Abstract. Undifferentiated pleomorphic sarcoma not otherwise specified belongs to the heterogeneous group of soft tissue tumors. It is preferentially located in the upper and lower extremities of the body, and surgical resection remains the only curative treatment. Preclinical animal models are crucial to improve the development of novel chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma. However, this approach has been hampered by the lack of reproducible animal models. The present study established two xenograft animal models generated from stable non-clonal cell cultures, and investigated the difference in chemotherapeutic effects on tumor growth between undifferentiated pleomorphic sarcoma in vivo and in vitro. The cell cultures were generated from freshly isolated tumor tissues of two patients with undifferentiated pleomorphic sarcoma. For the in vivo analysis, these cells were injected subcutaneously into immunodeficient mice. The mice were monitored for tumor appearance and treated with the most common or innovative chemotherapeutic agents available to date. Furthermore, the same drugs were administered to in vitro cell cultures. The most effective tumor growth inhibition in vitro was observed with doxorubicin and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA), also known as vorinostat. In the in vivo xenograft mouse model, the combination of doxorubicin and the tyrosine kinase inhibitor pazopanib induced a significant tumor reduction. By contrast, treatment with vorinostat did not reduce the tumor growth. Taken together, the results obtained from drug testing in vitro differed significantly from the in vivo results. Therefore, the novel and reproducible xenograft animal model established in the present study demonstrated that in vivo models are required to test potential chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma prior to clinical use, since animal models are more similar to humans, compared with in vitro cell cultures.

Introduction

Soft tissue sarcoma (STS) is a heterogeneous group of neoplasms arising from degenerated cells of mesenchymal origin (1). Currently, STS is differentiated into >20 distinct subtypes, which are classified according to their tissue of origin (2). The most common type of STS observed in adults is undifferentiated pleomorphic sarcoma not otherwise specified (NOS), which was previously known as malignant fibrous histiocytoma, and presents five histological subtypes (3-5). Currently, surgical resection remains the only method of curative treatment for undifferentiated pleomorphic sarcoma NOS, which often occurs at a high malignancy grade, possesses a high risk of metastasis, and exhibits resistance to radiotherapy and chemotherapy (6-8). Previous studies have demonstrated that additional radiation therapy improves local tumor control; however, chemotherapy remains palliative, since there are...
no effective chemotherapy drugs (9,10). Due to the limited number of patients with specific subtypes of undifferentiated pleomorphic sarcoma NOS, clinical studies often present limitations, and the majority of the data available may not apply to certain subtypes. The application of an anthracycline-based chemotherapy, including doxorubicin alone or in combination with ifosfamide, is often the first-line treatment of undifferentiated pleomorphic sarcoma NOS, and there are no widely recognized second-line therapies available (11-13). Recently, improved second-line drugs have been developed, including histone deacetylase (HDAC) inhibitors, trabectedin and tyrosine kinase inhibitors such as pazopanib, which are more effective than those currently available (14-19). With these drugs, a progression-free survival time of 3-5 months may be achieved (14-19). However, the overall survival time is not increased. Therefore, to prolong the survival time of patients with undifferentiated pleomorphic sarcoma NOS, additional improvements are required.

Cell lines and animal models are powerful tools for the development of innovative therapeutics. In the past recent years, numerous cell lines derived from undifferentiated pleomorphic sarcoma have been generated and characterized (20-24). A number of these cell lines were injected subcutaneously into immunodeficient mice, and 4 weeks subsequent to injection measurable tumor tissue was formed (20,21,24). However, investigations comparing the effect of therapeutic approaches in vivo and in vitro have not been performed thus far. In other studies using xenotransplantation models, original human tumor tissue was transplanted into immunodeficient mice (25-27). These animal models appear to be more similar to the tumors observed in humans, but hypoxia following tissue transplantation remains a problem (25-27).

In the present study, two stable cell cultures were generated from two patients with undifferentiated pleomorphic sarcoma. These cells were subcutaneously injected into immunodeficient mice, whereby remained tumorigenic. Following chemotherapeutic treatment of the tumor, clear differences were observed between the in vitro and in vivo models, which confirms that it is imperative to test innovative chemotherapeutics in appropriate animal models prior to clinical use.

