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RAPID COMMUNICATION

PANCREATIC TUMORS SHOW HIGH LEVELS OF HYPOXIA

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Purpose: Because of the dismal outcomes of conventional therapies for pancreatic carcinomas, we postulated that hypoxia may exist within these tumors.
Methods and Materials: Seven sequential patients with adenocarcinomas of the pancreas consented to intraoperative measurements of tumor oxygenation using the Eppendorf (Hamburg, Germany) polargraphic electrode.
Results: All 7 tumors demonstrated significant tumor hypoxia. In contrast, adjacent normal pancreas showed normal oxygenation.
Conclusion: Tumor hypoxia exists within pancreatic cancers. **© 2000 Elsevier Science Inc.**

Hypoxia, Oxygenation, Pancreas, Adenocarcinoma.

INTRODUCTION

Hypoxia, or a low oxygen environment, is a major determinant of local, regional, and distant failure after anticancer therapy. Hypoxic cells are approximately threefold more resistant to radiation than aerobic ones (1). They may also be resistant to chemotherapeutic agents because of their relatively slow rate of proliferation. Laboratory studies suggest that hypoxia results in the development of a more aggressive tumor phenotype by selecting for p53 mutations which can lead to further deregulation of cell growth and resistance to hypoxia induced apoptosis (2).

Direct oxygen measurements in human tumors have confirmed regions of hypoxia in multiple tumor sites including glioblastoma multiforme, soft tissue sarcomas, and carcinomas of the head and neck, breast, cervix and prostate (3–9). The presence of hypoxia within some of these tumors predicts for response to radiotherapy, distant metastases, and survival (4–8).

Pancreatic cancer is a near fatal disease in which 70%–80% of patients have locally advanced or metastatic disease at the time of diagnosis (10). Local disease progression is a significant clinical problem that may profoundly reduce quality of life by causing symptoms such as fatigue, pain, jaundice, malnutrition, hemorrhage, and duodenal obstruction. Chemoradiotherapy has been demonstrated to modestly improve survival in pancreatic cancer (11). However, multiple single-agent and multi-agent chemotherapy trials have not shown any substantial benefit (10). Despite the treatment selected, nearly all of these patients will die of their disease within 1 year.

We postulated that significant tumor hypoxia may exist in pancreatic cancer. We tested this hypothesis with direct intratumoral measurements of oxygenation and compared these measurements with the oxygen levels of adjacent normal pancreatic tissue. These findings demonstrate that pancreatic tumors contain regions of extremely low pO_2. This is the first report of tumor hypoxia in pancreatic cancer.

METHODS AND MATERIALS

All patients with pancreatic cancer at Stanford University Medical Center were evaluated by a multidisciplinary gastrointestinal tumor board after a complete staging evaluation. This study analyzed seven consecutive patients with radiographically resectable tumors of the head of the pancreas. The risks, rationale and benefits of the research study were reviewed in detail with the patient. All participants signed an investigational review board approved consent form.

After enrollment in the study, all patients underwent a planned pancreaticoduodenectomy in the standard manner. When adequate exposure of the tumor was achieved, intra-

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Table 1. Patient characteristics

Patient	Age/ Gender	Hemoglobin	Arterial pO_2	CA19-9	CEA	Location	Tumor size	Nodal status	Histology	LVI	Perineural invasion
1	76/F	9.0	121.7	3600	NA	HOP	3.1	0/7	MD ACA	neg	neg
2	69/M	9.3	250.3	1	NA	HOP	4.0	5/14	MD ACA	neg	neg
3	51/F	10.7	192.8	360	1.1	HOP	4.0	1/15	MD ACA	neg	pos
4	71/M	11.4	231.8	76	4	HOP	5.0	0/14	MD ACA	neg	neg
5	56/M	11.3	140.3	1530	9.7	HOP	4.0	1/25	MD ACA	pos	pos
6	67/M	11.9	205.5	1650	NA	HOP	3.0	0/18	MD ACA	pos	pos
7	55/F	11.6	373.1	NA	NA	HOP	3.0	0/10	MD ACA	neg	pos

