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Mini-review

Cancer stem cells in osteosarcoma

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ABSTRACT

Osteosarcoma is the most common primary bone tumour in children and adolescents and advanced osteosarcoma patients with evidence of metastasis share a poor prognosis. Osteosarcoma frequently gains resistance to standard therapies highlighting the need for improved treatment regimens and identification of novel therapeutic targets. Cancer stem cells (CSC) represent a sub-type of tumour cells attributed to critical steps in cancer including tumour propagation, therapy resistance, recurrence and in some cases metastasis. Recent published work demonstrates evidence of cancer stem cell phenotypes in osteosarcoma with links to drug resistance and tumorigenesis. In this review we will discuss the commonly used isolation techniques for cancer stem cells in osteosarcoma as well as the identified biochemical and molecular markers.

Cancer stem cells and tumour heterogeneity: what do we know about osteosarcoma?

Osteosarcoma predominantly initiates in the metaphysis of the long bones with a high prevalence in children and young adults. The origin of a tumour is potentially a single cell located in the bone marrow, which will eventually give rise to a polyclonal, heterogeneous tumour mass. Analysis of tumour heterogeneity allows us to decipher the steps that were taken from the initiating cell to the development of a heterogeneous tumour mass comprised of an array of distinguishable sub-clones. Indeed, osteosarcoma initiates as a monoclonal disease, which quickly develops into a polyclonal disease and is considered one of the most complex cancers in terms of molecular aberration. Deeper insight into this diversity therefore holds great promise to identify markers associated with the most aggressive tumour cells within a tumour mass. The vast heterogeneity found in osteosarcoma is shown in an exome sequencing study in which multiple pathways (14 driver genes) were identified [1]. The authors suggest that no single driver gene can be pinpointed to be the cause of the majority of investigated tumours and that several oncogenic pathways cause genetic instability in osteosarcoma development. Importantly, this high level of heterogeneity adds increased complexity for effective treatment strategies, which is clinically reflected in refractory and recurrent disease.

The increasing knowledge of the cancer genome through in depth analysis using for example deep sequencing has significantly added to the understanding of intra-tumour heterogeneity and an evolutionary pattern of a subset of clones within a tumour has been reported [2]. New technologies now allow us to view heterogeneity also on a single cell level. This has clearly increased the tumour complexity over performing analysis on bulk tissue showing even deeper levels of intra-tumour heterogeneity in many cancer types [3–6]. Single cell analysis on CSCs in osteosarcoma has, to our knowledge, currently not been reported but could significantly help to understand the diversity of these cells.

Cells-of-origin in osteosarcoma and properties of cancer stem cells

In osteosarcoma several cell-of-origin models have been proposed including transformation of undifferentiated mesenchymal stem cells (MSCs) as well as more committed osteogenic progenitor

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cells [7] (Fig. 1). Osteosarcoma is a bone forming tumour invading frequently the surrounding soft tissues as revealed by conventional imaging associating X-ray and Magnetic Resonance Imaging (MRI) (Fig. 1A–C). Osteosarcoma is a vascularized tumour characterized by typical osteoid matrix formed by cancer cells (Fig. 1D and E). Evidence comes predominantly from *in vivo* studies using MSCs and/or osteoprogenitors in which for example mutations in genes such as *P53* and *RB* and/or aberrant Hedgehog and NOTCH signalling were shown to induce osteosarcoma [7–9]. The terminology of 'cancer stem cells' is still under debate with the association to stem cells remaining controversial. Indeed, cancer stem cells and cancer initiating cells are often used interchangeably although they may indeed exhibit different properties. In a cancer stem cell model, tumours are thought to be hierarchically organised with a subpopulation of self-renewing cells at the basis of tumour progression [10]. These cancer stem cells (CSCs) are proposed to be unique subsets of clones within a tumour mass attributed with tumour propagation, resistance to therapy and have in some studies been attributed to initiate metastases. Evidence exists in osteosarcoma that patients may present with distant metastases decades after completion of their first treatment [11] potentially further highlighting the tumorigenic characteristics of CSCs although this currently remains speculative. Similar to the origin of osteosarcoma, the existing hypotheses for the origin of CSCs in general include the transformation of undifferentiated stem cells or more committed cells to gain aberrant self-renewal properties [12]. Remarkably, a stem cell transcription factor (Sox2) has been shown to maintain osteosarcoma CSCs [13] through inhibition of the Hippo pathway [14]. The complex self-renewal process of normal stem cells [15] is partly regulated by external signals from the surrounding microenvironment, the stem cell niche. Such a specialised microenvironment has also been proposed for CSCs, influencing

