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INTERSTITIAL FLUID PRESSURE IN HUMAN MELANOMA XENOGRAFTS

Relationship to fractional tumor water content, tumor size, and tumor volume-doubling time

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The interstitial fluid pressure (IFP) has been shown to be elevated in malignant tissue, but the possibility that IFP might be related to other pathophysiological parameters of the tissue has not been fully explored. The purpose of the study here reported was to measure the IFP in human melanoma xenografts and to search for possible correlations between tumor IFP and fractional tumor water content, tumor wet weight, or tumor volume-doubling time. Tumors of four melanoma lines (A-07, D-12, R-18, U-25), grown orthotopically in BALB/c-nu/nu mice, were included in the study. Tumor IFP, measured by using the wick-in-needle technique, ranged from 2 to 10 mm Hg (D-12), from 2 to 15 mm Hg (A-07 and U-25), and from 2 to 30 mm Hg (R-18). Statistically significant correlations between tumor IFP on the one hand and fractional tumor water content, tumor wet weight, or tumor volume-doubling time on the other were not found, whether the tumor lines were analyzed individually or together. These observations suggest that simple general relationships between the IFP and the other pathophysiological parameters measured here, might not exist in tumors.

Since the first measurements of interstitial fluid pressure (IFP) in neoplastic tissue in the early 1950s (1), several investigators have shown that the IFP is higher in tumors than in most normal tissues (2–12). The elevated IFP in tumors is mainly due to the lack of a functional lymphatic system (13), high vascular permeability (14) and hydraulic conductivity (15), and high viscous (16) and geometric (17) resistance to blood flow. The microvascular hydrostatic pressure is the principal driving force for the elevated IFP in tumors (7). Theoretical and experimental studies have suggested that in tumors growing as a single nodule, the IFP is relatively uniform throughout the tumor and drops precipitously to normal tissue values at the tumor–normal tissue interface (5, 18). High IFP in tumors might be indicative of resistance to some treatment modalities. Thus, inverse relationships have been demonstrated between IFP and oxygen tension both in experimental (6) and human (9) tumors. Moreover, the elevated IFP in tumors has been shown to restrict the access of macromolecular therapeutic agents to the neoplastic cells (19, 20).

The possibility that the IFP might be related to other pathophysiological parameters in tumors, however, has not been fully explored. Some studies have been performed both in experimental and human tumors, but with conflicting results. Thus, studies of rodent tumors have revealed significant positive correlations between IFP and fractional water content at small but not at large tumor volumes (6). The IFP has been found to increase with increasing tumor size, but only in some tumor lines (5, 19). In other tumor lines, the IFP showed significant positive correlations to time after implantation, without showing significant positive correlations to tumor size (7). Moreover, clinical studies have revealed significant positive correlations between IFP and tumor volume in malignant melanoma (8) and squamous cell carcinoma of the head and neck (10), but not in breast carcinoma (11) and liver metastases of colon carcinoma (11). The IFP was found to increase with

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increasing differentiation grade in human squamous cell carcinoma of the uterine cervix (9), but tended to decrease with increasing differentiation grade in human breast carcinoma (11).

A thorough understanding of the relationships between tumor IFP and other, more well-explored tumor parameters, might be of help in defining a potential role for IFP measurements in prediction of tumor treatment resistance. The objective of the work reported here was to measure the IFP in human melanoma xenografts and to search for possible correlations between tumor IFP on the one hand and fractional tumor water content, tumor wet weight, or tumor volume-doubling time on the other. Tumors of four different lines, grown orthotopically in athymic mice, were subjected to IFP measurements by using the wick-in-needle technique.

Material and Methods

Mice and tumors. Adult BALB/c-nu/nu mice (8–10 weeks old), bred at our research institute, were used as host animals for xenografted tumors. The mice were maintained under specific pathogen-free conditions at constant temperature (24–26°C) and humidity (30–50%). Sterilized food and tap water were given ad libitum. Four human melanoma lines (A-07, D-12, R-18, U-25) were included in the study (21). Xenografted tumors were initiated from exponentially growing monolayer cultures in passages 75–100. Monolayer cells, cultured in RPMI-1640 medium (25 mM HEPES and L-glutamine) supplemented with 13% fetal calf serum, 250 mg/l penicillin, and 50 mg/l streptomycin, were detached by trypsinization (treatment with 0.05% trypsin/0.02% EDTA solution at 37°C for 2 min). Approximately 3.5×10^5 cells in 10 μl of Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution were inoculated intradermally in the flanks of the mice by using a 100 µl Hamilton syringe (21).

