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Experimental Murine Model of Primary High Grade Undifferentiated Pleomorphic Sarcoma Not Otherwise Specified

DANIEL J. TILKORN[1], INGO STRICKER[2], JOERG HAUSER[1], ANDREJ RING[1], INGE SCHMITZ[2], LARS STEINSTRAESSER[1], HANS-ULRICH STEINAU[1], ADRIEN DAIGELER[1] and SAMMY AL-BENNA[1]

[1]Reference Centre for Soft Tissue Sarcoma, Department of Plastic Surgery and [2]Institute of Pathology, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, North Rhine-Westphalia, Germany

Abstract. *Background: Undifferentiated pleomorphic sarcoma not otherwise specified (NOS) is a malignant neoplasm of uncertain origin arising both in the soft tissue and the bone. The WHO classified this tumour in 2002 but controversy has plagued this entity due to limited availability of tissue for study. The aim of this study was to establish a reproducible xenograft model of primary human undifferentiated pleomorphic sarcoma NOS. Materials and Methods: Primary human sarcoma samples were divided into tumour fragments and transplanted subcutaneously in mice. Sarcoma xenografts were analysed histolomorphologically (light/electron-microscopy; immunohistochemistry). Results: All tumours resulted in viable sarcoma NOS xenografts demonstrating similar histological patterns. In both the original tumours and the xenografts, tumour necrosis was found ranging from 15% to 25%. The background stroma of the xenografts was hyalinised like the primary sarcoma. Electron microscopical analyses showed good maintenance of ultrastructure. Conclusion: Implantation of intact tumor fragments yielded in a complete tumor take rate. The development of new cancer therapeutics requires animal models that closely resemble the human patient. This study provides ideal animal models for the research of pathogenesis and pathobiology of primary human undifferentiated pleomorphic sarcoma NOS.*

Undifferentiated pleomorphic sarcoma not otherwise specified (NOS) is a malignant neoplasm of uncertain origin that arises both in the soft tissue and the bone. It is the most common soft tissue sarcoma in adults. Malignant fibrous histiocytoma (MFH) was introduced as a diagnosis in 1961, and was the prior terminology for undifferentiated pleomorphic sarcoma NOS (1). Prior to 1961, tumours of this type were classified as rhabdomyosarcoma or fibrosarcoma. Undifferentiated pleomorphic sarcoma NOS typically occurs in the extremities of elderly patients, principally during the sixth to eighth decade of life and is slightly more common in men and Caucasians. Usually dermal or subcutaneous, it involves deeper soft tissues in only one third of cases. The most common location is the lower extremity, specifically the thigh, followed by the upper extremity and the retroperitoneum. Despite much study, MFH has remained an enigma and the cell of origin is still a matter of debate between a histiocyte and a mesenchymal cell. This is a soft tissue sarcoma noted for its pleomorphic histological appearance. It is postulated that the origin cell is a histiocyte (2). This new terminology has been supported by a compelling body of evidence over the last decade to suggest that MFH represents a final common pathway in tumours that undergo progression towards dedifferentiation (3-5). The histopathological together with the immunohistochemical examination is of vital importance in the establishment of the positive diagnosis and furthermore in the establishment of the differential diagnosis from carcinoma, plasmacytoma, osteosarcoma, fibrosarcoma and lymphosarcoma. Yet, it remains unclear how to most accurately organise these tumours. Undifferentiated pleomorphic sarcoma NOS is a diagnosis of exclusion in which ancillary methods fail to demonstrate a specific line of differentiation. The mechanisms associated with the development of sarcoma NOS remain largely unclear because of the rarity of the disease, the large number of histological subtypes and its varied clinical behaviour.

