



Invited Review

Apoptosis and rotator cuff tears: scientific evidence from basic science to clinical findings

Leonardo Osti[†], Matteo Buda[‡], Angelo Del Buono[§], Raffaella Osti[‡], Leo Massari[‡], and Nicola Maffulli^{**††,*}

[†]Unit of Arthroscopy and Sports Medicine, Hesperia Hospital, Via Arqua', Modena, Italy, [‡]Department of Trauma and Orthopaedic Surgery, University of Ferrara, S.Anna Hospital, Via Aldo Moro, Ferrara, Italy, [§]Department of Orthopedic and Trauma Surgery, Fidenza Hospital, Via Tincati, Fidenza, Italy, ^{**}Department of Trauma and Orthopaedic Surgery, University of Salerno, Italy, and ^{††}Centre for Sports and Exercise Medicine Queen Mary University of London Barts and The London School of Medicine and Dentistry, Mile End Hospital London, UK

*Correspondence address. Department of Trauma and Orthopaedic Surgery, University of Salerno, Italy.
E-mail: n.maffulli@qmul.ac.uk

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Abstract

Introduction: Excessive apoptosis has been hypothesized as possible cause of tendinopathy and tear in the tendons of the rotator cuff (RC). Different mechanisms and molecules play a key role in cell regulation. Biological interventions can affect the process of apoptosis to control the tendinopathy process, and may be useful to design new treatments.

Source of data: We identified basic science, *in vitro* and *in vivo* preclinical and clinical studies listed in the Pubmed Google Scholar, CINAHL, Cochrane Central and Embase Biomedical databases in English, Spanish, Italian and French concerning the effects of apoptosis on RC tendons.

Areas of agreement: The homeostasis between the apoptotic and inflammatory processes is dynamic and controlled by pro- and anti-apoptotic mechanisms and signals, with variable balance in different areas of the RC tendons in human specimens.

Areas of controversy: Apoptosis can be identified along the whole tendon, not only in the area of the lesion. Therefore, it is not necessary to undertake wide debridement of the torn edges of the tendon when undertaking a repair.

Growing points: The identification of the various factors that control apoptosis and its mechanisms can help to design new treatments and exert positive effects in the recovery from tendon tears.

Areas timely for developing research: Further studies are needed to produce clear guidelines to determine how to balance the apoptosis process to reduce the failed healing response found in non-traumatic RC tears.

Key words: rotator cuff, tendons, apoptosis, shoulder, tenocytes, tendinopathy

Introduction

Apoptosis, or programmed cell death, is an important component in the control of cells proliferation by regulating embryogenesis, organogenesis and tissue morphogenesis, and maintaining homeostasis in many adult tissues.¹ The pathogenesis of atraumatic rotator cuff (RC) tendon tears, in which the disease is the result of a combination of intrinsic tendon and extrinsic factors, is still controversial.²

Of the intrinsic factors, apoptosis seems to may play a key role in the regulation and development of the lesions.

Yuan *et al.*³ in 2002 postulated excessive apoptosis as a primary cause of tendinopathy and tears within the supraspinatus tendon, rather than tears being a secondary effect of degeneration, identifying apoptotic cells in supraspinatus tendon tears compared with control samples. Furthermore, excessive apoptosis seems to involve the entire RC in the presence of a supraspinatus tear.⁴

Apoptosis is triggered by exogenous and endogenous stimuli that initiate two different highly complex and sophisticated pathways: intrinsic and extrinsic.

The mitochondrial or intrinsic pathway is triggered by different factors (i.e. oxidative stress, genetic damage, high concentration of cytosolic calcium ions) within the cell that increase mitochondrial membrane permeability. Bax protein, migrating to the surface of the mitochondrion and inhibiting the protective effect of anti-apoptotic Bcl-2 protein, inserts itself into the outer mitochondrial membrane, perforating it. This results in an opening of the mitochondrial permeability transition pore and release of two main groups of normally sequestered pro-apoptotic proteins from the

intermembrane space into the cytosol: cytochrome c and the serine protease.

Cytochrome c binds to the protein Apaf-1 (apoptotic protease activating factor-1) and together form apoptosomes which bind to and activate caspase-9. Caspase-9 cleaves and, in so doing, activates other caspases (caspase-3 and -7).

The activation of these 'executioner' caspases creates an expanding cascade of proteolytic activity (similar to what happens in blood clotting and complement activation) which leads to digestion of structural proteins in the cytoplasm, degradation of chromosomal DNA and phagocytosis of the cell.