Tumor volume (V) was calculated as $V = \pi/6 \times ab^2$, where a is the longer and b the shorter of two perpendicular tumor diameters, measured with calipers. Tumor volume-doubling time was derived from the tumor growth curve. Tumor weight was measured immediately after tumor excision and refers to the wet weight. Fractional tumor water content was determined by drying the tumor tissue at 40°C until a constant weight was reached.

Interstitial fluid pressure. Tumor IFP was measured by using the wick-in-needle technique (22). A 23 gauge needle (Microlance, Dublin, Ireland), filled with multifilamentous nylon thread, was connected to an Abbott Transpac II pressure transducer (Abbott Ireland Ltd., Sligo, Ireland) by a polyethylene tubing filled with sterile heparinized (70 units/ml) saline. The nylon thread increased the contact area and improved the fluid communication in the needle. The pressure transducer was connected to a model 13-6615-50 preamplifier (Gould Inc., Cleveland, OH, U.S.A.) and a model TA240 Easygraf dual-channel chart recorder (Gould Inc., Cleveland, OH, U.S.A.). The equipment was calibrated by determining the linear relationship between imposed pressure and measured pressure. The pressure of 30 cm of saline was maintained for 5 min to test for possible leaks in the system.

The mice were kept under anaesthesia during the IFP measurements. Propanidid (Gedeon Richter Ltd., Budapest, Hungary), fentanyl/fluanisone (Janssen Pharmaceutika, Beerse, Belgium), and diazepam (Dumex, Copenhagen, Denmark) were administered intraperitoneally in doses of 400 mg/kg, 0.24/12 mg/kg, and 4 mg/kg, respectively. The body core temperature of the mice, measured with a rectal probe, was kept at 36–38°C by using a heating pad. The needle was inserted in the central region of the tumor for measurement of IFP. The IFP was recorded for at least 5 min. The fluid communication between the tumor and the pressure transducer was checked by compressing and decompressing the tubing between the needle and the transducer, using a screw clamp. Measurements were discarded if the readings following compression and decompression differed by more than 1 mm Hg. Tumor IFP was determined by calculating the mean of these two readings. The IFP measured in the subcutaneous tissue of tumor-free dorsal skin served as an internal control.

Statistical analysis. Statistically significant correlations were searched for by linear regression analysis. Statistical comparisons of data were performed by non-parametric analysis using the Kruskal-Wallis H-test. A significance criterion of $p < 0.05$ was used.

Results

The IFP was confirmed to be elevated in tumor tissue relative to normal tissue. A typical example of an IFP recording in tumor tissue is illustrated in Fig. 1. The reading showed a sharp rise immediately after needle insertion, followed by a stable level which lasted until the needle was withdrawn. The readings before and after the compression and decompression tests were similar, suggesting that the fluid communication between the tumor and the pressure transducer was adequate. The IFP measurements were verified to be highly reproducible by performing repeated measurements in the same tumors. The IFP in the subcutaneous tissue of the dorsal skin was measured to range from -1 to $+1$ mmHg.

The IFP and the fractional water content differed considerably between individual tumors of the same line (Fig. 2). The IFP ranged from 2 to 10 mmHg (D-12), from 2 to 15 mmHg (A-07 and U-25), and from 2 to 30 mmHg (R-18) (Fig. 2a). Significant differences between the tumor lines were not detected ($p > 0.05$). However, some R-18 tumors showed a substantially higher IFP than the D-12 tumors. The fractional water content ranged from 77 to

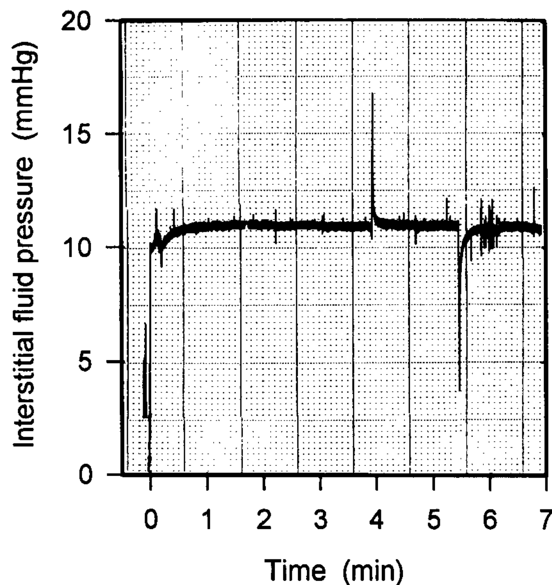


Fig. 1. Example of an IFP recording in an A-07 tumor growing intradermally in an athymic mouse. A stable value of approximately 11 mmHg was reached immediately after needle insertion. The tubing connecting the needle and the pressure transducer was compressed approximately 4 min later, resulting in a sharp increase in the reading followed by an exponential decrease towards the pre-compression value. Decompression of the tubing was performed approximately 1.5 min later, resulting in a sharp decrease in the reading followed by an exponential increase towards the pre-decompression value.

