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Radiofrequency Ablation of Experimental Bone Metastases in Nude Rats

D. PROSCHEK[1], M.G. MACK[2], A.A. KURTH[1], P. PROSCHEK[2], B. MARTIN[3], M.L. HANSMANN[4] and T.J. VOGL[2]

Departments of [1]Orthopaedic and Trauma Surgery, Friedrichsheim,
[2]Diagnostic and Interventional Radiology,
[3]Molecular Gynecology, and
[4]Pathology, University of Frankfurt, Frankfurt am Main, Germany

Abstract. *Purpose: Animal models are indispensable to investigate bone metastasis and to test different preclinical therapy options. Radiofrequency ablation is an upcoming technique for palliating pain from bone metastases. The aim of this study was to generate osteolytic lesions and to enable a technique to achieve access to the bone to successfully carry out radiofrequency ablation. Materials and Methods: Human breast cancer cell line MDA-MB-231 (10^5 tumor cells) was implanted into the femur of 10 nude rats using a drill hole after arthrotomy of the knee joint and opening of the femur through the notch. Weekly CT- and MRI-scans were performed to document number and size of bone metastases. Radiofrequency ablation (22G bipolar and impedance-controlled RF-applicator, 2-4 Watt, 3 min application time) was carried out. One week after RFA, the animals were sacrificed and macroscopic and histological examination followed. For statistical analysis, paired comparison procedures were used. Results: Inoculation of the tumor cells was well tolerated. The mean time of the surgical procedure was 6 minutes. All animals developped local bone metastases. Mean time to metastasis was 8 weeks (range 7-10 weeks) after tumor cell implantation. No leakage of tumor cells and no soft part metastases occurred. Radiofrequency ablation was performed without complications. Imaging showed a complete ablation of the bone tumor in all rats Histological findings confirmed a circular necrosis with an extensive destruction of tumor cells leaving a necrosis cavity. Conclusion: The experimental model presented here describes the first time the ability to carry out radiofrequency ablation in nude rats with intrafemoral induced osteolytic metastases of human breast cancer. RFA in human breast cancer cell line in nude rats is a feasible and useful possibility to evaluate and to test different RF-procedures. Additional treatment options like local chemotherapy or chemoembolization can be performed.*

Correspondence to: Dr. Dirk Proschek, Department of Orthopaedic and Trauma Surgery, University of Frankfurt, Marienburgstr. 2, 60528 Frankfurt am Main, Germany. Tel: +069 6705 0, Fax: +069 6705 856, e-mail: d.proschek@friedrichsheim.de

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Breast cancer is the most common site of origin of metastatic deposits in the skeleton. The metastasis of human breast cancer to bone is one of the most significant cause of morbidity and mortality in this disease. About 211,240 women in the United States will be found to have invasive breast cancer in 2005. More than 40,000 women die from this disease every year (1). Due to the improvements in medical treatments of this cancer, the patients are living much longer and the prognosis has become much better than in the past. Further advances are essential to improve the prognosis of this disease considerably. Therefore animal models are necessary to test different *in vivo* strategies for prevention and treatment of human breast cancer and therefore to increase the survial and life quality of the involved patients.

Heat has been used in medicine as long as history. Ancient Hindu medicine produced heated metal bars and the Greeks heated stones to stop bleeding. Electrocautery has been used for decades in surgery to stop bleeding, to cauterize and cut tissue. The radiofrequency generator forms an electric current. Resistance of biologic structures causes local ions to vibrate. This ionic agitation results in friction around the electrode tip as ions attempt to pursue changes in direction of the alternating current and create heat to the point of dessication (2, 3). Radiofrequency thermal ablation differs from electrocautery in that the tissue around the electrode, rather than the electrode itself, is the primary source of heat. Radiofrequency ablation depends upon the transfer of electrical energy to tissue (4). Generator-applicator-systems can be divided into monopolar or bipolar systems. When using monopolar systems, the patient is made into an electrical circuit by

placing grounding pads on the thighs. With bipolar systems, no grounding pads are necessary.