The death-receptor mediated or extrinsic pathway is activated by transmembrane receptor-mediator ligand involving receptors which are members of the tumor necrosis factor (TNF) gene. Fas Ligand (FasL), binding the TNF receptor, induces apoptosis activating caspase 8 and starting a cascade of caspase activation leading to phagocytosis of the cell. Nitric oxide induces apoptosis by dissipating the membrane potential of mitochondria, and therefore makes the mitochondrial wall more permeable.⁵

The mechanism responsible for the modulation of apoptosis may be a combination of different factors such as withdrawal of positive signals (i.e. growth factors for neurons or Interleukin-2) or receipt of negative signals (i.e. increased levels of oxidants within the cell, damage to DNA by oxidants, death activators as TNF- α , TNF- β and FasL). Furthermore, hypoxia, extracellular matrix (ECM), cytokines, genetic factor and other proteins interact in the apoptotic pathway.⁶

Basic science studies focus on both physiological and pathological processes regulating apoptosis, and external factors which can decrease or balance the apoptosis.

This review analyzes the relationship between the factors which have been identified as possible regulators of the apoptotic process in RC tendons, and evaluates how they can influence apoptosis.

Methods

Search strategy

In August 2016, a systematic search was conducted in the online PubMed, Google Scholar, CINAHL, Cochrane Central and Embase Biomedical databases using various combinations of the following key terms and MesH terms: RC, tendons, tenocyte, tendinopathy, apoptosis and shoulder, with no limits regarding the year of publication and following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.⁷ Our PRISMA checklist is presented in Figure 1. Two authors (M.B. and L.O.) screened all the articles identified for title, abstract and full text concerning the effects of apoptosis in RC. All *in vitro*, *in vivo* preclinical and clinical studies in English, Spanish, Italian and French were evaluated and analyzed in this review.

Results

Genetic influence

Genetic factors play a role in the development of full thickness tears of the RC.^{8,9} Siblings of patients with RC tear had more than twice the relative risk to develop a RC lesion, and nearly five times the risk of experiencing symptoms.¹⁰ Tashjian *et al.*¹¹ identified two single-nucleotide polymorphism associated with RC tears. They were located within two genes SAP30BP (on chromosome 17) and SASH1 (on chromosome 6); both these genes play a marked role in apoptosis, regulating tendon cell apoptosis and predisposing individuals to RC tears.

Hypoxia

Tenocytes are capable of withstanding acute and repetitive reductions of blood perfusion, and therefore are likely able to tolerate transient hypoxia. Hypoxia promotes the expression of pro-inflammatory cytokines, which are key apoptotic mediators, and drives matrix component synthesis towards a collagen type III profile by human tenocytes.^{12,13}

Millar *et al.*¹⁴ noted that hypoxia could start and regulate early tendinopathy by showing in apoptotic cells an increase of hypoxia-inducible factor 1 α (HIF1 α), a transcription factor that regulates several genes to promote survival in low oxygen,

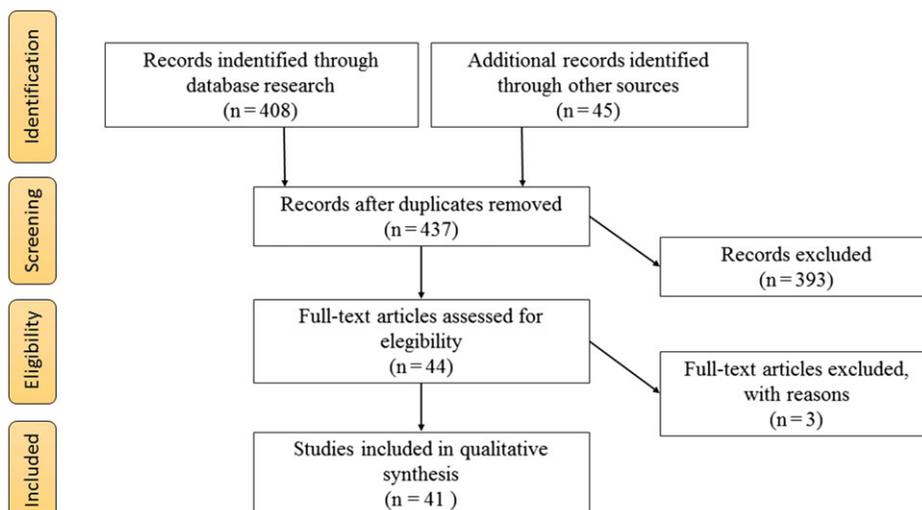


Fig. 1 PRISMA 2009 flow diagram.

Bcl-2, a regulator protein and clusterin, a molecular chaperone and a member of the small heat shock protein (HSP) family. Hypoxia reduces the ERK (extracellular signal regulated kinase) and p38 production, increasing collagen III synthesis compared with type I. The final result is a decrease of the resistance of tendons to tensile forces.

