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Advances in Brief

Tumor Oxygenation Predicts for the Likelihood of Distant Metastases in Human Soft Tissue Sarcoma[1]

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Abstract

This study was performed to explore the relationship between tumor oxygenation and treatment outcome in human soft tissue sarcoma. Twenty-two patients with nonmetastatic, high-grade, soft tissue sarcomas underwent preoperative irradiation and hyperthermia and pretreatment measurement of tumor oxygenation. The 18-month actuarial disease-free survival was 70% for patients with tumor median oxygen pressure (pO_2) values of >10 mm Hg but only 35% for those with median pO_2 values of <10 mm Hg ($P = 0.01$). There were eight treatment failures; the first site of recurrence was lung in all patients. Median pO_2 was 7.5 mm Hg for metastasizing tumors *versus* 20 mm Hg for nonmetastasizing tumors ($P = 0.03$). Potential mechanisms and implications for clinical trial design are discussed.

Introduction

In 1953, Gray *et al.* (1) postulated that tumor hypoxia played a significant role in determining the radiocurability of many cancers because of the well established role that oxygen has in modifying radiation damage. The actual relationship between tumor oxygenation and treatment outcome has remained unclear, however. Gatenby *et al.* (2) measured the oxygenation of cervical lymph nodes in patients with squamous cell carcinoma of the head and neck and showed a relationship between the volume of a tumor with a pO_2[3] of <8 mm Hg and the probability of achieving a clinically complete response after radiotherapy. Höeckel *et al.* (3) demonstrated that patients with advanced-stage cervical carcinoma, treated either surgically or by irradiation, who had tumors with a median pO_2s of <10 mm Hg had significantly worse survival and relapse-free survival than those who did not. These latter results suggest that hypoxia may identify tumors with an aggressive phenotype, independent of the therapy received.

A program consisting of preoperative irradiation and hyperthermia for the treatment of soft tissue sarcomas was instituted at Duke University Medical Center in 1984 (4). Studies designed to evaluate tumor biology and to attempt to predict treatment outcome have been carried out in conjunction with the clinical protocol. One of these is the measurement of tumor oxygenation. This study reports the relationship between pretreatment tumor oxygenation and treatment outcome. We explore possible mechanisms to explain the data and consider the implications for future clinical trial design in soft tissue sarcoma.

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[3] The abbreviations used are: pO_2, oxygen pressure; CT, computed tomography; DFS, disease-free survival; FAK, focal adhesion kinase.

Materials and Methods

Patients and Treatment. Between November 1992 and October 1995, 35 patients with nonmetastatic, high-grade, soft tissue sarcomas received a course of preoperative irradiation and hyperthermia. All patients received 50 Gy at 2 Gy/day and hyperthermia once or twice weekly (4). Tumor volume was determined by magnetic resonance imaging or CT.

Measurement of Tumor pO_2. Tumor oxygenation was measured in resting, awake patients using a polarographic device (Eppendorf Netheler Hinz, GmbH, Hamburg, Germany). All probe placements and measurements were made under direct CT scan guidance (model 9800; General Electric Corporation, Milwaukee, WI). This technique has been described previously (5, 6). Probe placements were oriented to avoid traversing radiographically hypodense, necrotic zones of tumor (7, 8). The CT procedure also ensured that all pO_2 measurements were obtained from tumor and not normal tissue. A minimum of two measurement tracks were obtained from each patient, but the majority of patients had two measurement tracks obtained from each of two physically separate sites within the tumors (four tracks total). Measurement track lengths ranged from 20 to 35 mm. This multiple track sampling strategy has been used by others (9).

Statistical Considerations. DFS was measured by the Kaplan-Meier product limit method (10). The log rank technique was used to compare the DFS of different groups of patients. Single factor ANOVA was used to compare tumor median pO_2 and tumor volume in those patients who relapsed *versus* those who did not. Spearman correlation coefficients were calculated to examine the relationship between tumor volume and tumor oxygenation.

