



Apoptosis in rotator cuff tendonopathy

Jun Yuan, George A.C. Murrell^{*}, Ai-Qun Wei, Min-Xia Wang

Orthopaedic Research Institute, St George Hospital Campus, 4-10 South Street, University of New South Wales, Sydney, NSW 2217, Australia

Received 15 June 2001; accepted 12 April 2002

Abstract

The aim of this study was to investigate the involvement of apoptosis (programmed cell death) in the pathogenesis of rotator cuff disorders. The edges of torn supraspinatus rotator cuff tendons were collected from patients with rotator cuff tear ($n = 25$). Samples of the intra-articular portion of subscapularis tendons were collected from patients without rotator cuff tear as control ($n = 6$). To minimize individual variance, we also collected six pairs of supraspinatus tendon and subscapularis tendon from six patients with rotator cuff tears. Apoptosis was detected by in situ DNA end labelling assay and DNA laddering assay. Immunohistochemical staining was performed to identify cells undergoing apoptosis. Control subscapularis tendon had normal morphology. Tendon from torn supraspinatus rotator cuff showed significant mucoid degeneration. Within the areas of degeneration, there were large numbers of apoptotic cells. The percentage of apoptotic cells in the degenerative rotator cuff (34%) was significantly higher than that in controls (13%) ($p < 0.001$). The excessive apoptosis detected in degenerative rotator cuff tissue was confirmed by DNA laddering assays. This is the first report of excessive apoptosis in degenerating rotator cuff tendon. Cells undergoing apoptosis in rotator cuff were mainly fibroblast-like cells. These findings indicate that apoptosis may play an important role in the pathogenesis of rotator cuff degeneration. © 2002 Orthopaedic Research Society. Published by Elsevier Science Ltd. All rights reserved.

Introduction

The rotator cuff is the primary dynamic stabilizer of the shoulder joint and is placed under significant stress during overhead sport and contact sport [7]. Rupture of the rotator cuff results in significant pain and difficulty with overhead activities. In clinical practice, a ruptured rotator cuff tendon rarely heals without surgical reposition [23]. The causes of failure of rotator cuff tendon are undetermined. Degenerative changes have been found in the tendon matrix of ruptured rotator cuff [6,28,41,43]. Mucoid degeneration and loss of mechanical strength are early changes in a rat rotator cuff overuse model [12]. Despite the clinical significance of tendon degeneration, the degenerative processes are poorly understood from a cellular and molecular perspective [26].

Apoptosis, or programmed cell death, is an important component of embryogenesis, organogenesis, and tissue morphogenesis as well as in the maintenance of homeostasis in many adult tissues [17,51]. It is a physi-

ological process contributing to the control of cell population [34]. Apoptosis removes damaged or virus-infected cells and is essential for the regulation of normal development. For example, millions of autoreactive T and B lymphocytes produced by the immune system are eliminated by apoptosis every day. However, uncontrolled apoptosis may be pathogenic. Excessive apoptosis is associated with neurodegeneration [25], osteoarthritis [2,14,33] and rheumatoid arthritis [4]. Impaired apoptosis is also characteristic of cancer [10]. The involvement of apoptosis in tendon degeneration has not been evaluated. The aim of this study was to examine the involvement of apoptosis in rotator cuff tendonopathy.

Materials and methods

Reagents

Potassium acetate, phenol:chloroform:isoamyl alcohol (25:24:1, v:v:v), chloroform:isoamyl alcohol (24:1, v:v), 3 M sodium acetate, Tris-HCl and EDTA were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). DNase-free RNase, RNase-free DNase, terminal transferase enzyme (TdT), cobalt chloride, proteinase K and biotinylated-11-dUTP were obtained from Roche Molecular Biochemicals (Mannheim, Germany). Streptavidin-HRP, streptavidin peroxidase, diaminobenzidine tetrahydrochloride (DAB) solution,

^{*} Corresponding author. Tel.: +61-2-93502827; fax: +61-2-93503967.

E-mail address: murrell.g@ori.org.au (G.A.C. Murrell).

64 Target Retrieval Solution, LINK solution, anti-human macrophage
65 antibody (CD68), and anti-human fibroblast antibody were obtained
66 from DAKO Corporation (Carpinteria, CA, USA). [α^{32} P]-ddATP was
67 purchased from GeneWorks Pty Ltd. (Adelaide, SA, Australia), and
68 30% (v/v) H₂O₂, sucrose, and ammonium acetate from Merck Pty Ltd.
69 (Whitehouse station, NJ, USA).

70 *Patients and tissue collection*

71 Human tissue collection was approved by the South Eastern Syd-
72 ney Area Health Service Ethics Committee, Australia. As illustrated in
73 Table 1, 25 supraspinatus rotator cuff tendon samples were collected
74 from patients with rotator cuff tear undergoing shoulder surgery. The
75 mean age of the rotator cuff ruptured patients was 61 years (age range
76 32–86 years). The samples consisted of the edges of torn supraspinatus
77 tendon excised prior to surgical reattachment. The rotator cuff repair
78 surgery was performed via a deltoid splitting approach. The size of the
79 tear was recorded at the time of the operation. As a control, six
80 samples of subscapularis tendon were collected from patients under-
81 going arthroscopic shoulder surgery for shoulder stabilization without
82 rotator cuff tear. The absence of a rotator cuff tear in these patients was
83 confirmed by arthroscopic examination. The mean age of the rotator
84 cuff normal patients was 24 years (age range 17–57 years). Six pairs of
85 subscapularis tendon tissue and degenerating supraspinatus tendon
86 tissue were also collected from six supraspinatus rotator cuff ruptured
87 patients (age range 35–86 years, mean age 66 years).