**Materials and methods**

**Animals.** In total, 4 immunodeficient non-obese diabetic (NOD) severe combined immunodeficiency (SCID) gamma (NSG) mice (NOD.Cg-Rplcscid Il2rgtm1Wjl/SzJ) (2 male and 2 female; 8 weeks-old) were purchased from the Jackson Laboratory (Sacramento, ME, USA), and bred in the animal facility at the Johannes Gutenberg University of Mainz (Mainz, Germany). The mice were bred, maintained and manipulated under specific pathogen-free conditions. All the food, water and litter were sterilized prior to use. The temperature and humidity were controlled at 20-24°C and 45-65%, respectively. Daily light cycles consisted of 12 h light and dark cycles. The cages were fully cleaned once or twice per week. Mice that were 6-8 weeks-old were used for subcutaneous injections. All animal procedures were conducted in accordance with the Institutional Guidelines of the Johannes Gutenberg University of Mainz, and approved by the responsible national authority (National Investigation Office Rheinland-Pfalz; Koblenz, Germany; approval no. 23 177-07/G 13-1-027).

**Isolation of sarcoma samples and cell culture.** Stable oligoclonal cell cultures, termed MZ-UPS-1 and MZ-UPS-2, were generated from freshly isolated tumor tissue from two patients diagnosed with undifferentiated pleomorphic sarcoma NOS. These two patients were treated in 2011 and 2012, respectively, at the University Medical Center in Mainz and had undergone surgeries where the local tumor tissue was removed. Immediately following resection, the tumor tissue was minced, placed onto 6-well plates (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and cultivated at 37°C in a humidified atmosphere with 5% CO₂ with Gibco® Dulbecco's modified Eagle's medium [Nutrient Mixture F-12 containing GlutaMAX™ Supplement (DMEM/F12; Thermo Fisher Scientific, Inc., Waltham, MA, USA)], 1% sodium pyruvate (100 mM; Thermo Fisher Scientific, Inc.), 10% fetal calf serum (FCS; GE Healthcare Life Sciences, Chalfont, UK) and 1% penicillin-streptomycin (Invitrogen™; Thermo Fisher Scientific, Inc.). The cells were expanded to form a sub-confluent layer of adherent cells 2-4 weeks subsequent to initial seeding. Subsequently, the adherent tumor cells were digested using Accutase (GE Healthcare Life Sciences) and transferred into 175 cm² cell culture flasks (Sigma-Aldrich Chemie GmbH). Since the MZ-UPS-1 cells were a fast-growing culture, they were passaged and serially subcultured at a dilution of 1:3-1:5 every week. By contrast, the MZ-UPS-2 cells were passaged every two weeks and serially subcultured at a dilution of 1:2. The two undifferentiated pleomorphic cell cultures MZ-UPS-1 and MZ-UPS-2 were maintained in vitro for ~30 passages for >1 year.

The two patients provided written informed consent for biobanking. The present study was approved by the Ethics Committee of the University Medical Center of Johannes Gutenberg University of Mainz [Mainz, Germany; approval no. 837.250.13 (8935)].

**Chemotherapeutic treatment in vitro and cell viability assay.** MZ-UPS-1 and MZ-UPS-2 cells from the fourth passage were harvested, washed in phosphate-buffered saline (PBS; Thermo Fisher Scientific, Inc.) and resuspended in DMEM/F12-GlutaMAX™ Supplement with 10% FCS. One day prior to chemotherapeutic treatment, the cells were seeded onto 96-well plates (1x10⁴ cells/100 µl medium/well; Greiner Bio-One GmbH, Frickenhausen, Germany) to ensure adherence. On day 1, the supernatants were discarded, and a cell viability assay was conducted with alamarBlue™ Cell Viability Assay Reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer’s protocol. Briefly, alamarBlue™ solution was diluted 1:10 in DMEM/F12-GlutaMAX™ Supplement, and 100 µl/well was added to the cells, which were incubated for 1-2 h. Subsequently, the supernatants were removed, and the absorbance was measured at 570 nm (reference wavelength, 600 nm) in a multipe plate spectrophotometer (Sunrise™; Tecan Group Ltd., Männedorf, Switzerland). In parallel assays, the plated tumor cells were cultured in 200 µl DMEM/F12-GlutaMAX™ Supplement in the presence of the appropriate concentrations of the chemotherapeutic agents for 1, 2, 4, 7, 10 and 14 days. alamarBlue™ assays were performed...
at each of the above time points to determine cell viability. At least three experiments were performed.