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Materials and Methods

Animals and tumor cells. Ten congenitally athymic female nude rats (rnu/rnu), 8 weeks old, were obtained from Harlan-Winkelmann (Harlan-Winkelmann Borchen, Germany). All animals were kept in single cages and under pathogen-free conditions and had free access to tap water and were fed with a standard chow containing 1 g calcium and 0.8 g phosphorus/100 g weight. After arriving in our laboratory, the animals were examined and adapted to the laboratory conditions for one week before entering the study. All experiments were approved by the governmental Animal Ethics Committee of the Regierungspräsidium Darmstadt (Hessen / Germany).

In this experiment, we used the human oestrogen-independent breast cancer cell line MDA-MB-231. The cell line was kindly provided by the Department of Molecular Gynecology (University of Frankfurt, Germany). Cells were cultured in a standard DMEM (Boehringer Mannheim, Germany) at humidified atmosphere (37˚C) and 5% CO_2. A concentration of 10^5 tumor cells was used in this trial. All animals were killed on day 60 and the femora and tibiae collected for histopathological examination.

Inoculation of the tumor cells. All animals underwent clinical and radiological examination before entering the trial. The tumor cells were washed with PBS and suspended in 0.5 ml of PBS. All procedures were performed under general anesthesia using 100 mg/kg body weight of ketamine hydrochloride *s.c.* (Parker-Davis, Berlin, Germany) and 10 mg/kg body weight of 2% xylazine hydrochloride (Bayer, Leverkusen, Germany). After careful desinfection and preparation of the knee- and leg-area, an arthrotomy of the left knee was carried in all animals. A longitudinal medial and parapatellar incision was made and the patella dislocated laterally with the knee in flexion of 90˚. The notch was displayed and a hole into the femur was drilled through the notch using a k-wire. After preparing the femoral bone, the inoculation of the tumor cells was carried out, using 10^5 tumor cells. 0.2 ml of the tumor cell suspension was injected into the femural drill hole using a small catheter (0.2 mm in diameter). After this procedure, the drill holes were sealed with bone wax in order to prevent a leakage of tumor cells into the soft tissue. After finishing the inoculation, the knee joint and the soft tissue were reconstructed surgically.

RFA procedure. All animals were screened weekly for metastases using CT- and MRI-scans. After detecting bone metastases with a tumor size bigger than 3 mm in diameter in the distal femur, thermal ablation using radiofrequency was carried out. Again, the knee joint was opened using an arthrotomy and preparation of the notch. The former drill hole was re-opened with a k-wire and the radiofrequency electrode was introduced. The correct position of the electrode in the center of the bone metastasis was controlled with a CT-scan. Radiofrequency ablation was carried out. An impedance-controlled and bipolar RF-generator with a 22 G applicator electrode was used. Tumor coagulation was finished when the continuing energy-flow was stopped due to an arising of the impedance. The knee joint and the soft tissue were reconstructed surgically. Postprocedural CT- and MRI-scans were performed and confirmed the successful tumor ablation.

Postinterventional assessments. All animals were examined clinically (inspection, evaluation of the ingestion, measures of body weight) on days -7, 0, 3, 6 and followed every 6th day unti day 60. CT scans (Siemens Somatom, Germany, UHR-spine program) were carried out in general anesthesia on days 14, 22, 30, 38, 46, 54 and 60. MRI-scans (Siemens Symphony, Germany, hand wrist coil, T1/T2 weighted and fat suppressed imaging) were carried out on day 28 and 54 and 60. Radiographs were analysed by two different and experienced investigators. The changes in the perimeter (mm) and the area of osteolytic bone (mm^2) were evaluated subsequently and documented by both investigators. Before finishing the trial, all animals were scanned for a systemic tumor spread using CT. Immediately after radiofrequency ablation and 1 week after the intervention a CT- and MRI-scan was performed to evaluate the changes in the perimeter (mm) and the area of metastatic bone (mm^2).

Histology. Both the femora and tibiae were collected for histopathological examination. Bones were fixed in 10% neutral buffered formalin and decalcified in an EDTA-solution. After finishing the decalcification, bones were embedded into paraffin and sections were cut at a distance of 200 nm. The osteolytic areas were measured and examined at a maximal magnification of x400. All procedures were carried out by an experienced investigators of the Department of Pathology, University of Frankfurt, Germany.

