

Osteopontin, inflammation and myogenesis: influencing regeneration, fibrosis and size of skeletal muscle

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Abstract Osteopontin is a multifunctional matricellular protein that is expressed by many cell types. Through cell-matrix and cell-cell interactions the molecule elicits a number of responses from a broad range of target cells via its interaction with integrins and the hyaluronan receptor CD44. In many tissues osteopontin has been found to be involved in important physiological and pathological processes, including tissue repair, inflammation and fibrosis. Post-natal skeletal muscle is a highly differentiated and specialised tissue that retains a remarkable capacity for regeneration following injury. Regeneration of skeletal muscle requires the co-ordinated activity of inflammatory cells that infiltrate injured muscle and are responsible for initiating muscle fibre degeneration and phagocytosis of necrotic tissue, and muscle precursor cells that regenerate the injured muscle fibres. This review focuses on the current evidence that osteopontin plays multiple roles in skeletal muscle, with particular emphasis on its role in regeneration and fibrosis following injury, and in determining the severity of myopathic diseases such as Duchenne muscular dystrophy.

Keywords Osteopontin · Regeneration · Skeletal muscle · Whole muscle grafting · Myoblast · Neutrophil · Macrophage

Skeletal muscle structure, injury and repair

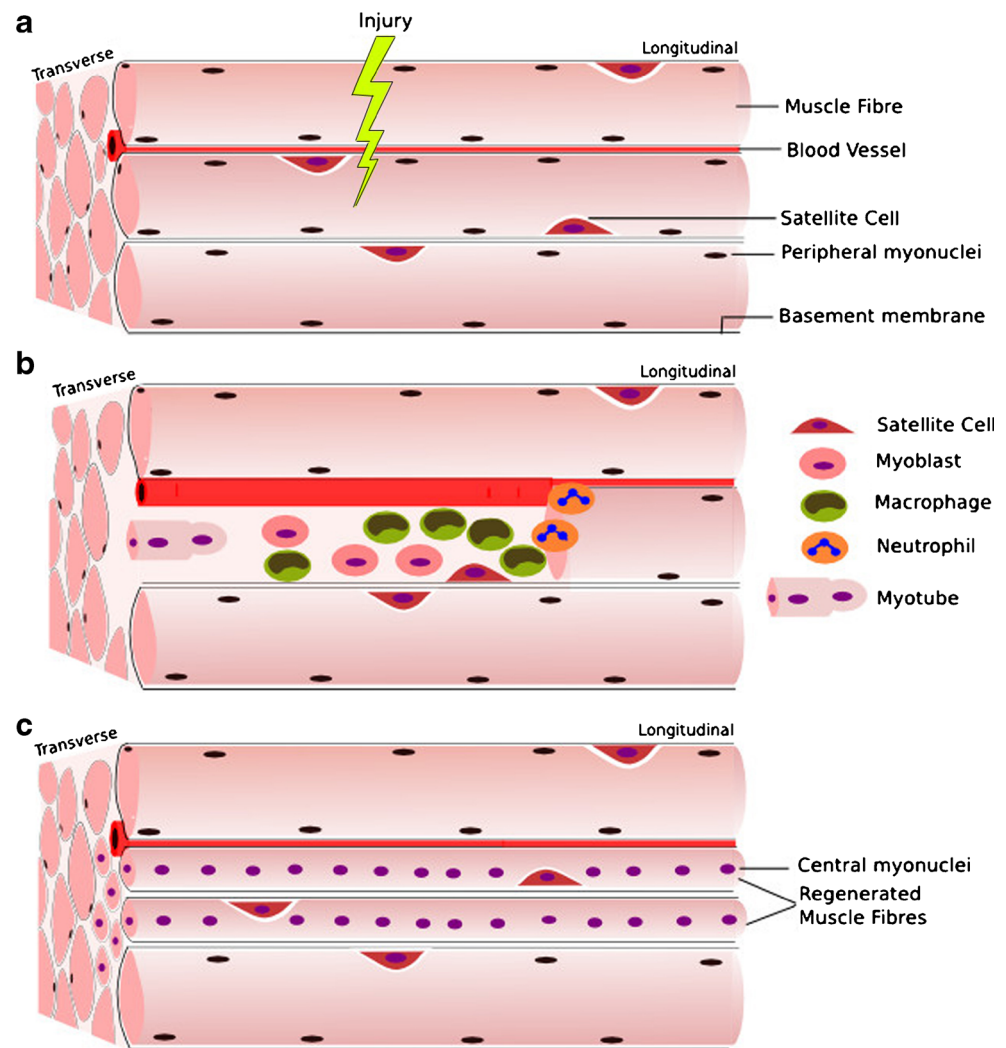
Skeletal muscle is largely composed of muscle fibres that are elongated cells, each containing multiple post-

