

Current advances in animal model of chondrosarcoma and related research (Review)

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Abstract. Chondrosarcoma is a type of malignant cartilage tumor with a high local recurrence. Due to its resistance to chemo- and radiotherapy, current treatment is limited to surgical resection. Animal model is one of the most important approaches to studying this disease, although systematic reporting on its development is rare. In this review, we summarized the elements involving animal model establishment. On the basis of these elements, we further classified chondrosarcoma animal models into various types. In addition, we compared various measurements for evaluating the animal model. Finally, its specific applications were discussed.

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1. Introduction

Chondrosarcoma is the second most common primary malignant bone tumor that predominantly occurs in adults >40 years of age (1,2). Due to its poor response to chemo-, as well as radiotherapy, at present surgical resection is an effective treatment for chondrosarcoma (3,4). This mesenchymal

malignancy has a poor prognosis with local recurrence and a 5-year survival rates of 24-33 and 64-77%, respectively (5,6). The predilection sites of chondrosarcomas are pelvic bones and femur, nevertheless, the majority of tumors grow slowly. Although metastasis is infrequent, the lung is the most common site of metastasis in chondrosarcoma (7-9).

2. Elements of chondrosarcoma animal model

The experimental animal model that closely resembles human chondrosarcoma in clinic is considered to be one of the most important approaches to studying tumor growth, progression and metastasis. Three key factors including experimental animal, cell lines for transplantation and graft sites should be taken into account when building the chondrosarcoma animal model.

3. Experimental animals

Mice and rats are considered to be optimal choices in animal experiments. There are many different strains that we can utilize. Sprague-Dawley rats have been used as implanted animal models for a long time. In their study 45 years ago, Maibenco *et al* (10) reported that an 18 month-old female Sprague-Dawley rat (SD rat) developed a spontaneous chondro-blastic-osteogenic tumor in the thoracic and lumbar vertebrae. The tumor was resected surgically to establish a chondrosarcoma cell line designated as Swarm rat chondrosarcoma (SRC) for transplantation (11). Varying types of rats deliver varying results to a certain extent. Mason and Bansal (12) demonstrated that the tumor weight in Lewis rats was two times bigger compared to Wistar rats at the same time point. Due to lack of host immune response, nude mice, such as Balb/c nude mice and severe combined immune deficiency (SCID) mice have been increasingly used by scientific researchers in recent years. Human chondrosarcoma cells can thus be implanted without host immune rejection.

4. Cell lines for transplantation

Cell lines for graft are mainly composed of animal and human chondrosarcoma cell lines, according to different sources (13). The animal chondrosarcoma cell lines primarily refer to SRC derived from a female SD rat with spontaneous tumor, as

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mentioned previously (11). SRC-JWS and SRC-TRO cell lines are mostly used in animal-derived chondrosarcoma cell lines. Stevens (11) compared the differences in subcutaneous tumor growth using these two cell lines. The first palpable tumor can be detected 7 days earlier in the SRC-JWS compared to the SRC-TRO group. Tumors (35 and 11 g) were harvested on days 21 and 35, for the SRC-JWS and SRC-TRO cell lines, respectively (11). The SRC-JWS cell line exhibited a more rapid growth and aggressive behavior compared to SRC-TRO. Similar to the experimental model selection, selection of cell line also influences tumor growth in animal models. A number of additional human chondrosarcoma cell lines are also being investigated, including OUMS-27, HCS-2/A, HCS-TG, NCDS-1, SW1353, JJ012, FS090 (1,13). To the best of our knowledge, apart from being two unique human chondrosarcoma cell lines for orthotopic xenograft models, JJ012 and SW1353 are the most frequently applied in human chondrosarcoma transplantation.

