Establishment of a Synovial Sarcoma Model in Athymic Nude Mice

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Abstract. Background: Synovial sarcoma represents one of the most frequent malignant tumours of soft tissues. Its prognosis is poor because of chemoresistance and high metastatic potential. Improvement of synovial sarcoma outcome requires well-characterized animal models in which to evaluate novel therapeutic options. The aim of this study was to establish a reproducible synovial sarcoma model in athymic nude mice with the SW-982 cell line. Materials and Methods: The SW-982 cell line was cultured in vitro and 5×106 cells undergoing exponential growth were collected and subcutaneously inoculated as a 0.1 ml suspension into the left flank of athymic nude mice. The sarcoma xenografts were subsequently analysed by histological and immunohistochemical staining. Results: All the mice transplanted with SW-982 cells engrafted, leading to solid tumours of up to 2105 mm3 volume after 4 weeks. Histological and immunohistochemical staining confirmed the mouse xenografts as synovial sarcoma. Conclusion: An easily established in vivo xenograft model of synovial sarcoma is presented.

Synovial sarcoma is a malignant high-grade soft tissue neoplasm (1). They constituted 6.4% of the 2,056 soft tissue sarcomas evaluated at the Operative Reference Centre for Soft Tissue Sarcoma, BG University Hospital Bergmannsheil, Bochum from 1990 until 2008 and is the most common malignant nonrhabdomyosarcoma soft-tissue sarcoma in children, with approximately 1 in 5 cases occurring in the first two decades of life (2). This malignancy usually involves the extremities, especially the lower extremities around the knees and ankles (1, 2). Its microscopic resemblance to developing synovium was suggested early in the literature, but its origin from preformed synovial tissues has never been substantiated and the origin of synovial sarcoma is unclear (3). Due to the similarity between cells of this tumour and primitive synoviocytes, the term synovial sarcoma has been used (3). Histologically, synovial sarcoma is characterized by epithelial-like and spindle cell components arranged in a monophasic or biphasic pattern (4). The pathological features of synovial sarcoma are identical in children and adults. Although synovial sarcoma can be graded according to mitotic index, percentage necrosis, and tumor differentiation, it should always be considered a high-grade sarcoma (3-5).

Synovial sarcoma is characterized by the immunohistochemical demonstration of both epithelial and mesenchymal filaments, a diagnosis of exclusion in cases in which ancillary methods fail to demonstrate a specific line of differentiation (5). Synovial sarcomas are composed of two morphologically and immunophenotypically distinct cell types: spindle cells, uniform and relatively small, with oval nuclei and scarce cytoplasm, forming solid sheets, and epithelial cells, characterized by true epithelial differentiation (5).

Synovial sarcomas are locally aggressive and have a high metastatic potential than other soft tissue sarcomas and the overall prognosis of patients is poor because of systemic metastasis (1-3). Local recurrence and/or metastatic disease is found in approximately 80% of patients (1-3). Surgical resection with an adequate surgical margin is the definitive choice of treatment for primary tumours and has been shown to control local recurrence (1-3). Reports of current therapy have shown a 5-year survival rate of around 50% to 68% (1-3). The efficacy of adjuvant chemotherapy is still a matter of debate, and the development of a new therapeutic approach is required to improve the prognosis since successful treatment is limited by the high rate of radio- and chemoresistance in recurrent sarcoma (1-3).
Despite the advances made in the last few years in the diagnosis and intensive multimodal treatment of patients, the five-year survival rate after diagnosis of synovial sarcomas has hardly changed for several decades (3-4). The majority of these patients are young and thus their synovial sarcomas are a significant public health problem despite their low incidence (1-3). The synovial sarcoma research field has long been hampered by the limited availability of representative model systems. In vitro studies with cell lines have been a mainstay of sarcoma research and drug screening efforts (6). Molecular alterations in cell biology have recently been identified and the translocation t(X;18) has been noted in more than 90% of cases (3, 5). The tumour cell characteristics include independence from growth factors, evasion of apoptosis and maintenance of genomic integrity (4, 5). A model allowing the study of these characteristics would expedite therapeutic opportunities. In addition, improved understanding of the different synovial sarcoma subtypes would allow for more strategic and selective treatment regimens for individual patients. The objective of this study was to establish a reproducible xenograft model of synovial sarcoma in athymic nude mice.

Materials and Methods

Animals. Congenitally athymic nude mice (nu/nu) were obtained from Harlan Winkelmann GmbH (Borchen, Germany). Five sexually mature males, 6 weeks old and weighing 20-25 g were used. They were individually housed in ventilated cages, in pathogen-free conditions under 12 h light-dark photoperiodicity and with controlled humidity and temperature (20±2°C). Boxes, bedding, food and water were sterilised. Sterility was maintained during the surgical procedures used for the inoculation of the cells and for subsequent removal and transplantation of tumours. The procedures were carried out under general anaesthesia, animal care and manipulation was in agreement with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (14).

Sarcoma cells. The human cell line SW-982, initiated by A. Leibovitz in 1974 from a surgical specimen of a synovial sarcoma removed from a 25-year-old female Caucasian, was obtained from Cell Lines Service (Éppelheim, Germany) and last authenticated via nonaplex PCR in July 2008. The cell line was cultured as a monolayer in 150 cm² flasks in Dulbecco’s modified Eagle’s medium (DMEM; PAA Laboratories GmbH, Coelbe, Germany) in a humidified atmosphere at 37°C with 5% CO₂. The media were changed three times a week. As soon as confluency (close to 100%) was reached, the cells were detached from the tissue-culture flasks with 0.05% trypsin and 0.02% EDTA in PBS for 2 min at 37°C. They were then resuspended in complete DMEM growth medium and were counted via a subcutaneous injection into its left flank. The matrix solidifies at 37°C and thus ensures that the cells remain in situ. The animals were weighed and the tumour was measured twice a week with a caliper. The mice were sacrificed when signs of suffering were noted or when the subcutaneous tumour reached a diameter larger than 20 mm. The mice that remained healthy were sacrificed 4 weeks after inoculation. Pathogenesis of the tumour was analysed via histological and immunohistochemical staining.

