least square routines for multiple analysis of variance within the framework of general linear models (general linear model procedure, SAS; SAS Institute, Cary, NC). Maximal oxygen and glucose consumption values were estimated with a nonlinear maximum likelihood least-squares routine assuming optimum supply conditions (22).

RESULTS

Tumor Perfusion and Metabolism. A total of 294 "tissue-isolated" preparations of the various tumor lines were investigated. The xenografts of various tumors types exhibited very different growth properties, reaching tumor sizes between 0.3 and 14.6 g after growth periods up to 6 weeks. Perfusion values and metabolic rates varied considerably both within and in between different tumor lines. In all tumors, blood flow per unit weight was higher in small transplants than at advanced growth stages (as an example, see ovarian cancers in Fig. 1, top). The most pronounced flow decline already occurred before gross tissue necrosis was noted. At relatively constant arterial substrate concentrations, the oxygen and glucose supply was mostly determined by tumor perfusion. The oxygen consumption and glucose uptake rates generally followed the changes in the respective availabilities. Therefore, the decline of the oxygen consumption (Fig. 1, top) and glucose uptake with increasing tumor sizes almost paralleled the reduction in tumor perfusion (Fig. 1, bottom). Lactate release was mainly determined by the amount of glucose taken up (Fig. 1, inset). From these series of experiments, it can be concluded that, within tumors of the same cell line, tumor size is the primary determinant of tumor blood flow per unit weight at constant arterial blood pressure. Metabolic functions mainly depend on the substrate supply at a given tumor size, which, in turn, is governed by tumor perfusion.

Since tumor size greatly influenced weight-related tumor perfusion and metabolic functions, xenografts from different cell lines but of similar volumes were compared. The results presented hold qualitatively true for every tumor weight. Relevant statistical parameters for tumor masses around 2.5 g are given in Table 1.

Considering only tumors of comparable sizes, tumor perfusion varied markedly. The most pronounced variations were observed in smaller tumors (wet weights, <2 g) with 10-fold flow differences between the various tumor lines.

Similar to the finding in a single tumor cell line, tumor perfusion was the principal modulator of supply and consumption both of oxygen and glucose (tumor masses, about 2.5 g, i.e., 1% of body weight; Fig. 2, A and B). The amount of oxygen and glucose consumed by various tumors of identical volumes was largely governed by the substrate availability, which, in turn, was limited by tumor blood flow. Again, lactate release from a variety of human tumor xenografts (about 2.5 g) correlated well with glucose consumption (lactate release = 1.545 * glucose uptake - 0.040, r = 0.888, P < 0.001). Since glucose consumption was governed by tumor blood flow, lactate release increased as a function of tumor perfusion (Fig. 2C). In this study, tumor venous pH values close to arterial levels were observed in highly perfused tumors despite high lactate release rates (ΔpH 0.11; Fig. 2C). At perfusion rates <0.2 ml/g/min, tumor venous blood was acidified, and pH values as low as 7.1 occurred (Fig. 2C). The probability of no overall effect of the cell line on blood flow, oxygen consumption, glucose uptake, lactate release, and tumor venous pH values of different tumors at identical wet weights was <0.0001 using Wilks', Pillai's, Hostelling-Lawley's, and Roy's criterion. Considering tumor proliferation, high blood flow rates coincided with rapid growth, whereas low perfusion values were found in tumors with relatively long growth periods (Fig. 2D).

The oxygen and glucose uptake values, measured over wide supply ranges, were fitted to a hyperbolic function (22) in order to gain further insight into intrinsic metabolic properties of human tumor tissue in vivo. The curves were characterized by the maximum oxygen and glucose uptake at optimum supply rates (i.e., the respiratory and glycolytic capacities) and by the oxygen and glucose supply at half-maximal substrate uptake. In order to separate tumors with clearly different pathophysiological properties, medullary breast cancers with high and low perfusion rates were considered separately. The estimated consumption values within the supply range investigated are shown in Figs. 3 and 4. It is obvious that the respiratory capacities varied 9-fold (P < 0.001), whereas the glycolytic capacities differed only 4-fold (value not significant). The substrate availabilities at half-maximal consumption rates were significantly different for both oxygen and glucose (P < 0.001). The respiratory and glycolytic capacities were very high reflecting the relatively rapid growth rates of the tumors investigated. It was found that the capacity of human tumor xenografts to consume substrates was unrelated to their actual perfusion rates in situ, i.e., tumors with high or low blood flow do not necessarily
the tissue mass at much smaller volumes.