As such, preclinical models to dissect mechanisms underlying sarcoma development, progression and treatment are greatly needed (6, 7). The subcutaneous implantation of tumour fragments into immunocompromised nude mice is a widely accepted model for the study of various tumour types

Correspondence to: Daniel Tilkorn, MD, Department of Plastic Surgery, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, North Rhine-Westphalia, Germany. Tel: +49 2343026851 Fax: +49 2343026379, e-mail: d.tilkorn@web.de

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(8-10). Since the new classification of this tumour by the WHO, controversy has continued to afflict undifferentiated pleomorphic sarcoma NOS due to limited availability of tissue for study. In addition, previous xenograft models have not been consistently successful (11). The aim of this study was to establish a successful, reproducible xenograft model of primary human undifferentiated pleomorphic sarcoma NOS.

Materials and Methods

Animals. The animals used were NMR nude mice (Harlan Winkelmann GmbH, Borchen, Germany) (n=6). The mice were sexually mature males, 6 weeks old and weighing about 20-25 g. They were housed in ventilated racks, in pathogen-free conditions under a 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 sarcoma cells to give rise to solid tumours and for subsequent removal and transplantation of tumours. Animal care and manipulation was in agreement with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (12). All animal experiments were carried out under the guidelines and with the permission of the Ethics Committee of the Ruhr University Bochum. The tumour specimens were obtained with written informed consent of the patients and with the permission of the Ethics Committee of the Ruhr-University Bochum.

Isolation of sarcoma specimens for xenotransplantation. Primary human soft tissue sarcoma tissue of tumours resected from two patients were directly transferred from the operating theatres of our hospital to the laboratory. The tumour samples were taken under sterile conditions from a representative area of the original tumour mass. The sample was then divided into two adjacent parts, one for further histology (conventional light/electron microscopy and immunohistochemistry) and the other for the *in vivo* experiment. The tumour sample was sliced into 1 mm fragments prior to transplantation.

In vivo sarcoma xenograft model. All surgical procedures on mice were performed under sterile conditions and under general anaesthesia with enfluran 1.5%. Vertical incisions were performed on the back of the NMR nude mouse and a subcutaneous pocket was prepared with blunt dissection of the subcutaneous tissue with scissors. To produce xenografts at the inoculation site, tumour fragments were placed in the pocket and the pocket was then filled with Matrigel (BD Bioscience, Palo Alto, CA, USA). The matrix solidifies at 37˚C and thus ensures that the cells remain *in situ*. Finally the skin was closed with 4-0 Ethilon sutures (Ethicon, Inc., Somerville, NJ, USA). After an *in vivo* incubation of 3 weeks in which the animals were allowed to move freely and were fed a standard mouse chow *ad libidum*, animals were anaesthetised without recovery. The wounds were reopened and the subcutaneous tumour mass was resected. Macroscopic pictures were taken and the tissue was sliced into two sections, one for histological assessment the other one for electron microscopy.

Histological assessment. Tumour samples from the primary tumour, as well as the xenotransplant, were stored in 10% formaldehyde. At least five individual sections of the primary tumour were assessed. The primary tumour and the xenotransplant counterpart were sectioned into 5 μm-thick sections. Standard H&E staining was performed.

Electron microscopy. Tumour samples from the primary tumour, as well as the xenotransplant, were fixed in 2% glutaraldehyde and embedded in Epon 812. Uranyl acetate and lead citrate were added to the ultra-thin sections for contrast. Specimens were analysed with special focus on differentiation criteria. Microscopic features of the tumours such as cellularity, growth pattern, cytomorphology, vascularity, invasiveness, degree of differentiation, and necrosis were recorded.

Results

Two primary human sarcomas obtained from two individual patients who both had reference histology were the basis for this investigation. Tumour diagnosis and classification were determined according to WHO guidelines (2). Both patients were diagnosed with an undifferentiated pleomorphic sarcoma NOS. Histological, immunohistological and electron microscopic analyses were performed for each tumour.