mitotic nuclei constituting a syncytium (Fig. 1). During embryonic development and post-natal regeneration, muscle fibres form by the fusion of mononucleate, mitotically active and determined myogenic cells known as myoblasts. Myoblasts in postnatal skeletal muscle are derived from quiescent myogenic cells, known as satellite cells, that remain sandwiched between the surrounding basal lamina and the cell membrane of postnatal muscle fibres (reviewed in Zammit et al. 2006).

The ability of postnatal skeletal muscle to adapt to changing use or to regenerate in response to even very severe trauma is a remarkable characteristic of this tissue. In response to muscle injury, satellite cells become activated due to the action of mitogenic factors, including fibroblast growth factor 2 (FGF2) and hepatocyte growth factor, which are released from damaged muscle fibres (Allen et al. 1995; Chen et al. 1994; Tatsumi et al. 1998). As well as the activation of satellite cells, injury to skeletal muscles also induces their infiltration by inflammatory cells including neutrophils and macrophages (Nguyen and Tidball 2003). Inflammatory cells play crucial roles in promoting skeletal muscle regeneration (Lescaudron et al. 1999; Nguyen and Tidball 2003; Tidball 2005), and recent studies have highlighted the critical roles played by phenotypically distinct macrophages during the early (T_h1 dominated) and later (T_h2 dominated) inflammatory responses to muscle injury (Tidball 2011). As necrotic muscle fibres are removed from injured muscles, myoblasts formed by activation of satellite cells at the site of injury are induced to undergo myogenic differentiation, withdraw from the cell cycle, align and fuse to regenerate damaged segments of fibres (Fig. 1; Collins et al. 2005). The cues that induce myogenic differentiation in injured muscles are poorly understood, but undoubtedly involve components of the extracellular matrix and growth factors in the local microenvironment (Florini et al. 1991; von der Mark and Ocalan 1989).

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Fig. 1 A schematic representation showing the processes involved in regeneration of skeletal muscle following injury. **a** Mature skeletal muscle is composed of closely packed elongated muscle fibres which contain peripherally located, post-mitotic myonuclei sharing a common cytoplasm. Quiescent mononucleated satellite cells are located between the sarcolemma and the basement membrane of muscle fibres. **b** In response to damage, injured muscle fibres undergo necrosis, resulting in activation of endogenous satellite cells to form a pool of mitotic myoblasts, and infiltration of the site of injury from the vasculature by inflammatory cells including neutrophils and macrophages. Eventually, myoblasts align, fuse and terminally differentiate to form primitive muscle fibres called myotubes. **c** As skeletal muscle regeneration proceeds, whole muscle fibres or new segments of injured muscle fibres are formed by the terminal differentiation of myoblasts. In murine muscle, the nuclei of regenerated fibres remain centrally located