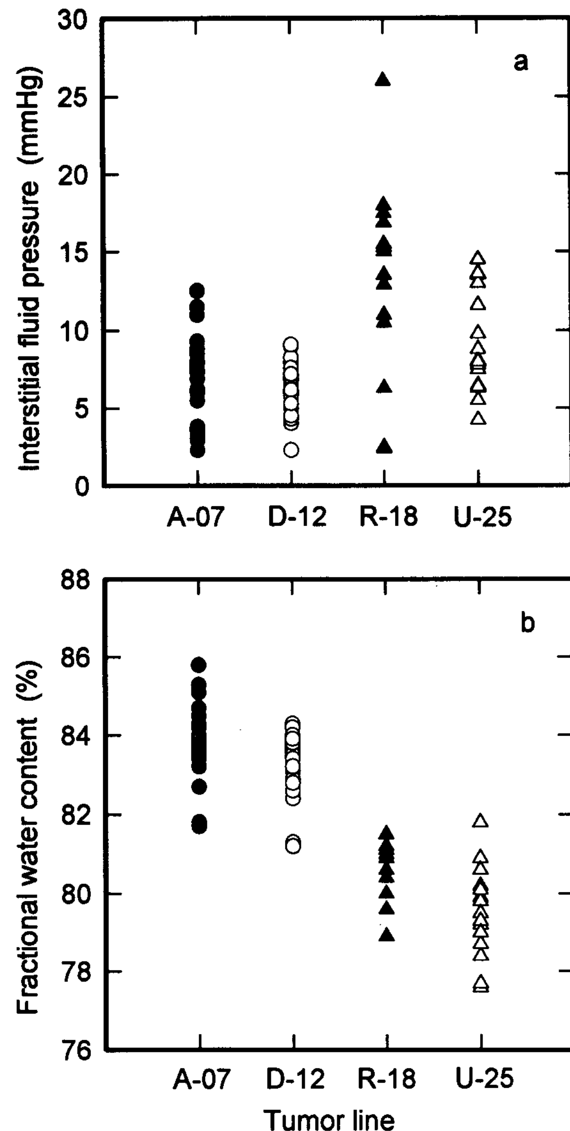


Fig. 2. Pathophysiological parameters of human melanoma xenografted tumors growing intradermally in athymic mice. a) Interstitial fluid pressure; b) Fractional tumor water content. Points, single tumors.

82% (R-18 and U-25) and from 81 to 86% (A-07 and D-12) (Fig. 2b). The values were significantly higher for the A-07 and D-12 tumors than for the R-18 and U-25 tumors ($p < 0.05$). There was no correlation between tumor IFP and fractional tumor water content, as verified by analyzing the tumor lines individually ($p > 0.05$ for all lines) and together ($p > 0.05$) (Fig. 3).

The tumors subjected to measurement of IFP had wet weights ranging from 100 to 1100 mg. The volume-doubling time of the tumors at the size at which the IFP measurements were performed, ranged from 2 to 4 days (A-07), from 2 to 6 days (D-12), from 5 to 12 days (R-18), and from 8 to 19 days (U-25). Significant correlations between tumor IFP and tumor wet weight or tumor volume-doubling time were not found, whether the tumor lines were analyzed individually ($p > 0.05$ for all lines, both for wet weight and volume-doubling time) or together ($p > 0.05$, both for wet weight and volume-doubling time) (Fig. 4).

Discussion

The wick-in-needle method was confirmed to be a reliable and simple method for assessment of the IFP in tissue. Thus, highly reproducible results were achieved both in tumors and subcutis. Moreover, since the IFP is relatively uniform throughout a tumor growing as a single nodule, a random central IFP measurement is representative of the entire tumor (5, 18).

The melanoma xenografts showed IFP values (2–30 mmHg) similar to those reported for rodent tumors (2–6) and melanomas in man (8, 12). The IFP differed substantially between individual tumors of the same xenograft line. Significant differences between the lines were therefore not detected. In contrast, it has been demonstrated that experimental tumor lines of different histologies might show significant differences in IFP (23).