Histological and electron microscopic morphology of the primary tumour. The primary undifferentiated pleomorphic sarcoma NOS tumour in all cases demonstrated a storiform-pleomorphic growth pattern with a discernible increase of nuclear size. Microscopically, they exhibited concurrent cells showing marked pleomorphism admixed with bizarre giant cells, spindle cells, and variable foamy cells. There were many atypical mitoses. Certain areas of the resected heterogeneous tumours showed extensive myxoid change and a partial hyalinised stroma was noted. A mean mitotic count of 15 mitoses per 10 high power fields were counted and 20% tumour necrosis was found. No line of differentiation was noted on immunohistochemical analysis. These tumors were also negative for epithelial, melanocytic, and haematopoietic markers, including CD34, desmin, (EMA), pancytokeratins (MNF116), S100 and smooth muscle actin but were positive for vimentin. The percentage of MiB-1-positive cells was 25%. Ultrastructurally, the undifferentiated pleomorphic sarcoma NOS were characterized by heterogeneity of cell size and shape. Nuclei tended to be small, euchromatic, with a peripherally located small stratum of heterochromatin, of elliptical shape and slightly irregular. Glycogen was unobtrusive. It was noted that the cell membranes were often irregular and frequently formed ruffles. Mitochondria appeared small but were focally numerous (Figures 1 and 2).

Histological and electron microscopical morphology of the xenotransplants. A total 6/6 specimens of dedifferentiated pleomorphic sarcomas NOS from two human primary sarcomas were analysed after implantation in 6 mice. Specimens exhibited a similar histological pattern to the

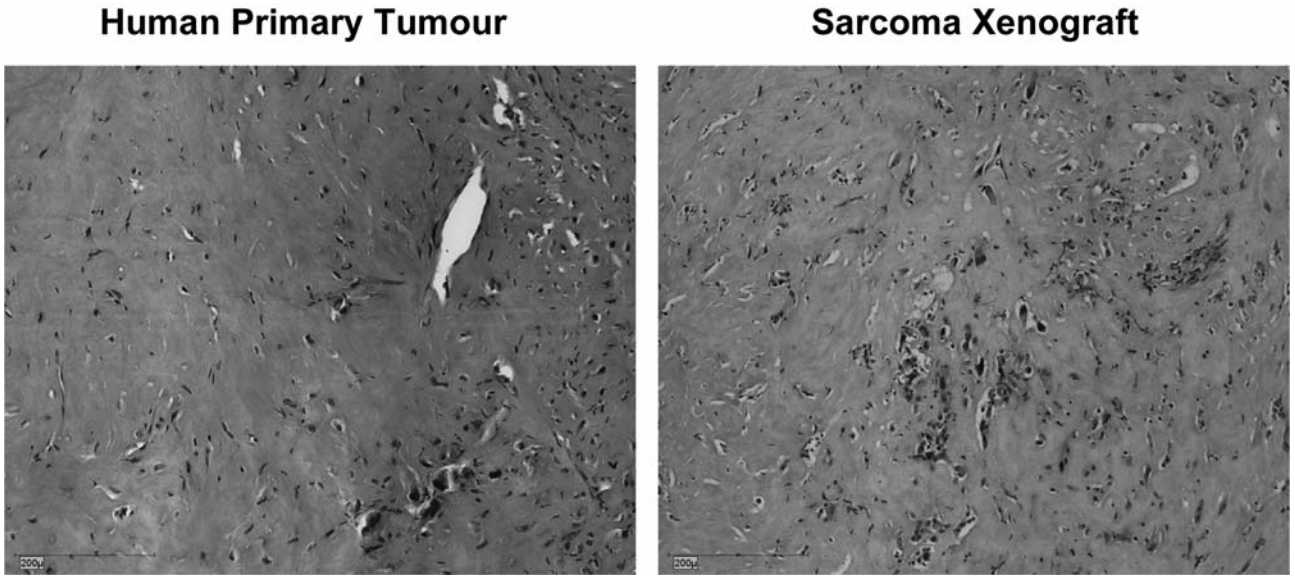

Figure 1. *Histolomorphological appearance (haematoxylin and eosin staining) of the pleomorphic sarcoma NOS in the human primary tumour and the corresponding xenograft tumour. Note: Partial storiform and pleomorphic growth pattern, marked increase of nuclear size and both pleomorphic bizarre cells and spindle cells.*