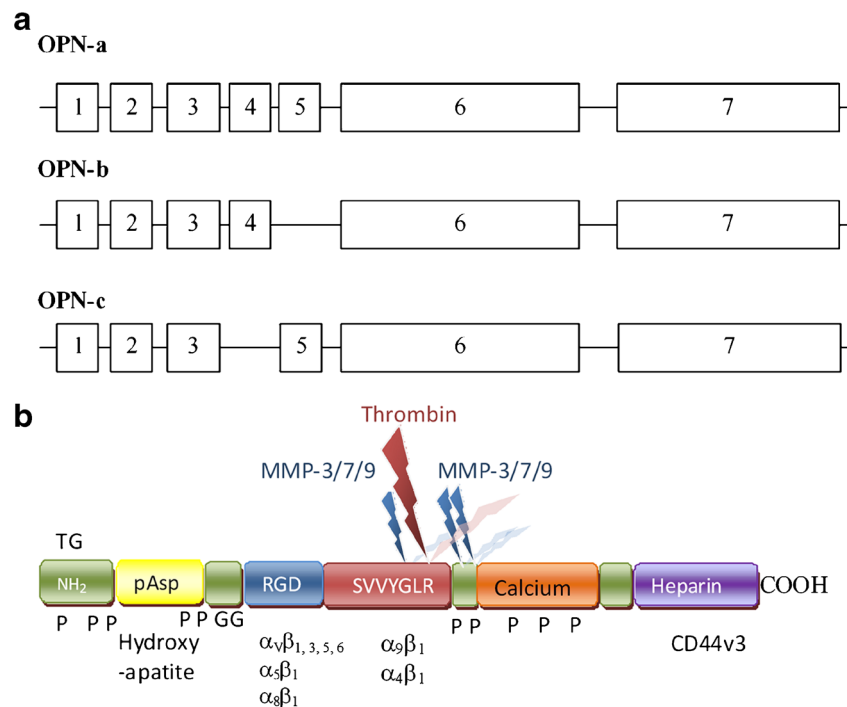
Osteopontin structure & function

Osteopontin, also known as early T-lymphocyte activation 1 (ETA1) protein, secreted phosphoprotein 1 (SPP1) and bone sialoprotein 1 (BSP1), is an acidic glycoprotein that is a member of the small integrin-binding N-linked glycoprotein family of proteins. Expression of osteopontin has been reported in a wide range of cells including macrophages, smooth and skeletal muscle cells, endothelial cells, lymphocytes, osteoblasts and tumour cells (Chambers et al. 1996; O'Brien et al. 1994; Patarca et al. 1989; Weinreb et al. 1990; Uaesoontrachoon et al. 2008). Whilst osteopontin is predominantly a secreted molecule, an intracellular form of the molecule has been reported (Inoue and Shinohara 2011; Zohar et al. 1997). The secreted form of osteopontin can exist both as a soluble molecule with cytokine-like functions and as an immobilised matricellular protein (O'Regan et al. 1999) that can interact with certain variants of the hyaluronan receptor CD44 and with a variety of integrins (Denhardt et al. 2001) to activate intracellular signalling pathways and mediate cell-cell

and cell-matrix interactions. The effects of binding of osteopontin to target cells include promoting attachment, proliferation, migration, chemotaxis and apoptosis of cells (Denhardt et al. 2001; Giachelli et al. 1998; Standal et al. 2004), thereby regulating many pathological and physiological processes, including tissue repair, inflammation and fibrosis (Berman et al. 2004; Denhardt et al. 2001; Duvall et al. 2007; Giachelli et al. 1998; Hashimoto et al. 2007; Liaw et al. 1994; Mori et al. 2008; O'Regan and Berman 2000; Sam et al. 2004,).

Alternative splicing of the human, but not mouse, *SPP1* gene has been reported (Saitoh et al. 1995). The full length isoform (OPNa) is encoded by 7 exons, whereas OPNb lacks exon 5 and OPNc lacks exon 4 (Fig. 2a). Alternative splicing is thought to influence the degree of phosphorylation of the isoforms and thus their biological properties (Gimba and Tilli 2013). Osteopontin consists of a number of functional domains (Fig. 2b; Kazanecki et al. 2007), and is extensively posttranslationally modified by glycosylation, phosphorylation and sulphation and may be subject to further processing by cross-linking and proteolytic cleavage. Specific posttranslational modifications and processing of

Fig. 2 a Genomic structure and alternative splicing of human *SPP1* gene. Exons 1 to 7 of *SPP1* are represented by *open boxes*. The exons contained in the three identified human osteopontin transcripts are shown. **b** A schematic diagram of osteopontin structure showing approximate sites of glycosylation (*G*), reactive glutamines (*Q*) phosphorylation (*P*) and proteolytic cleavage by thrombin and matrix-metalloproteinases. Sites responsible for RGD-dependent (*RGD*) and RGD-independent (*SVVYGLR*) integrin binding, as well as hydroxyapatite (*pAsp*), calcium and heparin binding are indicated



osteopontin alter the properties of the molecule and have functional significance. For example, the degree of phosphorylation regulates the adhesive properties of osteopontin (Christensen et al. 2007); moreover a cryptic binding site revealed by thrombin's proteolytic cleavage of osteopontin increases its adhesive properties by allowing binding to integrins in a non-RGD dependent manner (Yokosaki et al. 1999). Thus, alternative splicing, posttranslational modification, cross-linking and proteolysis of osteopontin give rise to a potential array of different isoforms of osteopontin that differ in the extent and type of their modifications and, therefore, their biological properties.

