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Review article

Small animal models for the study of bone sarcoma pathogenesis:characteristics, therapeutic interests and limitations

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ABSTRACT

Osteosarcoma, Ewing sarcoma and chondrosarcoma are the three main entities of bone sarcoma which collectively encompass more than 50 heterogeneous entities of rare malignancies. In contrast to osteosarcoma and Ewing sarcoma which mainly affect adolescents and young adults and exhibit a high propensity to metastasise to the lungs, chondrosarcoma is more frequently observed after 40 years of age and is characterised by a high frequency of local recurrence. The combination of chemotherapy, surgical resection and radiotherapy has contributed to an improved outcome for these patients. However, a large number of patients still suffer significant therapy related toxicities or die of refractory and metastatic disease. To better delineate the pathogenesis of bone sarcomas and to identify and test new therapeutic options, major efforts have been invested over the past decades in the development of relevant pre-clinical animal models. Nowadays, in vivo models aspire to mimic all the steps and the clinical features of the human disease as accurately as possible and should ideally be manipulable. Considering these features and given their small size, their conduciveness to experiments, their affordability as well as their human-like bone-microenvironment and immunity, murine pre-clinical models are interesting in the context of these pathologies. This chapter will provide an overview of the murine models of bone sarcomas, paying specific attention for the models induced by inoculation of tumour cells. The genetically-engineered mouse models of bone sarcoma will also be summarized.

1. Introduction

The injection of a cell suspension of murine (allograft) or human (xenograft) cancer cells, in orthotopic sites (in close contact to the bone or into the bone medullary cavity) is the most common methods used to induce bone sarcomas in mouse [1,2]. It has also been possible more recently to utilise the limited material available from patient biopsies (e.g. needle biopsies), and implant such tumour material into immunodeficient [e.g. Patient-Derived Xenografts (PDX)] [3] or immunocompetent animals [4,5]. The advantage of these PDX bearing mouse models is the possibility of expanding the tumour tissues by retaining the original tumour architecture.

The cell-injection close to the bone is called "paraosseous induction", in contrast to the "intraosseous model" that consists in cell inoculation into the femur or fibula diaphysis. Immunocompetent (e.g. syngeneic model in C57/BL6 mice or Sprague-Dawley rats) or immunocompromised (xenografts in Nude or SCID mice) models can be used according to the main objective of the studies (Fig. 1). Other heterotopic cell injections are also described in the literature (e.g. subcutaneous, under the renal capsule) however, they do not engage the vicious cycle established between cancer cells and the bone microenvironment and do not mimic all steps of tumour development.

The choice of the model will depend on the goal of the study (e.g. analysis of local tumour growth, imaging of lung metastases). In addition, financial aspects (e.g. relative inexpensive models based on injection of established cell lines versus genetically-engineered models) and availabilities of research tools (e.g. antibodies) are also key parameters that could influence the choice. Independently of their costs,

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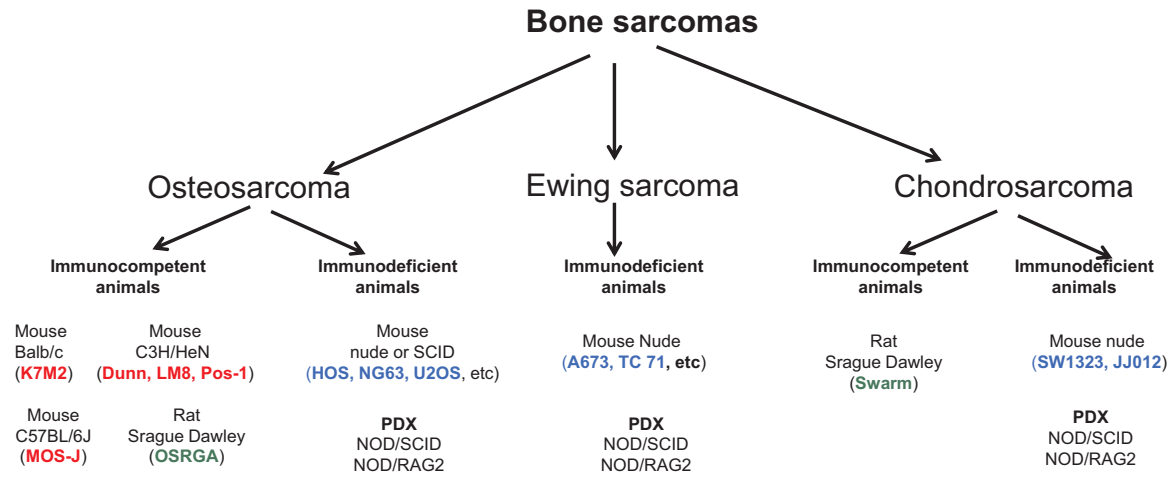

Fig. 1. Smal animal models available in the literature for the study of primary bone tumours. Cell lines: human (in blue), mouse (in red), rat (in green) orgin. PDX: Patient derived xenograft. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

each of these models have several advantages and limitations: i) inoculation of established cell lines may not represent the genetic heterogeneity of the human tumours; ii) genetically engineered models characterised by a spontaneous tumour development can mimic the natural history of the disease with an adapted tumour microenvironment (murine cancer cells in a murine microenvironment); iii) PDX models can maintain the cellular heterogeneity of the initial tumour fragments in a non human microenvironment. The current state of the art concerning the murine strains, the cell lines used, the number of cells injected per animal and some other specific technique-related features will be described in the paragraphs below.