original tumour. In all specimens, tumour necrosis was found, ranging from 15% to 25%. In addition, the background stroma was hyalinised like the primary tumour. Specimens had marginal inflammation. Electron microscopical analyses showed good maintenance of ultrastructure in three quarters of the specimens. In the others, some necrotic signs were found analogous to those of the human primary tumour. Heterogeneity of tumour cells in size and shape and nuclear irregularities were observed. Light and dark lipid droplets were found. Nucleoli were increased in size and number. Mitotic figures were seen in some specimens. Nuclei were often euchromatic, with few peripheral located heterochromatins. Rough endoplasmic reticulum was elongated and cisternae were often dilated. (Figure 3).

Discussion

Animal models are indispensable tools for the study of sarcoma NOS as a paucity of clinical samples makes large-scale analysis of human samples challenging. Until recently, studies of sarcoma biology were limited to human cell lines and xenografted tumours. An in-depth understanding of the tumour biology of such sarcoma NOS is warranted to develop and improve treatment, thus demanding a well characterized and reproducible *in vivo* tumour model. Xenotransplantation of tumour cells into immune-deficient mice is well recognised and is a useful experimental model in cancer research (6, 7, 11). The subcutaneous skin fold chamber is an established animal model, but the high failure rate after transplantation, as well as the instability regarding phenotype, differentiation and characteristics of the xenograft, remains an unsolved problem (6, 11). A 50% take rate of human tumour xenograft has commonly been reported (6, 11, 13). A variety of techniques to create a reproducible xenograft model of primary human soft tissue sarcoma in nude mice have been described. In our view, all are associated with potential shortcomings. Implantation of intact tumour fragments yielded in a complete tumour take rate. Direct injection of cell suspensions into the subcutis has previously not always resulted in a complete tumour take (6, 11, 13). In our approach, 3 to 4 small tumor fragments were implanted into the subcutis of nude mice and were fixed with Matrigel. This technique, enabled a complete coverage of the tumour fragments. No dislocation of tumor fragments from the implantation site was seen, and it should be emphasized that the tumour take rate was 100%, although the number of mice was low. This technique, thus, enabled complete coverage of the tumour fragments and no dislocation of tumour fragments from the implantation site was seen. Essential for the high tumor take rate was the fact that only vital tumor fragments from the periphery of donor tumours were transplanted. Larger tumors tend to develop necrotic areas, predominantly in the centre. A meticulous and systematic macroscopic evaluation at autopsy and confirmation by histopathological analysis at our Reference Centre for Soft Tissue Sarcoma were carried out in order to yield reliable and reproducible results.

Thus, this study has demonstrated a less traumatic technique for the implantation of small tumour xenografts