Results

Inoculation procedure. Inoculation of tumor cells was well tolerated as animals recovered quickly from the surgical procedure. General anesthesia was carried out without any side effects. There were no restrictions in the mobility of the operated knee in all animals. Figures 1-2 show the preparation of the femoral bone before inoculation of the tumor cells. The mean time of the surgical procedure was 5 minutes. A concentration of 10^5 tumor cells was used in this trial. The drill hole was sealed using bone wax. There were no technical and postprocedural complications.

Local and systemic tumor effects. All animals developed local metastases and did not show weight loss or any signs of walking with a limb. No cachexia occurred. Lytic lesions were detected by X-ray in some animals as early as 3 weeks after tumor cell implantation. Mean duration until first osteolytic lesions on CT-scans was 34 days (range 22-38). The mean lesion size is expressed as product of area and mean gray scale of the measured lesions in order to take the extent of bone lysis into consideration. The mean lesion area on first detection was 19 mm^2. The overall growth rate

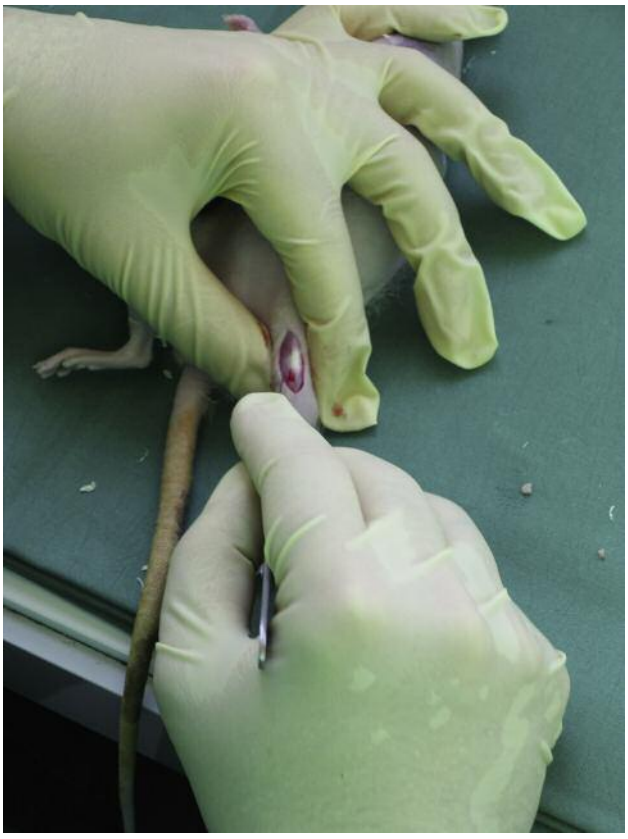

Figure 1. *Knee joint of the nude rat after medial skin incision and before arthrotomy.*

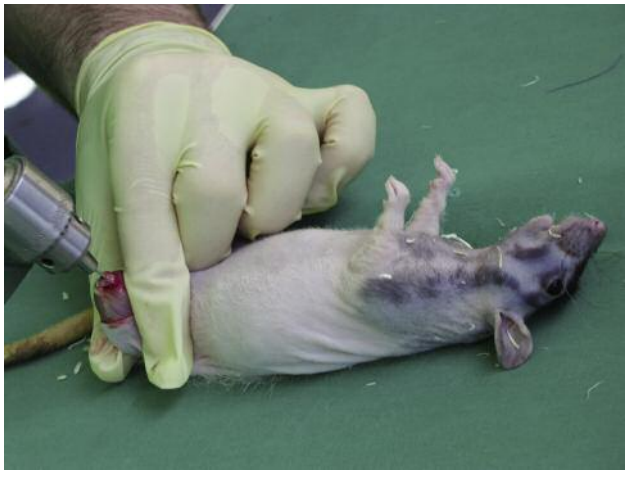

Figure 2. *Knee joint after arthrotomy and lateral luxation of the patella. Opening of the femoral bone using a K-wire.*