pO2 readings were already detected in more than one-half of maximal radiation sensitivity, are regarded hypoxic. This is in contrast to findings in poorly perfused tumors, where "hypoxic" values below 3 mm Hg, i.e., below those necessary for half-sizes exceeding 5% of the body mass were investigated. Hereby, exceed 7% of the measured tumor volume even when tumor "hypoxic" tumor areas in these well perfused xenografts did not contrast, the tissue oxygenation of human tumor xenografts of size, a worsening of the tissue oxygenation was noted at pO2 levels <5 mm Hg, i.e., in tissue areas with oxygen levels in well perfused tumors as a function of pHTumor supply Glucose utilization Glucose of transplants Tumor growth period (days) Tumor sizes around 2.5 g; values are means ± SE. " Tumor sizes around 2.5 g; values are means ± SE.

**DISCUSSION**

The perfusion of human tumor xenografts varied markedly even if tumors of the same cell line, of comparable sizes, and at the same implantation site were considered (Figs. 1 and 2). These differences indicate large variations in angiogenesis, blood vessel morphology, and tumor microcirculation. This finding is in good agreement with previous data on primary and metastatic human tumors (25-28) and on human tumor xenografts (29). The flow variations were within the range previously observed for tumors in patients (21) and for isolated transplanted rodent tumors (30). Comparison of the perfusion of xenograft and patient tumors shows that the blood flow through human tumors lies within the range of that of normal tissue at different stages of activity (Fig. 6). Preliminary histological data indicate that the mean intercapillary distance in small xenografts of highly perfused tumor lines was generally <100 μm whereas mean distances >200 μm were usually observed in poorly perfused transplants. However, the tumor microvessels were not homogeneously distributed throughout the tumor mass but densely vascularized areas were often adjacent to hypovascularized areas (31, 32).

With increasing tumor sizes, tumor perfusion declined due to severe morphological and functional alterations of the tumor microcirculation (14). As a consequence, the substrate availability is reduced in all tumor lines (Fig. 1). In order to assess the impact of the implantation site on the results obtained (33), data from DS-carcinosarcomas implanted as a "tissue-isolated" preparation into the groin are compared with those from "tissue-isolated" kidney tumors (34). It is obvious that the graft tumors have an approximately 2 times higher perfusion rate than those from the inguinal implantation site. The data from the comparison site were obtained from tumors included in Table II. The tumors at the same implantation site were considered (Figs. 1 and 2).

**HUMAN TUMOR XENOGRAFTS**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Breast carcinoma</th>
<th>Breast carcinoma</th>
<th>Breast carcinoma</th>
<th>Breast carcinoma</th>
<th>Ovarian carcinoma</th>
<th>Ovarian carcinoma</th>
<th>Melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transplants</td>
<td>8</td>
<td>12</td>
<td>3</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Tumor growth period (days)</td>
<td>31 ± 1</td>
<td>27 ± 1</td>
<td>34 ± 1</td>
<td>33 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Tumor blood flow (μl/min)</td>
<td>72 ± 18</td>
<td>87 ± 22</td>
<td>143 ± 38</td>
<td>145 ± 35</td>
<td>156 ± 26</td>
<td>179 ± 46</td>
<td>214 ± 52</td>
</tr>
<tr>
<td>Oxygen supply (μO2/g/min)</td>
<td>11.3 ± 3.7</td>
<td>15.9 ± 3.9</td>
<td>25.6 ± 7.5</td>
<td>20.9 ± 5.2</td>
<td>25.7 ± 4.6</td>
<td>30.8 ± 7.6</td>
<td>40.3 ± 9.3</td>
</tr>
<tr>
<td>Oxygen consumption (μO2/g/min)</td>
<td>4.5 ± 1.5</td>
<td>7.1 ± 1.3</td>
<td>8.3 ± 3.6</td>
<td>6.9 ± 1.4</td>
<td>11.8 ± 2.6</td>
<td>11.3 ± 2.3</td>
<td>9.4 ± 1.4</td>
</tr>
<tr>
<td>Oxygen utilization (%)</td>
<td>39 ± 7</td>
<td>52 ± 5</td>
<td>30 ± 4</td>
<td>40 ± 5</td>
<td>46 ± 6</td>
<td>43 ± 7</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Glucose supply (μmol/g/min)</td>
<td>0.65 ± 0.23</td>
<td>0.78 ± 0.16</td>
<td>0.81 ± 0.26</td>
<td>1.22 ± 0.21</td>
<td>1.21 ± 0.23</td>
<td>1.19 ± 0.26</td>
<td>1.05 ± 0.29</td>
</tr>
<tr>
<td>Glucose consumption (μmol/g/min)</td>
<td>0.25 ± 0.09</td>
<td>0.26 ± 0.04</td>
<td>0.22 ± 0.05</td>
<td>0.43 ± 0.09</td>
<td>0.43 ± 0.11</td>
<td>0.25 ± 0.07</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Glucose utilization (%)</td>
<td>36 ± 4</td>
<td>38 ± 4</td>
<td>29 ± 5</td>
<td>38 ± 3</td>
<td>34 ± 5</td>
<td>25 ± 4</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Lactate release (μmol/g/min)</td>
<td>0.24 ± 0.06</td>
<td>0.35 ± 0.07</td>
<td>0.39 ± 0.15</td>
<td>0.35 ± 0.09</td>
<td>0.34 ± 0.12</td>
<td>0.26 ± 0.06</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.40 ± 0.02</td>
<td>7.32 ± 0.02</td>
<td>7.41 ± 0.03</td>
<td>7.35 ± 0.01</td>
<td>7.32 ± 0.03</td>
<td>7.41 ± 0.02</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>Tumor venous pH</td>
<td>7.14 ± 0.03</td>
<td>7.10 ± 0.04</td>
<td>7.27 ± 0.03</td>
<td>7.16 ± 0.02</td>
<td>7.14 ± 0.04</td>
<td>7.28 ± 0.02</td>
<td>7.35 ± 0.01</td>
</tr>
</tbody>
</table>