Xenotransplantation. Cultured MZ-UPS-1 and MZ-UPS-2 cells from the fourth passage were harvested by detachment with Accutase and washed twice in PBS. Live cells were counted using trypan blue staining (Sigma-Aldrich Chemie GmbH) and a Neubauer counting chamber (Sigma-Aldrich Chemie GmbH). In total, 1x10⁶ cells were injected subcutaneously into the right flank of NSG mice. Tumor growth was verified 4 weeks later. The effectiveness of the xenograft transplant was 100% for the two undifferentiated pleomorphic sarcoma cell cultures MZ-UPS-1 and MZ-UPS-2.

Histology. Samples from the original tumors of the two patients and isolated xenografts from the mice were fixed in 4% phosphate-buffered formaldehyde solution (Roti®-Histofix; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and embedded in paraffin (Sigma-Aldrich Chemie GmbH). The tissue sections (5 µm) were subsequently deparaffinized using xylol (Sigma-Aldrich Chemie GmbH) and a descending sequence of ethanol (100, 90 and 70% and distilled water), using xylol (Sigma-Aldrich Chemie GmbH) and a descending sequence of ethanol (100, 90 and 70% and distilled water), stained with hematoxylin and eosin (Sigma-Aldrich Chemie GmbH) and viewed under a microscope (Axioskop 40; Zeiss GMBH, Jena, Germany).

Immunohistochemical staining were performed according to manufacturer's protocol on a Ventana BenchMark XT platform (Ventana Medical Systems, Inc., Tucson, AZ, USA). Following deparaffinization, antigen retrieval was performed using peroxidase and alkaline phosphatase blocking reagent (Dako, Glostrup, Denmark) for 10 min at 95-99°C. The tissue sections (5 µm) were subsequently deparaffinized using xylol (Sigma-Aldrich Chemie GmbH) and a descending sequence of ethanol (100, 90 and 70% and distilled water), stained with hematoxylin and eosin (Sigma-Aldrich Chemie GmbH) and viewed under a microscope (Axioskop 40; Zeiss GMBH, Jena, Germany).

Xenografts were inoculated subcutaneously into NSG mice. Tumors from the MZ-UPS-1 cells grew and the values were multiplied to calculate the tumor area.

Results

Two undifferentiated pleomorphic sarcoma cell lines were established. Histology of the biopsies and resected tumors of the two patients provided a diagnosis of undifferentiated pleomorphic sarcoma NOS grade 3, according to the FNCLCC classification (28). The tumors were located in the left axillary region or the gluteus maximus. The two primary tumor tissues histologically exhibited a storiform and pleomorphic growth pattern with specific myxoid regions (Fig. 1).

Immunohistochemical staining of the MZ-UPS-1 cells and the original tumor from which they were derived, revealed that the cells expressed vimentin and actin and possessed a Ki-67 index of >20%. The MZ-UPS-2 cells and the cells from their original tumor expressed vimentin and possessed a Ki-67 index of >20% (data not shown). The features of the two undifferentiated pleomorphic sarcoma cell lines are revealed in Table II.

Table I. Chemotherapeutic treatment regimen of xenograft mouse models of undifferentiated pleomorphic sarcoma.

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Body concentration, mg/kg</th>
<th>Type of application</th>
<th>Application mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>6</td>
<td>Intravenous</td>
<td>Weekly</td>
</tr>
<tr>
<td>Suberoylanilide hydroxamic acid</td>
<td>50</td>
<td>Intraperitoneal</td>
<td>Daily</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>100</td>
<td>Oral</td>
<td>Daily</td>
</tr>
</tbody>
</table>

Ready-to-use doxorubicin was purchased at a concentration of 2 mg/ml (Hexal AG, Holzkirchen, Germany), and suberoylanilide hydroxamic acid (SAHA; Sigma-Aldrich Chemie GmbH) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich Chemie GmbH) at a concentration of 25 mg/ml. Pazopanib was purchased as 400 mg capsules Votrient® (GlaxoSmithKline, Brentford, UK). One Votrient® capsule was ground using a pestle and mortar, and dissolved in DMSO at a concentration of 30 mg/ml upon mixing overnight. The mice were weighed and treated as described in Table I. Tumor size was measured every 2 days.