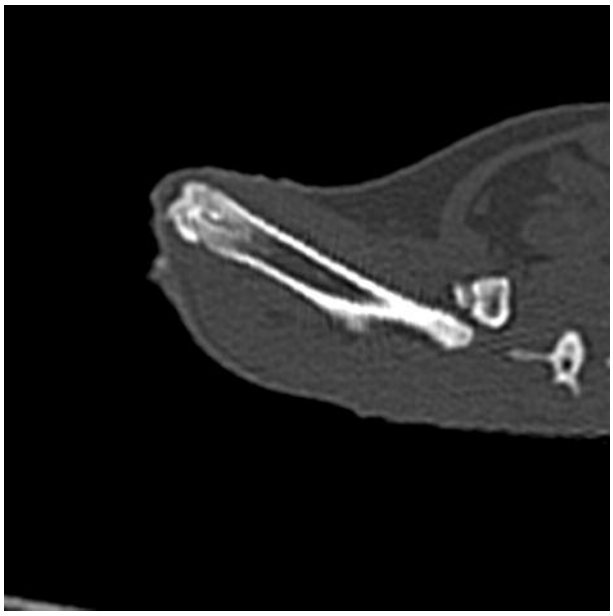

Figure 3. *Nude rat at 22 days after tumor inoculation. Coronar reconstruction of a CT-scan (UHR-spine program). Note the former drill hole and the tumor infiltration around the drill hole.*

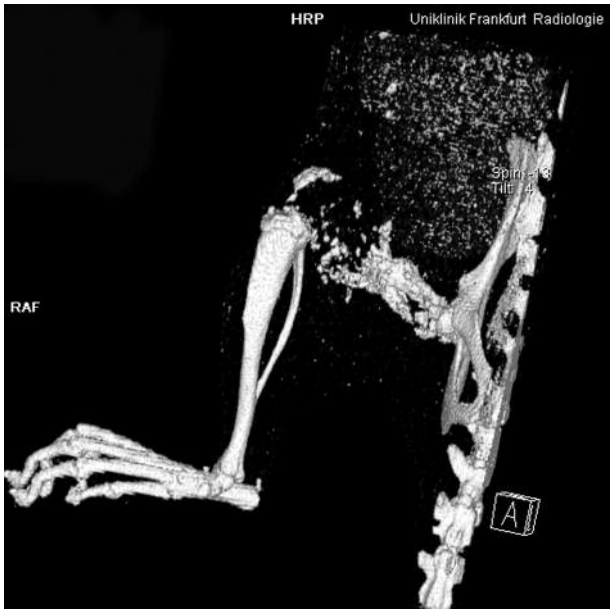

Figure 4. *Nude rat at 46 days after tumor inoculation. 3d reconstruction of a CT-scan (UHR-spine program). Note the tumor infiltration of the femur with an additional infiltration of the knee structures.*

was 0.16 mm^2/day. At study termination a complete CT-body-scan was carried out in order to detect metastases. No systemic tumor spread was seen in all animals.

Radiological examination. CT scans were carried out in general anesthesia on days 14, 22, 30, 38, 46, 54 and 60. Figure 3 shows a coronary reconstruction of a CT-scan on

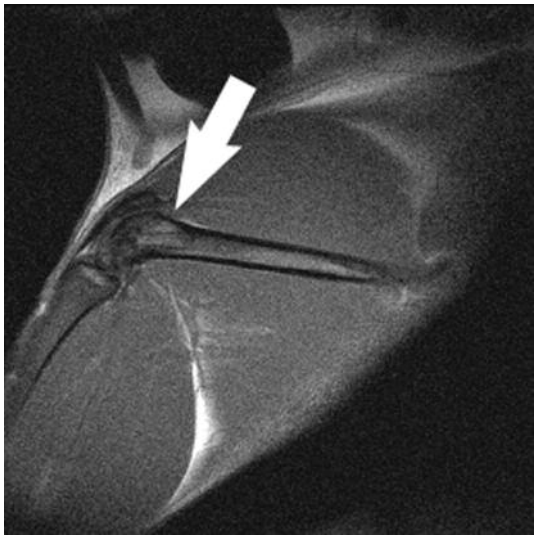

Figure 5. *Nude rat at 28 days after tumor inoculation. Sagittal reconstruction of an MRI-scan (T1 weighted, after application of contrast media). Note the local tumor infiltration and tissue enhancement (arrow) in the distal femur.*

Figure 6. *Nude rat at 42 days after tumor inoculation. After detection of local bone metastasis, radiofrequency ablation was carried out. The knee joint was re-opened by an arthrotomy (arrow 1) and the bipolar electrode introduced (arrow 2).*

