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MONITORING OXYGENATION DURING THE GROWTH OF A TRANSPLANTED TUMOR

Anna Bratasz, Ramasamy P. Pandian, Govindasamy Ilangovan, and Periannan Kuppusamy*

1. INTRODUCTION

Several studies with human tumors have shown a strong correlation between oxygenation status and treatment outcome.[1,2] Tumors, in general, are known to be poorly oxygenated (hypoxic) and oxygen deficiency is associated with increased resistance of the tumor cells to treatments such as chemotherapy or radiation.[3,4] Another confounding factor that may potentially interfere with the prediction of the treatment outcome is the non-uniform distribution of oxygenation within the same tumor.[5-7] Many tumors are characterized with regions of hypoxia, even in well-oxygenated tissue. Thus, information on the distribution of tumor oxygenation and its alterations as a function of tumor size (growth), response to therapeutic stress (e.g., radiation), and preconditioning (e.g., hyperoxic treatment) would be valuable to help understand and develop effective treatment strategies for targeted cancer-cell killing. This information would require the availability of tools and procedures capable of repetitive noninvasive imaging of oxygen concentration in living tissues. Of the variety of methods that are available for tissue oximetry,[8] the magnetic resonance-based methods, such as MRI, electron paramagnetic resonance (EPR) spectroscopy and imaging (EPRI), have the advantage of noninvasive imaging of oxygen concentration in tissues.[9-12]

The EPR-based method, known as "EPR oximetry", uses spin probes whose EPR lines are broadened by molecular oxygen. The oxygen-induced broadening is usually linear with respect to pO_2, and hence the measured line-width can be converted to oxygen concentration using appropriate calibration curves. The measurements can be performed noninvasively and repeatedly over periods of months in the same site. This approach uses particulate such as lithium phthalocyanine (LiPc), and lithium butoxy-naphthalocyanine (LiNc-BuO) whose EPR line-widths are highly sensitive to local oxygen

* Center for Biomedical EPR Spectroscopy and Imaging, Davis Heart and Lung Research Institute, Department of Medicine, The Ohio State University, 420 West 12th Avenue, Room 114, Columbus, OH 43210. Tel: 614-292-8998; Fax: 614-292-8454; e-mail: kuppusamy.1@osu.edu

concentration.[13,14] These probes are stable in tissues, nontoxic, and biocompatible. They can be implanted at the desired site or, with a suitable coating, they can be infused into the vasculature for targeted delivery to tissues.[15] In addition, these probes can be internalized in cells enabling measurement of intracellular pO_2 with high accuracy.[13]

In this manuscript, we report the development of a novel procedure for *in situ* monitoring of oxygen concentration in growing tumors by EPR-based oximetry using embedded paramagnetic particulates. Unlike the existing methods of oxygen measurement wherein the oximetry probes (needle electrodes, optical probes, or EPR implants) are physically inserted during measurement, the new approach uses spin probes that are permanently embedded in the tumor. A particular advantage of this procedure is that it is noninvasive, both in terms of implantation of the probe, as well as when obtaining readouts of oxygen.

2. MATERIALS AND METHODS

2.1 Reagents

Cell culture medium (RPMI 1640), fetal bovine serum, penicillin/streptomycin, sodium pyruvate, trypsin, and phosphate-buffered saline (PBS) were purchased from Gibco BRL (Grand Island, NY). In vitro MTT Toxicology Assay Kit was obtained from Sigma (St. Louis, MO). Lithium phthalocyanine (LiPc) and lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO) probes were synthesized as reported[13].

2.2 Mice

Female C3H mice were obtained from Frederick Cancer Research Center Animal Production (Frederick, MD). The animals were housed five per cage in a climate- and light-controlled room. Food and water were allowed *ad libitum*. The animals were 50-days old and weighed about 25 g at the time of the experiment. Animals were anesthetized with ketamine and xylazine (i.p.). The animals breathed either room air (21% O_2) or carbogen (mixture of 95% O_2 and 5% CO_2) delivered through a nose cone. During the measurements, the body temperature of the animal was maintained at $37\pm1^{o}C$ by an infra-red lamp placed just above the animal. The body temperature was monitored using a rectal thermistor probe.

2.3 Tumor Growth and Implantation of the Oxygen Probe

Radiation-induced fibrosarcoma-1 (RIF-1) cells were used in the present study. The cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in an atmosphere of 95% air and 5% CO_2 at 37°C. Cells were trypsinized, centrifuged and suspended in PBS without calcium and magnesium ions. The oxygen-sensing probes were implanted in the tumor in three different ways. (i) *Surgical implantation:* One million RIF-1 cells in 0.04 ml PBS were injected into the hind limbs of C3H mice. The tumors were allowed to grow to about 8 mm diameter. About 10 μg of LiPc microcrystalline powder (size: 5 – 50 μm) was implanted in the tumor tissue at a