ABSTRACT INTRODUCTION:
Clear cell sarcoma (CCS), also called malignant melanoma of soft parts, is an aggressive malignant soft-tissue tumor that usually develops in the tendons and aponeuroses of the extremities of young adults (Enzinger, 1965). The origin and pathogenesis of this disease are poorly understood, and beneficial therapy—except for surgical resection—has not yet been developed. The high rates of local recurrence and metastasis result in a 5-year overall survival rate of less than 50%. The characteristic t(12;22)(q13;q12) chromosomal translocation seen in CCSs leads to a fusion gene that encodes the Ewing’s sarcoma (EWS)/activating transcription factor 1 (ATF1) fusion protein (Bridge et al., 1990). Previous studies have shown that EWS/ATF1 has oncogenic potential in CCS in vitro, but the effect of EWS/ATF1 on sarcoma formation in vivo is not yet known (Davis et al., 2006; Jishage et al., 2003). By analyzing EWS/ATF1 transgenic mice, we aim to determine whether EWS/ATF1 expression is sufficient for CCS induction.

METHODS:

Establishment of inducible expression of EWS/ATF1 in mice

To test the effect of EWS/ATF1 expression on tumorigenesis, we established a mouse model in which reversible EWS/ATF1 expression was induced in various somatic tissues by using a tetracycline-inducible system (Beard et al., 2006).

Doxycycline induction
Mice were fed 50 µg/ml doxycycline added to drinking water supplemented with 2 mg/ml sucrose.

Histological analysis
Tissue samples were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin or used for immunohistochemical analysis.

Establishment of an EWS/ATF1-inducible sarcoma cell line
To establish a cell line from EWS/ATF1-induced tumors, a tumor sample was obtained from the primary tumor on the ventral trunk of an EWS/ATF1-inducible mouse that had been treated with 50 µg/ml doxycycline. For cultured cells, doxycycline was used at a concentration of 0.2 µg/ml.

Tumor formation assay
A total of 5.0 × 10^6 tumor cells were subcutaneously inoculated through a 26-gauge needle into the posterior flanks of the mice. Three weeks after the inoculation, tumor diameters were measured using digital calipers, and the tumor volume (mm^3) was calculated using the formula: volume = (width)^2 × length/2.

RESULTS SECTION:
EWS/ATF1 induced one or more tumors in all the transgenic mice (N = 38). The macroscopic tumors were located in the hypodermis of the following parts: trunk (N = 31), legs (N = 9), head (N = 6), whisker pad (N = 6), and tail (N = 1). Macroscopic tumors could not be detected in other sites. Histological analysis showed that the macroscopic tumors consisted of round-to-polygonal cells with clear cytoplasm. Immunohistochemical studies showed that the tumor cells expressed S100, sex determining region Y-box 9 (Sox9), sex determining region Y-box 10 (Sox10), and microphthalmia-associated transcription factor (Mitf). To elucidate the function of the EWS/ATF1 fusion protein in the sarcomas, we harvested and performed in vitro culture of tumor cells. The proliferation of the tumor cells depended on the in vitro expression of EWS/ATF1. In contrast, EWS/ATF1 expression in mouse embryonic fibroblasts (MEFs) decreased cell proliferation. The results indicated that EWS/ATF1 induces transformation in only specific cell types. Moreover, EWS/ATF1 induction in the tumor cells that were injected in nude mice caused tumorregenesis. EWS/ATF1 suppression in the tumor cells did not lead to tumorigenesis.

DISCUSSION:
We have shown that expression of the fusion protein EWS/ATF1 induces sarcoma formation in EWS/ATF1 transgenic mice. The histological features of the tumors in EWS/ATF1 transgenic mice were found to be consistent with those of human clear cell sarcomas. Immunohistochemical analysis showed the expression of neural crest-associated markers such as S100, Mitf, Sox9, and Sox10, in addition to that of the EWS/ATF1 fusion protein.

In this study, the results clearly indicate that EWS/ATF1 expression is sufficient for sarcoma formation. All the EWS/ATF1-inducible mice developed multiple sarcomas in only 4–6 weeks after induction by doxycycline. Our results suggest that additional genetic changes such as p53 mutation or epigenetic changes are not indispensable for transformation. Moreover, the fact that EWS/ATF1 expression in MEFs suppressed cell proliferation but did not cause any morphological change suggest that EWS/ATF1 causes transformation only in specific types of cells.

The origin of CCSs and the mechanism underlying sarcoma formation caused by EWS/ATF1 expression are unclear, and more elaborate studies involving mice are necessary in this regard.

SIGNIFICANCE:
In this study, we report an EWS/ATF1 transgenic mice model where a CCS-like sarcoma was induced by EWS/ATF1. EWS/ATF1 is a strong initiator of sarcoma formation.

REFERENCES: