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RAPID COMMUNICATION

Detlef Rohde · Christoph Wiesner · Dirk Graf
Johannes Wolff · Laszlo Füzesi · Gerhard Jakse

Interstitial fluid pressure is increased in renal cell carcinoma xenografts

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Abstract The purpose of this study was to test the hypothesis that renal cell carcinoma (RCC) exhibits an increased intratumoral interstitial fluid pressure (IT-IFP). Therefore, resected tumors from human primary ($n = 23$) or metastatic RCC ($n = 3$) were xenografted in SCID mice. The IFP of single tumor nodules ($n = 65$) and normal mouse tissue ($n = 195$) was measured by means of the "wick-in-needle" technique. Data demonstrate that the mean IT-IFP at neoplasia was 35 times greater than in normal tissue, and decreased precipitously at the tumor boundary. IT-IFP values tended to increase with the grade of malignancy of the tumor cells and tumor size. The mean IT-IFP of xenografts derived from primary RCC was twice as high as that from metastatic RCC tissue. These findings indicate a biophysical barrier to drug delivery in RCC; this may, in concert with cellular-based drug resistance mechanisms, be an additional explanation for resistance of the tumor to certain blood-borne anticancer therapies.

Key words Drug delivery system · Mice, nude · Neoplasm, metastasis, physiopathology · Pressure · Neoplasm, kidney

Introduction

Renal cell carcinoma (RCC) is refractory to treatment with various anticancer drugs. Hormonal or cytotoxic chemotherapy of metastatic disease results in tumor remission rates of 6% [34]. Resistance of RCC to systemic treatment remains only partially explained. Intrinsic or acquired cellular drug resistance mechanisms, such as overexpression of the mdr-1 gene product gp-170 efflux pump [10], as well as activation of gluthathione-S transferase or downregulation of topoisomerase-2 [33], have been identified for a few anticancer drugs. However, further investigation is warranted to gain further insight into the common property of the tumor to resist certain anticancer therapies.

Several barriers to drug delivery in solid tumors have been discussed [14, 15]. Mathematical modeling [1, 13] hypothesized an increased interstitial fluid pressure (IFP) in solid tumors which might contribute to ineffectively low intratumoral concentrations of anticancer drugs. Interstitial fluid transportation is dependent on the local microvascular pressure (MVP), hydraulic permeability of the vascular wall, and the hydraulic conductivity and compliance of the interstitial compartment [1, 27]. As the tumor grows, an increased IFP is suggested to result from an increased MVP, due to an imbalance of the induction of neoangiogenetic, leaky capillary vessels and an inadequate development of draining lymphatic vessels [2, 3, 5, 27]. The resulting pressure gradient is believed to induce flow stasis, retard passage of molecules into the interstitium and support a convective flow radially out of the tumor ("washout of drugs"). Thus delivery and perturbation of therapeutic agents are insufficient.

Biophysical measurements in experimental solid rodent and human tumors [2, 3, 5, 6, 16, 17, 18, 19, 22, 23, 24, 28, 32, 35, 36] and in cancer patients [4, 6, 7, 11, 20, 25, 29] confirmed the predicted elevated intratumoral IFP (IT-IFP) compared with the surrounding normal tissue. Random measurement of IT-IFP in a central tumor region is representative, since pressure is uniformly distributed within a single nodule [2, 13].

Measurement in one patient with a recurrent RCC, who had been pretreated with chemotherapy and immunotherapy, demonstrated a higher IT-IFP (38 mmHg) compared with most patients with other malignancies [14, 20]. However, detailed experiments

D. Rohde (✉) · C. Wiesner · D. Graf · J. Wolff · G. Jakse
Department of Urology, Medical Faculty,
University of Aachen,
Pauwelsstrasse 30, 52057 Aachen, Germany

L. Füzesi
Department of Pathology, Medical Faculty,
University of Aachen,
Pauwelsstrasse 30, 52057 Aachen, Germany

on RCC are lacking. Therefore, the objective of this preclinical study was to determine whether an increased IT-IFP is a common finding in RCC, whether IT-IFP of metastatic tissue differs from IT-IFP of primary cancer and to assess parameters that influence IT-IFP values.

