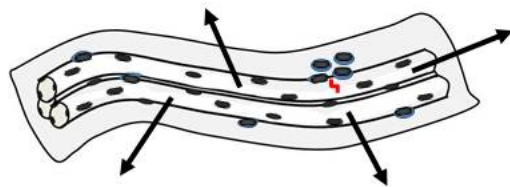


Serum osteopontin as a novel biomarker for muscle regeneration in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is an X-linked muscular disease, affecting boys in principle. It is caused by mutations in the gene coding for dystrophin, a membrane-related structural protein in skeletal and cardiac muscles. The lack of dystrophin results in the fragility of muscle fibers against mechanistic stress, and muscle fibers are easy to degenerate. Consequently, DMD boys suffer from progressive muscle wasting, leading to wheel chair dependency and early death by cardiac or respiratory failure at age 20-40.

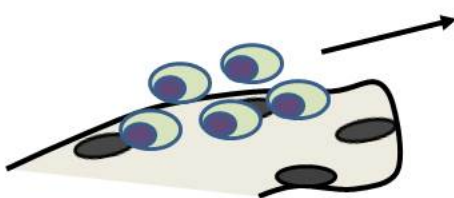
1) Muscle fiber degeneration



creatine kinase

Drastic elevation due to leakage from degenerating muscle fibers.

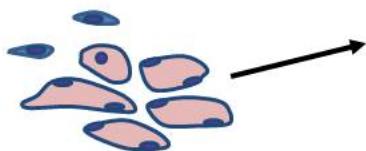
2) Secondary inflammation



OPN, MMP-9

Synthesized by inflammatory cells (e.g. macrophages).

3) Muscle fiber regeneration



OPN

Synthesized by regenerating muscle fibers.

Fig. 1. Serum biomarkers in the dystrophic pathology.

1) Muscle fibers are shown as elongated tubes containing many nuclei. Dystrophin deficient muscle fibers are result in degeneration due to the fragility against mechanical stress.

2) Inflammatory cells including macrophages are attracted and repair of fibers is initiated.

Osteopontin (OPN) as well as other factors mediate these events. The macrophages clear muscles of dead cells and debris using, among other tools, matrix metalloproteinase (MMP)-9.

3) OPN has functions to stimulate repair by promoting differentiation and fusion of stem cells with existing fibers.

Biomarkers are ideal tools to classify and predict the clinical conditions of DMD patients. Serum creatine kinase (CK) is an established primary biomarker for muscle damaging, however it is insufficient for evaluating therapeutic effects due to a limitation as a biomarker; too sensitive to stimuli, such as exercise. Therefore, biomarkers devoid of this problem are being explored by researchers worldwide.

Osteopontin (OPN) is a potential new candidate biomarker for DMD. OPN has various functions, including activation of immune cells as well as of muscle progenitor cells, such as myoblasts. In dystrophic muscles undergoing degeneration, OPN promotes inflammation and collagen deposition as well as muscle regeneration. Using a canine model of DMD, namely the Canine X-linked muscular dystrophy in Japan (CXMD_J), we found that OPN is produced in the muscles during the early dystrophic phase (Nakamura *et al. Sci Rep.* 3:2183. 2013). It appeared that OPN has the potential to become a readily quantifiable biomarker for disease activity. Note that CXMD_J dogs were bred in our Institute in 2000 by crossing golden retriever muscular dystrophy (GRMD) with normal beagles.

In a recent publication (Kuraoka *et al. Am J Pathol.* 186:1302-1312. 2016), we monitored serum OPN levels in CXMD_J and healthy beagles at different ages, and compared the values obtained to those of other biomarkers.

Serum OPN levels in CXMD_J dogs were elevated before and 1 hour after birth and at the age of 3 months as the early dystrophic phase. OPN has also been observed to be synthesized by infiltrating macrophages, a major cell type taking part in damaging muscle, as well as by embryonic Myosin heavy chain positive regenerating muscle fibers. Increased OPN expression was also observed during the process of muscle regeneration induced by cardiotoxin injection.

The serum elevation pattern of OPN was different from other biomarkers, including CK and matrix metalloproteinase-9 (MMP-9), an enzyme causing tissue destruction and inflammation. Regarding a role as biomarkers, it appears that OPN reflects muscle regeneration, whereas CK and MMP-9 monitor muscle damage and inflammation, respectively. Importantly, serum OPN levels were well correlated with the clinical severity of CXMD_J dogs at the onset of muscle weakness, whereas other markers displayed no such correlation.

In conclusion, OPN is a promising biomarker for muscle regeneration during the onset of the dystrophic process and has the potential to be applicable to DMD boys. We expect that serum OPN will be used as a surrogate endpoint in clinical trials of drugs that counteract the dystrophic pathology.

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