88 The subscapularis tendons were harvested arthroscopically from
89 the superior border of the tendon 1 cm lateral to the glenoid labrum.
90 The supraspinatus tendons were harvested from the torn edge of su-
91 praspriatus during the open portion of the surgery prior to surgical
92 repair. The tendon tissues were snap frozen and stored at -70 °C until
93 DNA extraction was performed. For immunohistochemical staining
94 and in situ DNA end labelling assays, the tissue samples were im-
95 mediately fixed in 10% (v/v) formalin for 4–6 h and then embedded in
96 paraffin.

97 *In situ end labelling of fragmented DNA*

98 One of the early features of apoptosis is DNA cleavage in cell
99 nuclei [52]. In our study, DNA cleavage was assessed by the terminal
100 deoxynucleotidyl transferase (TdT) mediated dUTP-biotin nick label-
101 ing reaction, using a modified method described by Gavrieli et al. [20].
102 In brief, paraffin sections of tendon tissue (3 μ m thick) were carefully
103 dewaxed with xylene and graded ethanol, and dehydrated in TBS
104 buffer (20 mM Tris-HCl pH 7.6, 140 mM NaCl) for 15 min. The tissue
105 was digested with 20 μ g/ml proteinase K for 10 min at room temper-
106 ature, incubated with 3% (v/v) H₂O₂ to inactivate endogenous per-
107 oxidase, and equilibrated with TdT buffer (30 mM Tris-HCl pH 7.2,
108 140 mM sodium cacodylate, 1 mM cobalt chloride). Fragmented nu-
109 cleic DNA on each section was end labelled with biotinylated-11-
110 dUTP using TdT for 90 min at 37 °C. After incubation with strepto-
111 avidin-HRP for 30 min at room temperature, the colour was developed
112 with DAB solution for 5 min at room temperature. Cells were
113 counterstained with Harris haematoxylin. Negative controls consisted
114 of slides incubated with reaction mixture lacking TdT enzyme. Positive
115 control slides were included in each assay. These were generated by
116 DNase (0.5 μ l) digestion of tendon tissue for 20 min at room tem-
117 perature. Positive nuclei stained brown and negative nuclei stained
118 blue. The percentages of apoptotic cells in tissue sections were assessed
119 by two researchers blinded to the source of tissue. In each section,
120 counts were made of 500 cells distributed in each of three areas with

distinct morphological appearances. The degenerative tendon tissue
mainly contained collagen fibers [16,29] (<100 fibroblast-like cells per
0.04 mm²), granular material (proteoglycans and glycosaminoglycans),
proliferative fibroblast-like cells [29] (>100 fibroblast-like cells per 0.04
mm²) and vascular tissue [16,29].

Analysis of DNA fragmentation by agarose gel electrophoresis

The DNA laddering assay was performed to determine if there
were DNA laddering patterns characteristic for apoptosis [49]. To
extract sample DNA, the tissue was milled to a fine power in liquid
nitrogen using a mortar and pestle and lysed in a lysis buffer containing
300 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 20 mM EDTA,
200 mM sucrose and 0.5% (v/v) sodium dodecyl sulfate (SDS) at 65 °C
for 30 min. 8 M potassium acetate was then added to the reaction mix
and incubated on ice for 60 min. Samples were centrifuged at 5000 \times g
for 10 min at 4 °C. The supernatant was extracted with an equal
volume of phenol:chloroform:isoamyl alcohol (25:24:1, v:v:v) and
chloroform:isoamyl alcohol (24:1, v:v). The upper aqueous phase was
collected and precipitated by addition of 2.5 \times volume ice-cold 100%
(v/v) ethanol. The precipitated nucleic acids were collected by centri-
fugation at 14,000 \times g for 30 min at 4 °C. RNA was removed by
incubation of the extract with DNase-free RNase. The DNA sample
was reprecipitated, resuspended in purified water and quantitated by a
spectrophotometer at 260 nm. To label the extracted sample DNA, 500
ng DNA was incubated with a reaction mixture containing 1 μ l (25 U)
TdT, 5 \times TdT buffer, 1 mM cobalt chloride, and 5 μ l [α^{32} P]-ddATP (17
pmoles; 50 μ Ci) for 60 min at 37 °C. The reaction was terminated by
0.25 M EDTA (pH 8.0). Unlabelled radioisotopes were removed by 2
cycles of ammonium acetate precipitation. Labelled DNA was frac-
tionated on a 2% (w/v) agarose gel at 50 V for 3 h. The gel was dried in
a slab-gel drier (SGD 2000, Savant, Farmingdale, NY) for 2 h and
subsequently exposed to an X-ray film.

Immunohistochemical identification of tendon cells

CD68 and anti-human fibroblast antibody were used as makers for
macrophages and fibroblasts respectively. Paraffin sections were de-
waxed with xylene and graded ethanol. Antigen retrieval was achieved
using DAKO Target Retrieval Solution as per the manufacturer's in-
structions. Endogenous peroxidase activity was scavenged with 3% (v/
v) H₂O₂, and non-specific antibody binding blocked with 10% (w/v)
milk in TBS buffer for 15 min. Tissue slides were incubated with pri-
mary antibody (CD68 or anti-human fibroblast antibody) diluted 1:50
in 1% (w/v) BSA/TBS at room temperature for 60 min. After two
washes, slides were incubated with DAKO LINK solution consisting
of biotinylated anti-mouse and anti-rabbit Ig. The slides were washed
and incubated with streptavidin-peroxidase, followed by extensive
washing and exposure to DAKO liquid DAB for 5 min. Finally, the
sections were counterstained with haematoxylin. An irrelevant mouse
monoclonal antibody was included in each assay as negative control.