Osteopontin as a link between inflammation and myogenesis during regeneration of injured skeletal muscles

Induction of osteopontin expression following skeletal muscle injury, associated with infiltrating inflammatory cells, necrotic muscle fibres and endogenous myogenic cells (Fig. 3), has been reported by a number of studies (Barbosa-Souza et al. 2011; Hirata et al. 2003; Hoffman et al. 2013; Uaesoontrachoon et al. 2013). One study measured an approximately 100-fold increase in expression of osteopontin mRNA over baseline within 12 and 24 h after muscle injury, returning to baseline between 14 and 16 days post injury (Hoffman et al. 2013). In vitro, FGF2, has been shown to strongly induce osteopontin expression by myogenic cells, and recombinant osteopontin was found to significantly affect myoblast attachment, proliferation and migration (Uaesoontrachoon et al. 2008). Furthermore, the form in which the molecule was presented to myogenic cells determined the

nature of the response; osteopontin immobilised onto tissue culture substrata promoted adhesion and differentiation but not proliferation of cells of the myogenic cell line C2C12, whereas

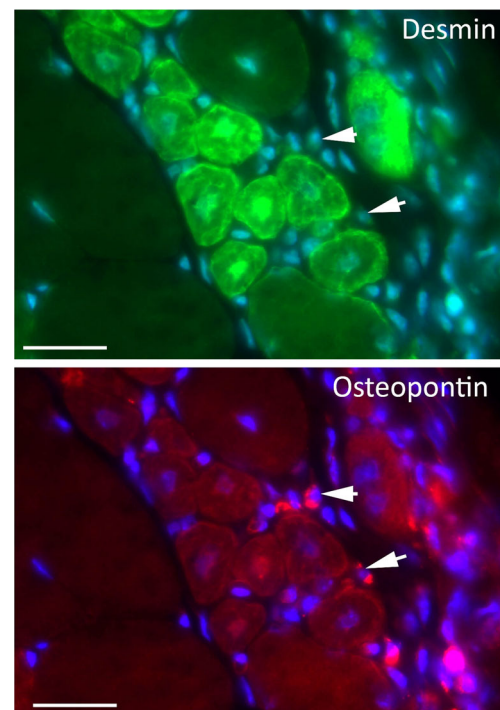


Fig. 3 Co-immunolocalisation of desmin and osteopontin in the *tibialis anterior* muscle of a 12 week old *mdx* mouse. Strong desmin staining is observed in regenerated (centrally nucleated) muscle fibres as well as in single, infiltrating cells (arrowed). Osteopontin expression is low in mature and regenerated muscle fibres and high in single, infiltrating cells (arrowed). Nuclei counter stained with 4',6-diamidino-2-phenylindole, bar=50 μ m

osteopontin presented in solution stimulated adhesion to fibronectin coated substrata and proliferation of C2C12 cells as well as inhibiting differentiation and migration of these cells (Uaesoontrachoon et al. 2008).

The rapidity and magnitude of the induction of osteopontin expression in response to muscle injury coupled with its known effects on inflammatory and myogenic cells suggests that osteopontin is an important factor during the degenerative and regenerative events that follow skeletal muscle injury. This hypothesis was tested using a whole muscle autografting model of muscle injury that causes a single severe injury, leading to acute osteopontin expression, a characteristic pattern of inflammatory infiltration, and muscle fibre degeneration and regeneration in wild type mice (Uaesoontrachoon et al. 2013). In autografted muscles of osteopontin null mice, inflammatory infiltration (macrophages and neutrophils) and degenerative changes were reduced 3 days after injury, and the early regenerative response (5 days post-injury) was delayed, compared to wild type autografted muscles (Uaesoontrachoon et al. 2013). These observations indicate that osteopontin is involved in the inflammatory, degenerative and regenerative events that occur in early skeletal muscle regeneration. Based on these studies we have proposed a model for osteopontin's effect on muscle

