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RAT XENOGRAFT CHONDROSARCOMA DEVELOPMENT BY HUMAN TISSUE FRAGMENT

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Chondrosarcoma is one of the most difficult types of cancers to diagnose and treatment. Therefore, the development of a reliable animal model for chondrosarcoma would be a helpful tool to study of the tumor's growth and progression. ***Aim*****: We conducted this study to develop a chondrosarcoma on rat by graft of human chondrosarcoma tumor tissue.** ***Methods*****: Fourteen male Sprague-Dawley rats equally divided in xenograft-implanted and control groups. On the lateral side of the right femur distal 1/3, 5 mm incision was done on the skin after animal anesthesia. Then, was drilled 3 mm on the bone and implanted the xenograft in the bone. Radiography was taken from the operated femur weekly until the fourth week and monthly for 3 months. Four animals of each group were sacrificed after 4 weeks of operation; femur was harvested for histopathological study.** ***Results*****: Radiological images showed sclerotic area on the implanted bone after 4 weeks of operation. Sections from tumoral areas reveal cartilage forming hypercellular neoplastic tissue with lobular pattern of growth and foci of adjacent tissue invasion such as bone trabeculas and bone marrow.** ***Conclusion*****: the present study showed that rat xenograft chondrosarcoma can develop by human chondrosarcoma fresh tissue fragments.**
Key Words**: human chondrosarcoma, xenograft, rat.**

Chondrosarcoma is the second most frequent malignant primary bone tumor in human. Orthopedic oncologists believe that chondrosarcoma is one of the most difficult types of cancer to diagnose and treatment; it is also highly resistant to ionizing radiation action and chemotherapy. In experimental musculoskeletal oncology animal models are routinely used to assess the efficacy of new and innovative treatment methodologies for these tumors [1–3].

Quite a few animal models of chondrosarcoma have been developed and described in literature, such as (i) inoculation chondrosarcoma cell line (MCS-1) [4–5], (ii) allograft tumor tissue fragments on rats [6], (iii) human tumor xenograft implanted cell line into the nude mice [7–8]. In 1960's allograft chondrosarcoma models were developed based on rat tumor implantation [9]. The chondrosarcoma model was established with repeated transplantation. However, these models could not show the human tumor behavior. Therefore, the development of a reliable animal model for chondrosarcoma would be a helpful tool to study tumor growth and progression.

To have a more similar experimental human tumor, human chondrosarcoma development on animal cases is needed. Yet, such a tumor may not be entirely representative of spontaneously developing chondrosarcoma [10]. Since animal model of human xenograft would be feasible and reproducible. We conducted this study to develop a chondrosarcoma model on Sprague-Dawley rat by fresh human chondrosarcoma tissue fragments.

Animals. Fourteen, four-week-old male Sprague-Dawley rats (weighing 90±5 g) were taken from Razi Vaccine and Serum Research Institute of Iran, kept in separated cages at 21–23 °C, humidity 50% and 12 h light-dark cycle. They had free access to rat chow and water. Animals were equally divided in xenograft-implanted and control groups. The implanted group received tumoral fresh tissue fragments. The human chondrosarcoma tissue was obtained from a 37 year-old female diagnosed with left leg chondrosarcoma (Tumor bank number 328, File number 822585, Cancer Institute, Emam Khomeini Hospital, Tehran, Iran). The fresh sample had atypical and anaplastic cells as a low-grade type of tumor. All experimental procedures were in accordance with the guidelines of the animal and human ethical committee of Tehran university of Medical Sciences.

Surgical procedure. Animals were anesthetized by a mixture of ketamine (60 mg/kg i.p) and xylazine (10 mg/kg i.p). First the lateral skin of the right femur distal 1/3 was incised 5 mm and then drilled 3 mm on the bone. Fresh chondrosarcoma tumoral segments were taken from operation room, immersed in normal saline immediately. The segments were divided into very small fragments less than 0.5 mm and inoculated with gage 14 needle into femoral intramedullary space [11]. The control group was injected normal saline.

Cyclosporine A (10 mg/kg i.p) has been given for 4 weeks from implantation day (day 0) to suppress graft versus host reactions [12]. Animals were weighted once a week throughout the experiment.

Radiological study. Post operative AP X-rays were taken every week for one month to assess the implantation site. X-rays were obtained monthly for three months after immunosuppressive discontinuation.

Histopathological study. Four weeks after the operation, four rats were sacrificed of each group to harvest their femur bones and tumoral samples immediately fixed in a 10% formalin solution for 24 h and then put in a nitric acid solution (10%) for five days to decalcify bone mass. Samples were embedded in

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paraffin and sliced into 5 μm serial sections for staining Hematoxylin and Eosin (H & E). For the pathologic classification of chondrosarcoma, cellularity, nuclear format cells and necrosis were examined.

Statistical analysis. Nonparametric fisher exact test was used to compare between two groups ($p < 0.05$).

The radiological image showed sclerotic area on the implanted group (Fig. 1 *a*). The cortical bone presents signs of invasion and thickening of the periosteum. A low-grade chondrosarcoma showed by disruption of trabeculae, intralesional calcification and periosteal reaction. Around the implantation area was seen more opacity compared to control group (Fig. 1 *b, c*).