Statistical analysis

All values in the test and figures are expressed as mean \pm SEM of *n*
observations. Statistical analysis among the different areas in degen-
erative rotator cuff group was performed using analysis of variance
(ANOVA). Statistical comparison between normal subscapularis ten-
don and degenerative supraspinatus tendon from the different patients

Table 1
Clinical characteristics of the patients

Tendon samples	Condition of group	Number of patients	Age	Sex (M/F)	History of trauma (+/-)	Duration of symptoms (months)
Subscapularis tendon	Control	6	25 \pm 8	4/2	4/2	10 \pm 3
Supraspinatus tendon	Rotator cuff tear	25	61 \pm 3	16/9	18/7	18 \pm 4
Paired supraspinatus and subscapularis tendon	Rotator cuff tear	6	63 \pm 8	3/3	3/3	20 \pm 2

174 was performed using unpaired two-tailed Student's *t*-tests. Statistical
175 analysis of subscapularis tendon and supraspinatus tendon from the
176 same rotator cuff ruptured patients was performed using paired two-
177 tailed Student's *t*-tests. The confidence limit was predetermined at an
178 alpha level of 0.05.

179 Results

180 Apoptosis in ruptured rotator cuff tendon

181 With the in situ DNA end labelling method, apop-
182 totic cells stain dark brown, the negative cells stain blue.
183 Sections of rotator cuff tendon prepared with the omit-
184 ted TdT enzyme showed a blue colour (Fig. 1a), con-
185 firming that there was no non-specific binding.

186 Subscapularis tendon had a normal appearance with
187 a dense collagenous matrix interspersed with normal
188 fibroblast-like cells. Only a few of the cells stained
189 brown (Fig. 1b). Tendon from torn supraspinatus ten-
190 don had mucoid degeneration. There were changes in
191 the cellular components, collagen fibers and blood ves-
192 sels that were visible by hematoxylin and eosin (H&E)
193 staining on light microscopy. This histological appear-
194 ance was similar to that described by Kannus and Clark
195 [16,29]. In our study, a large number of dark brown cells
196 was found in section of degenerative supraspinatus

tendon (Fig. 1c and d). The apoptotic cells were evenly
197 distributed in tendinous, cellular and peri-vascular areas
198 in the degenerative supraspinatus tendon.
199

200 There were no significant differences in the propor-
201 tions of apoptotic cells among the tendinous, cellular
202 and vascular areas of the degenerative supraspinatus
203 rotator cuff tendon group (30% vs 37% vs 34%,
204 $p = 0.27$). Therefore, we combined data from these three
205 areas to provide a single mean value for the percentage
206 of apoptotic cells in each supraspinatus tendon sample.
207 As shown in Fig. 2, there was more than a twofold
208 increase in the percentage of apoptotic cells in supra-
209 spinatus tendon (34%) compared with subscapularis
210 tendon (13%). To evaluate the apoptosis in normal and
211 degenerative tendon from the same host, paired tissue
212 samples from the edges of degenerative torn supraspinat-
213 us tendon and normal subscapularis tendon were
214 collected from rotator cuff ruptured patients. The per-
215 centage of apoptotic cells in the degenerative tissue
216 (36%) was significantly higher than that in the normal
217 tissue (21%) ($p < 0.001$) (Fig. 3). To evaluate the
218 amount of apoptosis in normal tendon tissue under
219 different shoulder conditions, normal subscapularis
220 tendon tissue was collected from rotator cuff normal
221 patients and rotator cuff ruptured patients. There was a
222 significantly higher percentage of apoptotic cells in

