Geographical mapping of metabolites in biological tissue with quantitative bioluminescence and single photon imaging*

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Summary

This article features a novel technique for measuring the spatial distribution of metabolites, such as ATP, glucose, and lactate, in rapidly frozen tissue. Concentration values are obtained in absolute terms and with a spatial resolution of single-cell dimension. The method is based on enzymatic reactions that link the metabolite of interest to luciferase with subsequent light emission. Using a specific array, cryosections are brought into contact with the enzymes in a well-defined, reproducible way inducing a distribution of light across the section with an intensity that is proportional to the metabolite concentration. The emitted light can be visualized through a microscope and an imaging photon counting system, and the respective image can be transferred to a computer for image analysis. Measurements in spherical cell aggregates with central necrosis demonstrate a close correlation between the distribution of ATP and of cellular viability at a microregional level. Similarly, ATP and glucose are correlated with the geometrical arrangement of more viable and more necrotic tissue regions in human melanomas xenografted in nude mice. Lactate did not show such a structure-related distribution in these tumours. Structure-related distributions of ATP, glucose, and lactate are found in cervix tumours of patients. In contrast to the heterogeneous distributions in tumours, the distribution patterns were much more homogeneous in normal tissues. Regional differences were present, but were much more gradual than in malignancies. This was illustrated for heart muscle where ATP concentrations were found that agreed with data in the literature, and that showed a decrease in periventricular areas.

Introduction

The functional state of living tissue may be characterized, among other parameters, by the steady-state distribution of oxygen and various metabolites that are known to be of biological relevance. It is generally assumed that normal organs in a physiological state are sufficiently supplied with oxygen and nutrients and that metabolic waste is efficiently drained from these tissues. In contrast, malignant tumours often show restrictions in tissue oxygenation, in nutritive supply, and in the removal of metabolic products (Vaupel et al., 1989). The distribution of metabolites in normal biological tissues may be rather homogeneous which reflects a physiological balance between the delivery and the consumption of oxygen and nutrients in the entire organ considered. This is contrasted by a pronounced heterogeneity in the physiological state of malignant cells within solid tumours. The differences in the metabolic state between normal organs and malignancies can be attributed mainly to a vastly different vascularity. The vascular pattern in normal tissue is characterized by a hierarchical arrangement of blood vessels with a well defined geometry leading to an efficient nutritive blood flow at a microregional level. Unlike this physiological situation, tumours often show a chaotic vascularity with normal and extremely abnormal blood vessels in close proximity. The abnormality of tumour vessels includes deviation from the normal architecture of the vessel wall, large variations in vessel length, diameter, and density, as well as the emergence of arteriovenous shunts. As a consequence, solid tumours

often consist of regions with restricted blood perfusion not matching the metabolic requirements of that area next to regions of abundance of nutritive blood supply (Vaupel et al., 1981, 1989).

Knowledge about these peculiarities of solid tumour growth was initially obtained from pathohistological investigations (Thomlinson & Gray, 1955; Rubin & Casarett, 1966; Tannock & Steel, 1969; Falk, 1977). Such studies were supplemented by detailed investigations on corrosion casts and by quantitative measurements using morphometric techniques (Grunt et al., 1986; Konerdng et al., 1989; Lametschwandtner et al., 1990; Skinner et al., 1990). Recently, quantitative characterization of tumour vasculature has unequivocally demonstrated correlations between vascular geometry in tumours and biological as well as therapeutic behaviour of malignant lesions (Streffer et al., 1989; Monschke et al., 1991). First approaches to relating vessel geometry to blood flow at a regional level were made by the use of transparent chamber preparations of tumours in laboratory animals in combination with intravital microscopy (Algie & Chalkley, 1945; Goodall et al., 1965; Reinhold et al., 1977; Endrich et al., 1982). Although the majority of these studies was rather descriptive and qualitative, fundamental information on tumour microcirculation could be gained with such systems, such as the existence of irregular blood perfusion with presta-

s, stasis, and reversal of flow which may occur even at an early stage of malignant growth. Quantitative data on regional tumour blood flow come mainly from the detection of washout kinetics of radioactive tracers or heat (e.g. reviewed in Vaupel et al., 1989). However, the spatial resolution of these techniques is not sufficient for the assessment of heterogeneities as observed in chamber preparations which are typically found in the millimetre and sub-millimetre dimension. For instance, autoradiography with $^{14}$C-iodoantipyrine has been successfully applied to detect heterogeneous distributions of blood perfusion in such small tumour microregions (Blasberg et al., 1985; Tozer et al., 1990; Mies et al., 1990).