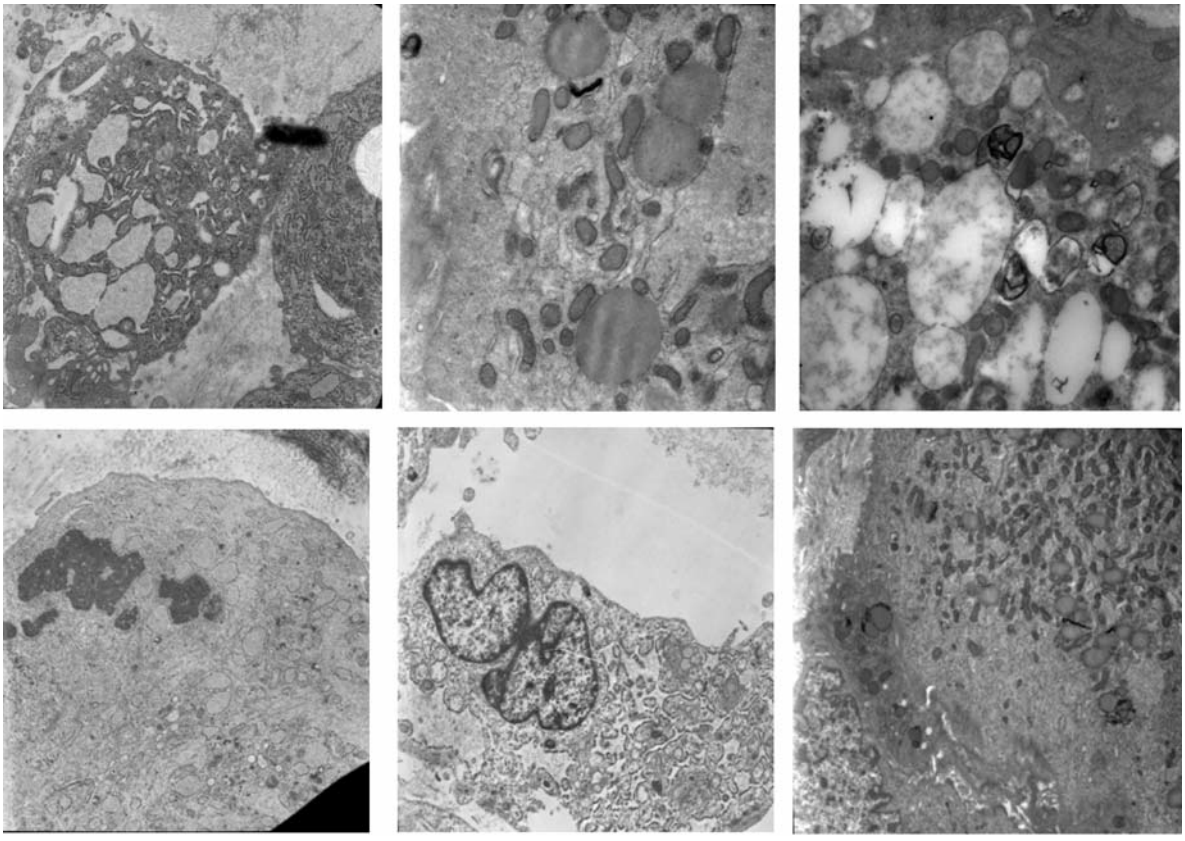

Figure 2. *Light/electron microscopic appearance of human primary pleomorphic sarcoma NOS. Note: Heterogeneity of cell size and shape; small euchromatic nuclei of elliptical shape with a peripherally located small stratum of heterochromatin; irregular and frequently ruffled cell membranes; small mitochondria.*

into the subcutis which resulted in a 100% tumour take rate. This is in contrast to other studies where around half of the human sarcoma xenografts grew subcutaneously and only a third resembled those of the human primary (13). In contrast, in the present study there was a strong resemblance between both NOS xenotransplants and their respective primary tumour. We hypothesize that certain subtypes of undifferentiated sarcomas are more susceptible to hypoxia or other environmental cues.

In the present study, tumour fragments were transplanted directly after tumour resection to be able to compare better the growth pattern to that of the original human sarcoma. A potential disadvantage to the use of these tumour fragments includes the heterogeneity of the tumour fragment and therefore the lack of standardization. No metastases were observed in any experiment. The short three week observation period may explain this.

Undifferentiated pleomorphic sarcoma NOS is a malignant neoplasm of uncertain origin that arises from mesenchymal tissue. Sarcoma NOS is a diagnosis of exclusion in which ancillary methods fail to demonstrate a specific line of differentiation. There is developing evidence to suggest that NOS represents a final common pathway in tumours that undergo progression towards dedifferentiation, leading to the ambiguous morphology which is characteristic of these sarcomas. Lack of primary tumour tissue for experimental studies hinders research into the oncogenesis of this entity. Familiarity with the clinicopathological features of soft tissue sarcoma will provide critical information for future accurate analysis. Preclinical testing of novel therapeutic strategies in animal models also requires a meticulous assessment of the effects of treatment on tumour growth.

