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Scanning Acoustic Microscopy—A Novel Noninvasive Method to Determine Tumor Interstitial Fluid Pressure in a Xenograft Tumor Model[1]

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Abstract

Elevated tumor interstitial fluid pressure (TIFP) is a prominent feature of solid tumors and hampers the transmigration of therapeutic macromolecules, for example, large monoclonal antibodies, from tumor-supplying vessels into the tumor interstitium. TIFP values of up to 40 mm Hg have been measured in experimental solid tumors using two conventional invasive techniques: the wick-in-needle and the micropuncture technique. We propose a novel noninvasive method of determining TIFP via ultrasonic investigation with scanning acoustic microscopy at 30-MHz frequency. In our experimental setup, we observed for the impedance fluctuations in the outer tumor hull of A431-vulva carcinoma–derived tumor xenograft mice. The gain dependence of signal strength was quantified, and the relaxation of tissue was calibrated with simultaneous hydrostatic pressure measurements. Signal patterns from the acoustical images were translated into TIFP curves, and a putative saturation effect was found for tumor pressures larger than 3 mm Hg. This is the first noninvasive approach to determine TIFP values in tumors. This technique can provide a potentially promising noninvasive assessment of TIFP and, therefore, can be used to determine the TIFP before treatment approach as well to measure therapeutic efficacy highlighted by lowered TFP values.

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Introduction

Elevated tumor interstitial fluid pressure (TIFP), as already shown in the early works of Young et al. in the 1950s, has been identified as a major barrier for transmigration of larger molecules into the interior of solid tumors [1]. TIFP is outwardly directed and hampers mainly the transport of big molecules as they are dependent on convectional flow rather than diffusional effects [2–4]. Moreover, a heightened TIFP not only has significant effects on molecular transport but also plays a role in inducing mechanical stress on the tumor capsule, thereby triggering cell proliferation; in turn, the lowering of TIFP accounts for beneficial effects on tumor progression [5,6]. Measuring

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the TIFP has been performed by invasive, hydrostatically coupled techniques such as the wick-in-needle or the micropuncture method [7,8].

Still, nowadays, one of the biggest problems in tumor therapy is how to effectively transport high-molecular substances like monoclonal antibodies to its desired target location. The knowledge of the TIFP as a "physical biomarker" to presume the efficacy of drug uptake would help the clinicians to determine tumor therapies. Therefore, it is desirable for future clinical and preclinical applications to develop a noninvasive measuring method which relies on merely acoustic signal acquisition. Multiple tissues have been characterized with high-frequency acoustic techniques to quantify the biomechanical properties of cells and internal cellular structures in the past years [9,10]. In regard to biophysical effects of TIFP, our group has employed scanning acoustic microscopy (SAM) in an early stage of development to measure TIFP values [11]. In a recent work, we proposed SAM as a putative method of choice for the noninvasive investigation of TIFP using an improved setup to measure TIFP.

Material and Methods

Cell Culture and Drugs

A431 epidermoid vulva carcinoma cell line was purchased from the American Type Culture Collection (ATCC/LCSC Standards, Wesel Germany) and cultured in low-glucose Dulbecco's modified Eagle's medium (Invitrogen, Karlsruhe, Germany) containing 10% fetal calf serum (Gibco, Paisley, UK). Cells were cultured for several days or a few weeks until a density of 12×10^7 cells per 25-ml flask was reached. After trypsin/EDTA (Gibco, Paisley, UK) treatment, cells were harvested and centrifuged. Cells were then subcutaneously injected into immune-suppressed mice (see below) at a density of 5×10^6 cells per flank.

Tumor Models

For the required tumors to grow, female Naval Medical Research Institute nude mice (5-6 weeks, 18-22 g; Janvier Labs, Le Genest-Saint-Isle, France) were subcutaneously injected on both flanks with 5×10^6 A431 cells. The animals were kept in a pathogen-free environment, and the experiments were approved in accordance with the German animal welfare regulations (Regierungspräsidium Darmstadt, FK/1002). The mice had access to sterilized food and tap water *ad libitum*. For quick *in situ* tumor volume measurements, anesthesia was carried out under isoflurane (Forene, Abbott, Wiesbaden, Germany) vaporizer stream (2%). For tumor excision, ketamine/xylazine (100/10 mg/kg, intraperitoneally; Pharmacia, Erlangen, BayerVet, Leverkusen, Germany) injections were applied. Tumor excision was performed when a size no larger than 20% of the total body weight of mice was reached individually. After excision, anesthetized mice were sacrificed.