Despite a relatively even distribution of blood flow, normal tissue may exhibit spatial variations in the metabolic state of cells, yet these variations presumably occur at a larger distance scale, as compared with tumours, and the range of variation may be less than that in malignant tissue. In some organs, differences in cellular metabolism can be related to the histological architecture of the tissue; on the other hand, some organs that are rather homogeneous in their tissue structure, such as liver, may still show regional differences in metabolism (Schmidt et al., 1978). This metabolic zonation of living tissue in a normal or pathophysiological state has been subject to intensive research and is still the focus of interest of numerous scientists (reviewed by Klinger et al., 1988; Jungermann & Katz, 1989). 'Classical' techniques for the quantification of metabolic zonation are the Lowry technique (Lowry & Passonneau, 1972) and the deoxyglu-

cose method according to Sokoloff (1978). Both techniques enable the detection of steady-state tissue concentrations of metabolites as well as enzyme activities or metabolic turnover rates yielding absolute values with a spatial resolution almost at the cellular level and in relation to the histological tissue structure. However, these techniques are very sophisticated in handling, very time-consuming to get the final result, and the latter technique requires the use of radioactivity in animals. More recent approaches in this field are imaging bioluminescence (Mueller-Klieser et al., 1988, 1989, 1990, 1991; Walenta et al., 1990) or non-invasive techniques such as positron emission tomography (PET; Miraldi, 1986; Lammertsma, 1987; Hawkins & Phelps, 1988; Wahl et al., 1991) or magnetic resonance spectroscopy (MRS; Evanochko et al., 1984; Bottomley, 1989; Steen, 1989; Kasimos et al., 1990) or imaging (MRI; Fishman et al., 1989). The non-invasive techniques are still lacking the spatial resolution required in many experimental and clinical studies and the calibration of the signal in absolute terms is still a problem particularly in heterogeneous tissue (Fishman et al., 1989; Bottomley, 1989; Lammertsma & Jones, 1992). In contrast, imaging bioluminescence has been advanced in the past few years to be a powerful technique for measuring absolute concentrations of metabolites at a high spatial resolution which will be subject of this article.

The comparison of microcirculation in normal organs with that in tumours suggests that global information on the metabolic state of malignancies may not reflect the specific situation of metabolism in some tumour micro-

areas. For example, the overall concentration of ATP as measured in tumour homogenates with HPLC is typically in a range of 0.5–2.0 mM (Koutcher et al., 1990; Mueller-

Klieser et al., 1991). Due to the heterogeneous micro-
circulation in tumours it is very likely that ATP concentration in vivo may be low in some distinct tumour regions with implications for metabolism, growth, viability, and therapeutic response of the respective cancer cells. It is well accepted that many tumours exhibit resistance to a large number of anticancer drugs owing to a membrane transport protein, the so-called glycoprotein P (Juranka et al., 1989). This multidrug resistance (MDR) protein is highly sensitive to ATP, i.e., within certain limits drug resistance is enhanced with increasing ATP. As a consequence, cancer cells with a high ATP content next to blood vessels may escape therapy with drugs, although these cells may be exposed to relatively high concentrations of drugs due to their vicinity to capillaries. Conversely, cells that are located at a distance from blood vessels may have less ATP and may thus be more sensitive to drugs, yet local drug levels may be low as a result of limited penetration into tumour tissue. This example illustrates that metabolic imaging in tumours has to include the correlation of the measuring values with histology. Tumours are often characterized by various necrotic areas that are randomly distributed across the malignant tissue. Since ATP is rapidly hydrolysed upon