operative measurements of the tumor and surrounding normal pancreas tissue was carried out using the Eppendorf pO_2 histograph (Eppendorf Inc., Hamburg, Germany), a computerized polargraphic needle electrode system that records multiple measurements of tissue oxygenation. The device is mounted on a fixed arm that can be manipulated into any position within the body cavity. The oxygen sensing device consists of a gold microcathode (17 μM in diameter) imbedded in a stainless steel shaft that was polarized against a silver-silver chloride anode placed on the skin of the patient. The resulting current (4–5 pA/mm Hg) was proportional to the oxygen partial pressure in the tissue. Calibration was performed before and after measurements using a phosphate buffered saline solution equilibrated with air or 100% nitrogen. The values were corrected for barometric pressure and body temperature. When adequate exposure of the tumor was achieved, the patients' inspired pO_2 was reduced to room air. An arterial blood gas was drawn at this time. Then a sterile Eppendorf probe was inserted into the tumor or normal tissue. The probe was advanced in an automatic stepwise fashion of 0.7 mm forward followed by 0.3 mm backward to prevent pressure artifact. No imaging technique was used for probe guidance during the procedure. The length and direction of the tracks was defined clinically by the surgeon. Two to three tracks through the normal pancreas tissue was recorded first and followed by a similar number of tracks through the pancreatic tumor as defined by the surgeon. This entire process took 15–30 min per patient.

A pO_2 histogram was then generated from these measurements. The data were analyzed as a median pO_2 value and as a percentage of values less than 2.5 mm Hg. Within each patient, comparisons were made of the cumulative pO_2 measurements between tumor and normal pancreas. Statistical analysis was completed according to the Mann–Whitney rank–sum test.

RESULTS

Seven patients with pancreatic tumors were measured intraoperatively. The patient characteristics are listed in Table 1. After completion of the oxygen measurements, all patients completed their planned pancreaticoduodenectomy. There were no complications related to the intraoperative Eppendorf measurements.

All pathologic tissue was reviewed by Stanford pathologists. Patients were staged pathologically according to the 1997 AJCC criteria.

The intraoperative Eppendorf measurements of the pancreatic tumors and the corresponding normal pancreatic tissue are described in Table 2. Multiple recordings through at least 2 different tracks were made of both the pancreatic tumors and the normal pancreas. The number of measurements ranged from 104 to 180 per patient. For each patient, a pO_2 histogram was generated for measurements of the tumor and the normal pancreas. The data are reported as both a median pO_2 value and as a percentage of values less than 2.5 mm Hg. Interestingly, the intratumor pO_2 levels remained extremely low despite the elevated arterial pO_2 values of the patients in this study.

The median pO_2 values of the pancreatic tumors matched with the normal pancreas measurements from each patient is depicted in Fig. 1A. A similar graph showing the percent of values less than 2.5 mm Hg is plotted in Fig. 1B. In every case, the median pO_2 value of the tumor was lower than that of the normal pancreas. Similarly, all of the tumors had a higher percentage of values that were below 2.5 mm Hg. The median pO_2

Table 2. pO_2 measurements in pancreatic tumors and normal pancreatic tissues

Patient	Med pO_2 tumor	%<2.5 mm Hg tumor	Med pO_2 normal	%<2.5 mm Hg normal
1	0	94	46.6	0
2	2.7	43	24.3	0
3	3.1	35	47.3	9
4	3.4	24	67.4	0
5	0.9	95	51.6	0
6	2	54	69.8	7
7	5.3	68	92.7	0