Results

Thirty of the 35 patients underwent assessment of tumor oxygenation prior to the initiation of irradiation and hyperthermia. Of these, 22 have undergone subsequent limb-sparing resection and also have a minimum of 6 months' follow-up. Median follow-up time is 9 (range, 6–28) months. The 18-month actuarial DFS is 50%. The DFS is 70% for patients whose tumor median pO_2s were >10 mm Hg but only 35% for those with median pO_2 values <10 mm Hg (Fig. 1; $P = 0.01$). The median DFS is 7 months for the patients with hypoxic tumors and has not been reached in the patients with well-oxygenated tumors.

There were eight treatment failures. The first and only site of failure was distant (lung) in seven patients. One other patient developed lung metastases and had a local recurrence subsequently as well. There were no other local failures. Median pO_2 values of tumors that recurred distantly were compared with those that did not recur. The average median pO_2 of 7.5 mm Hg for those that recurred was significantly lower than the average median of 20 mm Hg for those that did not ($P = 0.03$).

Median tumor volume was 167 (range, 4–844) cm^3. Tumor volume and median pO_2 were not correlated with one another ($r = -0.14$; P = not significant). There was no significant difference in the volumes of those tumors that relapsed (mean, 165 cm^3) *versus* the volumes of those that did not (mean, 220 cm^3).

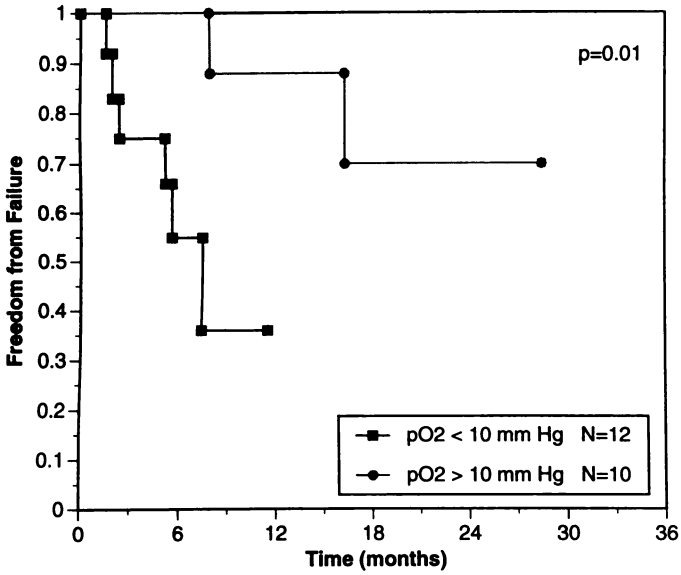

Fig. 1. Kaplan-Meier plot of freedom from distant failure comparing patients with tumor median pO_2 values of >10 mm Hg *versus* those with values of <10 mm Hg. The patients with the more hypoxic tumors have significantly worse prognoses.

Discussion

Mechanisms of Failure. Gatenby *et al.* (2) and Höeckel *et al.* (3) have demonstrated increased local-regional failure and a worse prognosis for head and neck and uterine cervical cancer patients who present with hypoxic tumors. The current study is unique, because it shows that the pretreatment oxygenation of soft tissue sarcomas predicted the likelihood of distant relapse, not local failure. Tumors that recurred were significantly more hypoxic than those that did not. The probability of distant metastases was twice as great for tumors with median pO_2s of <10 mm Hg *versus* those with pO_2s of >10 mm Hg.

Does hypoxia play a role in the development of distant metastases, or does it reflect merely more advanced disease with a worse prognosis? Schwickert *et al.* (11) showed in a pilot study that tumor lactate levels were significantly higher in biopsy specimens from cervical carcinoma patients who developed nodal metastases compared with patients who did not. Metabolic conditions, such as high lactate levels, would be expected to occur in a hypoxic environment.

Graeber *et al.* (12) demonstrated *in vitro* that oncogenically transformed cells expressing wild-type p53 (+/+) underwent apoptosis when incubated under hypoxic conditions. Cells expressing mutant-type p53 (−/−), however, were 3.5 times less likely to become apoptotic under the same conditions. Repeated hypoxic exposures to a mixed culture of −/− and +/+ cells resulted ultimately in the mutant-type cells overtaking the wild-type cells (12). These data imply that hypoxia protects against apoptosis and may provide a selective growth advantage in some tumors.