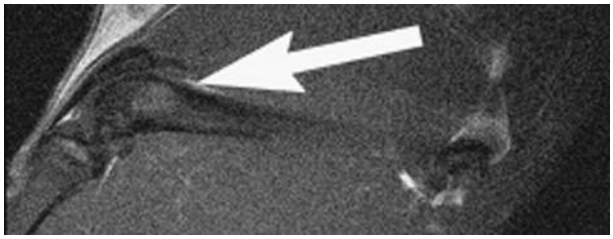

Figure 7. *Nude rat at 28 days after tumor inoculation. Sagittal reconstruction of an MRI-scan (T1 weighted, after application of contrast media). Note the local tumor infiltration and tissue enhancement (arrow) in the distal femur.*

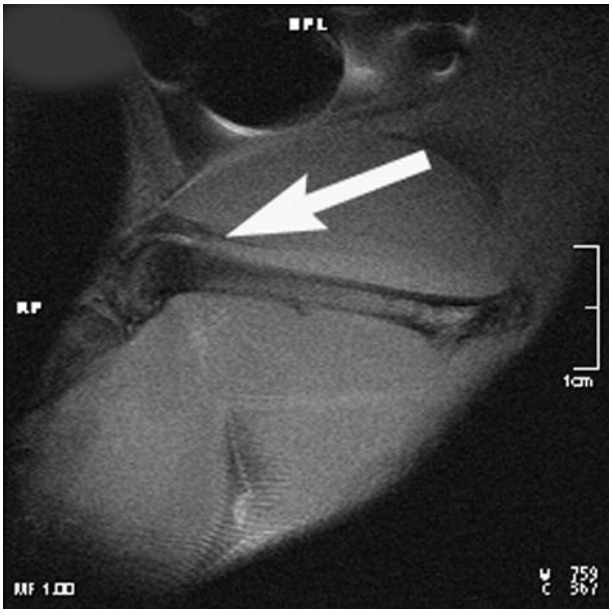

Figure 8. *Nude rat at 40 days after tumor inoculation and one week after radiofrequency ablation. Sagittal reconstruction of an MRI-scan (fat suppressed, after application of contrast media). Compare to fig. 7 and note the reduction of tissue enhancement after application of contrast media (arrow) in the distal femur.*

day 22. The former drill hole can still be seen. Cortical structures are intact, local tumor infiltration around the drill hole can be seen. Figure 4 shows a 3d reconstruction of a CT-scan on day 46. Note the tumor infiltration of the femur with an additional infiltration of the knee structures. MRI-scans (Siemens Symphony, Germany, hand wrist coil, T1/T2 weighted and fat suppressed imaging) were carried out on day 28 and 54 and 60. Figure 5 shows a sagittal reconstruction of an MRI-scan on day 28 (T1-weighted sequence with contrast media). A local tumor infiltration and contrast enhancement in the distal femor can be seen.

Radiofrequency ablation. After detecting bone metastases with a tumor size bigger than 3 mm in diameter in the distal femur, thermal ablation using radiofrequency was carried out. The former drill hole was re-opened with a k-wire and the radiofrequency electrode was introduced (see Figure 6). An impedance-controlled and bipolar RF-generator with a 22 G applicator electrode was used. Mean application power was 3 Watts (range 2-4) with a mean application time of 3 minutes (range 2:30-4:30). Tumor coagulation was finished

