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Review

The Hippo signal transduction pathway in soft tissue sarcomas

Abdalla D. Mohamed [a], Annie M. Tremblay [c,d,e], Graeme I. Murray [b], Henning Wackerhage [a,*]

[a] School of Medical Sciences, University of Aberdeen, AB25 2ZD Scotland, UK
[b] School of Medicine and Dentistry, University of Aberdeen, AB25 2ZD Scotland, UK
[c] Stem Cell Program, Children's Hospital, Boston, MA 02115, USA
[d] Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[e] Harvard Stem Cell Institute, Cambridge, MA 02138, USA

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ABSTRACT

Sarcomas are rare cancers (≈1% of all solid tumours) usually of mesenchymal origin. Here, we review evidence implicating the Hippo pathway in soft tissue sarcomas. Several transgenic mouse models of Hippo pathway members (*Nf2*, *Mob1*, *LATS1 and YAP1* mutants) develop various types of sarcoma. Despite that, Hippo member genes are rarely point mutated in human sarcomas. Instead, *WWTR1-CAMTA1* and *YAP1-TFE3* fusion genes are found in almost all cases of epithelioid haemangioendothelioma. Also copy number gains of *YAP1* and other Hippo members occur at low frequencies but the most likely cause of perturbed Hippo signalling in sarcoma is the cross-talk with commonly mutated cancer genes such as *KRAS*, *PIK3CA*, *CTNNB1* or *FBXW7*. Current Hippo pathway-targeting drugs include compounds that target the interaction between YAP and TEAD G protein-coupled receptors (GPCR) and the mevalonate pathway (e.g. statins). Given that many Hippo pathway-modulating drugs are already used in patients, this could lead to early clinical trials testing their efficacy in different types of sarcoma.

Contents

1. Introduction

The Hippo pathway was discovered as a result of tumour suppressor screens in the fruit fly (*Drosophila melanogaster*) and was later found to be conserved in mammals. In flies, inactivation of Hippo members resulted in an overgrowth that resembles the skin of a Hippopotamus, thereby naming the pathway [1]. Hippo pathway members are rarely point mutated in cancer [2,3] but the experimental mutation of Hippo members usually causes overgrowth in fruit flies [4] and tumours in mice, including sarcomas [2,3,5]. Here, we review the role of the Hippo pathway specifically in soft tissue sarcomas excluding osteosarcomas

* Corresponding author.
E-mail address: h.wackerhage@abdn.ac.uk (H. Wackerhage).

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and viral-mediated sarcomas such as Kaposi's sarcoma. After introducing sarcomas and the Hippo pathway, we review animal models where Hippo pathway-related transgenesis results in sarcomas. We then discuss findings and hypotheses implicating the Hippo pathway in sarcoma signal transduction, development and pathology. Finally, we review the current possibilities for Hippo pathway-targeting treatments in sarcoma and highlight areas for future research.

2. Pathology of soft tissue sarcomas

Soft tissue sarcomas (from Greek *sarx* flesh) represent a biologically, clinically, pathologically, and genetically [6] diverse array of malignant tumours arising mostly from mesenchyme-derived tissues. Sarcomas can occur in both adults and children. They are rare cancers and account for about 1% of solid tumours in adults and for a significantly higher proportion of cases in children (Cancer Research UK, 2015). In 2014, it was estimated that 790 new cases of rhabdomyosarcoma and bone tumours, including osteosarcomas and Ewing sarcomas, would be diagnosed in children 0–14 years old, representing 7% of all childhood cancers (American Cancer Society, 2014). The most common types of soft tissue sarcomas include leiomyosarcoma, liposarcoma, fibrosarcoma, rhabdomyosarcoma and angiosarcoma (see Fig. 1 [7]).

The current classification of soft tissue sarcomas is based on a combination of tumour morphology, immunophenotype and molecular pathology. Genetically, sarcomas can show either aberrant, chimeric transcription regulators as a consequence of fusion genes such as *PAX3/7-FOXO1* [6,8], somatic point mutations of well-known cancer genes such as oncogenic *RAS* isoforms, *PIK3CA* or *TP53*, or DNA copy number gains or losses [6,9–12]. As we will show later, there is no evidence for recurrent Hippo gene point mutations in sarcomas, while there is abundant evidence for mutations of typical cancer genes [13,14] that can cross-talk to the main members of the Hippo pathway. Additionally, fusion genes involving *WWTR1* or to a lesser extent *YAP1* are found in nearly all cases of epithelioid haemangioendothelioma [15,16], whereas *YAP1* and *VGLL3* copy number gains have been reported for some types of sarcoma, especially rhabdomyosarcoma [17–19]. It is unclear to this date whether VGLL3, which is associated with tumour suppression in ovarian cancer [20], is a *bona fide* Hippo pathway member.