This study provides a pertinent animal model for the study of the pathobiology of primary human undifferentiated pleomorphic sarcoma NOS. With careful development and controlled experimentation, this model affords the prospect for analysis of biological aspects of human sarcoma NOS cells in tissue that possesses the same morphological characteristics as the primary tumour.

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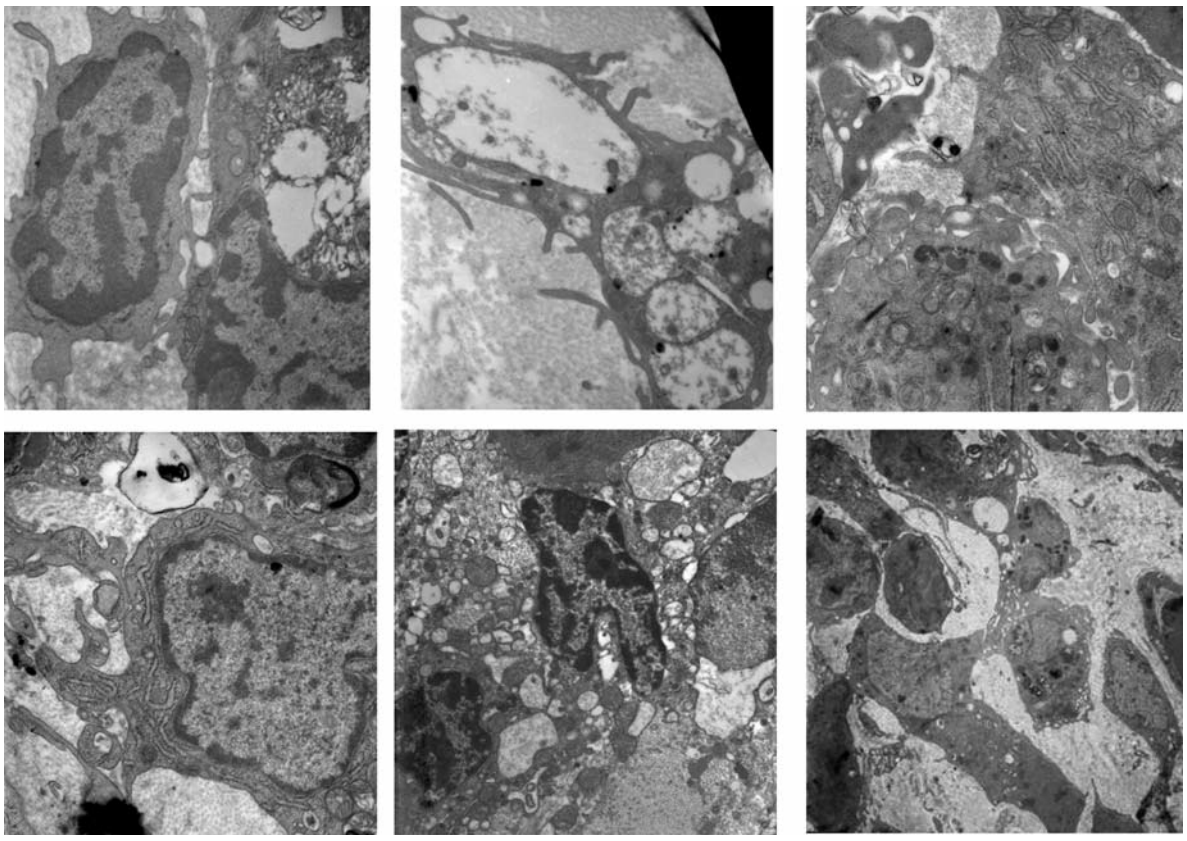

Figure 3. *Light/electron microscopic appearance of the pleomorphic sarcoma NOS xenograft: Note: heterogeneity of tumour cells in size and shape and nuclear irregularities; light and dark lipid droplets; euchromatic nuclei with few peripheral located heterochromatin; elongated rough endoplasmic reticulum with dilated cisternae.*

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