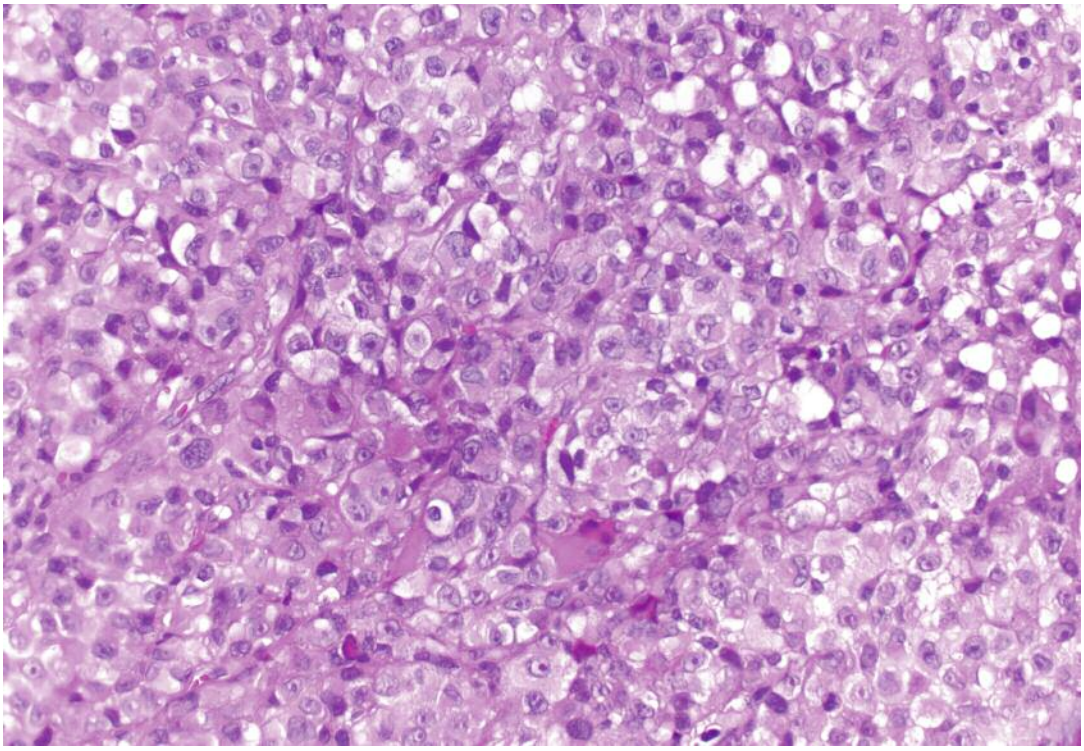

Figure 9. *Histological examination (HE, magnification x400) of the left distal femur of a nude rat. The trabecular bone is destroyed and the bone marrow cavity is replaced by metastatic MDA-231 breast cancer cells.*