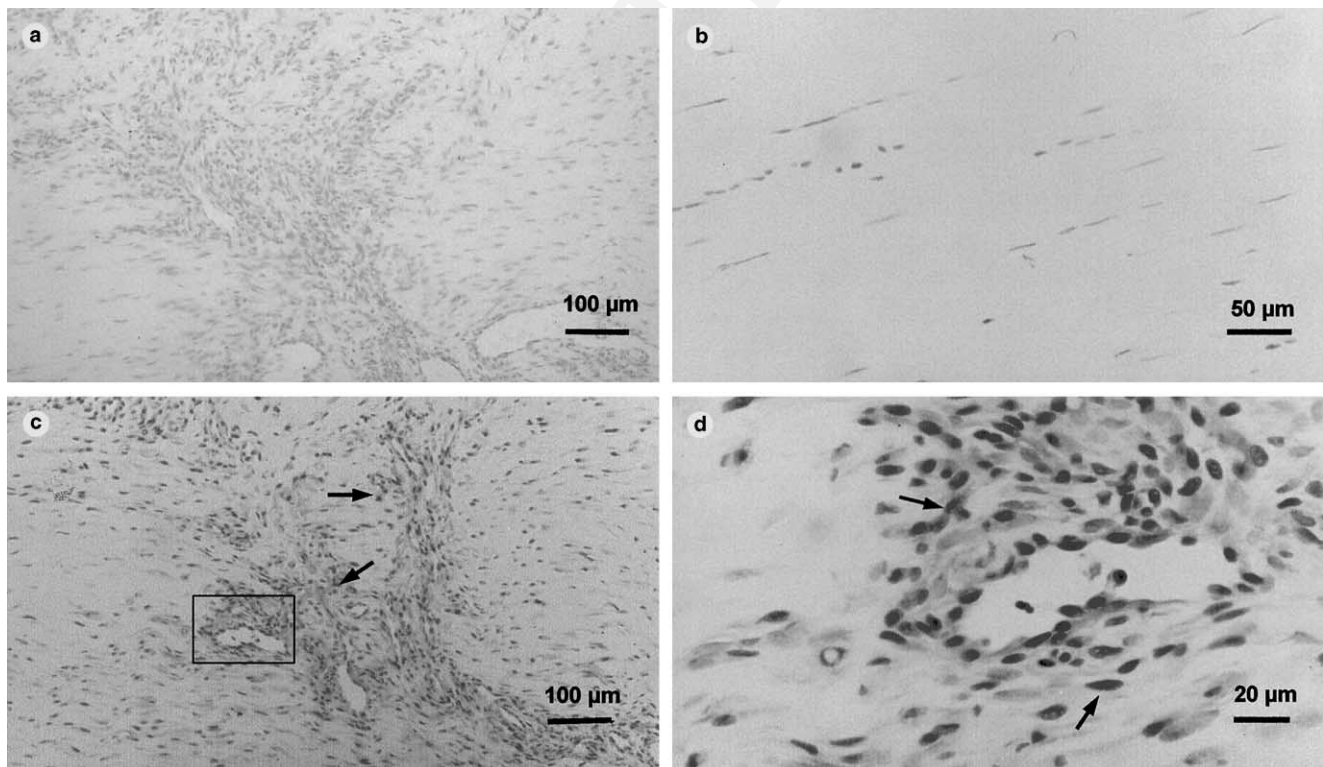


Fig. 1. Apoptosis in rotator cuff tendon was assessed by in situ DNA end labelling assay. (a) Negative control section of degenerative supraspinatus tendon tissue from a ruptured rotator cuff patient generated by omitting TdT from reaction. (b) Subscapularis tendon from a rotator cuff normal patient showing only a few apoptotic cells. (c) A large number of apoptotic cells were identified in degenerative supraspinatus rotator cuff tissue (arrows). (d) High-power view of boxed area in (c).

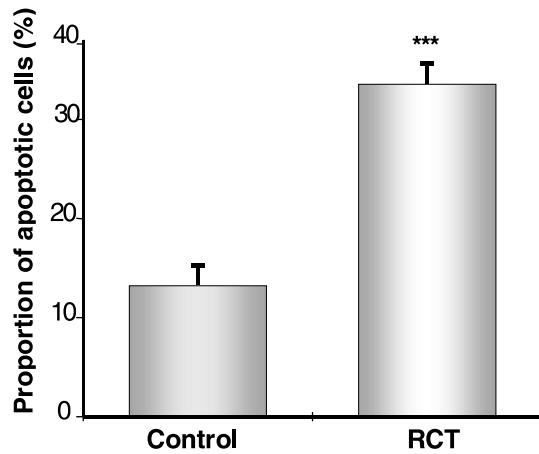


Fig. 2. Comparison of proportion (%) of apoptotic cells in supraspinatus rotator cuff tendon (RCT) from rotator cuff ruptured patients and subscapularis tendon (control) from rotator cuff normal patients. All tissues were stained by DNA end labelling assay. Blinded assessment of percentage of apoptotic cells was performed. In each section, 500 cells were counted in tendinous, cellular and vascular areas. Mean \pm SEM, $n = 25$ for RCT group, $n = 6$ for control group. *** $p < 0.001$ when compared with control group using unpaired two-tailed Student's t -tests.

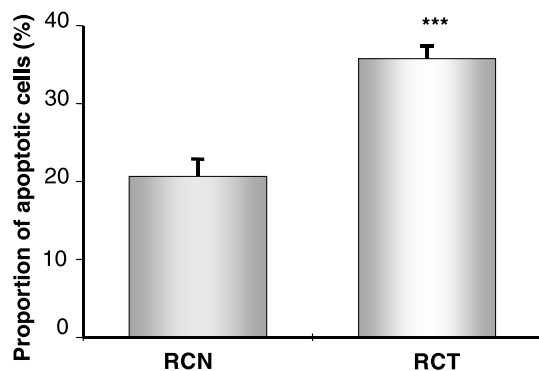


Fig. 3. Comparison of proportion (%) of apoptotic cells in supraspinatus rotator cuff tendon (RCT) and normal subscapularis tendon (RCN) from the same patients. Paired RCT and RCN were collected from the same rotator cuff ruptured patients. All tissue was stained by DNA end labelling assay, and the percentages of apoptotic cells were calculated. Mean \pm SEM, $n = 6$ for each group. *** $p < 0.001$ when compared with RCN group using paired two-tailed Student's t -tests.

223 subscapularis tendon tissue (21%) taken from rotator
 224 cuff ruptured patients than from rotator cuff normal
 225 patients (13%) ($p < 0.05$) (Fig. 4).
 226 Excessive apoptosis detected in degenerative rotator
 227 cuff tissue were confirmed by the DNA laddering assay.
 228 Degenerative supraspinatus tendon samples from rota-
 229 tor cuff ruptured patients not only showed excessive
 230 apoptosis by DNA end labelling assay but also showed a
 231 characteristic DNA ladder pattern on agarose gel. DNA
 232 extracted from subscapularis tendon did not show this
 233 pattern (Fig. 5a). To minimize variance caused by in-
 234 dividual differences, the same methods were used to

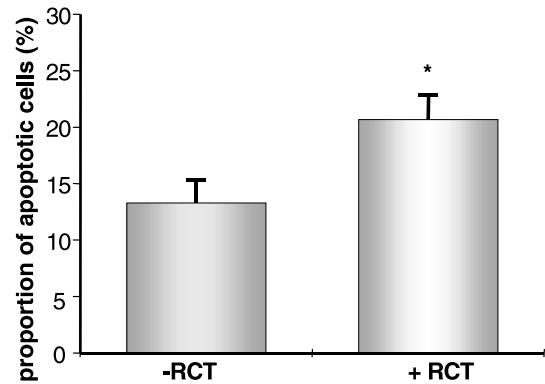


Fig. 4. Comparison of proportion (%) of apoptotic cells in subscapularis tendon from rotator cuff ruptured patients and rotator cuff normal patients. Subscapularis tendon tissues collected from the patients with (+RCT) or without (-RCT) rotator cuff tear were stained by DNA end labelling assay. The percentage of apoptotic cells was calculated. Mean \pm SEM, $n = 6$ for +RCT group, $n = 6$ for RCT group. *** $p < 0.05$ when compared with RCT group using unpaired two-tailed Student's t -tests.

