Correlation of High Lactate Levels in Human Cervical Cancer with Incidence of Metastasis

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Abstract

Tissue concentrations of ATP, glucose, and lactate in cervical cancer biopsies that were taken before a conventional radiation treatment were imaged quantitatively with a bioluminescence technique. Concomitantly, a number of clinically relevant data, such as local tumor control, patient survival, metastatic spread, etc., were documented. There was no correlation between staging or grading and any of the metabolic parameters measured. Local correlations between ATP, glucose, and lactate on a pixel-to-pixel basis were generally positive, with respective Spearman’s correlation coefficients being lower in patients without clinically documented metastasis compared to those with metastatic spread. Lactate concentrations were significantly higher and scattered over a wider range in tumors with metastatic spread in comparison to malignancies in patients without metastasis. Thus, high local lactate levels of ≥20 μmole/g appear to be associated with a high risk of metastasis, at least in the ten human cervical tumors investigated to date.

Introduction

Numerous investigations on experimental tumors have documented severe disturbances of the microcirculation, which occur already at an early state of growth (1, 2). The abnormalities of the tumor vasculature include a loss of the natural hierarchy of blood vessels, changes in the vascular density, and loss of the physiological regulation of blood perfusion. Perhaps the most striking difference between solid tumors and healthy organs is the emergence of pronounced spatial and temporal heterogeneities in tumor blood supply (3, 4). As a consequence, solid tumors often exhibit regions with insufficient blood perfusion adjacent to areas with luxurious blood supply. Relevant factors that modulate tumor growth and therapeutic sensitivity, such as local oxygen pressure and concentrations of metabolites and waste products, may, thus, vary largely within malignancies (5, 6). Recently, such heterogeneities in the metabolic milieu have been demonstrated not only for experimental tumors but also for malignant disease in patients (7, 8). The significance of tissue oxygen pressure as a parameter that is predictive of patient survival after radiation therapy has been clearly shown for cervical cancer in patients in a systematic clinical study (8).

The present study was undertaken to characterize the metabolic milieu in human cervical cancer more in detail. The working hypothesis was that pathophysiological parameters, such as oxygen tension or acid base status in tumors, may be correlated with clinical parameters that are relevant for the progression of the disease, e.g., tumor size or incidence of metastasis. The ultimate goal was to evaluate the clinical relevance of metabolic parameters, e.g., the local concentrations of metabolites, and to develop an experimental protocol for the assessment of pathophysiological prognostic parameters in the malignancies investigated.

Measurements were carried out on tumor biopsies that were routinely taken for pathohistological classification of the malignancies at the Norwegian Radium Hospital (Oslo, Norway). The biopsies were rapidly frozen in liquid nitrogen and transferred to our laboratory on dry ice where metabolic measurements were made. To characterize metabolites in these human biopsies, a technique using quantitative bioluminescence and single photon imaging was applied (9–12).

Materials and Methods

A total of 11 tumor specimens from 10 patients with cervical cancer were obtained from the Norwegian Radium Hospital. The material was part of biopsies that were taken from patients in stage 2 or 3 [International Federation of Gynecologists and Obstetricians (FIGO)] of the disease. The biopsies which were randomized with regard to the anatomical site of removal were taken from the tumors with a curette before a conventional radiation treatment. Relevant patient data, such as treatment protocol, tumor shrinkage, patient survival, incidence and location of metastasis, etc., were documented.

For metabolic imaging, cryostat sections were made from the frozen tumors and were subsequently adhered to the upper side of a cover glass. The cover glass was laid upside down on a glass slide with a casting mold. The mold was filled with a liquid reaction solution containing various enzymes to link the substrate of interest to the luciferase light reaction. Different mixtures of enzymes and luciferases were used for the detection of ATP, glucose, and lactate, yet the use of 20-μm thick serial sections allowed for the determination of the different metabolites at quasi-identical locations within the biopsies. The casting mold carrying the cover glass and the tissue section was transferred to a microscope stage in an air-conditioned environment. The temperature of the array was adjusted to 22°C (± 1°C), resulting in reproducible kinetics of the enzyme reactions. The spatial distribution of the bioluminescence intensity within the tissue section was registered directly by using an appropriate microscope (Axioptot; Zeiss, Oberkochen, Germany) and an imaging photon-counting system (ARGUS 100; HAMAMATSU, Herrsching, Germany). The photon intensity was calibrated by appropriate tissue standards so that density distributions were obtained representing the distribution of ATP, glucose, and lactate in volume-related tissue concentrations (μmole/g wet weight). These values were routinely validated by independent measurements with HPLC and enzymatic standard assays, respectively.

The digitized images of the different substrate distributions, as well as of an adjacent tissue section stained with hematoxylin and eosin were transferred to a personal computer with commercial image software. By optical overlay of the metabolite distributions with the histological section, metabolites were evaluated in tumor regions with densely packed viable cancer cells assigned "neoplasia," in areas with necrosis and eventually in stromal tissue elements. Furthermore, a computer algorithm allowed for the pixel-to-pixel correlation among the images of the different substrates (13, 14). Between 7 and 9 sections for each metabolite and for histological analysis were made from each tumor. To obtain mean substrate concentrations (± SD) for individual tumors, pixel values of each section were averaged first, and then these section means were averaged by taking into account differences in section areas. Correspondingly, mean concentration values of selected tumor regions were calculated under...
consideration of the size of the respective tumor region. Additional details on the technique of bioluminescence and imaging photon counting have been published elsewhere (10, 12).