2. Induction of primary bone tumour by cell injections in heterotopic sites

2.1. Induction of bone sarcoma by subcutaneous cell injections

Given the mesenchymal and bone/joint origin of bone sarcomas, their initiation through heterotopic subcutaneous cell injection does not take account of the proper interactions between the tumour cells and their normal bone/muscle/cartilaginous microenvironment. However, this model has the advantage of being technically easy to carry out, a large panel of cancer cell lines and diverse injection sites can be used and the resulting tumours are easily and directly accessible for experiments. Importantly, however, this approach can also address whether the transformed cells have the potential to form tumours in a cell-autonomous way in the absence of their normal environment. In the context of osteosarcoma, the human 143B cell line as well as several c-Fos-transgenic mouse osteosarcoma cells were reported to form tumour masses containing bone after subcutaneous injection [6,7]. Cancer cells have been also incorporated into acellular Matrigel™ based-matrix to provide an active bio-molecule scaffold from murine origin and facilitate cell engraftment. Utilising such an approach, Duan et al. established osteosarcoma tumours subcutaneously by resuspending KHOS osteosarcoma cells in a 1:1 Matrigel™ volume ratio and injected an amount of 2×10^6 cells per mouse [8]. The use of the Saos-2 human osteosarcoma cells combined with Matrigel™ was also reported. A recent study reports the injection of 3×10^6 cells resuspended in 100 µL of Matrigel™ mix (1:1) in this case [10]. Syngeneic models of osteosarcoma are also available. The Dunn cell line and its derivate LM8 subline are the most frequently used. Dunn cells were originally reported with a low metastatic profile in contrast to its LM8 subline which is highly metastatic. LM8 was initially obtained after 8 successive cycles of in vivo selection [10,11]. $1–10 \times 10^6$ Dunn or LM8 cells resuspended in 200–300 µL of PBS are inoculated subcutaneously into the flank of C3H mice (5- to 8- weeks-old) [12,13]. The inoculation of LM8 cells results in the development of a primary local tumour and the formation of metastases to the lungs within 4 weeks with an incidence of 100%. Finally, genetically-engineered osteosarcoma cells have also been reported to efficiently grow after subcutaneous injection [13–17] (Fig. 2). For instance, the low metastatic mouse RF43 osteosarcoma cells and their stable genetically-modified counterparts expressing sFRP2-were injected into nude mice[17] Similar studies have also been reported with Ewing Sarcoma cells, with A673 cells being one of the most commonly reported, for drug screening [18]. One to three million A673 cells are sufficient to generate a tumour mass after subcutaneous implantation into the flank or in the inguinal region of nude mice [19,20]. TC71 and SK-N-MC cell lines were also described to reproduce relevant non-osseous Ewing sarcoma models [21]. Similarly to osteosarcoma, Ewing sarcoma cells (5×10^6 of TC32 cells) suspended in 30% Matrigel™ have been inoculated subcutaneously [22]. Finally, the subcutaneous injection method is also employed to generate chondrosarcomas, as shown by Li et al. [23] and Wang et al. [24], who injected 5×10^6 of SW1353 cells and 10^6 c-Fos-transformed murine chondrosarcoma cells, respectively, into the hind limbs of nude mice. One million JJ012 human chondrosarcoma cells resuspended in 200 µL of serum-free medium [25] or diluted in 100 µL of medium,

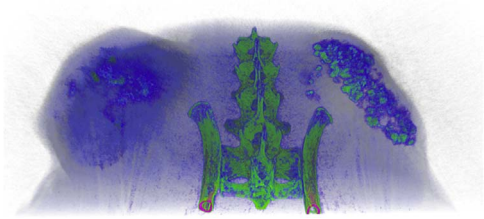

Fig. 2. Typical view of microCT image of luciferase expressing murine OS cell lines grown on the back flank of Balb/c nu/nu mice. Cells were implanted subcutaneously in matrigel. Mass on the left is control cells (control shRNA) and those on the right is expressing an shRNA directed against Pthr1. Pseudo coloring indicates intensity of the gray scale density of the tumour with green being most dense and blue least dense. (image generated by A. Goradia/M. Russell/C Walkley [13]). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)