degeneration and regeneration (Fig. 4). The ability of factors such as FGF2, which is released by injured muscle fibres (Chen et al. 1994), to induce osteopontin expression by myogenic cells (Uaesoontrachoon et al. 2008), suggests that these cells present at the site of injury may be some of the first cells to express osteopontin directly in response to muscle injury. Local secretion of soluble osteopontin may contribute to both the retention of a pool of proliferative myoblasts at the site of injury and infiltration of the injured muscle by macrophages and neutrophils (Giachelli et al. 1998; Banerjee et al. 2006; Koh et al. 2007). As macrophages in particular are a rich source of osteopontin (Giachelli et al. 1998; Hirata et al. 2003), osteopontin expression by these inflammatory cells could play an equally crucial part in osteopontin's role in muscle regeneration by helping to amplify the initial osteopontin expression in the muscles. At later stages in muscle regeneration, osteopontin may increasingly become incorporated into the remodelled extracellular matrix, for example by binding to fibronectin and others of its constituents (Mukherjee et al. 1995). As an immobilised molecule osteopontin may then promote attachment of myoblasts to the extracellular matrix, and support their terminal differentiation into new muscle fibres (Uaesoontrachoon et al. 2008).

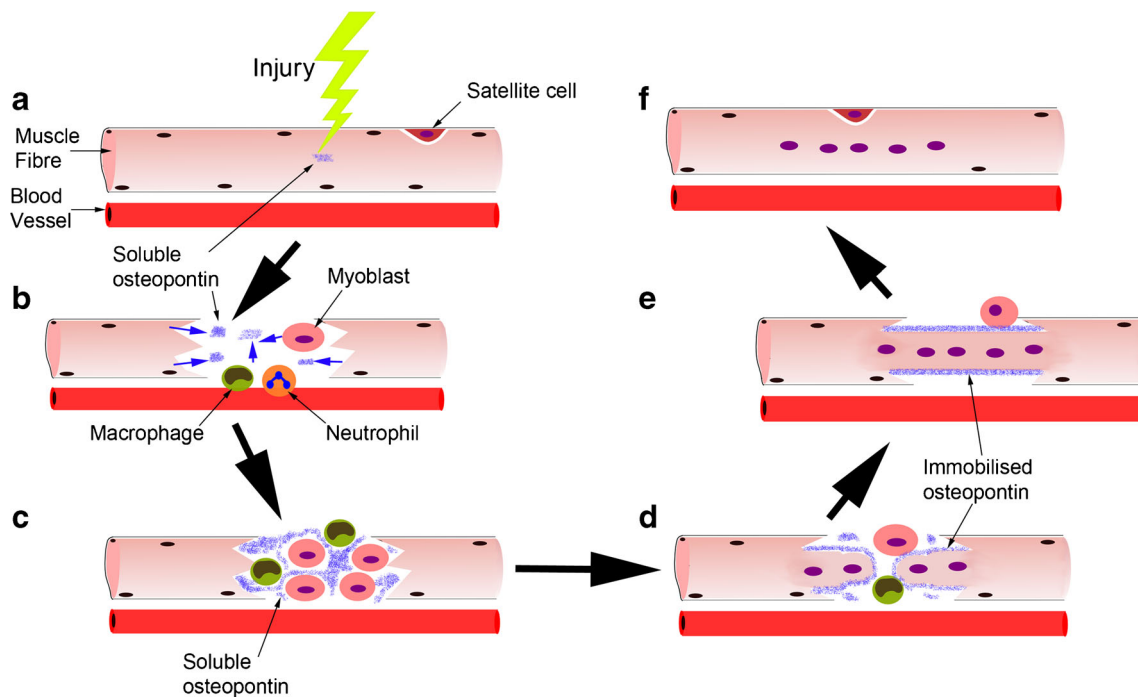


Fig. 4 Schematic diagram showing a proposed model of role for osteopontin during skeletal muscle repair. **a** Osteopontin expression is induced in response to injury to mature skeletal muscle. **b** Release of osteopontin from the site of injury promotes proliferation of myoblasts, formed from activated satellite cells, and infiltration of the muscle by blood-borne inflammatory cells including macrophages and neutrophils. Osteopontin expression at the site of injury is further augmented by its release from myoblasts and inflammatory cells. **c** High levels of soluble osteopontin at the site of injury helps retain a pool of proliferative myoblasts at the site of

injury and promotes further infiltration of the muscles by inflammatory cells. **d** As osteopontin is immobilized by incorporation into the extracellular matrix terminal differentiation and fusion of myoblasts is favoured over proliferation, thereby promoting the formation of myotubes. **e** As skeletal muscle regeneration proceeds soluble osteopontin and inflammatory cells are lost from the site of injury, whilst immobilised osteopontin and myoblasts are retained. **f** During maturation of the regenerated muscle fibre, osteopontin is lost from the site of injury