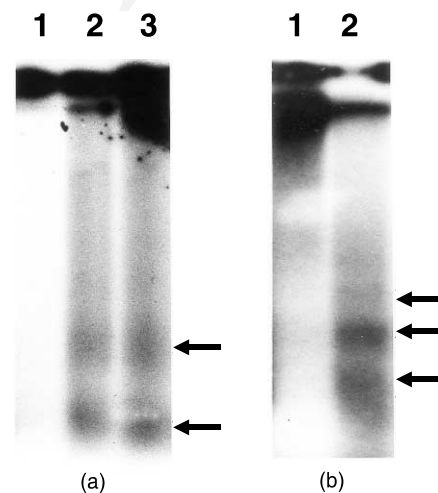


Fig. 5. DNA laddering assay. (a) Laddering bands (arrows) unique for apoptosis were detected in degenerative supraspinatus tendon tissues from rotator cuff ruptured patients (lane 2-3). Subscapularis tendon (control) from a rotator cuff normal patient did not show the laddering patterns (lane 1). (b) Paired subscapularis tendon and supraspinatus tendon from three rotator cuff ruptured patients were analyzed. Laddering bands (arrows) were detected in degenerative supraspinatus tendon tissue of patient (lane 2), but not in normal subscapularis tendon tissue of the same patient (lane 1).

235 evaluate apoptosis in normal and degenerative tendon
 236 tissue collected from the same patients. As shown in Fig.
 237 5b, DNA from degenerative supraspinatus tendon tissue
 238 had much more intensive laddering bands than that
 239 from subscapularis tendon tissue, indicating the differ-
 240 ence of apoptosis detected in both samples was reliable.
 241 Tissue from degenerative supraspinatus tendon with a
 242 higher proportion of apoptotic cells also showed more
 243 intensive laddering bands.

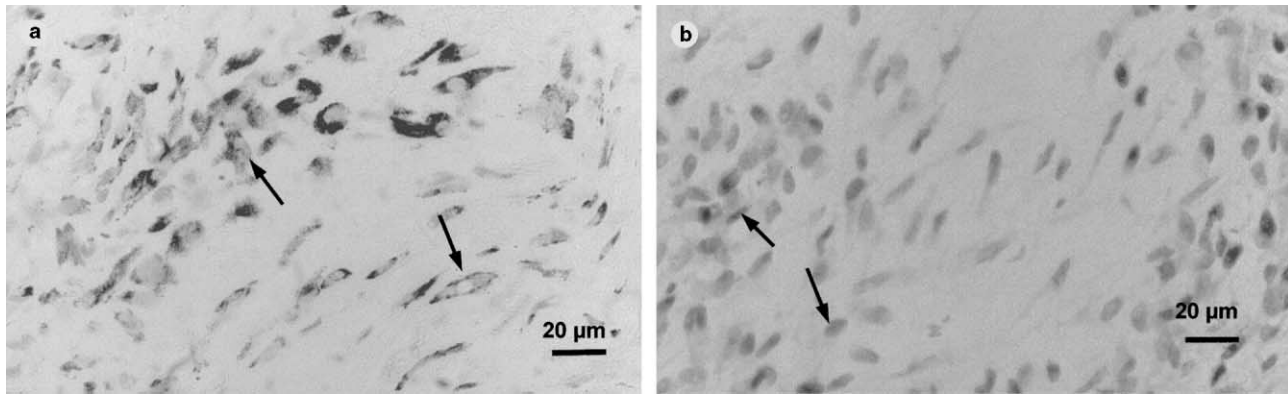


Fig. 6. Immunohistochemical identification of fibroblast-like cells. In degenerative supraspinatus tendon from a rotator cuff ruptured patient, fibroblast-like cell cytoplasm (arrows) stained positive with the anti-human fibroblast antibody (panel a). On a serial section, fibroblast-like cell nuclei stains positive with in situ DNA end labelling assay (panel b).

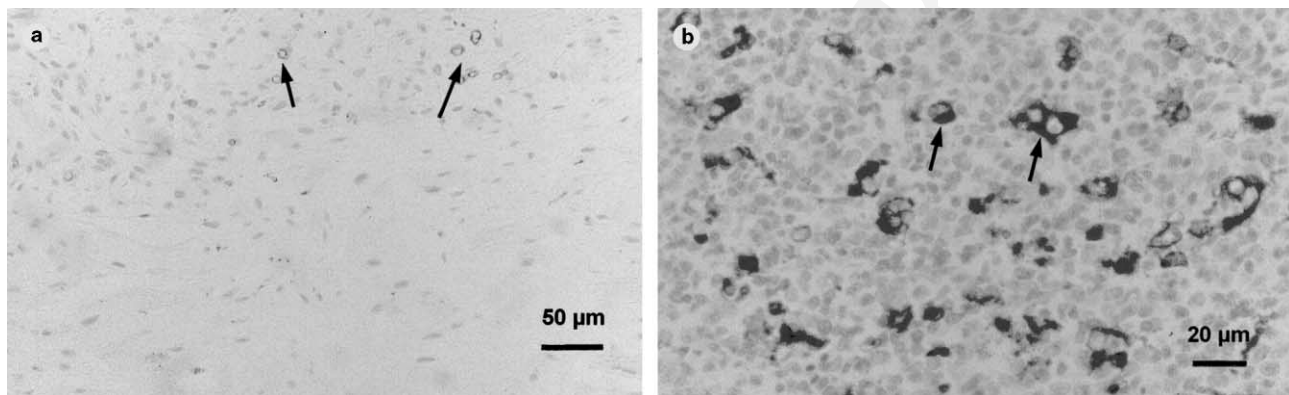


Fig. 7. Immunohistochemical identification of macrophages. CD-68 stained a few macrophages (arrows) in degenerative supraspinatus tendon from a rotator cuff ruptured patient (panel a). The same antibody stained macrophages in a human tonsil section as positive control (panel b).

244 *Excessive apoptotic cells in ruptured rotator cuff are*
245 *fibroblast-like cells*

246 Immunohistochemical staining was performed to
247 identify cell types undergoing apoptosis using CD-68
248 and anti-human fibroblast antibody. As shown in Fig. 6,
249 the majority of apoptotic cells stained with anti-human
250 fibroblast antibody, i.e. were fibroblast-like cells. Only a
251 few cells were CD-68 positive (Fig. 7).