Benson *et al.*¹⁵ showed that the expression of HIF-1 α was greatest in mild impingement and in association with different stages of RC tendinopathy (partial, small, medium and large RC tears). Bcl-2 Nineteen KiloDalton interacting protein (BNip3) expression, a pro-apoptotic member of the Bcl-2 family with a role in hypoxia-induced death in many cell types, increased significantly in RC tears, but was reduced in massive tears. This is possibly a consequence of the reduction of BNip3-positive cells from apoptosis. Excessive apoptosis was found within full thickness tears of the RC, with almost a threefold increase compared with the control or mild RC tear groups. Furthermore, there was a statistically significant association between the proportion of apoptotic cells and the age of the patients. This is contrary to Yuan *et al.*³ study, in which there was no association between the proportion of apoptotic cells and age, duration of symptoms or size of tear. This is a likely consequence of the relatively small number of patients studied and the limited age range (50–70 years).

Nuclear factor-kB (NF-kB) also plays a role in the pathogenesis of RC tears. Evaluating the tear margins of 63 patients with non-traumatic RC tears (mean age 65 years, range: 52–74), it was shown that NF-kB, activated by hypoxia, was increased on the margins of the tendon tear with increasing size of the lesion. NF-kB seems to lessen the progression of the damage, supporting the healing response, and lessening the rate of tendon cells death.¹⁶

Cytokines and caspases

Cytokines are important molecular messengers in the response of soft tissue from injury to wound healing, and play an important role in oxidative-stress induced cellular apoptosis.¹⁷

The caspases are a family of cysteine proteases for aspartic acid residues, which cleave specific intracellular substrates and regulate cell death induced by apoptosis.¹⁸

Millar *et al.*¹⁹ confirmed the presence of the cytokines IL-18, IL-15, IL-6 and macrophage inhibitory factor (MIF) in both rat and human models of tendinopathy. They identified significantly increased levels of key mediators, caspases 3 and 8 and of Fas-Ligand apoptosis in the margins of full thickness tears of the RC compared with matched subscapularis tendon and normal control tendon. In another study, Millar *et al.*²⁰ showed an increase of IL-17A in early RC tendinopathy. This cytokine seems to regulate and modulate the first phases of tendon degeneration by regulating inflammatory, apoptotic pathways and collagen matrix synthesis, as it increases collagen III and decreases collagen I synthesis.

Voloshin *et al.*²¹ showed an elevated expression of IL-1, IL-6 and TNF- α in the subacromial bursa in 10 patients with full thickness tears the RC comparing them to eight patients (control group) ($P < 0.001$).

Blain *et al.*²² found an increase of IL-1, IL-6, TNF, COX-1, COX-2, MMP-1 and MMP-9 in 14 patients with subacromial bursitis undergoing mini-open RC repair compared to four patients undergoing surgery for shoulder instability.

Lundgreen *et al.*²³ comparing biopsies from nine patients (five men and four women; mean age 54) with partially torn supraspinatus tendons and the matched intact subscapularis tendons, and 10 reference subscapularis tendons (five women and five men, mean age 43.9, undergoing an arthroscopic labral repair and who demonstrated no RC pathology), showed a significant increase of apoptotic tenocytes, and cells expressing p53 and caspase c in both the supraspinatus and subscapularis tendons of patients presenting partial-thickness tears. In this study, however, the control group was significantly younger than the patient group, and two different types of tendons were compared.

Fabis *et al.*,^{24,25} in nine patients, noted a reduction of caspases 3, 8, 9 and Bax (pro-apoptotic molecules) along a 1 cm section taken from the edge

of a torn supraspinatus tendon ($P < 0.05$). TNF- α decreases in a distal to proximal direction within 1 cm of the edge of a torn supraspinatus tendon, contrary to an increase of the anti-apoptotic molecules Bcl-2 and expression of the anti-inflammatory cytokine IL-10, which showed an opposite trend ($P < 0.05$).

Furthermore, the expression of type I collagen, IL-4 and IL-13 significantly increased, and IL-1b and IFN- γ decreased from the distal to the proximal parts of the tendon edge ($P < 0.05$). The homeostasis between the apoptotic and inflammatory processes can be dynamic and is highly dependent on the distance from the edge of the tear. The authors recommended careful resection of the torn edges of the RC tear, as resection 4–7 mm from the edge of the torn supraspinatus tendon may enhance the healing process.

Lee *et al.*²⁶ quantified the extrinsic and intrinsic apoptosis pathways by examining the activities of caspase 3/7, 8 and 9 and cytochrome c in tendon biopsies collected from 14 patients undergoing arthroscopic RC repair for RC tears. When these biopsies were compared with tendon biopsies from seven patients undergoing surgery for proximal humeral fractures, the activity of caspase 9 and cytochrome c was more than double that of other proteins. The increase of caspase 9 and cytochrome c activity suggests that the intrinsic (mitochondrial) pathway might be more important in the apoptosis mechanism of RC tears. They noted a significant difference in the apoptotic activities harvesting the samples from three different sites of the tendons. This suggests that apoptosis is a generalized phenomenon throughout the entire tendon. They hypothesized that apoptosis could start from the lateral aspect of the RC, and then propagate in all directions.