The importance of macrophages in osteopontin's effects on the response of skeletal muscle to injury has been highlighted in a study that has reported an age-related increase in osteopontin expression associated with a reduction in the regenerative capacity of skeletal muscle in response to injury (Paliwal et al. 2012). This study reported that osteopontin expression was elevated in serum and in myoblasts and macrophages isolated from injured muscles of old mice (22–24 months) compared to serum, myoblasts and macrophages isolated from injured muscles of young (2–3 months) mice. Furthermore, macrophages, exogenous recombinant osteopontin or osteopontin-neutralising antibodies significantly influenced myoblast proliferation, leading the authors to conclude that osteopontin expression is altered with aging and that increased osteopontin expression contributes to reduced skeletal muscle regeneration in older muscle by reducing the myogenic response of the endogenous myoblasts (Paliwal et al. 2012). Interestingly, this study also reported that injection of an osteopontin neutralising antibody into injured muscles of old mice significantly increased the number of regenerating fibres in muscles 5 days post injection. Conversely, injection of recombinant osteopontin into injured muscles of young mice significantly reduced the number of embryonic myosin positive regenerating fibres in muscles 5 days post injection. These findings appear to contradict the observation of greater numbers of centrally nucleated (regenerated) fibres 5 days post injury in the injured muscles of wild type mice compared to injured muscles of osteopontin-null mice at the same time point (Uaesoontrachoon et al. 2013). However, the difference in the timing of regeneration between the two models used and the transient nature of expression of the marker of regeneration used by (Paliwal et al. 2012) means that at the single time point studied, such comparisons are possibly misleading. Intriguingly, however, the use of recombinant osteopontin and osteopontin neutralising antibodies suggests that modulating osteopontin levels in injured muscles *in vivo* may be possible and may provide a novel approach to influencing the outcome of muscle repair following injury.

Osteopontin and disease severity in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a chronic muscle disease that invariably results in the death of affected individuals during the second decade of life, due to wasting and fibrosis of the muscles involved in respiration. The primary defect in DMD, and in the C57BL/10ScSn-*Dmd*^{mdx} (mdx) strain of mouse, is the lack of the cytoskeletal protein dystrophin in the muscle fibres of affected individuals (Hoffman et al. 1987); dystrophin links the cytoskeleton of muscle fibres with the extracellular matrix and thus stabilises the sarcolemma (Ervasti 2007). The absence of dystrophin results in increased

fragility and damage to muscle fibres in response to contraction (Petrof et al. 1993). The pathology of DMD and mdx muscles is characterised by persistent cycles of spontaneous focal necrosis of muscle fibres, inflammatory infiltration of sites of injury, phagocytosis of damaged sections of fibres by invading macrophages, and muscle fibre regeneration. In the muscles of patients with DMD, muscle fibre regeneration progressively fails, and as a result the muscles develop the typical severe secondary pathology of DMD that is characterised by fibrosis and fibro-fatty replacement of muscle fibres (Partridge 1997). With the exception of the diaphragm, the majority of the muscles of mdx mice retain the ability to regenerate efficiently and do not develop the severe secondary pathology associated with muscle fibrosis (Stedman et al. 1991).

Osteopontin has been described as a component of the inflammatory milieu of dystrophin-deficient muscles (Haslett et al. 2002; Porter et al. 2002; Uaesoontrachoon et al. 2008; Zanotti et al. 2011), and muscles of individuals with dystrophies caused by deficiencies of other proteins (Turk et al. 2006). As osteopontin promotes inflammation and fibrosis in a wide range of tissues (Mori et al. 2008; Pardo et al. 2005), Spencer and colleagues tested the hypothesis that osteopontin may influence inflammation and fibrosis in dystrophin deficient muscles by crossing dystrophin-deficient mdx mice with osteopontin-null mice to produce double mutant mice (DMM), lacking both dystrophin and osteopontin (Vetrone et al. 2009). This study reported that osteopontin deficiency decreased neutrophils, but not macrophages, natural killer T-cells (NKT)-like cells and increased the number of CD3⁺/CD4⁺ cells in the inflammatory infiltrate of mdx muscles. The increase in CD3⁺/CD4⁺ cells was found to accompany an increase in FoxP3 mRNA expression, suggesting that these cells may represent a population of regulatory T-cells (Vetrone et al. 2009). Functionally, young (4 and 8 week old) DMM mice were found to have greater grip strength and a greater proportion of regenerating fibres in the quadriceps muscles than littermate mdx control mice. By 6 months of age, no significant difference in muscle strength was observed between DMM and mdx mice, however, at this time point collagen type 1 deposition in the diaphragm muscles and cardiac muscle as well as expression of TGFβ, a known fibrotic modulator (Andreotta et al. 2006), were significantly reduced in the DMM muscles. Thus, in mice ablation of osteopontin in dystrophin deficient muscles altered the composition of the inflammatory infiltrate, improved muscle strength and reduced muscle fibrosis, suggesting that chronic overexpression of osteopontin contributes to some of the most damaging aspects of the pathology of dystrophic muscles.