The IFP has been reported to be positively correlated to the fractional water content in FSaII tumors with volumes less than 500 mm^3 (6). This observation led to the sugges-

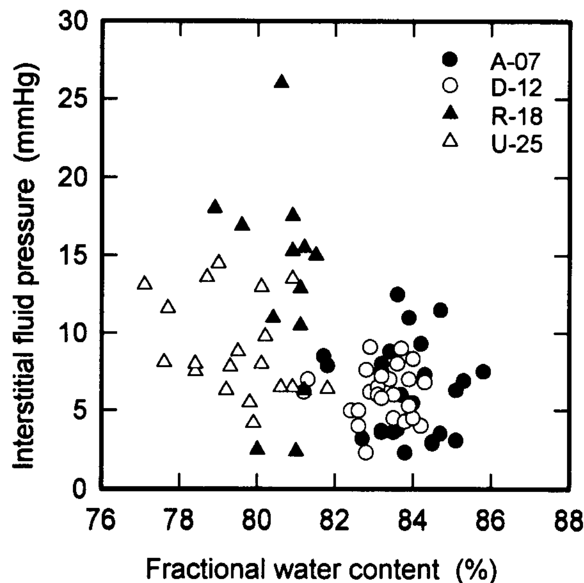
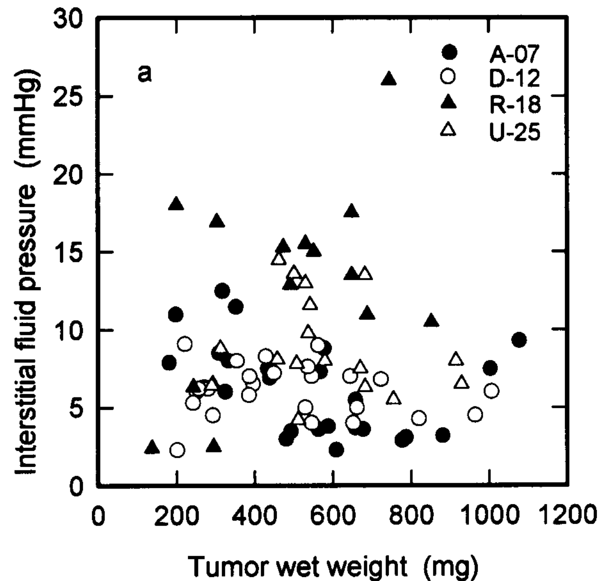


Fig. 3. Interstitial fluid pressure vs fractional tumor water content in human melanoma xenografted tumors growing intradermally in athymic mice. Points, single tumors.



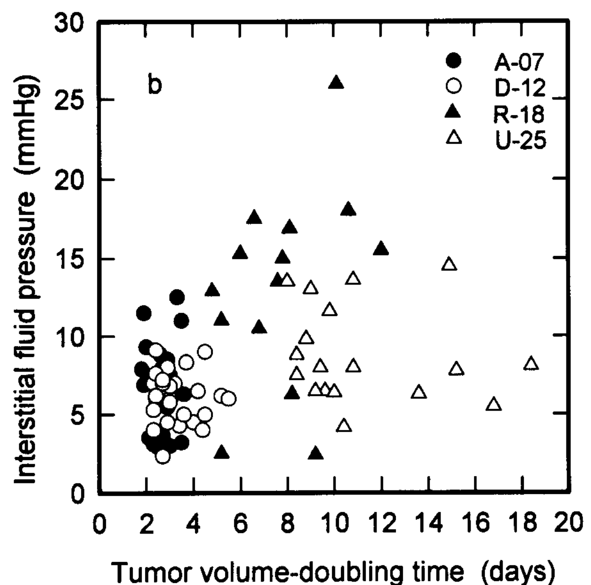


Fig. 4. Interstitial fluid pressure vs a) tumor wet weight and b) tumor volume-doubling time in human melanoma xenografted tumors growing intradermally in athymic mice. Points, single tumors.

tion that the IFP in tumors might be measured non-invasively by ^{1}H-NMR imaging (6). However, significant correlations between IFP and fractional water content were not found for any of the xenograft lines studied here. Moreover, some rodent tumor lines have been reported to show positive correlations between tumor IFP and tumor size (2–5) whereas others have been found to show positive correlations between tumor IFP and time after implantation (7). In contrast, none of the xenograft lines studied here showed significant correlations between tumor IFP and tumor wet weight or tumor volume-doubling time.

The discrepancy between our data on human melanoma xenografts and those reported by others on rodent tumors is probably due to biological differences between the tumor model systems. The xenografted human tumors were in contrast to the rodent tumors grown in orthotopic sites in the mouse (21). Several essential biological properties of the donor patients' tumors, including angiogenic, vascular, histopathological, and pathophysiological parameters, have been shown to be retained in our orthotopic human tumor model systems (21), suggesting that they are more relevant models of human cancer than the rodent tumor models used in previous studies of tumor IFP.

In conclusion, significant correlations between tumor IFP and fractional tumor water content, tumor wet weight, or tumor volume-doubling time were not found, suggesting that simple general relationships between the IFP and the other pathophysiological parameters measured here, might not exist in human melanoma xenografts growing orthotopically in athymic mice.

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