Yuan *et al.*²⁷ showed that oxidative stress, exposing to hydrogen peroxide (H_2O_2) human tenocytes from patients undergoing surgery for RC repair, induced apoptosis most likely through releasing cytochrome c from mitochondria to cytosol, and activating caspase-3 in a time and dose-dependent manner. Lower concentration of H_2O_2 (50 μ M) led to tenocytes apoptosis, while there was

a significant increase in necrotic cell death with higher concentrations of H_2O_2 .

Scott *et al.*²⁸ examined apoptosis using antibodies against fragmented DNA in 32 male Sprague-Dawley rats randomly divided into controls (standard cage care) and runners (standard cage care plus treadmill running which induced supraspinatus tendinopathy). They did not find consistent results regarding apoptosis in the primary stages of tendinopathy, concluding that apoptosis might play a secondary role in the more advanced stages of injury such as fibrosis or after frank tendon tears.

Heat shock protein

HSPs protect cells from the cytotoxic effects of cytokines and apoptotic mediators, and play a protective role as molecular chaperones in cells by facilitating the folding, intracellular transport, assembly and disassembly of other proteins.²⁹

Millar *et al.*³⁰ using 24 rat and 17 torn supraspinatus tendons collected from patients with RC tears undergoing shoulder surgery (mean age 57 years but with wide range, 39–75 years) suggested that HSP plays a central role in caspase dependent apoptotic cell signaling and in the cascade of failed healing response found in tendinopathy. Over-expression of HSP 27 is essential in preventing cells from undergoing apoptosis, inhibiting specifically the cytochrome C and ATP-triggered activity of caspase 9 on the apoptotic pathway. Furthermore, HSP 27 indirectly interferes with cell death because of its ability to modulate intracellular glutathione, while HSP 70 interacts with Apaf-1, thereby preventing its interaction with the caspases, preventing apoptosis and protecting cells from heat stress, the cytotoxic effects of TNF α and from nitric oxide.

Extracellular matrix

The ECM of tendons is composed largely of type I collagen, which provides structural integrity and accounts for mechanical strength. Disruption of the collagen matrix is characterized by loss of its normal longitudinal alignment. Fragmentation of collagen bundles decreases the mechanical property of the tendon, and may result in tendon rupture.³¹

Wu *et al.*³² demonstrated that the percentage of cells undergoing apoptosis increased gradually with the degree of ECM breakdown. The increase of apoptotic cells changes severely the tendon through the loss of fiber structure, hyalinization and loss of parallel arrangement, with no discernible orientation of the fiber. A reduced cell population might affect the rate of collagen synthesis and capacity of healing after injury by contributing to matrix deterioration and promoting further cell death and cell differentiation.

The increase of type III collagen is expression of tendinopathy and hypoxia changes, and alters collagen synthesis by tenocytes, with a disproportionate concentration of type III collagen compared with type I, with detrimental changes in the ECM.¹⁴

Exogenous substance and hormones

Hyaluronic acid (HA) has an anti-inflammatory and anti-adhesive activity in tendons and in synovial fibroblasts derived from RC tear without evident adverse effect.^{33,34} Osti *et al.* in *in vitro* studies in RC degenerative torn tendon samples demonstrated a decreased rate of apoptosis when the tendon-derived cells were exposed to HA, stimulating and reducing the synthesis of collagen type I and type III respectively. The reduction in collagen type III seems to be a protective factor for tendons. The molecular weight of HA seems not to affect this process, although the presence of sorbitol 4% increases the expression of collagen I, limiting HA degradation.³⁵

The effectiveness of corticosteroid (CS) injections results from their analgesic and anti-inflammatory properties, but they exert profound negative effects on human tenocytes. CSs induce early tendon fibroblasts death and inhibit their proliferation at the injection site, and decrease the biomechanical properties of the injected tendons.³⁶ High doses of CS (1.0 mg/mL of triamcinolone acetonide) cause irreversible effects on the human RC-derived tenocytes by prolonging the suppression of their viability and promoting apoptosis. CS increase mRNA expressions of caspase-3, 7, 8 and 9, indicating that both the endogenous and the exogenous apoptosis pathways are activated. Lower concentrations of CS

(0.1 mg/mL of triamcinolone acetonide) seem to have reversible effects on the tendons in 3 weeks, suggesting intervals longer than 3 weeks for safe CS re-administration.^{37,38}

Furthermore, the use of lidocaine with the injection causes cytotoxicity, decreases biomechanical properties, induces apoptosis and delays collagen organization in torn RC tendons both *in vivo* and *in vitro* increasing reactive oxygen species production regulated by extracellular signals and activation of caspase-3/7.³⁹