*Table I Growth, perfusion, and metabolism of xenografted human tumors*
leading to significantly higher oxygen and glucose supply rates. However, the kidney tumors achieve a comparable oxygen uptake by doubling the oxygen utilization. In contrast, the glucose uptake and the lactate release are low in kidney tumors due to relatively low glucose extraction ratios. Based on the flow data it can be concluded that the fat pedicle in the groin is a better "soil" for the implantation of "tissue-isolated" tumors than the renal site despite the high kidney perfusion.

It has been estimated that the maximal respiratory and glycolytic metabolism by the xenografted human tumors varied independently of their actual substrate delivery (Figs. 3 and 4). However, under in vivo conditions, the substrate supply and not the metabolic demand of the cancer cells limited the oxygen and glucose uptake by the xenografts. This is probably due to the fact that the high intratumor flow resistance even of the most highly vascularized tumors permitted usually less than half-maximal substrate uptake due to restricted supply rates. This finding infers steep gradients for glucose, lactate, and ATP in the intercapillary space of human tumor xenografts (22). Furthermore, glutaminolysis can be expected only in the close vicinity of tumor microvessels (35).

In s.c. xenografts of highly perfused tumors, tissue oxygenna is comparable to that of normal liver (Fig. 5). This is most probably due to an even better vascularization of s.c. implants as compared with "tissue-isolated" preparations (33). At larger sizes, the tumor outgrows its vasculature (36) and hypoxic and anoxic tissue areas develop (37). In poorly perfused tumors, such areas are already present at early growth stages (Fig. 5) and enlarge with tumor growth (21). In such tissue areas, decreased radiation response and altered sensitivity towards some antiproliferative drugs have to be expected (7, 16–18). There is no evidence for tissue acidosis even at high lactate
tumors. This indicates that the proliferation rate of these tumors is limited by the substrate delivery leading to a high cell loss factor in poorly perfused tumors (39). At the moment, the experimental data are derived from relatively fast-growing tumors transplanted into rodents. However, both local progression and therapeutic response of tumors with the same histology and similar clinical grading and staging might also depend on perfusion-related parameters (40, 41). In order to individualize treatment, the assessment of substrate delivery or turnover by human tumors should supplement histological and clinical information. Further development of relatively noninvasive technology (magnetic resonance imaging, magnetic resonance spectroscopy, or positron emission tomography) might permit such monitoring (42–44).

ACKNOWLEDGMENTS

The authors are very pleased to thank H. P. Fortmeyer for the immunodeficient animals, H. Gabbert for the histological classification of the human tumor xenografts, and S. Skates for expert statistical advice.

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