In contrast to the findings of Spencer and colleagues, a recent human genome wide association study found evidence that suggested higher levels of osteopontin expression may be beneficial to the muscles of patients with Duchenne muscular dystrophy. The study in question identified a single nucleotide

polymorphism (rs28357094), which is found 66 bp upstream of the transcriptional start site of the human *SPP1* gene, as a strong genetic modifier of DMD. The SNP rs28357094 had previously been characterised in human cell lines transfected with promoter constructs linked to a luciferase reporter (Giacopelli et al. 2004). In this system the T allele of rs28357094 was shown to bind SP1/SP3 transcription factors with higher affinity and to lead to higher transcriptional activity than the alternative G allele. Patients carrying the rarer G allele (G/T or G/G) were found to have lower grip strength and decreased age to loss of ambulation than patients homozygous for the T allele (T/T) and the association between genotype and grip strength was found to be strongest in steroid-treated non-ambulatory patients (Pegoraro et al. 2011). Whereas this study identified *SPP1* as a significant modifier of disease it did not attempt to identify the mechanism by which *SPP1* genotype influenced disease progression in patients with DMD. The most obvious explanation, given the observations by (Giacopelli et al. 2004), was that the T/T genotype results in higher levels of osteopontin than the G/G and G/T genotypes and higher levels of osteopontin had a protective or pro-regenerative effect on dystrophic muscle. Osteopontin expression at the level of mRNA and protein was measured in muscle biopsies from a cohort of patients with DMD who had previously been genotyped with respect to rs28357094 (Piva et al. 2012). Whilst this study found no significant association between the genotype of the SNP rs28357094 and osteopontin expression in muscle it did report significantly higher numbers of CD4⁺ and CD68⁺ cells in muscle biopsies from patients of the T/T genotype.

Together these studies have identified apparent roles for osteopontin in the progression of muscular dystrophy, but the precise mechanisms of such effects are still unclear. While the disease-modifying effect of the SNP rs28357094 cannot be attributed to the level of osteopontin expression in muscles from patients with DMD, it is possible that the SNP either influences osteopontin expression levels in other tissues or influences transcription in some other way. Whilst the mechanism of the effect of the *SPP1* gene on muscle strength and disease progression in DMD remains elusive, one consequence of the finding that *SPP1* genotype influences progression of Duchenne muscular dystrophy is the recommendation that in order to establish functionally homogenous study populations, patients involved in clinical trials should be stratified on the basis of *SPP1* genotype (Bello et al. 2012).

Does osteopontin influence skeletal muscle development and postnatal growth?

Recent gene association studies have also identified the SNP rs28357094 as a significant modifier of muscle size in humans (Hoffman et al. 2013). This study attempted to measure the

association between rs28357094 and a number of skeletal muscle phenotypes, including muscle size in untrained muscles and the response of muscles to either resistance training or eccentric exercise in a multicentre cohort of young males and females. The study reported that females within the study cohort with the less common combined G/T and G/G genotype of rs28357094 had significantly greater volume of untrained upper arm muscles than females with the more common T/T genotype. No significant associations were observed in males and no association between osteopontin genotype and muscle strength or responses of the upper arm muscle to resistance training were observed in either male or female subjects. Furthermore, this study also reported that in female osteopontin null mice, of the seven muscle groups studied five were found to be significantly smaller than in female wild type controls of the same age. In male osteopontin null mice two of the muscle groups analysed were significantly smaller than in male wild type controls, suggesting that osteopontin contributes to post-natal muscle size in a sexually dimorphic fashion in both mice and humans (Hoffman et al. 2013).