Materials and methods

Tumor origin

Tissue specimens were freshly prepared from resected tumors of patients ($n = 57$) with renal tumor disease (46 primary RCC, 8 metastases of RCC, 3 oncocytoma). Specimens were washed with RPMI cell culture medium (Gibco BRL/Life Technologies, Eggenstein, Germany), supplemented with 1% (v/v) penicillin/streptomycin, and cleared of necrotic and hemorrhagic areas under sterile conditions.

Animals

Six- to 8-week-old female severe combined immune deficiency (CB. 17-scid/Hsd SCID) mice (Bomholdgard/Breeding and Research Centre Ry, Denmark) were kept under pathogen-free housing conditions at 25°C and fed a sterile standardized diet (Altromin, Lage, Germany) and water ad libitum. Animal experiments were carried out in accordance with the Principles of Laboratory Animal Care and the German law on the protection of animals.

Induction of xenografts

Mice were anesthetized by inhalation of isoflurane (Hypnorm; Forene Abbott, Wiesbaden, Germany). Within 30–60 min after operation, cleared tumor specimens ($8 \times 8 \times 6$ mm) were subcutaneously implanted in the shaved upper back of the animals. The volume of the growing tumors was measured once weekly with calipers and determined as: $V_t = a \times b^2/2$ (V_t = tumor volume, a = longer perpendicular axis, b = shorter perpendicular axis) [4].

Measurement of IFP

IFP was measured by use of a modified wick-in-needle (WIN) technique [4, 8, 11, 19]. In brief, a 23 gauge needle was prepared with a 2–3 mm long side-hole 3 mm distant from the tip. The needle was filled with five 6–0 nylon surgical sutures (Ethilon; Ethicon, Norderstedt, Germany). The needle was connected to a pressure transducer (DTP pressure transducer; pvb Medizintechnik Kirchseeon, Germany) by a polyethylene tube. Needle and tube were filled with sterile, heparinized (70 units/ml) isotonic saline. Pressure measurements were continuously amplified and recorded by a Dantec 23G01 Menuet system (Dantec Medical, Skovlunde, Denmark) with integrated software (Dantec software 4.00). Zero reference was simultaneously obtained by a second needle system at tumor height.

After calibration of the pressure transducer setup by imposed pressures, the WIN was first placed in subcutaneous tissue. The needle was then inserted in the center of the xenograft and retrieved to the periphery (0.5 mm distant from the boundary). The needle was inserted without external fixation. IFP was determined after equilibration of the measurement, i.e., when IFP reached a steady-state plateau (approximately 5–10 min after insertion of WIN). Finally, IFP was measured in normal liver and kidney tissue of the mouse after the abdominal cavity had been opened by median laparotomy. Experiments were performed under controlled temperature conditions. At the end of the experiments, the animals were killed by an overdose of narcotics.

Integrity of each measurement, i.e., fluid communication, was determined by standardized elevation of the mouse after measurement of IFP reached its steady state. The measurement was taken as valid if IFP increased according to the artificial hydrostatic load and returned to is previous IFP signal at the end of the maneuver.

Histopathology

Resected tumors of the patients were routinely classified according to the TNM system [12]. The grade of malignancy (nuclear grading) was determined in a masked procedure by an independent pathologist (L.F.) and classified as well differentiated (grade 1), moderately differentiated (grade 2) or poorly differentiated (grade 3).

Subcutaneous xenografts were excised, fixed in 8% (v/v) PBS-buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin by standard techniques.

Statistics

Statistical evaluation of parameters was restricted to samples sized $n \geq 5$. For statistics on tumor take rate, Fisher's exact test was used. Single comparisons of independent IFP measurements were performed using the Wilcoxon range test. P values ≤ 0.05 were considered significant. Correlation between xenograft volume and IFP was carried out by Pearson's correlation coefficient. Significant correlation was acquired at corr ≥ 0.45. All statistical calculations were performed on the SAS system (SAS Institute, Cary, N.C., USA).

Results

Twenty-three of 46 primary RCC induced subcutaneous xenografts (take rate: 50%), as well as three of eight metastases from RCC (take rate: 37.5%). Oncocytomas ($n = 3$) could not be established on SCID mice. The tumor take rate tended to rise with tumor stage and grade of malignancy of the primary RCC (Table 1). However, statistically significant differences in tumor take rate could only be demonstrated with regard to the grade of malignancy. The take rate of primary RCC from patients with concomitant distant metastases (M1) did not differ from the take rate of primary RCC from patients without metastases (M0).