Developmentally, more advanced disease might be reflected in the grade of the tumor, because a higher-grade tumor presumably would have more derangement in its genome than a low-grade tumor. Grade is the primary component of the sarcoma clinical staging system. Because all of the tumors in this study were high grade, it stands to reason that hypoxia is marking something independently of the standard assessment of advanced stage.

Tumor volume is also an important prognostic parameter in some tumors, but we have not been able to demonstrate previously that it has any importance in this patient group, aside from the fact that extremely large tumors have to be excluded from this treatment protocol, because we do not have a device that is capable of heating them. The fact that tumor volume was not correlated with either median pO_2 or tumor recurrence makes it unlikely that volume is a confounding variable. Other investigators have failed to show a correlation between tumor volume and oxygenation in soft tissue sarcoma (9).

Young *et al.* (13) showed in a murine model that anaerobic culture of fibrosarcoma and melanoma cells followed by reoxygenation led to both significant DNA overreplication and an increased level of distant metastases. Entry of hypoxic cells such as these into the systemic circulation and subsequent sequestration into the oxygen-rich environment of the lungs could explain the results of the present study partially.

Young *et al.* (13) did not identify which genes were up-regulated after hypoxia and reoxygenation, but work from other laboratories may shed some light on this issue. Vascular endothelial growth factor is expressed in the setting of hypoxia (14, 15). This substance promotes tumor angiogenesis, and the tumor neovasculature could provide the portal of entry into the systemic circulation for cells that are shed from the tumor. Tumor angiogenesis and vascular density have been demonstrated to be independent risk factors for the development of distant metastases in both breast and prostate cancer (16, 17).

Another possible substance that could increase the risk of metastasis is *FAK*. This gene was isolated initially from high-grade, human soft tissue sarcomas (18). It plays a role in cellular adhesion and can be activated when a cell's integrin receptors contact the extracellular matrix. Weiner *et al.* (18) studied *FAK* mRNA expression in normal tissues and in a series of benign and malignant human tumors. They found no expression in the normal tissue or benign tumors. However, 85% of the invasive tumors and 100% of the metastatic tumors expressed the gene. Matched biopsies from four colon cancer patients showed progressively increasing levels of *FAK* expression from normal tissue to metastatic tumor (18). Whether or not *FAK* is up-regulated by hypoxia is not known.

Down-regulation of cell-cell adhesion molecules, such as E-cadherin, could enhance the ability of individual cells to break free from the primary tumor mass and to enter the systemic circulation. Expression of this molecule is reduced in high-grade tumors compared with low-grade tumors (19). Reduced cadherin expression predicts poor prognoses independently in high-grade prostate, colorectal, and thyroid carcinomas (19–21). Because all tumors in the current study were high grade, it is unlikely that differential cadherin expression would be responsible for the different rates of distant metastases. Whether hypoxia affects cadherin expression is unknown, however.

Potential Clinical Significance. The long-term DFS of high-grade, soft tissue sarcoma of the extremities is ~50% (22). Aggressive combinations of surgery and irradiation, including the regimen used in this study, result in local control rates of ≥80% (23). The predominant mode of relapse is distant metastases, with rates approaching 50%. The follow-up in the current study is short; therefore, the results must be considered preliminary. However, the data are qualitatively similar to those of studies with longer follow-up.

The efficacy of adjuvant chemotherapy in soft tissue sarcoma is a controversial topic. The largest randomized trial from the European Organization for the Research and Treatment of Cancer showed no benefit (24). Smaller studies either have been inconclusive or suggested a small improvement in DFS and overall survival (25). With distant failure rates of ~50%, nearly half of the patients entered into such studies do not require the adjuvant therapy. Inclusion of these patients dilutes the power of these trials to detect a treatment advantage if there actually is one.

The data presented here indicate that tumor hypoxia may be useful as a marker of biologically aggressive disease and may help select

those sarcoma patients who are at greatest risk of having metastases. These patients would constitute the most appropriate group to enroll in studies of adjuvant therapy. Confirmation of our results by others is needed. Further investigation is also required to define the mechanism(s) by which tumor hypoxia leads to distant metastases. Such knowledge could improve oncologists' ability to select patients for clinical trials properly and could lead to a more rational design of adjuvant treatment programs.

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