Recently, platelet rich plasma (PRP), a blood fraction containing a high concentration of platelets, has been used to improve healing in tendon injuries, stimulating cell proliferation and total collagen production in human tenocyte cultures. Kim *et al.* showed that self assembled peptide (SAP) + PRP can be effective in healing a RC tear by enhancing the collagen arrangement and inhibiting inflammatory changes and apoptosis.⁴⁰ Muto *et al.* confirm that the deleterious effect and apoptosis of CS injections can be prevented by adding PRP *in vitro*.³⁸ On the other hand, Carr *et al.*⁴¹ in a randomized controlled trial including 60 patients (age: 35–75 years) showed that the co-application of PRP during arthroscopic acromionplasty did not improve clinical outcomes at any time of the study ($P < 0.001$). In that study, PRP caused potential deleterious effects on healing tendons, decreasing blood vessel number and tendon cellularity and increasing the levels of p53 protein in tendon biopsies taken 12 weeks after acromionplasty. The authors concluded that PRP treatment could actually be pro-apoptosis.

Three studies showed how oxidative stress increased autophagy and RC tears, and how antioxidants protect against autophagic cell death.⁴² Anthocyanins, a natural antioxidant, inhibiting intracellular ROS production and reducing the H₂O₂-induced phosphorylation of ERK1/2, JNK and p38, plays an important anti-apoptotic role in the prevention of RC tendinopathy.^{43,44}

Nevertheless, other studies are necessary to explain better their molecular interactions and how these molecules could be used to regulate the apoptosis process.

Table 1 Effects of exogenous substances

Author	Study type	Drug	Specimen	Results
Honda <i>et al.</i> ³⁹	<i>In vivo</i> <i>In vitro</i>	Lidocaine	<i>In vitro</i> : tenocytes from 14 torn human RC tendons cultured in monolayers at various concentrations of lidocaine <i>In vivo</i> : 33 rats underwent bilateral shoulder surgery	Lidocaine significantly inhibit cell proliferation and cause cell death in tenocytes, induce apoptosis, collagen necrosis and decrease biomechanical strength
Yamada <i>et al.</i> ³³	<i>In vitro</i>	HA	10 Patients with RC disease (age: 62 years, range: 44–72).	Treatment with various concentrations of HA significantly modulate cell proliferation decreasing the expression level of procollagen alpha1 (III) mRNA, but not that of procollagen alpha1 (I) mRNA
Osti <i>et al.</i> ³⁵	<i>In vitro</i>	HA	Supraspinatus tendons tear from 10 patients (mean age of 63,6 ± 6,9 years) operated arthroscopically for shoulder RC repair	HA increased viability and proliferation, in dose-dependent manner and reduce apoptosis at 24 h compared to control cells
Nakamura <i>et al.</i> ³⁶	<i>In vitro</i> <i>In vivo</i>	Corticosteroid and HA	16 Tendon specimens from patients with RCT; 60 adult bilateral shoulder Sprague-Dawley male rats (40 shoulders treated by CS or HA, 40 by CS or PBS, and 40 by HA or PBS)	CS induced the death of tendon fibroblasts and inhibited their proliferation with features typical of apoptosis and secondary necrosis. HA possesses anti-inflammatory and anti-adhesive activities in tendon and synovial fibroblasts derived from RCT without adverse effects
Harada <i>et al.</i> ³⁷	<i>In vitro</i>	Triamcinolone acetone	Human RC-derived cells isolated from the torn edges of human supraspinatus tendons of two men aged 61 and 54 years, and one woman aged 72 years	A 0.1 mg/mL dose of TA temporarily decreased cell viability and increased cell apoptosis, which was recovered at 21 days, however, 1 mg/mL of TA caused irreversible damage to cell morphology and viability
Muto <i>et al.</i> ³⁸	<i>In vitro</i>	PRP and triamcinolone acetone (TA)	Human RC-derived cells isolated from the torn edges of human supraspinatus tendon of three men aged 61, 54 and 32 years and one woman aged 72 years	Exposure to TA significantly decreased cell viability and increase the number of apoptotic cells, but cell viability and apoptosis did not decrease when PRP was added with TA. The deleterious effect of TA was prevented by PRP
Carr <i>et al.</i> ⁴¹	<i>In vivo</i>	PRP	60 Randomized patients with RC tendinopathy (55% women, 35–75 years). Patients were randomized to arthroscopic acromionplasty (AA) alone or in combination with an injection of autologous PRP into the subacromial bursa (AA + PRP).	PRP significantly alters the tissue characteristics in tendons after surgery reducing cellularity and vascularity and increasing levels of apoptosis