Phantom Models and TIFP Measurements

Phantom models were established using balloon and Fogarty thrombectomy catheters (320808 F, Edwards Lifesciences, Unterschleißheim, Germany) as spherical/ellipsoid reflectors of 13 mm diameter and maximum liquid capacity of 2.25 ml 0.9% NaCl solution (B. Braun, Melsungen, Germany).

For simultaneous, invasive calibration measurements of TIFP, a digital pressure transducer including digital amplifier TAM-D (Hugo-Sachs-Elektronik, Harvard Apparatus GmbH, March-Hugstetten, Germany) was used. In the tumors, the cannulae (size 27G) were injected instantaneously via a proprietary spring-controlled injection system which was hydrostatically coupled to the pressure transducer and amplifier. *Aqua dest.* respectively 0.9% NaCl solution were used as coupling fluids and heated up to a stable temperature of 37°C (±0.1°C accuracy) via a P.I.D. controlled heating system (Watlow GmbH, Kronau, Germany). A self-developed vacuum fixation device with a pump flow rate of 3 l/h at 20 rpm was used to keep the submersed tumors immovable. For measurement, excised tumors were put in a cone-shaped Perspex holder with a filter-covered bottom outlet (Ø 5 mm) for the vacuum pump.

Acoustic Microscopy

Scanning acoustic microscopes (SAM 100/AM300, PVATePla, Aalen, Germany), equipped with variable detachable transducers, were used. The piezoelectric transducer, which yielded optimal compromise between penetration depth and resolution, had a center resonating frequency of 30 MHz and was comprised of ZnO with a sapphire backing lens with half opening angle at 30°, focusing with a lateral resolution of 20 µm. The signal has a bandwidth of 30 MHz @ 2 dB and was sampled with a resolution of 8 bits at 500 MS/s prior to storage on the microscope internal hard drive. The radiofrequency output gain curve was determined on a 13-mm-diameter phantom model and subsequently adjusted at a fixed value throughout the experiments to obtain good topological imaging without increasing systematic noise. The typical acquisition time was set for C-scan at 40 seconds in an imaging mode with 500 × 500 pixels.

Image Acquisition and Image Analysis

Images were acquired in B- and C-operational modes of the acoustic microscope with Windows-based software (WinSam) at given maximum *x-y* resolution. Image data were stored in SAM and TIFF data output formats and analyzed by SAMnalysis software 2.1 (PVATePla, Aalen, Germany) and Image-J (NIH, Bethesda, MD).

Results

To show the feasibility of extracting intensity and contrast information changes out of B- and C-mode sonographic images, the complex echogenic situation in a real three-dimensional tumor was reduced to a simplified phantom model system. A fluid-filled balloon and Fogarty thrombectomy catheter system with stepwise-controllable inflation/deflation operation modes were used. Internal

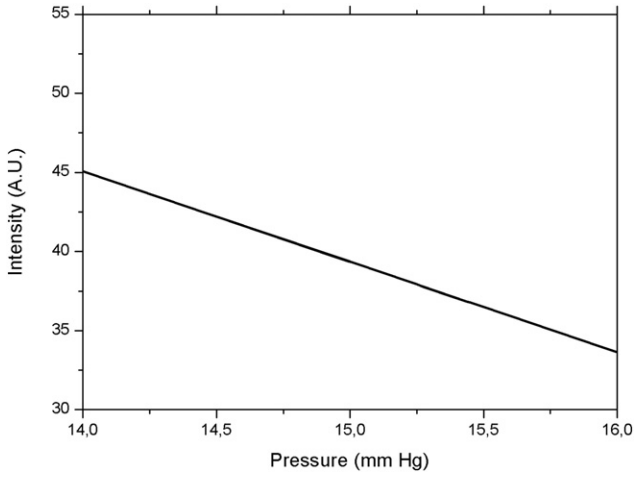

Figure 1. Graph displaying the relationship between hydrostatically recorded TIFP values and obtained B-mode image gray value intensity for the hull of a 13-mm balloon catheter phantom model.