Visualization of ectopic calcification in skeletal muscle of mdx mice

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It has been demonstrated that osteogenic differentiation of skeletal muscle cells are induced by osteogenic factors, such as bone morphogenetic proteins, both in vitro and in vivo. Spontaneous ectopic bone formation in vivo has also been reported in, for instance, fibrodysplasia ossificans progressiva, which is a rare bone disorder. Another example of self-generating ectopic calcification has been found in skeletal muscle of mdx mouse, a model of Duchenne muscular dystrophy (DMD).

We observed the ectopic calcification in mdx mouse thigh muscle by using x-ray micro CT-scanner SkyScan-1074, which gives resolution of 22um. The x-ray images were reconstructed into three dimensional visions. Calcifications were found as spicular structures running parallel to the muscle fiber. Paraffin sections of the regions found by X-ray microtomography were Von Kossa stained to confirm calcium deposits. We also detected ectopic calcifications of living mice with mouse-whole-body x-ray CT-scanner of Aloka. No ectopic calcification was observed in skeletal muscle of B10 mouse, which possesses normal dystrophin gene and was used as a negative control. Calcified regions of mdx mouse thigh did not overlap with the Evans blue-positive areas but corresponded to some of the regenerating areas. Thus, ectopic calcification can be a diagnostic marker of muscle regeneration in mdx mice, and be available to determine the effects of drug or gene therapies.