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Activation of mTORC1 in chondrocytes does not affect proliferation or differentiation, but causes the resting zone of the growth plate to become disordered

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ABSTRACT

There are several pitfalls associated with research based on transgenic mice. Here, we describe our interpretation and analysis of mTORC1 activation in growth plate chondrocytes and compare these to a recent publication (Yan et al., Nature Communications 2016, 7:11151). Both laboratories employed TSC1-floxed mice crossed with collagen type 2-driven Cre (Col2-Cre), but drew substantially different conclusions. It was reported that activation of mechanistic target of rapamycin complex 1 (mTORC1) *via* Tsc1 ablation promotes the hypertrophy of growth plate chondrocytes, whereas we observe only disorganization in the resting zone, with no effect on chondrocyte hypertrophy or proliferation. Here, we present our data and discuss the differences in comparison to the earlier phenotypic characterization of *TSC1* ablation in cartilage. Importantly, we detect Col2-Cre activity in non-cartilaginous tissues (including the brain) and discuss it in relation to other studies reporting non-cartilaginous expression of collagen alpha(1) II. Altogether, we conclude that mouse phenotypes following genetic ablation using Col2-Cre should be interpreted with care. We also conclude that activation of mTORC1 by *TSC1* ablation in postnatal chondrocytes with inducible Col2-Cre (Col2-CreERt) leads to disorganization of the resting zone but causes no changes in chondrocyte proliferation or differentiation.

1. Introduction

mTOR is a serine/threonine kinase activated by complex formation and this complex (mTORC) 1 coordinates anabolic (protein and lipid synthesis) and catabolic activities (autophagy) (Laplante and Sabatini, 2012). Both the function and sequence of mTOR have been highly conserved during evolution. For example, the yeast homolog of mTOR, TOR, regulates cell growth in response to nutrient supply (Laplante and Sabatini, 2012). The drosophila homolog, dTOR, also controls cell growth, as well as regulating body size (Oldham, 2011). With respect to mammals, mTORC1 signaling controls cell size in isolated systems, including liver, and in both skeletal and cardiac muscle (Lee et al., 2007). Genetic ablation of *MTOR* or *RPTOR* in the whole limb results in these structures being extremely small (Chen and Long, 2014).

We previously reported that activation of mTORC1 signaling stimulates bone growth *in vitro* (Newton et al., 2015) and aimed to explore its role *in vivo* by using Col2-Cre mice to ablate the gene encoding a key inhibitor of mTORC1, tuberous sclerosis 1 (*TSC1*). Although these mice displayed severe growth retardation starting after 2 weeks of age, we were dismayed that they also developed chronic wasting and seizures associated with premature death, which might be attributed to leakage of Cre in non-cartilaginous tissues. At the same time, a recent study based on this same model came to the conclusion that mTORC1 coordinates chondrocyte growth, proliferation and differentiation, in part *via* regulation of PTHrP (Yan et al., 2016).

Here, we suggest an alternative explanation to the phenotype described by Yan et al. (2016) based on actions of Tsc1 in cells other than chondrocytes. Furthermore, our present considerations are of key relevance to all experimental approaches involving tissue-specific gene ablation.