5. Graft sites

Regardless of the types of chondrosarcoma cell lines chosen, subcutaneous and tibia are the two most common locations for implantation. Due to the easy application, subcutaneous injection had been widely adopted over the past decades, especially with SRC cell lines. With the deepening of cognition, more attention is paid to the implantation of tumor cells into tibia, where chondrosarcomas often occur. In addition, in their study, Clark *et al* (1) made a comparison of two transplantation sites using intratibial and periosteal injections. Their findings demonstrated that more time was required to reach the same tumor size in the intratibial compared to the periosteal group. They argued that this discrepancy may be due to the fact that vascular supply was more direct through the adjacent femoral vessels by the periosteal compared to the intratibial injection (1). Additionally, blood supply has been generally considered to be involved in tumorigenesis (14).

6. Classification of the chondrosarcoma animal model

Allograft and xenograft models. Transplantation animal models can be divided into allograft and xenograft models, depending on the origin of the chondrosarcoma cell lines used. The process that implants animal-derived cells, such as SRC, in most cases into animal, is termed allograft. Similarly, xenograft refers to the process whereby human chondrosarcoma cell are injected into animals. SD rats as usual experimental animals have been utilized in allograft models for a long time. When using this model, tumor growth and lung metastases were only 2/9 and 1/24, separately (15,16). Due to lack of relevance to human disease, allograft models are now infrequently employed. Although this model has several weaknesses, in their study, Clark *et al* (13) suggested allograft models to be useful for the evaluation of chondrosarcoma growth and histology rather than the development of novel therapeutic agents. The use of nude mice made xenograft models possible. Without host rejection response, human chondrosarcoma cells can be implanted. Clark *et al* (1) injected JJ012 cells into the intramedullary canal of the left tibia and the periosteum of the anterior tibia in female, 5-week-old Balb/c nude mice. Their

findings demonstrated that the average of implanted tumors by periosteal injection was 174.2 mm³ at 7 weeks, and 198.5 mm³, by intratibial at 10 weeks. Seven weeks after implantation, lung metastases rates were 5/5 and 2/4, respectively, which is markedly better compared to the allograft model. In their study, Klenke *et al* (17) injected SW1353 cells subcutaneously into the left flank of SCID mice to assess the inhibitory effect of tyrosine kinase inhibitor SU6668 on chondrosarcoma growth *in vivo*.

Heterotopic and orthotopic models. Depending on the graft sites, transplantation animal models comprise heterotopic and orthotopic models. Subcutaneous implantation is the most common method in heterotopic models. SRC, the animal-derived chondrosarcoma cell, has mostly been used as a subcutaneously implanted graft (13). Orthotopic transplantation mainly refers to the injecting of chondrosarcoma cells into the marrow cavity of the tibia, where primary chondrosarcoma often occurs. Transplantation sites have been shown to affect the malignancy of the tumor i.e., the grade of the tumor formed by orthotopic transplantation is higher compared to that of a tumor formed by heterotopic transplantation (16). In their study, Hamm *et al* (18) verified the varying effect of SRC on tumor growth and metastasis at different transplantation sites. Their findings suggested that after 3 weeks the average weight of tumors induced by heterotopic and orthotopic transplantations was 35.05 g and 75.22 mg, respectively. Lung metastasis can be detected in 50% of the orthotopic group animals, whereas in none in the heterotopic group animals. Although the former induced obviously larger tumors compared to the latter, the orthotopic transplantation resulted in more aggressive tumors that were capable of invading the surrounding bone tissue (16). The tumors exhibited various growth and histologic characteristics, depending on transplantation sites (18). This is the reason that a growing number of investigators prefer to build orthotopic models (Fig. 1).

Evaluation of chondrosarcoma animal models. Tumor formation and lung metastasis rates are two important indicators when assessing chondrosarcoma animal models. Implantation tumors growth condition can be evaluated by tumor weight and volume in most animal experimental studies. Tumor weight can be easily measured by scales subsequent to resection, while the traditional method to measure tumor volume is using a caliper. Its volume value can be calculated by the formula: $V=0.5 \times L \times S^2$, where L and S stand for the largest and smallest perpendicular tumor diameters, respectively (19). Whether or not lung metastasis occurs is mainly assessed by hematoxylin and eosin (H&E) staining and immunohistochemistry of the lung tissue harvested from sacrificed animals. Accumulating evidence has demonstrated that bioluminescent imaging (BLI) is a promising technique used widely in the field of pre-clinical oncology research (20). It provides rapid, non-invasive monitoring of tumor growth and regression in animals, quantitative and sensitive analysis of tumor growth and metastasis, assessment of tumor development and responses to drug therapies *in vivo* (21). In their study, Comstock *et al* (22) compared BLI and the caliper approach for the measurement of primary tumor volume in nude mice. Their findings showed that there was a good linear correlation

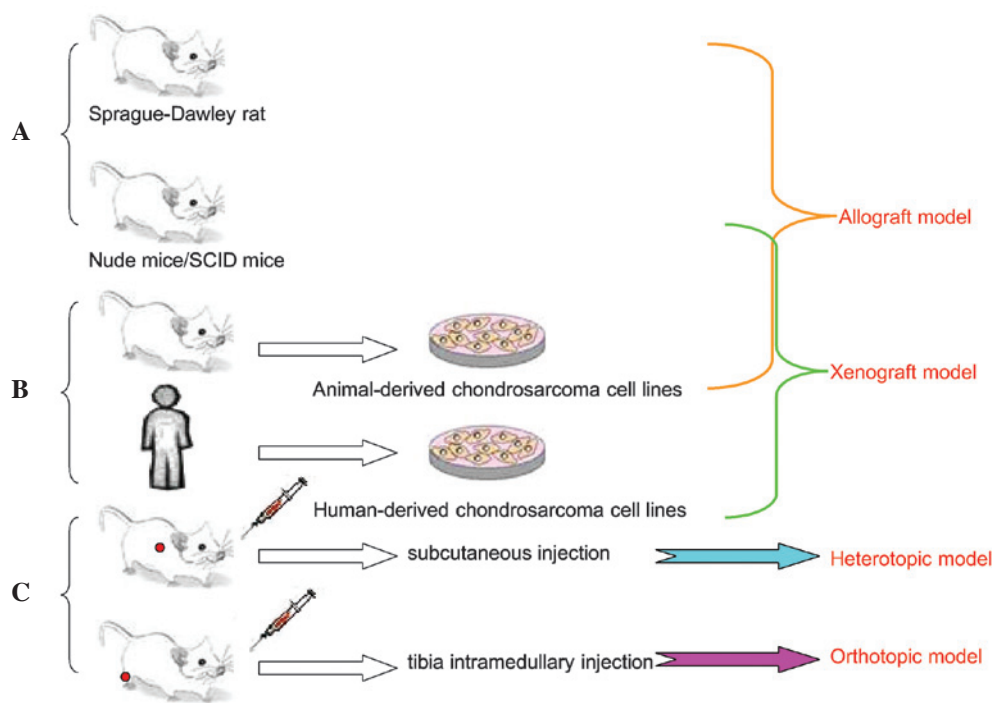


Figure 1. Elements and classification of the chondrosarcoma animal model are shown. (A) Experimental animals involved in chondrosarcoma animal model establishment mainly comprise Sprague-Dawley rat and nude mice/severe combined immune deficiency (SCID) mice. (B) Cell lines for transplantation are mostly divided into animal- and human-derived chondrosarcoma cell lines. (C) Graft sites primarily consist of subcutaneous and tibia intramedullary injection. Allograft model is established by injecting animal-derived chondrosarcoma cell lines into Sprague-Dawley rats. The xenograft model is built by injecting human-derived chondrosarcoma cell lines into nude or SCID mice. Heterotopic and orthotopic models are constructed by subcutaneous and tibia intramedullary injection, respectively.