The increasing use of immunohistochemistry and the application of sophisticated molecular techniques combined with a better understanding of soft tissue sarcoma biology have led to a continued refinement of the classification of soft tissue sarcomas. Some previously recognised types of soft tissue sarcoma, most notably malignant fibrous histiocytoma, are now being reclassified. Most tumours previously classified as malignant fibrous histiocytomas but showing no specific immunophenotype would now be regarded as undifferentiated pleomorphic sarcomas. The majority of soft tissue sarcomas arise in the limbs of older people although rhabdomyosarcoma, especially the embryonal subtype, tends to occur predominantly in young children [21]. Most soft tissue sarcomas arise sporadically but muscle injury, most likely by increasing the number of activated satellite cells, enhances the penetrance and shortens the latency of rhabdomyosarcoma phenotypes in mice [18,22]. In addition, known risk factors for the development of specific types of sarcoma in humans include exposure to radiation (e.g. post-radiation angiosarcoma) or exposure to environmental/occupational carcinogens (e.g. vinyl chloride-associated angiosarcoma). Sarcomas, in contrast to carcinomas, have a predilection for showing a vascular pattern of spread with the lungs generally being one of the predominant sites of metastasis.

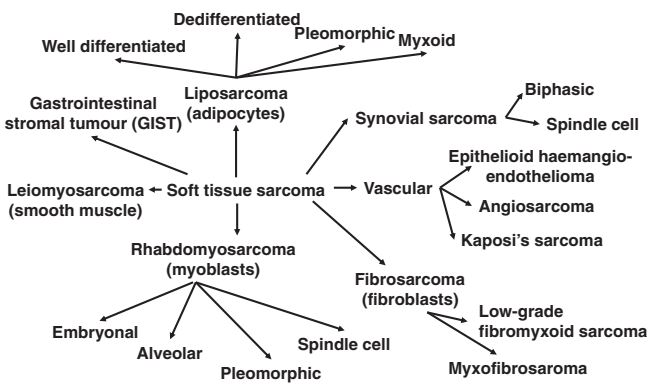

Fig. 1. Classification of soft tissue sarcomas on the basis of their differentiation.

A combination of surgery, cytotoxic chemotherapy and radiotherapy are the mainstays of current treatment with some types of sarcoma being treated with neoadjuvant chemo-radiotherapy prior to definitive surgery [23–25]. Immunotherapy and targeted, novel biologic therapies are also now being evaluated in the treatment of specific types of sarcoma [26–29] and as we show below, targeting the Hippo pathway in sarcoma should already be possible with existing drugs.

The prognosis and outcome of soft tissue sarcomas depend on a range of factors including the particular subtype of tumour, the anatomical location, size and grade as well as tumour stage at time of diagnosis [7,30,31]. The overall 5-year survival rate for soft tissue sarcomas has gradually been improving, especially in children, but the outcome varies with the specific type of soft tissue sarcoma and depends on the prognostic factors that have been outlined above. Overall the 5-year survival rate for adults with soft tissue sarcomas is approximately 60% while it is higher in children, reaching approximately 70% (Cancer Research UK, 2015).

3. Hippo pathway & cancer

The fruit fly (*D. melanogaster*) has been used over many decades as a model organism to identify genes whose knockout results in cancerous growth [32]. This research has led to the discovery of a set of genes that encode two interacting kinases and auxiliary proteins, now defined as the core Hippo pathway (see Fig. 2). The main function of the Hippo pathway is to inhibit proliferation and to promote apoptosis, thereby limiting organ growth [4]. In the conserved mammalian Hippo pathway, the STE20-like protein kinases 1 and 2 (MST1 and MST2, gene symbols: *STK4* and *STK3*) regulate the large tumour suppressor kinases 1 and 2 (protein name and gene symbol: *LATS1* and *LATS2*), through phosphorylation [33]. Active *LATS1* and LATS2 then interact through their PPxY motifs with the WW domains of the transcriptional co-factors YAP (gene symbol *YAP1*) or TAZ (gene symbol *WWTR1*; note that the gene *TAZ* encodes a protein termed Tafazzin which is not part of the pathway) [34]. This physical contact allows LATS1 and LATS2 to inhibit YAP [35] and TAZ [36] through the phosphorylation of multiple HXRXXS amino acid motifs. The phosphorylation of these motifs promotes the inactivation of YAP and TAZ through translocation from the nucleus into the cytosol and degradation. Additionally, YAP can be phosphorylated at Tyr357 by the tyrosine kinase YES1, which has resulted in its name Yes-associated protein (YAP) [37]. Nuclear and active YAP, which was first discovered by Marius Sudol [38,39], and its paralogue TAZ is believed to exert their tumourigenic functions mainly via the TEAD transcription factors [40]. Specifically, YAP and presumably TAZ de-repress and activate the TEAD transcription factors that otherwise recruit transcriptional repressors [41]. Additionally, YAP and TAZ are capable of co-regulating other transcription factors including those belonging to the Smad family [42] and Tbx5 in some contexts [37,43]. In addition to the Hippo kinases, extensive cross-talk mechanisms also regulate the activity of YAP and TAZ, notably mechanotransduction [44], WNT signalling [45,46], and G protein-coupled receptors [47].

The early studies in fruit flies and subsequent studies in mammals demonstrate that the upstream proliferation-inhibiting Hippo proteins and the proliferation-promoting Hippo transcriptional regulators act as potent tumour suppressors and oncogenes, respectively. For