Continued

Table 1 *Continued*

Author	Study type	Drug	Specimen	Results
Kim <i>et al.</i> ⁴⁰	<i>In vivo</i>	PRP + self assembled peptide (SAP) Cyanidin	27 Sprague-Dawley rats randomly divided in four groups (7 SAP, 7 PRP, 7 SAP-PRP and six control) Human supraspinatus tendon tissues collected from three patients (two males and one female, ages 50, 56 and 55 years)	SAP-PRP can be effective in healing a rotator cuff tear by enhancing the collagen arrangement and inhibiting inflammatory changes and apoptosis Cyanidin inhibits autophagic cell death
Nam <i>et al.</i> ⁴⁴	<i>In vivo</i>	Cyanidin and delphinidin	Supraspinatus tendons of adult male Sprague-Dawley rats	Cyanidin and delphinidin exerted dose-dependent anti-apoptotic effects ($P < 0.001$)
Park <i>et al.</i> ⁴³	<i>In vitro</i>	Anthocyanins	Supraspinatus tendons of male Sprague-Dawley	Anthocyanins has an inhibitory effect on H2O2-induced apoptosis by suppressing both the intracellular ROS production and activation of JNK and ERK1/2
Lundgreen <i>et al.</i> ⁴⁷	<i>In vivo</i>	Smoke	10 Smokers vs 15 non smokers with full-thickness supraspinatus tendon tears	The supraspinatus tendons from smokers have significantly more advanced degenerative changes with increased density of apoptotic cells, reduced tenocyte density and upregulation proliferative activity

Cigarette smoke has a negative effect on tendon healing, and many studies have remarked on the increase of the risk of RC tears in smokers; in particular, nicotine and carbon monoxide decrease microperfusion and tissue oxygenation, leading to tissue hypoxia.^{45,46}

Lundgreen *et al.*⁴⁷ showed that the supraspinatus tendons in smokers have significantly more advanced degenerative changes, with increased density of apoptotic cells, reduced tenocyte density and down regulation of proliferative activity.

Endogenous substances may also regulate apoptosis. Recent studies have investigate the role of hormones in the pathogenesis of tendinopathy, and it seems that hormonal imbalances cause negative effects on the healing and maturation process of tendon healing.⁴⁸

Oliva *et al.* investigated the presence of thyroid hormones receptors in RC tendons, and described their possible role in the proliferation and apoptosis of human tenocyte by enhancing tenocyte growth and counteracting apoptosis in healthy tenocytes.^{49,50}

Berardi *et al.* confirmed this hypothesis, in an *in vitro* study evaluating biopsies from five different healthy supraspinatus tendons, and showing how thyroid hormones modulate ECM proteins secretion (such as collagen I, biglycan and Cartilage Oligomeric Matrix Protein) in tenocytes.⁵¹

Maman *et al.*⁵² described the reaction of tenocytes to the estrogens and calciotropic stimulations: female sex steroids and vitamin D promoted tendon-derived cell proliferation via estrogen receptor α and vitamin D receptor.

The results are summarized in Table 1.

Conclusion

Apoptosis in RC tears may be induced by many stimuli, both endogenous and exogenous. *In vivo* and *in vitro* studies showed that repetitive stress and overuse in normal human tenocytes produce hypoxia, and start a typical pathway that induce apoptosis and contribute to tendon failed healing response.

In RC tears, the concentration of cytokines and caspases is increased. They play a key role in

cellular apoptosis and in the regulation of the homeostasis between the apoptotic and inflammatory processes in the RC tendon tears.

The pro-apoptotic signals increase moving away from the tear margins, and can play an important role during the surgical repairs of RC tear on the healing response of the tendon. It appears that wide debridement the edge of a tendon tear is not necessary to enhance the healing process.

Genetic influences increase the susceptibility to the earlier incidence of tears and progression of symptoms and tear size. Antioxidants could play a role in the development of RC tears, but other studies are necessary to confirm this. Understanding the pathological cascade should lead to the development of cell-target treatment modalities for early RC tendinopathy.

Several drugs are injected to manage RC tear. CS seems to reduce pain, but with deleterious effects on tendons. HA reduces apoptosis acting on the production of collagen type I and reduction of type III, but it is still unclear what are the most appropriate molecular weight and therapeutic protocol.

In conclusion, knowing which stimuli may trigger the cascade of RC tendinopathy and its modifiable mediators would allow to reduce the damage and increase the potential healing of such injuries.

Conflict of interest statement

The authors have no potential conflicts of interest.