between these two methods, while the tumors can be detected an average of 12.5 days earlier, when using BLI compared to caliper measurement (22). Consistent with their study, similar conclusions were drawn by Honigman *et al* (23) suggesting that malignant cells be visualized using BLI at least 13 days prior to palpable tumor formation in certain xenograft models. The advantages of BLI over the traditional approaches are: it permits real-time monitoring of tumor growth, spread, response to treatment in pre-clinical cancer models using the same animal without sacrifice (24,25), and the detection of unexpected micrometastases that are frequently too small to be visible and thus may be neglected using traditional methods (21). The prerequisite of BLI is the establishment of the chondrosarcoma cell lines, which can stably express luciferase by transfection. Factors affecting the overall sensitivity of BLI include signal depth, transgene expression level and the extent of background bioluminescence (20). Bioluminescent light emission attenuates with the increased tissue depth. Besides, the change in tumor shape during growth and tumor necrosis at later time points are also responsible for imprecise measurement when using BLI (22).

7. Application of the chondrosarcoma animal model

The chondrosarcoma animal models are mainly used to investigate novel effective therapies to treat this disease. Gouin *et al* (19) determined the effects of zoledronic acid (ZOL) on chondrosarcoma tumor progression. They constructed an allograft model of SD rats by placing a 10 mm³

SRC fragment. Four days subsequent to implantation, the rats were randomized into two groups: the ZOL-treated and control groups. In the ZOL-treated group each rat received 100 µg/kg ZOL subcutaneously twice a week. The average tumor volume in the ZOL-treated group was demonstrated to be markedly smaller compared to the control group on day 25 (4318±2278 and 10355±7414 mm³, respectively) and on day 27 (5253±4133 and 15092±10781 mm³, respectively). Survival rate in ZOL-treated group was 0.667±0.33 compared with 0.3±0.197 in the control group on day 40. Liu *et al* (26) developed a locoregional recurrence model of chondrosarcoma by injecting CS-1 cells, an aggressive human chondrosarcoma cell line, into the dorsum of 6 to 8-week-old female NU/J mice to evaluate the effect of paclitaxel-eluting polymer film on reducing locoregional recurrence rates and improving survival rates. Those authors did not perform an R0/R1 resection until the volume of primary tumors reached approximately 500 mm³. The mice were then randomized into four groups: implantation of Pax-film containing 300 µg paclitaxel, implantation of unloaded film, intravenous (IV) injection of 300 µg paclitaxel in Cremophor/ethanol and no additional treatment. Locoregional recurrence was observed in 2 of 12 Pax-film mice (17%), 9 of 13 unloaded-film mice (69%), 8 of 9 Pax IV mice (89%), and 7 of 8 untreated mice (88%) within 100 days. The median overall survival was 81, 64, 48 and 56 days, respectively. Their findings indicated that continuous local drug release by polymer films was a potential novel approach for the treatment of local aggressive chondrosarcoma (26).

In addition to discovering new remedies, chondrosarcoma animal models were utilized for other studies. In their study, Yonekawa *et al* (27) established an SRC animal model to investigate whether or not serum cartilage-derived retinoic-acid-sensitive protein (CD-RAP) could be used as a marker of tumor activity. Their findings demonstrated that there was a positive correlation between serum CD-RAP level and tumor growth. A marked decrease in the serum CD-RAP level was observed subsequent to tumor resection, which increased prior to tumor recurrence.

8. Conclusions

Chondrosarcoma is a primary malignant bone tumor that responds poorly to chemo- as well as radiotherapy. Despite adopting surgical resection, the therapeutic effect remains unsatisfactory and has high local recurrence and low 5-year survival rates. Management of this disease remains an ongoing challenge. The animal model, which closely mimics human chondrosarcoma in clinic, is considered to be an indispensable tool when exploring the pathogenesis, metastasis and drug resistance mechanism of chondrosarcoma or evaluating the therapeutic effect of novel treatments. Although as discussed previously, several types of chondrosarcoma animal models are available, orthotopic xenograft models remain the gold standard in oncology research. With the wide application of BLI, the animal model that can be used for *in vivo* imaging facilitates the non-invasive, rapid and precise detection of tumor growth.

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