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Smooth muscle features of mouse extraocular muscle

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We have been intrigued to find smooth muscle markers within mouse extraocular muscle (EOM) while studying contractile features of the aqueous humour drainage tissues by immunohistochemistry. Smooth muscle is known to be present in connective tissue fascial pulleys ensheathing EOM¹ but not in the EOM per se. The mouse has many available engineered strains, shares many biological similarities with primates, and is widely used to model human biology, including that of EOM.² Herein, we show that classic smooth muscle proteins of alpha-smooth muscle actin (α -SMA), myosin heavy chain (MHC), caldesmon, and tropomyosin are intimately associated with EOM fibre bundles, a finding that may be relevant to better understanding EOM control.

We studied C57BL/6 mice (2-3 months, Charles River, Wilmington, MA) that were housed in a temperature-controlled room with 12h light and dark cycles and fed ad libitum. Animal care and use complied with the Institutional Animal Care and Use Committee as well as the Association for Research in Vision and Ophthalmology guidelines.

Anterior (insertion) and posterior (retro-equator) portions of four rectus EOM of C57BL/6 mice (n=5) were carefully isolated, quickly embedded in OCT compound and snap-frozen in liquid nitrogen. Eight μ m-thick cryosections were fixed with 4% paraformaldehyde, permeabilised and blocked (0.3% Triton X-100+5% BSA), incubated with primary antibodies (Abcam) to α -SMA, MHC non-muscle, caldesmon, and tropomyosin overnight at 4°C, then secondary antibodies and Alexa 568-phalloidin. Vascular smooth muscle labeling of the same tissues represented positive controls, while normal IgG isotype labeling represented negative controls. Sections were analysed by Leica SP5 or Zeiss LSM 710 confocal microscopy. The same tissue slides were further processed for hematoxylin and eosin staining to confirm structure.

Figure 1 shows representative longitudinal and cross sectional immunohistochemistry images of the mouse anterior EOM at their globe insertions. F-actin labeling localized to contractile regions that were mostly consistent with striated muscle fibre bundles. Positive labeling for α -SMA, MHC, and caldesmon labeling was present in the periphery of phalloidin-positive muscle fibre bundles. Positive tropomyosin labeling was seen centrally

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and peripherally in muscle bundles, corresponding to regions of striated and presumed smooth muscle elements. Smooth muscle marker labeling partially overlapped with phalloidin labeling in fibre bundles.

Figure 2 shows the distribution of smooth muscle markers in representative longitudinal and cross sections of rectus muscles posteriorly. All recti showed this pattern. As in the anterior EOM, α -SMA, MHC, and caldesmon labeling localized to the periphery of phalloidin-labeled bundles, while tropomyosin was present peripherally and centrally. Positive immunolabeling using the same prior antibody panel was seen in vascular smooth muscle from the same tissues (not shown).

Orbital smooth muscle is present in superior and inferior palpebral muscles, inferior orbital fissure and EOM fascial pulleys¹ as part of a periorbital smooth muscle network.⁵ To our best knowledge smooth muscle has not been described in EOM, which is considered striated muscle. Here we describe classic smooth muscle markers within mouse EOM, mostly in the periphery of striated muscle fiber bundles. Smooth muscle was present in all rectus muscles at their insertions and retro-equatorially, indicating this organization is widely present in mouse EOM.

The EOM and their supporting structures represent a complex that helps orchestrate eye movement. Striated EOM are organized as global and orbital layers, each with different structural, vascular, neural, mechanical and metabolic features.³⁻⁴ Fascial sheaths and pulleys influence muscle actions on the globe¹ under possible smooth muscle modulation. As with autonomically-innervated palpebral smooth muscle that works with striated muscle to regulate eyelid position,⁵ we postulate that smooth muscle associated with striated EOM plays a role in determining eye position in mice, and possibly in humans.

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Figure 1.

Smooth muscle profile in the anterior part of 4 different rectus extraocular muscles (EOMs). Co-localisation of α-SMA, MHC, caldesmon, or tropomyosin with phalloidin in the anterior portion of EOM. All 4 different rectus EOMs demonstrated a similar in situ distribution and co-localisation of each marker. α-SMA: alpha-smooth muscle actin; MHC: myosin heavy chain, non-muscle.

Figure 2.

Smooth muscle profile of the posterior part of 4 different rectus EOMs. Co-localisation of α -SMA, MHC, caldesmon, or tropomyosin with phalloidin in the posterior portion of EOM was shown. All 4 different rectus EOMs demonstrated a similar in situ distribution and co-localisation of each marker. α -SMA: alpha-smooth muscle actin; MHC: myosin heavy chain, non-muscle.