References

1. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995;267:1456–62.
2. McFarland e.g., Maffulli N, Del Buono A, et al. Impingement is not impingement: the case for calling it 'Rotator Cuff Disease'. *Muscles Ligaments Tendons J* 2013;3:196–200.
3. Yuan J, Murrell GA, Wei AQ, et al. Apoptosis in rotator cuff tendinopathy. *J Orthop Res* 2002;20:1372–9.
4. Lundgreen K, Lian OB, Engebretsen L, et al. Tenocyte apoptosis in the torn rotator cuff: a primary or secondary pathological event? *Br J Sports Med* 2011;45:1035–9.
5. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007;35:495–516.
6. Maffulli N, Longo UG, Berton A, et al. Biological factors in the pathogenesis of rotator cuff tears. *Sports Med Arthrosc* 2011;19:194–201.
7. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;151:264–9.
8. Longo UG, Berton A, Papapietro N, et al. Epidemiology, genetics and biological factors of rotator cuff tears. *Med Sport Sci* 2012;57:1–9.
9. Chaudhury S, Carr AJ. Lessons we can learn from gene expression patterns in rotator cuff tears and tendinopathies. *J Shoulder Elbow Surg* 2012;21:191–9.
10. Harvie P, Ostlere SJ, Teh J, et al. Genetic influences in the aetiology of tears of the rotator cuff: sibling risk of a full-thickness tear. *J Bone Joint Surg* 2004;86-B:696–700.
11. Tashjian RZ, Granger EK, Farnham JM, et al. Genome-wide association study for rotator cuff tears identifies two significant single-nucleotide polymorphisms. *J Shoulder Elbow Surg* 2016;25:174–9.
12. Liang M, Cornell HR, Zargar Baboldashti N, et al. Regulation of hypoxia-induced cell death in human tenocytes. *Adv Orthop* 2012;2012:984950.
13. Petersen W, Varoga D, Zantop T, et al. Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1 alpha) in tendon fibroblasts. *J Orthop Res* 2004;22:847–53.
14. Millar NL, Reilly JH, Kerr SC, et al. Hypoxia: a critical regulator of early human tendinopathy. *Ann Rheum Dis* 2012;71:302–10.
15. Benson RT, McDonnell SM, Knowles HJ, et al. Tendinopathy and tears of the rotator cuff are associated with hypoxia and apoptosis. *J Bone Joint Surg Br* 2010;92:448–53.
16. Gumina S, Natalizi S, Melaragni F, et al. The possible role of the transcription factor nuclear factor-κB on evolution of rotator cuff tear and on mechanisms of cuff tendon healing. *J Shoulder Elbow Surg* 2013;22:673–80.
17. Nguyen MT, Lue H, Kleemann R, et al. The cytokine macrophage migration inhibitory factor reduces oxidative stress-induced apoptosis. *J Immunol* 2003;170:3337–47.
18. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harb Perspect Biol* 2015;5:a008656.
19. Millar NL, Wei AQ, Molloy TJ, et al. Cytokines and apoptosis in supraspinatus tendinopathy. *J Bone Joint Surg Br* 2009;91:417–24.
20. Millar NL, Akbar M, Campbell AL, et al. IL-17A mediates inflammatory and tissue remodeling events in early human tendinopathy. *Sci Rep* 2016;6:27149.