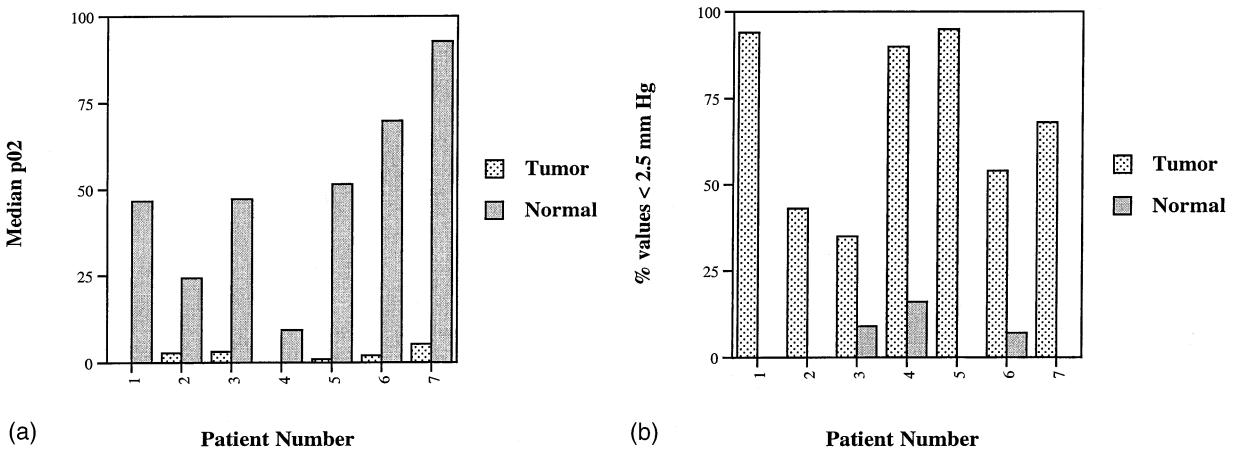

Fig. 1. A. Bar graph depicting the differences between the median pO_2 of pancreatic tumors and the median pO_2 of corresponding normal pancreas. B. Bar graph showing differences between the percent of values less than 2.5 mm Hg in the pancreatic tumors and in the normal pancreas.

values for the tumor ranged between 0 and 5.3 mm Hg and the median values for the normal pancreas ranged from 9.3 to 92.7 mm Hg ($p < 0.001$). The percent of values less than 2.5 mm Hg ranged from 24% to 94% for the tumors and 0%–16% for the normal pancreas ($p < 0.001$).

DISCUSSION

The presence of tumor hypoxia was first postulated by Thomlinson and Gray (12) in a classic paper in which they described areas of necrosis within tumor specimens. These necrotic regions occurred at distances from blood vessels that were beyond the diffusion capacity of oxygen. They hypothesized that the absence of oxygen leads to necrosis and that there was a steadily decreasing concentration of oxygen as one moves farther away from blood vessels.

Since this study, other investigators have reported that hypoxic tumors negatively impact local control after radiotherapy and in some cases may influence the development of distant metastases (4–8, 13). These factors all ultimately contribute to the survival of patients with hypoxic tumors.

The lethal effects of radiation are significantly diminished in the absence of oxygen. When radiation is administered in the absence of oxygen, the dose must be increased by a factor of 2.5–3.0 to achieve the same degree of cell killing as what one would expect under fully oxygenated conditions (1). In addition to the relative resistance of hypoxic tumors to radiation therapy, this population of tumor cells does not divide rapidly and therefore may also be relatively resistant to conventional chemotherapeutic agents (14).

The concept that hypoxia selects for tumor cells with a more malignant phenotype was suggested by Graeber *et al.* (2) who showed in a series of elegant experiments that hypoxia selects for p53 mutants leading to an apoptosis resistant population of cells. In addition, many other genes are induced by hypoxic stress and the analysis of these transcriptional changes may provide clues relating to the development of a more aggressive tumor phenotype (15). The molecular events that occur in response to hypoxia may ultimately form the basis for developing new treatment approaches to pancreatic tumors.

This report provides the first evidence that a significant population of tumor cells within pancreatic cancers are hypoxic. Although we only recorded pO_2 measurements from seven patients, the differences between the oxygenation of the tumor and normal pancreas were striking (despite the patient's elevated arterial pO2 values). Many current clinical strategies are directed at overcoming tumor hypoxia. Interventions that increase arterial pO_2 such as breathing increased levels of oxygen during radiotherapy may have only limited effectiveness (because tumor oxygenation may be independent of arterial pO_2). Whereas, novel chemotherapeutic agents such as tirapazamine, a hypoxia specific cytotoxin, which acts independent of arterial pO_2 may hold more promise. Our findings suggest that some of these approaches may have utility in the treatment of pancreatic cancer.

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