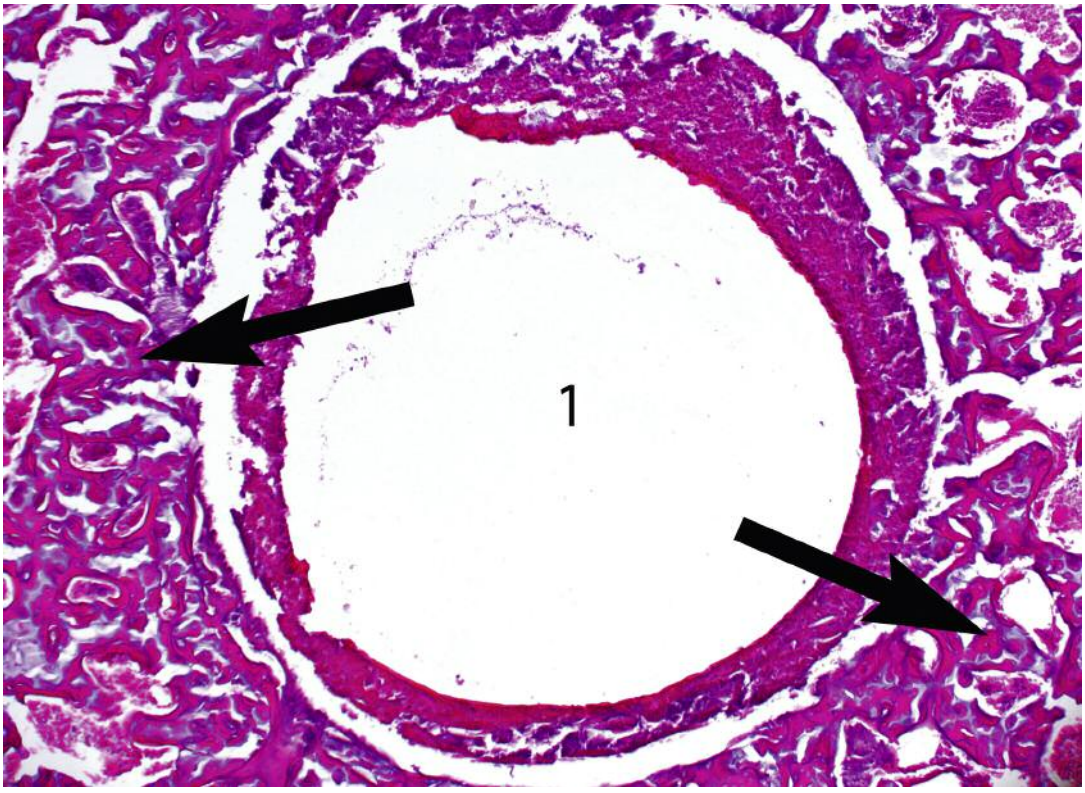

Figure 10. *Histological examination (HE, magnification x400) of the left distal femur of a nude rat after radiofrequency ablation. Note the former drill / electrode hole ("1"). The surrounding tissue shows a circular necrosis after thermal ablation (arrows). No tumor cells can be detected.*

when the continuing energy-flow was stopped due to an arising of the impedance. The knee joint and the soft tissue were reconstructed surgically. Postprocedural CT- and MRI-scans were performed and confirmed the successful tumor ablation. Figure 7 shows a nude rat at 28 days after tumor inoculation with a local tumor infiltration. One week after radiofrequency ablation and before termination of the study, an MRI-scan was carried out. Figure 8 shows a reduction of tissue enhancement after application of contrast media (arrow) in the distal femur. Radiofrequency ablation was carried successfully in all animals.

Histology. Both the femora and tibiae were collected for histopathological examination. All animals developed local bone metastases in the distal femur. Especially the areas around the former drill hole showed an extensive tumor infiltration (Figure 9). Medium size (diameter) of the bone metastases was 3 mm (range 2 mm to 5 mm). Figure 10 shows the histological examination (HE, magnification 400) of the left distal femur of a nude rat after radiofrequency ablation. Note the former drill / electrode hole (“1). The surrounding tissue shows a circular necrosis after thermal ablation (arrows). No tumor cells can be detected.

Discussion

After lung and liver, bone is the third most common metastatic site and is relatively frequent among patients with primary malignancies of the breast, prostate, and lung. Bone metastases often cause osteolysis resulting in pain, fractures, decreased mobility, and reduced quality of life. External beam irradiation often is the initial palliative therapy for osteolytic bone metastases. However, pain from bone metastases is refractory to radiation therapy in 20% to 30% of patients, while recurrent pain at previously irradiated sites may be ineligible for additional radiation due to risks of normal tissue damage. RFA has been investigated as another alternative and upcoming technique for palliating pain from bone metastases (5, 6). Many different animal models have been tested in the past to address the pathophysiology of bone metastases including different therapy options.

Inoculation of tumor cells. Reviewing the literature, syngeneic induction was the method most often used to induct bone metastasis. Different ways to implement tumor cells have been described and included co-injections of tumor cells with human bone tissue (7), intracardiac injections of tumor cells (8), orthotopic injection of tumor cells to bone (9, 10), or occlusion of the vena cava during intravenous injections of tumor cells (11). We used a surgical technique in which tumor cells were directly inoculated into the femoral bone. The mean time of the surgical procedure was 5 minutes. All animals developed multiple osteolytic metastases in the distal femora with a success rate of 100%. No comorbidity was seen. The intrafemoral application technique enables a simple and easy technique to successfully implement tumor cells directly into the bone and to avoid a systemic tumor spread and a high rate of comorbidity.

Radiofrequency ablation. The experimental model presented here describes the first time the ability to carry out radiofrequency ablation in nude rats with intrafemoral induced osteolytic metastases of human breast cancer. Other models are available to induce (12-16) and to test different medications in the therapy of human breast cancer in nude rats (1, 17-19). Radiofrequency ablation is a safe and minimal invasive tumor ablation therapy which rapidly reduces pain and improves quality of life in patients with bone metastases. Recent advances in different generator and applicator systems as well as in different guiding / monitoring technologies will overcome previous shortcomings. Especially due to the possibility to immediately evaluate the results of the treatment, radiofrequency ablation has the potential of an effective option in the local treatment of bone tumors. Therefore, it is important to test new combinations of generators and applicators in animals.

Conclusion

Animal models are indispensable to investigate bone metastasis and to test different preclinical therapy options. Our data show that this model will make further examinations of thermal ablation techniques possible and is valuable to further understanding of this disease and its treatment options.

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