Further evidence for a possible link between osteopontin and muscle size comes from a study that reported differential expression of osteopontin during primary and secondary myogenesis between embryos of two breeds of pigs associated with differing muscularity (Murani et al. 2007). This study reported that during embryogenesis the levels of osteopontin expression in the more muscular Pietrain breed was significantly lower than in the muscles of male and female pigs from the Duroc breed. The authors suggest that changes in the level of expression of a number of transcripts including osteopontin reflected a delay in primary myogenesis in the Pietrain breed possibly leading to an increased pool of myogenic cells that subsequently contribute to greater myogenesis and an increase in muscularity in postnatal animals of this breed. This study did not examine whether the differential osteopontin expression was influenced by gender and or whether it caused the different rates of myogenesis, but it does provide support for the hypothesis that osteopontin is involved in the determination of postnatal muscle size. Furthermore, in a separate study the same group reported a SNP in the 3' end of the first intron of the Duroc *Spp1* gene that encodes a C/EBP β binding site. The less frequent G allele at this SNP was found to disrupt C/EBP β binding, reducing transcriptional activity as well as influencing splice site selection leading to ubiquitous aberrant splicing of the gene (Murani et al. 2009).

The mechanism by which osteopontin may influence muscle development and or post-natal growth remains unclear. One possibility is that osteopontin functions either as a pro-myogenic growth factor such as IGF1, or as a negative regulator of myogenesis such as TGF β 1 or myostatin (Joulia et al. 2003; Ten Broek et al. 2010). However, the experiments to date suggest that osteopontin's effects on the size of skeletal muscles are subtle with a decrease in osteopontin expression

associated with increased muscle volume in human females and a total loss of expression in mice leading to a significant reduction in muscle weight. Whilst this may be as a result of differences in osteopontin's function between species, it may also be a reflection of a more complex role that osteopontin plays in determining the size of postnatal skeletal muscles or the influence of alternative forms of the molecule, generated by alternative splicing or post translational modification or processing in the local environment.

Conclusions and future perspectives

To date, studies on the role of osteopontin in skeletal muscle pose almost as many questions as they answer. Current evidence suggests that acute osteopontin expression is required for normal muscle regeneration following a single severe injury (Uaesoontrachoon et al. 2013), but that overexpression of osteopontin reduces the regenerative response to injury in aged muscle (Paliwal et al. 2012). Similarly, in dystrophic mouse muscles chronic overexpression of osteopontin appears to be related to decreased muscle strength and fibrosis in mice (Vetrone et al. 2009), whereas in muscles from patients with Duchenne muscular dystrophy a SNP associated with osteopontin overexpression in vitro, has been found to be a significant positive modifier of the disease (Pegoraro et al. 2011). Currently, little work has been focused on identifying the form or forms of osteopontin found in injured or diseased skeletal muscle. Therefore, one possible explanation for the apparently contradictory observations is that osteopontin plays many roles, some of them antagonistic to others, in injured muscle, and that posttranslational modification, processing, timing, as well as absolute level of osteopontin expression influence the role that it plays. For example, in studies using the whole muscle grafting model or cardiotoxin injection models of muscle injury the muscles are subjected to a single severe injury resulting in a short lived acute overexpression of osteopontin. In contrast, due to the persistent cycles of injury that characterise them, dystrophic muscles are subjected to chronic osteopontin overexpression, furthermore, at least one enzyme responsible for processing osteopontin is known to be up-regulated in the muscles of mdx mice (Porter et al. 2002). Thus limited acute osteopontin overexpression may be beneficial for muscle regeneration by initiating inflammatory infiltration and muscle fibre degeneration in injured muscle, whereas chronic overexpression and processing of osteopontin by enzymes present in dystrophic muscles may lead to chronic inflammation of injured muscles and eventually to fibrosis and functional impairment. Further analysis of the structure and function of osteopontin in injured skeletal muscle may also provide much needed insights into the diverse and at times apparently contradictory roles that osteopontin plays in injured and dystrophic muscles.

Another area of potential future interest arises from osteopontin's ability to induce expression of T_H1 -related cytokines by macrophages and T-cells (Ashkar et al. 2000; O'Regan et al. 1999). The importance of different macrophage phenotypes in the early, inflammatory, T_H1 dominated phase and the later, regenerative T_H2 dominated phase of the response of skeletal muscle to injury has only recently been described (Deng et al. 2012; Tidball 2011; Villalta et al. 2011). The possibility that osteopontin influences macrophage phenotype and therefore inflammation and regeneration in injured and diseased muscle warrants further investigation.

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