21. Voloshin I, Gelinas J, Maloney MD, et al. Proinflammatory cytokines and metalloproteases are expressed in the subacromial bursa in patients with rotator cuff disease. *Arthroscopy* 2005;21:1076.e1–1076.e9.
22. Blaine TA, Kim YS, Voloshin I, et al. The molecular pathophysiology of subacromial bursitis in rotator cuff disease. *J Shoulder Elbow Surg* 2005;14:84S–9S.
23. Lundgreen K, Lian Ø, Scott A, et al. Increased levels of apoptosis and p53 in partial-thickness supraspinatus tendon tears. *Knee Surg Sports Traumatol Arthrosc* 2013;21:1636–41.
24. Fabiš J, Szemraj J, Strek M, et al. Is resection of the tendon edge necessary to enhance the healing process? An evaluation of the homeostasis of apoptotic and inflammatory processes in the distal 1 cm of a torn supraspinatus tendon: part I. *J Shoulder Elbow Surg* 2014;23:1772–8.
25. Fabiš J, Szemraj J, Strek M, et al. Is resection of the tendon edge necessary to enhance the healing process? An evaluation of the expression of collagen type I, IL-1 β , IFN- γ , IL-4, and IL-13 in the distal 1 cm of a torn supraspinatus tendon: part II. *J Shoulder Elbow Surg* 2014;23:1779–85.
26. Lee HJ, Kim YS, Ok JH, et al. Apoptosis occurs throughout the diseased rotator cuff. *Am J Sports Med* 2013;41:2249–55.
27. Yuan J, Murrell GA, Trickett A, et al. Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. *Biochim Biophys Acta* 2003;1641:35–41.
28. Scott A, Cook JL, Hart DA, et al. Tenocyte responses to mechanical loading in vivo: a role for local insulin-like growth factor 1 signaling in early tendinosis in rats. *Arthritis Rheum* 2007;56:871–81.
29. Hartl FU, Martin J, Neupert W. Protein folding in the cell: the role of molecular chaperones Hsp70 and Hsp60. *Annu Rev Biophys Biomol Struct* 1992;21:293–322.
30. Millar NL, Wei AQ, Molloy TJ, et al. Heat shock protein and apoptosis in supraspinatus tendinopathy. *Clin Orthop* 2008;466:1569–76.
31. Del Buono A, Oliva F, Longo UG, et al. Metalloproteases and rotator cuff disease. *J Shoulder Elbow Surg* 2012;21:200–8.
32. Wu B, Chen J, Dela Rosa T, et al. Cellular response and extracellular matrix breakdown in rotator cuff tendon rupture. *Arch Orthop Trauma Surg* 2011;131:405–11.
33. Yamada T, Gotoh M, Nakama K, et al. Effect of hyaluronan on cell proliferation and mRNA expression of procollagens α 1(I) and α 1(III) in tendon-derived fibroblasts from patients with rotator cuff disease. *Am J Sports Med* 2007;35:1870–6.
34. Osti L, Buda M, Buono AD, et al. Clinical evidence in the treatment of rotator cuff tears with hyaluronic acid. *Muscles Ligaments Tendons J* 2016;5:270–5.
35. Osti L, Berardocco M, Di Giacomo V, et al. Erratum to: hyaluronic acid increases tendon derived cell viability and collagen type I expression in vitro: comparative study of four different hyaluronic acid preparations by molecular weight. *BMC Musculoskelet Disord* 2015;16:334.
36. Nakamura H, Gotoh M, Kanazawa T, et al. Effects of corticosteroids and hyaluronic acid on torn rotator cuff tendons in vitro and in rats. *J Orthop Res* 2015;33:1523–30.
37. Harada Y, Kokubu T, Mifune Y, et al. Dose- and time-dependent effects of triamcinolone acetonide on human rotator cuff-derived cells. *Bone Joint Res* 2014;3:328–34.
38. Muto T, Kokubu T, Mifune Y, et al. Platelet-rich plasma protects rotator cuff-derived cells from the deleterious effects of triamcinolone acetonide. *J Orthop Res* 2013;31:976–82.
39. Honda H, Gotoh M, Kanazawa T, et al. Effects of lidocaine on torn rotator cuff tendons. *J Orthop Res* 2016;34:1620–7.
40. Kim SJ, Lee SM, Kim JE, et al. Effect of platelet-rich plasma with self-assembled peptide on the rotator cuff tear model in rat. *J Tissue Eng Regen Med* 2017;11:77–85.
41. Carr AJ, Murphy R, Dakin SG, et al. Platelet-rich plasma injection with arthroscopic acromioplasty for chronic rotator cuff tendinopathy: a randomized controlled trial. *Am J Sports Med* 2015;43:2891–7.
42. Kim RJ, Hah YS, Sung CM, et al. Do antioxidants inhibit oxidative-stress-induced autophagy of tenofibroblasts? *J Orthop Res* 2014;32:937–43.
43. Park HB, Hah YS, Yang JW, et al. Antiapoptotic effects of anthocyanins on rotator cuff tenofibroblasts. *J Orthop Res* 2010;28:1162–9.
44. Nam DC, Hah YS, Nam JB, et al. Cytoprotective mechanism of cyanidin and delphinidin against oxidative stress-induced tenofibroblast death. *Biomol Ther (Seoul)* 2016;24:426–32.
45. Baumgarten KM, Gerlach D, Galatz LM, et al. Cigarette smoking increases the risk for rotator cuff tears. *Clin Orthop Relat Res* 2010;468:1534–41.
46. Carbone S, Gumina S, Arceri V, et al. The impact of preoperative smoking habit on rotator cuff tear: cigarette smoking influences rotator cuff tear sizes. *J Shoulder Elbow Surg* 2012;21:56–60.
47. Lundgreen K, Lian OB, Scott A, et al. Rotator cuff tear degeneration and cell apoptosis in smokers versus non-smokers. *Arthroscopy* 2014;30:936–41.
48. Oliva F, Piccirilli E, Berardi AC, et al. Hormones and tendinopathies: the current evidence. *Br Med Bull* 2016;117:39–58.

49. Oliva F, Berardi AC, Misiti S, et al. Thyroid hormones and tendon: current views and future perspectives. Concise review. *Muscles Ligaments Tendons J* 2013;3:201–3.
50. Oliva F, Berardi AC, Misiti S, et al. Thyroid hormones enhance growth and counteract apoptosis in human tenocytes isolated from rotator cuff tendons. *Cell Death Dis* 2013;4:e705.
51. Berardi AC, Oliva F, Berardocco M, et al. Thyroid hormones increase collagen I and cartilage oligomeric matrix protein (COMP) expression in vitro human tenocytes. *Muscles Ligaments Tendons J* 2014;4:285–91.
52. Maman E, Somjen D, Maman E, et al. The response of cells derived from the supraspinatus tendon to estrogens and calciotropic hormones stimulations: in-vitro study. *Connect Tissue Res* 2015;57:124–30.