Statistical analysis. Student's t test was used to compare the mean values between two experimental groups where appropriate using GraphPad Prism version 6.0f (GraphPad Software, Inc., La Jolla, CA, USA). For non-Gaussian distributions, the Mann Whitney U test was used for the calculation of statistical significance. Gaussian distribution was analyzed with the Kolmogorov Smirnov test. Data are expressed as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Animal treatment and tumor measurement. The size of the tumors in the mice were measured using a digital caliper 6-8 weeks subsequent to subcutaneous injection of 1x10⁶ MZ-UPS-1 or MZ-UPS-2 cells. Tumor size was measured from caudal to cranial and dorsal to ventral, and the values were multiplied to calculate the tumor area. Chemotherapeutic agents were prepared prior to application.
increased myxoid regions and a fluid-filled chamber. On the surface of the tumor and the surrounding tissue, neovascularization was clearly observed (Fig. 2). Similarly to the in vitro findings, the in vivo tumor formation of MZ-UPS-2 cells was slower, compared with MZ-UPS-1 cells. Histologically, the MZ-UPS-2 xenograft exhibited a predominantly storiform growth pattern; however, no myxoid regions or fluid-filled chambers were observed (Fig. 1), contrarily to the original MZ-UPS-2 tumor tissue. Similarly to the xenograft tumor from the MZ-UPS-1 cells, the MZ-UPS-2 tumor exhibited clear neovascularization on the surface.

Chemotherapeutic treatment of undifferentiated pleomorphic sarcoma cell cultures in vitro. Doxorubicin is commonly used as the first-line treatment for patients with undifferentiated pleomorphic sarcoma. There is no widely accepted second-line treatment. However, novel therapeutic approaches have been recently identified as second-line treatments for patients with recurrent or incurable sarcoma, including HDAC inhibitors and tyrosine kinase inhibitors, such as pazopanib, which has been approved in USA and Europe since 2012 for the treatment of distinct STS subtypes (14-19).

The two patients in the present study were administered doxorubicin; however, this treatment did not prevent tumor progression. Pazopanib was also used in the two patients as a second-line treatment, which resulted in the tumor becoming stable. During pazopanib treatment, symptom relief with increased tumor necrosis was observed in one patient (MZ-UPS-2), and in the other patient a mild tumor regression was observed (data not shown).

Prior to the in vivo experiments, the fourth passages of the two cell cultures, MZ-UPS-1 and MZ-UPS-2, were incubated with various concentrations of doxorubicin, pazopanib and the HDAC inhibitor SAHA, also known as vorinostat (Fig. 3). The anthracycline doxorubicin is a remarkably potent cytostatic drug, and caused the death of the MZ-UPS-1 cells within...
The viability of the MZ-UPS-2 cells began to decrease on day 7 following incubation with the highest concentration of doxorubicin tested (100 nM). On day 14, the viability of the MZ-UPS-2 cells was considerably decreased, even at a concentration of 10 nM doxorubicin. The viability of the two cell cultures was decreased on day 4 following incubation in the presence of 5 or 10 µM vorinostat. Pazopanib had no inhibitory effect on MZ-UPS-1 cells, and slightly inhibited the growth of MZ-UPS-2 cells. The combination of doxorubicin and pazopanib had no synergistic effect on MZ-UPS-1 cells, and only caused a small synergistic reduction in the viability of MZ-UPS-2 cells.

**Chemotherapeutic treatment in vivo.** Immunodeficient NSG mice with xenografts of MZ-UPS-1 or MZ-UPS-2 cells were generated in the present study to compare the efficacy of different chemotherapeutic treatments in vivo (which is expected to be comparable to human patient response) with the cytostatic effects demonstrated by these chemotherapeutic drugs in vitro.

### Table II. Two novel undifferentiated pleomorphic sarcoma cell lines.

<table>
<thead>
<tr>
<th>Features</th>
<th>MZ-UPS-1</th>
<th>MZ-UPS-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian</td>
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<td>Age, years</td>
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<td>48</td>
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<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Tissue</td>
<td>Mesenchymal</td>
<td>Mesenchymal</td>
</tr>
<tr>
<td>Morphology</td>
<td>Fibroblastic/myofibroblastic</td>
<td>Fibroblastic/myofibroblastic</td>
</tr>
<tr>
<td>Cell type</td>
<td>Pleomorphic sarcoma NOS, G3</td>
<td>Pleomorphic sarcoma NOS, G3</td>
</tr>
<tr>
<td>Growth properties</td>
<td>Monolayer</td>
<td>Monolayer</td>
</tr>
<tr>
<td>Culture medium</td>
<td>DMEM/F12-GlutaMAX™ Supplement with sodium pyruvate and 10% FCS</td>
<td>DMEM/F12-GlutaMAX™ Supplement with sodium pyruvate and 10% FCS</td>
</tr>
<tr>
<td>Split ratio</td>
<td>1:3-1:5 every week</td>
<td>1:2 every 2 weeks</td>
</tr>
<tr>
<td>Medium renewal</td>
<td>2-3 times weekly</td>
<td>2-3 times weekly</td>
</tr>
<tr>
<td>Tumorigenic</td>
<td>Yes, in NSG mice</td>
<td>Yes, in NSG mice</td>
</tr>
</tbody>
</table>