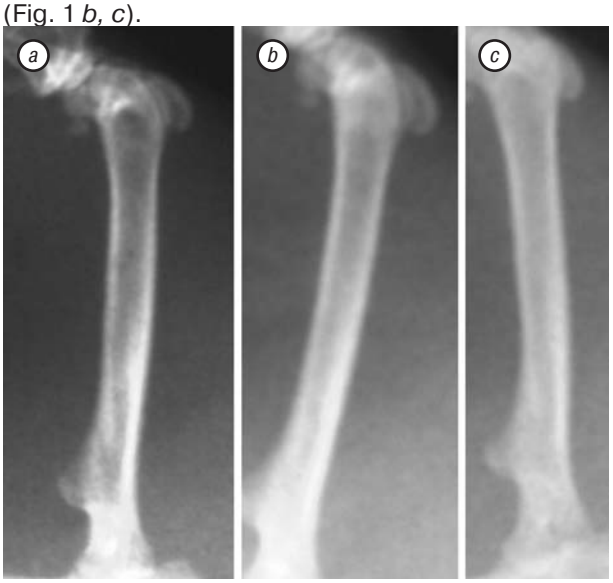

Fig. 1. Radiographies after chondrosarcoma tissue fragments implantation: *a*, period of the immunosuppressive treatment with abnormalities and bone resorption in implanted group; *b*, immunosuppressive agent discontinue showing bone formation improvement; *c*, control group

Typical signs of chondrosarcoma were observed four weeks after operation implanted group (Fig. 2 *a, c, d*). Sections from tumoral areas reveal cartilage forming hypercellular neoplastic tissue with lobular pattern of growth and foci of adjacent tissue invasion. Invasion occurred in bone trabeculas and bone marrow tissue as well as cellular criteria of chondrosarcoma (see Fig. 2 *c, d*). Section from implanted area of femur in control group showed normal pattern of tissue (Fig. 2 *b*).

In the present study, xenograft chondrosarcoma induction was established in rat femoral bone by fresh human chondrosarcoma fragments.

Poor prognosis of chondrosarcoma demands new therapeutic options to improve the overall rate of survival, especially in high-risk groups. Animal models of accurately reproduced human pathology, physiology and histology are needed to experience a new therapeutic strategy. Accordingly, animal xenograft cell line inoculation was done in nude mice, rats and hamsters during the last century [4–5, 13–15]. It will be more useful to have animal tumoral models behave very similar to the human cancerous cells with minimal manipulation.

A number of xenograft implantable human tumors including chondrosarcoma were prepared by treating the animals with radiation and cortisone. With the advent of athymic nude mice, animal immunosuppressant free cancerous cell models were adopted xenograft implantation [16]. In 1990's, chondrosarcoma cells of human were implanted in nude mice. Since, this technique has become popular, so that, most of chondrosarcoma models used during the last two decades involve human chondrosarcoma tissue or cell lines as ectopically implanted (within subcutaneous tissue) in various strains of immunocompromised mice [10]. However, transgenic mouse chondrosarcoma have been observed with unpredictability of tumor location, multiple tumors forming and varying phenotypes that makes this approach difficult to control as a function assessment model [10, 12].

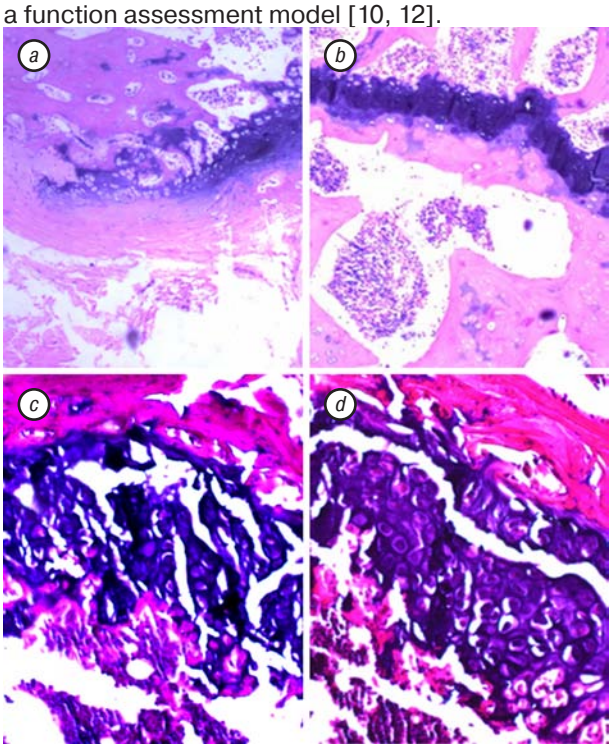

Fig. 2. Chondrosarcoma implantations after 28 days stain with hematoxylin and eosin: a — bone infiltration was seen obviously, 40X; *b* — control group, 40X; *c, d* — hyper cellularity, bone marrow and trabeculae infiltration in implanted group, 100X

In present study, radiographic findings show more opacity at the implantation region (Fig. 1 *a*) compared to control group (Fig. 1 *c*). Implanted region lag growth may result immunosuppressive agent discontinuation (Fig. 1 *b*, see Fig. 2 *a, c, d*). Cellular criteria of chondrosarcoma include lacunar spaces with more than one nucleus, atypical hyperchromatic nuclei and bone infiltration [10]. Note that penetration into the bone, hypercellularity and irregularity are characteristics of chondrosarcoma tumor. This investigation showed chondroid cell infiltration into the bone marrow and trabeculae (see Fig. 2 *a*), also irregularity and hypercellularity were seen in histopathological images of treated group (see Fig. 2 *c, d*).

In our study it seems the ectopic chondroid cells at the intramedullary bone region may be related to well-differentiated chondroid cells or benign tumor in focal site. Thus, the present study shows that animal xenograft chondrosarcoma can be produced from fresh human

fragments. The advantages of this kind of method are reproducibility, feasibility and cost effectiveness. Based on, chondrosarcoma xenograft can be a helpful model in human cancer studies and may be a good adjuvant to assess the efficacy of new and innovative treatment methodologies for chondrosarcoma tumors.

CONCLUSION

The present study showed that xenograft chondrosarcoma in rat can be developed by human chondrosarcoma tissue fragments.

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