Table 1 Tumor take rate of tissue from primary renal cell carcinoma (*RCC*) with regard to tumor stage (pT1–pT4), grade of malignancy (G1–G3) and the prevalence of concomitant metastasis (M1)

RCC	*n*	Take rate	*P* value
pT1	2	0/2	–
pT2	17	7/17 (41%)	pT2 vs pT3: $P = 0.523$
pT3	22	12/22 (54%)	pT3 vs pT4: $P = 0.618$
pT4	5	4/5 (80%)	pT4 vs pT2: $P = 0.311$
G1	12	3/12 (25%)	G1 vs G2: $P = 0.286$
G2	25	12/25 (48%)	G2 vs G3: $P = 0.05$
G3	9	8/9 (89%)	G3 vs G1: $P = 0.007$
pTx M0	31	13/31 (42%)	$P = 0.208$
pTx M1	15	10/15 (67%)	

Since serial transplantation was performed in some xenografts, IT-IFP could be measured in at least 65 single tumor nodules. The IFP of the surrounding subcutaneous tissue, normal liver and kidney was predominantly < 2 cm H_2O. Data analyses (Table 2) significantly demonstrated that: (a) the mean IT-IFP (10.97 ± 6.76) was always ≥3 cm H_2O and was increased 35-fold compared with normal tissue ($n = 195$; mean IFP: 0.31 ± 0.88), (b) the mean IFP was 3.5-fold decreased at the tumor periphery compared with the center of the tumor, and (c) the mean IT-IFP tended to increase according to the grade of malignancy of the xenografts. It is of interest that the mean IT-IFP in xenografts that were derived from primary RCC was twice as high as in xenografts from metastatic renal carcinoma tissue ($P = 0.02$).

Mean tumor volume of xenografts was 3000 mm^3. Regression analyses of single data suggested that IT-IFP increased with the tumor volume (Fig. 1), but statistical correlation did not reach significance (corr: 0.364). However, IT-IFP of xenografts ≤3000 mm^3 was significantly decreased compared with IT-IFP of tumors > 3000 mm^3 ($P = 0.01$).

Discussion

Measurements of IT-IFP were performed on xenografts derived from human primary and metastatic RCC. Similar to other solid tumors (melanoma, mammary and colon carcinoma, intracranial tumors) [3, 4, 5, 6, 15, 17, 21, 23, 24, 32, 35, 36], the present animal experiments demonstrate a significantly increased IT-IFP, which decreases precipitously at the boundary of the tumor [2, 13]. However, the observed ranges and mean IT-IFP values of RCC did not obviously differ from data on other solid tumors. Large intertumoral variations in IT-IFP [3–37 cm H_2O = 2.2–27.2 mmHg (1 mmHg = 1.36 cm H_2O)] [19] have been found and may result from a heterogeneous tumor microenvironment [2, 5].

The observed pressure gradient between tumor tissue and surrounding tissue is proposed as a pathophysiologic barrier, that is, in concert with cellular drug resistance mechanisms it is likely to explain the failure of anticancer therapy in RCC by insufficient drug delivery to tumor cells. In fact, it has been often noted that only disappointing responses could be attained after treatment of renal cancer patients with anticancer drugs that had displayed potent activity in cell culture [34].

In addition, our data corroborate previous reports that IT-IFP increases with tumor mass [4, 6, 11, 19, 25]. Similar to the findings in squamous cell carcinoma [11] and mammary tumors [20], IT-IFP tended to rise with grade of malignancy. Such findings are likely to further explain why (chemo)therapy is more effective with small tumor burden than with bulky carcinoma as well as in well-differentiated versus undifferentiated tumors.

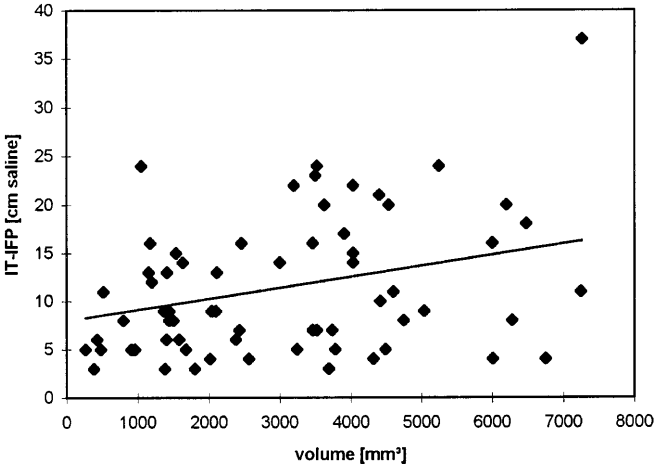

Fig. 1 Regression analysis ($y = 7.252141 + 0.001315x$) of xenograft volume and corresponding intratumoral interstitial fluid pressure (IT-IFP) value. Although statistical correlation did not reach levels of significance (corr: 0.364), the IT-IFP of xenografts >3000 mm^3 was significantly increased (see Table 2)