Results

Metabolite distributions in all the tumors investigated were extremely heterogeneous, but the distribution patterns of ATP, glucose, and lactate that were acquired in serial sections showed obvious similarities. This was reflected by positive correlations between the distribution patterns of the three metabolites, i.e., there was a high concentration of ATP at a location with high glucose and lactate and vice versa. As an example, Fig. 1 shows such a correlation between corresponding pixel values of ATP and glucose concentrations. The quality of the correlation was quantified by Spearman’s correlation coefficient, $r_s$, which was 0.5—0.7 for the best correlations obtained. Average correlation coefficients, $r_s$, from patients with clinically documented metastasis were 0.32, whereas values registered in patients without documented metastasis being at 0.24. Although this difference is not statistically significant, it represents a trend that a better correlation between ATP and glucose be an unfavorable prognostic parameter.

Pronounced concentration differences were obvious preferentially between vital and necrotic tumor regions, which is demonstrated in Fig. 2. The interindividual differences were even more pronounced than the intratumoral variability of the parameters investigated. This can be read from large SDs of the mean regional concentration values determined (see Fig. 2). For example, average SD within individual malignancies were 1.77 μmole/g, whereas the SD obtained from averaging the mean lactate concentration of all tumors investigated was 4.18 μmole/g. Similar differences were found for ATP and glucose. As a general observation, lactate was high next to the necrotic zones, yet dropped within these areas to levels far below those found in viable tumor regions.

Lactate concentrations averaged over the entire tumor sections investigated were significantly higher ($P < 0.05$) in tumors of patients with para-aortal and abdominal lymph-node metastases compared to malignancies of metastasis-free patients, as illustrated in Fig. 3. Whereas relatively low lactate concentrations within a relatively narrow concentration range were registered in malignancies of patients without metastasis, lactate concentrations obtained in patients with metastasis were scattered over a wide concentration range. Frequency distributions of measured pixel values of lactate obtained in patients without metastasis were tilted toward low lactate concentrations and rarely exceeded 20 μmole/g. In contrast, frequency distributions of lactate concentrations in tumors with metastatic spread were often extended between 0 and 40 μmole/g with a Gaussian shape or with two or more peaks. Whereas 13% of the lactate concentration values were above 20 μmole/g in primary tumors with metastatic spread, only 2% of the data acquired in metastasis-free patients exceeded this level. No such differences were found for mean intratumoral ATP or glucose concentrations (Fig. 3).

Discussion

The technique of quantitative bioluminescence and single photon imaging has been designed to be applicable to biopsies from human tumors in the clinic. The present study demonstrates that this is feasible, and that metabolic imaging with this method can be performed adequately with human tumor biopsies. The results of metabolic imaging can be compared with data obtained from the clinical protocol, such as patient survival or incidence of metastasis.

In the present and still preliminary study on human cervical cancer, there was no correlation between clinical staging or pathohistological grading of the tumors and any of the metabolic parameters measured. In general, local correlations between pixel values of ATP, glucose, and lactate were positive, and these correlations were better in patients with documented metastases compared to patients without metastatic spread. The regional evaluation in selected tissue areas demonstrates that concentrations of metabolites can be obtained in relation to the histological architecture of the tissue. As expected, ATP and glucose levels were less in more necrotic tumor regions compared to viable “tumor cell
nests." Low lactate levels in necrotic versus viable regions may indicate that removal of this metabolite is still effective in such necrotic areas; otherwise, these regions would equilibrate with the high lactate concentrations in their vicinity.

The most striking difference between patients with and without metastasis was the difference in the lactate distribution within tumor tissue, which was statistically significant with a 5% probability of error even in this limited study on ten patients. Whereas relatively low lactate concentrations within a relatively narrow concentration range was registered in malignancies of patients without metastasis, lactate concentrations obtained in patients with metastasis were scattered over a wide concentration range. Frequency distributions obtained in patients without metastasis were tilted toward low lactate concentrations and rarely exceeded 20 \( \mu \text{mole/g} \). In contrast, frequency distributions of lactate concentrations in tumors with metastatic spread were often extended between 0 and 40 \( \mu \text{mole/g} \) with a Gaussian shape or with two or more peaks. This finding indicates that high local levels of lactate within cervical cancers may be associated with a high risk of incidence of metastasis. This finding still has to be verified in a higher number of patients and also in different tumor entities. On the other hand, it is very challenging to investigate the mechanism underlying this phenomenon in future research. One hypothesis may be that increased levels of lactate and reduced pH values may decrease the adhesive properties of tumor cells within the malignant tissue and may, thus, enhance the spread of cells from the primary tumor. One other possible explanation may be that such spots with unfavorable metabolic conditions within tumor tissue may enhance neovascularization; this may be true for both blood vessels and lymphatic vessels (15, 16). Although still under debate, a number of investigations on angiogenesis in breast tumors and other tumor entities have demonstrated increased neovascularization, with regard to either blood or lymphatic vessels, to be positively correlated with the incidence of metastasis (17).

Definitely, the preliminary data obtained at present have to be verified on a more solid statistical basis in a larger number of patients. Also, the significance of the present finding for other tumor entities should be investigated. If the present results would be confirmed under these conditions, the local distribution of lactate within cervical cancer, and possibly other solid tumors, could be indicative of the risk of metastatic spread. Metabolic imaging in human cervical cancer may, therefore, provide additional information for the oncologist who has to decide how aggressive a potentially curative therapy should be designed.

References


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