252 *Apoptosis and its clinical association*

253 There was no correlation between the proportion of
254 apoptotic cells, subject age, the size of rotator cuff tear
255 and duration of symptoms in degenerative tissue from
256 supraspinatus rotator cuff ruptured patients. However,
257 there was a 1.5-fold increase in the percentage of
258 apoptotic cells in subscapularis tendons in the group
259 with rotator cuff tears, compared with the younger
260 group with no tears, indicating that age may be a factor
261 contributing to apoptosis and degeneration.

Discussion

A significant finding in this study was that there were
twice as many apoptotic cells in ruptured supraspinatus
tendon than normal subscapularis tendon. The apoptotic
cells were distributed evenly throughout the torn
edges of the supraspinatus tendon. This is the first evi-
dence of excessive apoptosis in rotator cuff tendon dis-
orders. To confirm this phenomenon, we collected
degenerative supraspinatus tendon and normal sub-
scapularis tendon from the same patients and performed
a paired experiment. Degenerative tendon had a much
higher proportion of apoptotic cells than normal tendon
which had relatively normal histology. This observation
was also confirmed by the DNA laddering assay.

Although there are no reports of apoptosis in de-
generative tendon, increased apoptosis has been found
in the several neurodegenerative diseases including
Alzheimer's disease, Huntington's disease, Parkinson's
disease, amyotrophic lateral sclerosis, ataxia telangiectasia,
and retinitis pigmentosa [9,18,24,39,47]. The en-
suing slower and more widespread degeneration that is

262

263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282

283 responsible for much of the damage in these conditions
284 has apoptotic characteristics [31,32]. In the musculo-
285 skeletal system, excessive apoptosis has been found in
286 osteoarthritis [2,33,35].

287 Using specific cell markers, we were able to identify
288 that the apoptotic cells in the rotator cuff were fibro-
289 blasts or fibroblast-like cells. The role of apoptotic cells
290 in rotator cuff tear is not clear. It is well known that
291 fibroblasts are the predominant cell type within tendon.
292 They are responsible for the production and mainte-
293 nance of tendon collagen and non-collagenous constitu-
294 ents [8]. Tendon fibroblast-like cells express the genes
295 for the matrix molecules present. Both the mRNA and
296 protein for collagen have been detected in tendon tissue
297 [44]. Loss of cellular activity and decreased extracellular
298 matrix synthesis are thought to be intrinsic causes of
299 tendon degeneration [42]. The DNA degradation de-
300 tected in degenerative tissue from the rotator cuff rup-
301 tured patients indicates that the fibroblast/fibrocyte
302 function may be affected by apoptosis. The reduced
303 number of functional fibroblasts/fibrocytes may con-
304 tribute to impaired collagen metabolism culminating in
305 rotator cuff degeneration.

306 In our study, we investigated the apoptosis in de-
307 generative supraspinatus tendons. The subscapularis
308 tendons from non-rotator cuff ruptured and rotator cuff
309 ruptured patients were served as control based on their
310 H&E appearance. Many investigators have shown that
311 there is a difference between the subscapularis and su-
312 praspinalis tendons in terms of their anatomy and
313 function. Clark et al. [16] further demonstrated that
314 both tendons fused into one structure at or near their
315 insertion into the humerus. They formed a cuff and
316 shared a similar environment.

317 Murrell and Watson [37] have showed that the
318 prevalence of rotator cuff tears increases with age. In our
319 study, there was no correlation between the proportion
320 of apoptotic cells, subject age and duration of symp-
321 toms. This is probably due to the relatively small num-
322 ber of patients studied, and/or the age of most patients
323 being limited in the range of 50-70 years old. However,
324 we did find a significant difference of apoptotic per-
325 centage in normal subscapularis tendon tissue between
326 the rotator cuff normal patients (mean age 25 years) and
327 the rotator cuff ruptured patients (mean age 66 years),
328 indicating that age may be a factor contributing to
329 apoptosis and degeneration.

330 Apoptosis is a physiological process which can be
331 identified in healthy adult tissue [13,38]. We have found
332 a surprisingly high figure of apoptotic cells (13%) in
333 subscapularis tendon from the control group. Two
334 possibilities could explain these finding: (1) All of our
335 control patients had other shoulder problems, therefore
336 they are not really normal. (2) Certain types of cells may
337 undergo apoptosis much more slowly than other cell
338 types. Therefore, more apoptotic cells can be identified

in these cell populations [30]. The degree of apoptosis in
normal tissue has varied considerably in recent investi-
gations. Adams and Horton identified 3-10% of cells
undergoing apoptosis in the articular cartilage of adult
animals [1]. While, Gruber and Hanley found that 73%
of the cells were apoptotic in normal human inter-ver-
tebral disc [22]. There was no laddering pattern detected
in subscapularis tendon (control) by DNA laddering
assay, although a high percentage of apoptotic cells was
identified by in situ DNA end labelling assay. DNA
laddering pattern is a hall-marker of apoptosis. This
method is more specific but less sensitive for apoptotic
detection comparing with in situ DNA end labelling
assay. The undetected apoptosis in the subscapularis
tendons by the DNA laddering assay is consistent with
similar findings that no laddering patterns were detected
in the normal tissue of brain, muscle and cartilage
[19,40].

With regard to the high percentage of apoptosis
(34%) in degenerative tendon, false positive stain needs
to be elucidated, as DNA end labelling assay also stains
necrotic cells [36]. Results from our DNA laddering
assay clearly showed the unique DNA laddering pattern
in degenerative tendon, suggesting apoptosis is a true
phenomenon in situ. The increased apoptosis in degen-
erative tendon could be the result of some risk factors,
such as, ischaemia [15,27,50], hypoxia [5], free radical
generation [21,45] and nutritional imbalances [11] in-
volved in other types of tissue degeneration. The
mechanism that initiates apoptosis also be a combina-
tion of factors.