Histological and immunohistochemical staining. Samples of the tumours were fixed in 5% buffered formalin (PathoMed, Viersen, Germany). For histological evaluation, 4 μm sections were deparaffinised, rehydrated and stained with haematoxylin and eosin following standard procedures. For immunohistochemical staining 4 μm sections were first deparaffinised and rehydrated. High temperature antigen retrieval in citrate buffer (Vector Laboratories, Burlingame, CA, USA) was performed. Endogenous peroxidase activity was blocked by incubating the slides in a 0.3% solution of hydrogen peroxide in phosphate-buffered saline for 10 min at room temperature. Subsequently the tissue sections were incubated for 20 min with blocking serum to avoid nonspecific binding of antibodies. Afterwards, the slides were incubated for 20 to 30 min with primary antibodies against alpha-smooth muscle actin, CD34, desmin, HIF-35, MNF116, vimentin (all DAKO, Hamburg, Germany) and S100 (DCS, Hamburg, Germany); all monoclonal and prediluted. Primary antibodies were detected by biotinylated secondary antibody followed by avidin-biotin complex and 3,3’-diaminobenzidine tetrahydrochloride. The slides were counterstained with haematoxylin. Internal positive control staining was seen in normal tissue, such as lymphocytes, endothelial cells, and fibroblasts within the tumour sections. Negative controls were performed for each sample by omitting the primary antibody. The results of the histological and immunohistochemical staining were evaluated by light microscopy.

Histological grade was determined according to the FNCLCC method based on differentiation, mitotic index, and necrosis (7). Regarding tumour differentiation, a score of 3 was automatically attributed to synovial sarcoma. The mitotic rate (m) was determined by counting the number of mitoses in 10 successive high-power (×400) fields (HPF) (m=1, 0 to 9; m=2, 10 to 19; m=3, more than 19 mitoses). Microscopic tumour necrosis was recorded only for areas of tumour necrosis, with or without leucocyte infiltration (n=0, absent; n=1, <50%; n=2, >50%). The final grade was determined by adding the score of each factor. Three grades were defined as grade 1 (total <4), grade 2 (total of 4 or 5), and grade 3 (total of 6, 7, or 8).

Results

The injected SW-982 cells led to the growth of a solid sarcoma xenograft in all five athymic nude mice (Figure 1). After 4 weeks, the tumour volumes reached 955 mm³ (sarcoma 1), 805 mm³ (sarcoma 2), 1047 mm³ (sarcoma 3), 2105 mm³ (sarcoma 4) and 904 mm³ (sarcoma 5) respectively (Figure 2).

Histological analysis revealed that all five xenografts were malignant mesenchymal tumours and they were characterized by a predominantly fasciculated growth pattern.
with monotonous spindle-shaped cells, with mostly sheets of uniform, relatively small ovoid nuclei and scarce cytoplasm (Figure 3). No epithelial formations, such as glandular structures were seen. Immunohistochemical analysis showed coexpression of cytokeratin MNF116 (Figure 4) and vimentin (Figure 5) in all cases, which was consistent with synovial sarcoma. Stains for alpha-smooth muscle actin, CD34, desmin, HHF-35 and S-100 were negative. According to the FNCLCC grading system, all tumours were grade 3 (high grade).
Discussion

This was the first study to demonstrate the establishment of a synovial sarcoma model with the SW-982 cell line, and as shown by the histological and immunohistochemical staining it is a good model for studying biological aspects of human synovial sarcoma for in vivo study. Subcutaneous tumours were easily established in 100% (5/5) of the nude athymic mice and the reduced immune response of the athymic mice did not prevent injected cells from developing into solid tumours. Tumours were generally palpable within 10 days of the injection of 5×10^6 tumour cells confirming that athymic nude mice offer a suitable microenvironment for the growth of inoculated SW-982 synovial sarcoma cells. The appearance of a solid tumour within one week and the subsequent survival of animals with growing tumours suggested that this model would be useful for an animal tumour therapy response study.

In this study, the tumours exhibited histologically limited invasiveness and like the original human sarcoma never metastasized. The lack of ability to metastasize might be a substantial drawback (8-10). In addition, nude mouse models are often more sensitive than the original tumour, which may be due to the subcutaneous environment regarding pH, vascularity, accessibility to drugs, etc. (8-10).

Most preclinical studies that have been performed using xenograft tumour models have used subcutaneous implantation of tumour cells and several studies have reported that they can predict the clinical activity of cytotoxic agents (11-13).

Only recently have a significant number of human synovial sarcoma xenograft models been established (14-23). The ‘classical’ model is the SYO-1 cell line (14), although a limited number of other synovial sarcoma cell lines are available including YaFuSS, HS-SY-II, SYO-1, Fuji, 1273/99 and KUSHI (15-23). Therefore, the addition of an easily established and reproducible synovial sarcoma xenograft model will add to the options for preclinical testing.

Xenografts have been instrumental in evaluating the extent of abnormal genetic changes and gene profiles in human synovial sarcoma by various research groups. Knowledge on the genomic profile of xenografts is crucial also for selecting the most appropriate preclinical model, especially in the new era of targeted therapies that require model systems with a specific molecular expression profile that mimic a specific disease stage or patient group. The SW-982 model described above is easily established and reproducible, and provides a means for studying these important subjects.

References