Table 2 Interstitial fluid pressure (*IFP*) was measured in 65 xenografts of human renal cell carcinoma (*RCC*), derived from primary tumor tissue or metastatic tissue, and in 195 normal tissue samples

Tissue	*n*	IFP		*P* value
		Range (cm H_2O)	Mean ± SD (cm H_2O)	
Xenografts				
G1	3	3–5	4.33 ± 0.94	–
G2	43	3–23	10.09 ± 5.70	0.0548
G3	19	4–37	14.00 ± 8.05	
Primary RCC (center)	55	3–37	11.73 ± 6.98	0.02
Metastatic RCC (center)	10	4–13	6.8 ± 2.93	
All RCC (center)	65	3–37	10.97 ± 6.76	0.0001
All RCC (periphery)	65	0–17	3.12 ± 3.09	
Tumor volume ≤3000 mm^3	32	3–24	8.75 ± 4.76	0.01
Tumor volume >3000 mm^3	33	3–37	13.67 ± 7.92	
Normal tissue				
Subcutaneous tissue	65	−1–2	0.05 ± 0.51	0.0001
Liver	65	−1–2	0.37 ± 0.80	0.0001
Kidney	65	−2–3	0.52 ± 1.14	0.0001

Some strategies are reported to reduce increased IT-IFP, such as radiotherapy [29, 36], hyperthermia [21] or photodynamic therapy [22]. Cycling systemic blood pressure by vasoactive substances [27] or the administration of dexamethasone [17], nicotinamide [19, 28], pentoxifylline [18], or vasodilatators such as hydralazine and nitroglycerin have been examined [35]. Delivery of molecules that are predominantly distributed by convection is assumed to be enhanced by this means. Moreover, bifunctional antibodies or prodrugs are supposed to accumulate within a tumor due to a self-perpetuating inward diffusion gradient [2] that is likely to overcome "washout" or flow stasis.

For treatment of advanced or metastatic RCC, immunotherapy-based regimens have proved more effective than chemotherapy [26]. Thus, it is speculated that the active migration of effector cells might represent an essential driving force to overcome the biophysical pressure gradients in RCC. Recent data additionally indicate that the cytokine tumor necrosis factor-alpha is itself capable of temporarily reducing IT-IFP in melanoma xenografts [16].

Finally, our findings show that metastatic tissue of RCC exhibited significantly lower IT-IFP values compared with primary RCC. These data are likely to support clinical experience on cytokine-based immunotherapy, which has demonstrated responses in metastatic sites but failure of therapy in the intact primary RCC in situ [9, 31]. Since activated effector cells are characterized by increased cell rigidity [30], we suspect that a borderline pressure exists to inhibit effective tumor cell cytolysis by invading immunocompetent effector cells, as well as decreased delivery of high-molecular-weight cytokines in RCC. Thus, tumor response is suggested to be restricted predominantly to low-pressure metastasis. Consistent with this hypothesis, Curti and coworkers [7] reported that the mean IT-IFP of metastatic nodules in patients with melanoma which had responded to interleukin-based immunotherapy was half that in nonresponding lesions (12.2 mmHg vs 24.4 mmHg).

Conclusions

In conclusion, the present xenograft experiments provide substantial evidence that human RCC are characterized by an elevated IT-IFP. These findings suggest that this property of tumors retards passage of molecules and effector cells into the interstitial matrix. Thus, IT-IFP seems an additional principal force for resistance of RCC to therapy with blood-borne anticancer therapy, although its definitive role in chemoresistance of RCC remains to be established.

On the basis of the present preclinical results, future investigation should focus prospectively on patients with RCC, so as to correlate individual, site-specific IT-IFP with the tumor recurrence rate, or to correlate IT-IFP with the tumor response to certain anticancer treatments. Such measurements can easily be performed with devices that are in use for urodynamic purposes.

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