At present, we do not know what induces apoptosis
in tendon degeneration. In a rat rotator cuff injury
model, it has been shown that repetitive stress (overuse)
causes tendonitis and degenerative changes [46]. Inves-
tigations from another group have demonstrated that
stress-activated protein kinases (SAPK), an upstream
regulator of apoptosis, were upregulated in tendon cells
under cyclic strain [3,48]. It is possible that repetitive
stress (overuse) may activate tendon cells to undergo
apoptosis and contribute to tendon degeneration.
Apoptosis has been found to contribute to tissue de-
generation in other degenerative conditions, such as,
neurodegeneration [25] and osteoarthritis [2,14,33].
Whether the apoptosis observed in this study is a cause
of degeneration or a sequella of the degenerative process
requires further investigation.

In summary, the present study is the first to reveal
that excessive apoptosis is present in rotator cuff tendon
disorders. The increased number of apoptotic tendon
cells in degenerative tendon tissue could affect the rate of
collagen synthesis and repair. Impaired or dysfunctional
protein synthesis may lead to weaker tendon tissue and
eventually increase the risk for tendon rupture. Further
research is necessary to see if this work will lead to a
better understanding of the cellular and molecular

339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394

395 mechanisms of tendon degeneration, and better treat-
396 ment strategies.

397 Acknowledgements

398 This study was supported by The St George Hospital/
399 South Eastern Sydney Area Health Service.

400 References

401 [1] Adams CS, Horton Jr WE. Chondrocyte apoptosis increases with
402 age in the articular cartilage of adult animals. *Anat Rec*
403 1998;250:418-25.
404 [2] Amin AR, Abramson SB. The role of nitric oxide in articular
405 cartilage breakdown in osteoarthritis. *Curr Opin Rheumatol*
406 1998;10:263-8.
407 [3] Arnoczky S, Tian, T, Schuler, P, Morse, P, Lavagnino, M,
408 Gardner, K. Upregulation of stress-activated protein kinases
409 (SAPK) in response to increased cytosolic calcium levels due to
410 cyclic strain: a potential cellular mechanism for repetitive stress
411 injuries in tendons. In: 47th Orthopaedic Research Society Annual
412 Meeting, February 25-28, 2001, San Francisco, CA.
413 [4] Asahara H, Hasunuma T, Obata T, Sumida T, Nishioka K.
414 Expression of Fas/Fas ligand and proto-oncogenes in rheumatoid
415 synovial tissues. *Nippon Rinsho* 1996;54:1960-4.
416 [5] Biagas K. Hypoxic-ischemic brain injury: advancements in the
417 understanding of mechanisms and potential avenues for therapy.
418 *Curr Opin Pediatr* 1999;11:223-8.
419 [6] Birch HL, Bailey JV, Bailey AJ, Goodship AE. Age-related
420 changes to the molecular and cellular components of equine flexor
421 tendons. *Equine Vet J* 1999;31:391-6.
422 [7] Blevins FT. Rotator cuff pathology in athletes. *Sports Med*
423 1997;24:205-20.
424 [8] Blevins FT, Djurasovic M, Flatow EL, Vogel KG. Biology of the
425 rotator cuff tendon. *Orthop Clin North Am* 1997;28:1-16.
426 [9] Bredesen DE. Neural apoptosis. *Ann Neurol* 1995;38:839-51.
427 [10] Bruckheimer EM, Kyprianou N. Apoptosis in prostate carcino-
428 genesis. A growth regulator and a therapeutic target. *Cell Tissue*
429 *Res* 2000;301:153-62.
430 [11] Cai J, Nelson KC, Wu M, Sternberg Jr P, Jones DP. Oxidative
431 damage and protection of the RPE. *Prog Retin Eye Res*
432 2000;19:205-21.
433 [12] Carpenter JE, Flanagan CL, Thomopoulos S, Yian EH, Soslow-
434 sky LJ. The effects of overuse combined with intrinsic or extrinsic
435 alterations in an animal model of rotator cuff tendinosis. *Am J*
436 *Sports Med* 1998;26:801-7.
437 [13] Carraro U, Franceschi C. Apoptosis of skeletal and cardiac
438 muscles and physical exercise. *Aging (Milano)* 1997;9:19-34.
439 [14] Chikanza I, Fernandes L. Novel strategies for the treatment of
440 osteoarthritis. *Expert Opin Invest Drugs* 2000;9:1499-510.
441 [15] Choi DW. Ischemia-induced neuronal apoptosis. *Curr Opin*
442 *Neurobiol* 1996;6:667-72.
443 [16] Clark JM, Harryman DT, 2nd. Tendons, ligaments, and capsule
444 of the rotator cuff. Gross and microscopic anatomy. *J Bone Joint*
445 *Surg Am* 1992;74:713-25.
446 [17] Cohen JJ. Apoptosis. *Immunol Today* 1993;14:126-30.
447 [18] Cotman CW, Anderson AJ. A potential role for apoptosis in
448 neurodegeneration and Alzheimer's disease. *Mol Neurobiol*
449 1995;10:19-45.
450 [19] Facchinetti A, Tessarollo L, Mazzocchi M, Kingston R, Collavo
451 D, Biasi G. An improved method for the detection of DNA
452 fragmentation. *J Immunol Meth* 1991;136:125-31.