2. Results

1. Ablation of Tsc1 using Col2-Cre causes severe developmental abnormalities

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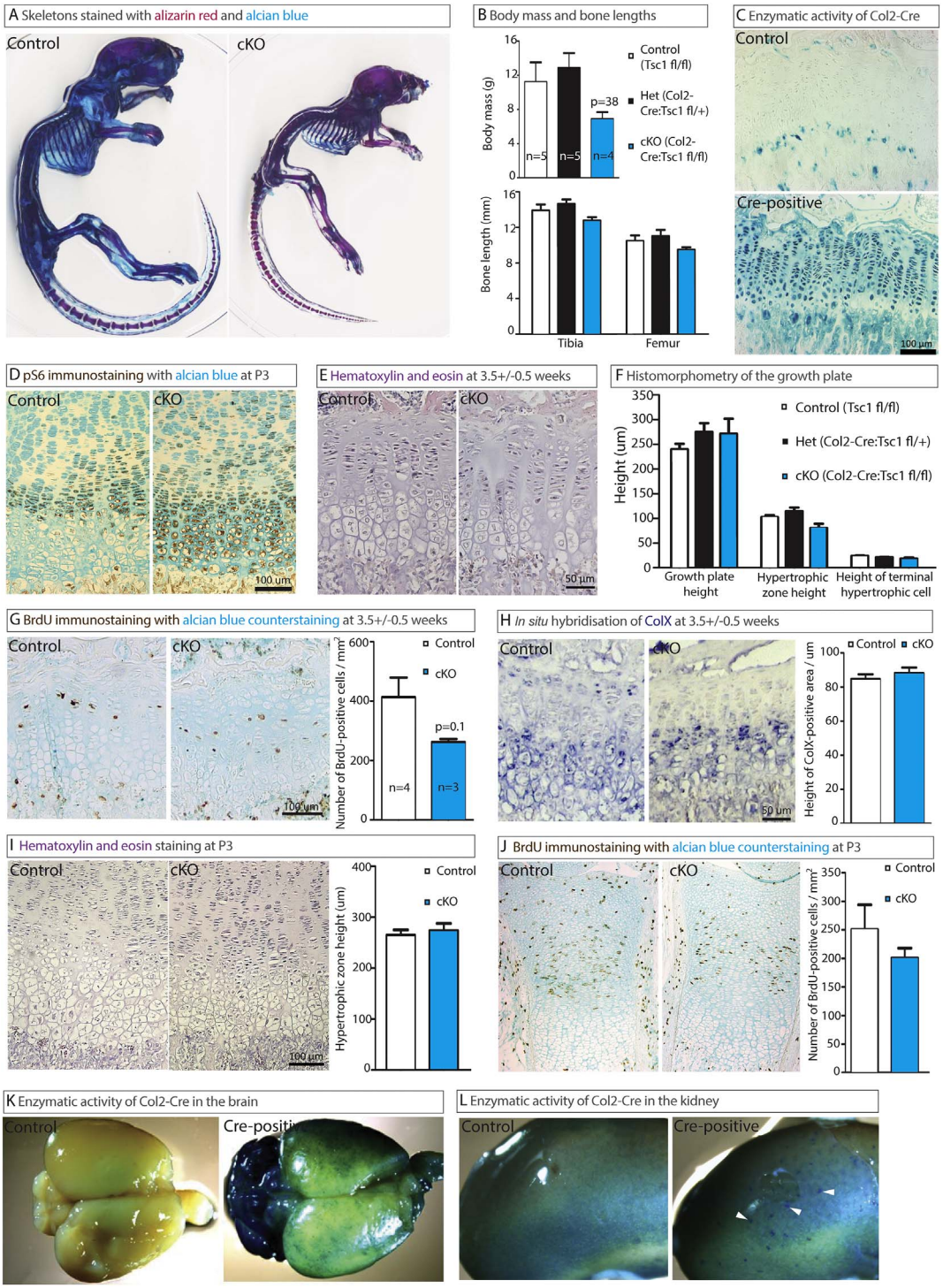

Fig. 1. Col2-Cre:*Tsc1* fl/fl mice develop abnormally, but their bones are relatively normal. (**A**) Skeletal preparations from one-month-old mice stained with alcian blue and alizarin red. (**B**) The body mass and bone length of mice at 3.5 ± 0.5 weeks of age. (**C**) The enzymatic activity of Cre in sections of growth plate from the three-week-old progeny of Rosa26 reporter mice crossed with Col2-Cre:*Tsc1* fl/fl mice (upper panel – no Cre; lower panel - Cre-positive). (**D**) Immunohistochemical staining for phosphorylated S6 in the growth plate of 1-day-old mice. (**E**) Staining of sections of the growth plate of 3.5 ± 0.5-week-old mice with hematoxylin and eosin (H&E) for (**F**) morphometric analysis. (**G**) Immunohistochemical quantification of BrdU (injected 2 h prior to sacrifice) and quantification in 3.5 ± 0.5-week-old mice. (**H**) *In situ* hybridization and quantification of COL10A1 transcription in the growth plates of mice at 3.5 ± 0.5 weeks of age. (**I**) H&E staining for histomorphometry of the growth plates of three-day-old mice. (**J**) Immunohistochemical quantification of BrdU (injected into 2 ± 1-day old mice 2.25 ± 0.25 h prior to sacrifice). (**K**, **L**) The enzymatic activity of Cre in the whole brain and kidney of Col2-Cre:Rosa26 reporter mice, as determined by staining with X-gal at one month of age. In this figure, all controls are Col2-Cre-negative mice and all cKO mice have the genotype Col2-Cre:*Tsc1* fl/fl, unless otherwise stated. The values presented are means ± SEM. No significant differences were detected between control and cKO mice (for G–J) utilizing either one-way ANOVA or an unpaired *t*-test.

We start by making direct comparisons below between our work and the similar experiments performed by Dr. Yan et al. (2016), we refer to our figures in **bold letters** and those of Yan and colleagues in *italics*.

Like Yan et al. (2016), we ablated *TSC1* in murine chondrocytes utilizing non-inducible Collagen2-driven Cre (Kobayashi et al., 2002) and observed similar growth retardation in the resulting mice (**Fig. 1A** and **B** *versus Fig. 3c* and *6b* (Yan et al., 2016)). Our ablation was