NOS, not otherwise specified; G3, grade 3; DMEM/F12, Dulbecco’s Modified Eagle’s Medium: Nutrient Mixture F-12; FCS, fetal calf serum; NSG, NOD SCID gamma; NOD, non-obese diabetic; SCID, severe combined immunodeficiency.

Figure 2. Tumor xenografts observed at 8-12 weeks subsequent to subcutaneous injection of undifferentiated pleomorphic sarcoma cells in NSG mice. Cultivated MZ-UPS-1 or MZ-UPS-2 cells were harvested and washed twice in phosphate-buffered saline. A total of 1x10⁶ cells of each culture were injected subcutaneously into the right flank of NSG mice. Solid tumor tissues of ≤1.5 cm³ in size were observed in all three pleomorphic sarcomas at 8-12 weeks following injection. NSG, NOD SCID gamma; NOD, non-obese diabetic; SCID, severe combined immunodeficiency.
Figure 3. Chemotherapeutic treatment of undifferentiated pleomorphic sarcoma cells in vitro. Cultured cells from two patients with undifferentiated pleomorphic sarcoma, MZ-UPS-1 and MZ-UPS-2, were seeded onto 96-well plates at a concentration of 1x10^4 cells/100 µl medium/well. Doxorubicin was added on day 0 and 7, while vorinostat and pazopanib were added daily. For the determination of cell viability, an alamarBlue™ assay was performed on days 1, 2, 4, 7, 10 and 14. The metabolized substrate was measured photometrically at 570 nm. All experiments were performed in triplicate. Data are presented as the mean ± standard deviation. *P≤0.01 vs. untreated cells.

Figure 4. Combined therapy with doxorubicin and pazopanib resulted in a reduction of tumor size in the two xenograft sarcoma mouse models. In vitro cultured MZ-UPS-1 and MZ-UPS-2 cells were washed twice in phosphate-buffered saline, and 1x10^6 cells were injected subcutaneously into NSG mice. Chemotherapeutic treatment was started 6–8 weeks following xenotransplantation. (A) The chemotherapeutic regimen administered to the xenograft sarcoma mouse models was as follows: Doxorubicin was injected intravenously, while suberoylanilide hydroxamic acid and pazopanib were administered intraperitoneally and orally, respectively. (B) The initial tumor size of each mouse was normalized to 100%. Tumor size was measured every 2 days, and compared to the initial size. Relative tumor growth was averaged in each group. Data are presented as the mean ± standard deviation. n=5–10/group. *P≤0.05 vs. untreated. M, measurement; s.c., subcutaneously; D, doxorubicin; S/AHA, suberoylanilide hydroxamic acid; P, pazopanib; i.p., intraperitoneally; i.v., intravenously.
Cells from the same passage were isolated and injected into NSG mice. The concentrations of the chemotherapeutic agents used and the frequency of application corresponded to treatment regimens that are normally employed to treat human patients with undifferentiated pleomorphic sarcoma (Fig. 4A), according to the guidelines from the German Society for Hematology and Medical Oncology (www.onkopaedia-guidelines.info/en/onkopaedia/guidelines). Tumor size from the mouse models was initially measured and normalized to 100% 6-8 weeks subsequent to the injection of the human undifferentiated pleomorphic sarcoma cells. The tumor size of MZ-UPS-1 mice that were not treated with chemotherapy had more than doubled within 8 days. By contrast, the tumor growth of mice treated with doxorubicin or SAHA was markedly decelerated, and treatment with pazopanib resulted in a stabilization of tumor size. Combined therapy with doxorubicin and pazopanib significantly reduced the tumor to <50% of its initial size (Fig. 4B).

The xenograft development of the MZ-UPS-2 cells was slower compared to that of MZ-UPS-1 cells (Fig. 4B), which was comparable to the growth of the MZ-UPS-2 cells in vitro. The tumor size of MZ-UPS-2 mice that were not treated with chemotherapy increased ~0.5 times in size 8 days subsequent to the initial measurement of the tumor. Following treatment with doxorubicin, SAHA and pazopanib, the pleomorphic tumor xenograft only marginally decreased in size. The combined therapy of doxorubicin and pazopanib led to a significant reduction in the tumor size, and therefore was the most promising therapeutic treatment in vivo. The in vivo results obtained in the present study are markedly different to the in vitro results, where doxorubicin and the HDAC inhibitor SAHA were the most potent chemotherapeutic agents, while pazopanib only marginally influenced tumor cell viability.

Discussion

Undifferentiated pleomorphic sarcoma is an extremely heterogeneous aggressive subgroup of soft tissue sarcoma (2). Due to the heterogeneity of undifferentiated pleomorphic sarcoma, numerous studies are focused on developing individualized therapeutic strategies for patients, instead of administering a standard chemotherapy to all patients (29). Since there is a limited number of patients affected by each of the undifferentiated pleomorphic sarcoma subgroups, no large clinical trial has been conducted to date to evaluate the efficiency of chemotherapeutic treatment. Therefore, it is crucial to develop preclinical tools that allow the evaluation of individualized therapeutic approaches. Previous studies have demonstrated that there are distinct histopathological differences between the different subtypes of undifferentiated pleomorphic sarcoma, and have revealed a panel of molecular markers that may significantly aid the development of an optimal management regimen for patients with undifferentiated pleomorphic sarcoma (30,31). Consequently, reliable and reproducible preclinical animal models are required, which are similar to the oligoclonal biological diversity observed in human patients, for testing various targeted therapeutic approaches for individual patients.

The present study established two xenograft animal models generated from stable undifferentiated pleomorphic sarcoma cell cultures to investigate the efficacy of chemotherapeutic regimens for the treatment of undifferentiated pleomorphic sarcoma in vivo vs. in vitro. The results of the present study demonstrated that there is a clear discrepancy between the in vitro cell culture and the in vivo xenograft model, which is comparable to a human treatment scenario. The mouse model reflects the local microenvironment of a human tumor, which appears to be crucial to allow a predictive analysis of treatment regimens in addition to monitoring direct cytotoxic effects of drugs (32).

Understanding the various biological sensitivities of the various histological subtypes of undifferentiated pleomorphic sarcoma may lead to the development of individual therapeutic targeted approaches (33).

In contrast to other approaches using solid tumor tissue or silicon chambers to place tumor fragments around the superficial epigastric vessels (26,27), the present study generated stable oligoclonal cell cultures from freshly isolated tumor tissue of two patients with undifferentiated pleomorphic sarcoma, which were similar to the oligoclonal variety exhibited by the original tumors. Additionally, these cultures were subcutaneously injected into immunodeficient mice to establish xenograft animal models. The present study observed that neovascularization was identical between the original tumor and the xenograft tumor. There were no regions of hypoxemia in the xenograft tumor, which is important, as it allows the analysis of anti-angiogenic therapeutic approaches and rules out the possibility of anomalous results that hypoxic conditions may generate during homing and engraftment of the tumor (34).

Furthermore, the present study treated the tumors in vivo and in vitro with the most common or innovative chemotherapeutic agents currently available. Only in the xenograft mouse model the results observed were comparable to the treatment results of the two patients from whom the original tumors were resected. Therefore, tumor derived cell cultures do not reflect the actual treatment condition that is observed in patients. Notably, the combination of doxorubicin and pazopanib significantly reduced the tumor size with an acceptable toxicity level, in terms of weight loss (<20%), movement disorder and apathy (data not shown).

In addition, the novel xenograft models allow chemotherapeutic analysis at various time points, which may lead to the identification of molecular mechanisms associated with pleomorphic sarcoma development and progression, and other local tumor-tissue interactions.

In conclusion, there is a discrepancy in tumor growth and cell viability between in vitro and in vivo models concerning chemotherapeutic treatments. The novel and reproducible xenograft animal models generated in the present study have demonstrated that in vivo models are required to test potential chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma, since they provide similar results to those observed in human patients, compared with in vitro models.

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References