[20] Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of pro- 453
grammed cell death in situ via specific labeling of nuclear DNA 454
fragmentation. *J Cell Biol* 1992;119:493-501. 455
[21] Gorman AM, McGowan A, O'Neill C, Cotter T. Oxidative stress 456
and apoptosis in neurodegeneration. *J Neurol Sci* 1996;139:45-52. 457
[22] Gruber HE, Hanley Jr EN. Analysis of aging and degeneration of 458
the human intervertebral disc. Comparison of surgical specimens 459
with normal controls. *Spine* 1998;23:751-7. 460
[23] Habermeyer P, Lehmann L, Lichtenberg S. Rotator cuff tears: 461
diagnosis and therapy. *Orthopade* 2000;29:196-208. 462
[24] Hartley A, Stone JM, Heron C, Cooper JM, Schapira AH. 463
Complex I inhibitors induce dose-dependent apoptosis in PC12 464
cells: relevance to Parkinson's disease. *J Neurochem* 465
1994;63:1987-90. 466
[25] Jacobson MD. Anti-apoptosis therapy: a way of treating neural 467
degeneration? *Curr Biol* 1998;8:R418-21. 468
[26] Jarvinen M, Jozsa L, Kannus P, Jarvinen TL, Kvist M, Leadbetter 469
W. Histopathological findings in chronic tendon disorders. *Scand* 470
J Med Sci Sports 1997;7:86-95. 471
[27] Jennings RB, Reimer KA. The cell biology of acute myocardial 472
ischemia. *Ann Rev Med* 1991;42:225-46. 473
[28] Jozsa L, Lehto M, Kvist M, Balint JB, Refly A. Alterations in dry 474
mass content of collagen fibers in degenerative tendinopathy and 475
tendon-rupture. *Matrix* 1989;9:140-6. 476
[29] Kannus P, Jozsa L. Histopathological changes preceding sponta- 477
neous rupture of a tendon. A controlled study of 891 patients. *J* 478
Bone Joint Surg Am 1991;73:1507-25. 479
[30] Kavantzias NG, Lazaris AC, Agapitos EV, Nanas J, Davaris PS. 480
Histological assessment of apoptotic cell death in cardiomyopa- 481
thies. *Pathology* 2000;32:176-80. 482
[31] Li Y, Sharov VG, Jiang N, Zaloga C, Sabbah HN, Chopp M. 483
Ultrastructural and light microscopic evidence of apoptosis after 484
middle cerebral artery occlusion in the rat. *Am J Pathol* 485
1995;146:1045-51. 486
[32] Linnik MD, Zobrist RH, Hatfield MD. Evidence supporting a 487
role for programmed cell death in focal cerebral ischemia in rats. 488
Stroke 1993;24:2002-8, discussion 08-9. 489
[33] Lotz M, Hashimoto S, Kuhn K. Mechanisms of chondrocyte 490
apoptosis. *Osteoarthritis Cartil* 1999;7:389-91. 491
[34] Macaya A. Apoptosis in the nervous system. *Rev Neurol* 492
1996;24:1356-60. 493
[35] Manolagas SC. Cellular and molecular mechanisms of osteopo- 494
rosis. *Aging (Milano)* 1998;10:182-90. 495
[36] Mesner Jr PW, Kaufmann SH. Methods utilized in the study of 496
apoptosis. *Adv Pharmacol* 1997;41:57-87. 497
[37] Murrell GA, Walton JR. Diagnosis of rotator cuff tears. *Lancet* 498
2001;357:769-70. 499
[38] Phaneuf S, Leeuwenburgh C. Apoptosis and exercise. *Med Sci* 500
Sports Exerc 2001;33:393-6. 501
[39] Portera-Cailliau C, Hedreen JC, Price DL, Koliatsos VE. 502
Evidence for apoptotic cell death in Huntington disease and 503
excitotoxic animal models. *J Neurosci* 1995;15:3775-87. 504
[40] Praul CA, Gay CV, Leach Jr RM. Chondrocytes of the tibial 505
dyschondroplastic lesion are apoptotic. *Int J Dev Biol* 506
1997;41:621-6. 507
[41] Riley GP, Harrall RL, Constant CR, Cawston TE, Hazleman BL. 508
Prevalence and possible pathological significance of calcium 509
phosphate salt accumulation in tendon matrix degeneration. 510
Ann Rheum Dis 1996;55:109-15. 511
[42] Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, 512
Hazleman BL. Glycosaminoglycans of human rotator cuff ten- 513
dons: changes with age and in chronic rotator cuff tendinitis. *Ann* 514
Rheum Dis 1994;53:367-76. 515
[43] Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, 516
Hazleman BL. Tendon degeneration and chronic shoulder pain: 517
changes in the collagen composition of the human rotator cuff 518
tendons in rotator cuff tendinitis. *Ann Rheum Dis* 1994;53:359-66. 519

- 520 [44] Robbins JR, Vogel KG. Regional expression of mRNA for
521 proteoglycans and collagen in tendon. *Eur J Cell Biol*
522 1994;64:264-70. 534
- 523 [45] Shackelford RE, Kaufmann WK, Paules RS. Oxidative stress and
524 cell cycle checkpoint function. *Free Radical Biol Med*
525 2000;28:1387-404. 535
- 526 [46] Soslowsky LJ, Thomopoulos S, Tun S, Flanagan CL, Keefer CC,
527 Mastaw J, et al. Neer Award 1999. Overuse activity injures the
528 supraspinatus tendon in an animal model: a histologic and
529 biomechanical study. *J Shoulder Elbow Surg* 2000;9:79-84. 536
- 530 [47] Thompson CB. Apoptosis in the pathogenesis and treatment of
531 disease. *Science* 1995;267:1456-62. 537
- 532 [48] Tian T, Arnoczky, S., Lavagnino, M., Gardner, K. Frequency vs
533 amplitude in the upregulation of stress-activated protein kinases
induced by cyclic strain in canine tendon cells: an in vitro
experimental study. In: 47th Orthopaedic Research Society
Annual Meeting, February 25-28, 2001, San Francisco, CA. 538
- [49] Tilly JL, Hsueh AJ. Microscale autoradiographic method for the
qualitative and quantitative analysis of apoptotic DNA fragmen-
tation. *J Cell Physiol* 1993;154:519-26. 539
- [50] Weinberg JM. The cell biology of ischemic renal injury. *Kidney
Int* 1991;39:476-500. 540
- [51] White E. Life, death, and the pursuit of apoptosis. *Genes Dev*
1996;10:1-15. 541
- [52] Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is asso-
ciated with endogenous endonuclease activation. *Nature*
1980;284:555-6. 542
- 543
544
545
546

UNCORRECTED PROOF