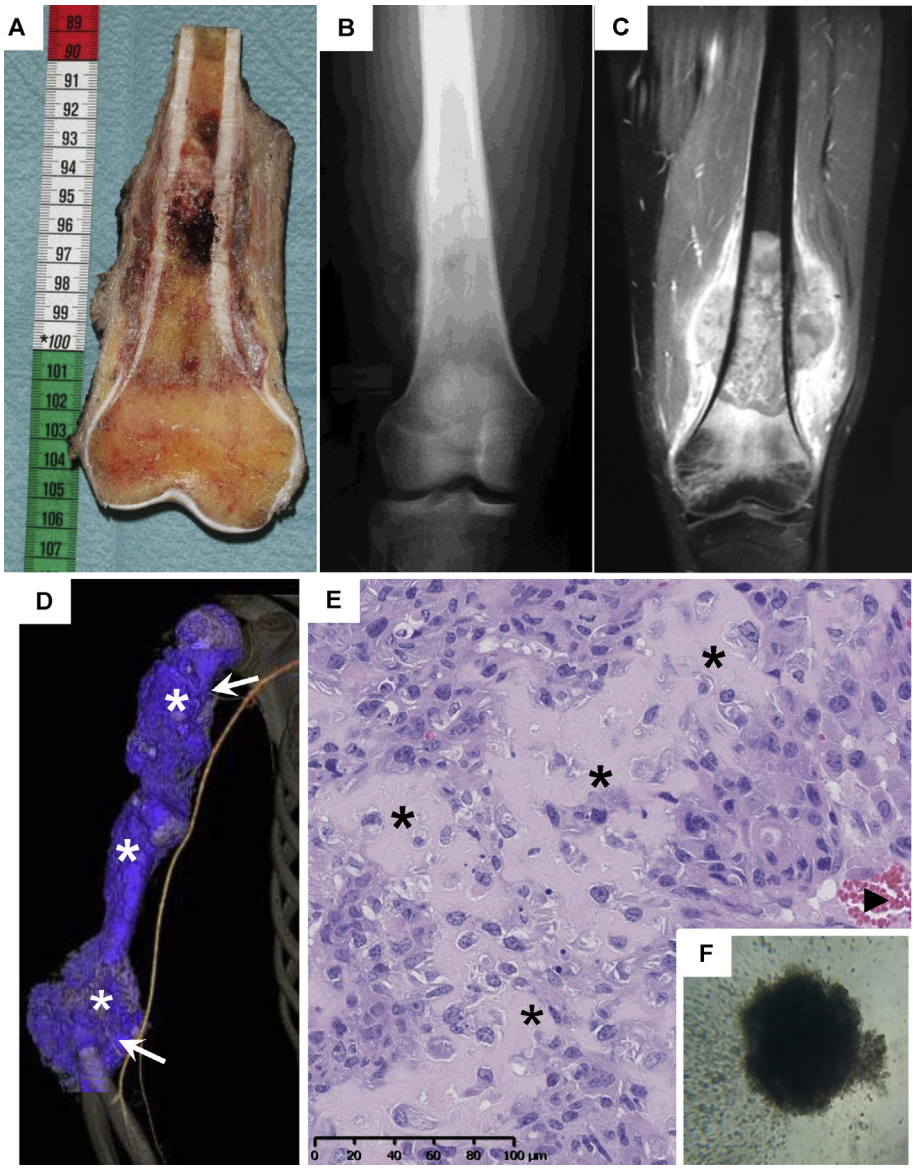

Fig. 1. Representative imaging of osteoblastic osteosarcoma. (**A**) Macroscopic view of a resected osteosarcoma infiltrating surrounding soft tissue. Conventional X-ray (**B**) and (**C**) Magnetic Resonance Imaging (MRI) of osteosarcoma. (**D**) Computed tomography of an osteosarcoma in a 15-year old patient (adapted from "Bone Cancer" 1st Edition, Ed. Heymann D., Academic Press, 2009). Tumour tissue is composed by mineralized component detectable to X-Ray (*) and is strongly associated with the vasculature (arrows). (**E**) Typical histological view showing osteoid extracellular matrix produced by osteosarcoma cells (*), tumour tissue is vascularized (arrow head). (**F**) Sarcosphere generated from the human MNNG-HOS osteosarcoma cell line under non-adherent serum-free conditions.