Quantitative analysis and study of the mRNA expression levels of apoptotic genes *BCL2*, *BAX* and *BCL2L12* in the articular cartilage of an animal model of osteoarthritis

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Background: Given that apoptosis of chondrocytes is one of the most important factors related to the pathogenesis of osteoarthritis (OA), the recent research interest adds progress not only to the knowledge of the molecular signals that mediate apoptosis but also to find new therapeutic targets. This study attempts to investigate the differential expression of *BCL2* family genes in the articular cartilage of an experimental animal model of OA.

Methods: In total, 26 New Zealand white rabbits underwent an anterior cruciate ligament transaction, 26 more were subjected to a placebo surgery and 18 specimens constituted the control non-operated group. Thirteen weeks later, samples of cartilage from the osteoarthritic and non-osteoarthritic knees were collected and subjected to analysis of the *BCL2*, *BAX* and *BCL2L12* gene expression at the mRNA level.

Results: Installed osteoarthritic alterations of varied intensity and of grade 1 up to grade 5, were confirmed according to the OARSI system. Contrary to the physiologically healthy samples, in the osteoarthritic samples the mRNA expression levels of *BAX* and *BCL2L12* genes were found significantly upregulated by signals which can activate apoptosis. However, the difference between *BCL2* mRNA expression levels in healthy and osteoarthritic samples was not supported statistically.

Conclusions: Since apoptosis is the main feature of the cartilage degeneration in OA, the effective inhibition of apoptosis of chondrocytes can provide novel and interesting therapeutic strategies for the treatment of OA. Therefore, *BAX* and *BCL2L12* are highlighted as potential therapeutic targets in OA.

Keywords: Apoptosis; osteoarthritis (OA); animal model; quantitative polymerase chain reaction (qPCR); statistical analysis

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Introduction

Since osteoarthritis (OA) is characterized mainly by the degeneration of the articular cartilage and the loss of chondrocytes, many studies have focused on the proposition that cell death plays a central role in the course of the disease progression (1). Apoptosis and the loss of chondrocyte survival in OA results in the collapse of articular cartilage. Of course, some of the researchers point to the possibility that although apoptosis undoubtedly happens in osteoarthritic cartilage, its contribution to the breakdown of cartilage may be limited (2).

Considering the above, the research interest on the molecular signals that mediate apoptosis of articular chondrocytes is well justified. Furthermore, among the array of intracellular signals that regulate cell activity, much of the research interest is focused on the role of the BCL2 family proteins. Thus, since 1984, when the first identification of the *BCL2* gene during the acute B-cell leukemia took place, a large volume of data on the function and regulation of proteins of the whole BCL2 family has been published. To this purpose, New Zealand white rabbits have been widely used as experimental animal models since they have gained favor for their numerous advantages.

Both *BCL2* and *BAX* genes, classic members of the family, have been widely shown to act as anti- and pro-apoptotic factors, respectively (3-6). Regarding the protein BCL2L12, after its discovery in 2001, apart from the scientific team who studied it first (7), several other researchers tried to identify its role among the proteins that are involved in the control of apoptosis. So far however, the precise role of this protein in apoptotic pathways is not known yet. So, for example, several studies have shown, with clarity so far, an anti-apoptotic activity of the full-length isoform of the protein BCL2L12 in human glioblastoma cells (8-11). On the other hand, the same member appears to have a pro-apoptotic activity in human breast cancer cells (12), in fetal mouse fibroblasts (13) and in Chinese hamster ovary cells (14). The existence of such differences regarding the real role of BCL2L12 in apoptosis, as anti- or pro-apoptotic factor, it has been suggested that is cell type-dependent (13). Also, it is important that some of the isoforms of the BCL2L12 protein can be categorized in the subgroup of "BH3-only" members of the BCL2 family and hence they could act in a pro-apoptotic way, in contrast to the full-length isoform, which is most likely to inhibit apoptosis. Certainly, there is a need for further exploration of the new *BCL2L12* transcripts that is necessary for our understanding about the exact role of distinct protein isoforms of BCL2L12 in apoptosis (15). In fact, a lot of protein-coding *BCL2L12* transcripts have been recently cloned by members of our group, using next-generation sequencing (NGS) technology (1). NGS has significantly pioneered transcriptomics, particularly the discovery of novel transcripts (16-20).

BCL2L12 mRNA expression has been suggested as a favorable prognostic biomarker in colon cancer (21), whereas it constitutes an unfavorable and independent prognostic indicator of short-term relapse in nasopharyngeal carcinoma (22). Its potential as a molecular biomarker has also emerged in laryngeal and tongue squamous cell carcinomas (23), similar to other members of the BCL2 family which represent important molecular biomarkers in head and neck tumors (24-27), as well as in bladder cancer (28). Moreover, BCL2L12 protein overexpression predicts a favorable outcome in diffuse large B-cell lymphoma patients in the rituximab era (29), as other key members of the BCL2 apoptosis-related family (30-32). On the contrary, high *BCL2L12* mRNA levels have been associated with advanced clinical stage and shown to predict shorter overall survival in chronic lymphocytic leukemia patients (33), similarly to other cancer-related genes (34-36). The prognostic significance of *BCL2L12* mRNA expression in several other malignancies has been studied intensively during the last decade, similarly to other cancer-related protein-coding genes (37-42) or microRNAs (miRNAs) (43-51), along with several other clinical markers, particularly in chronic lymphocytic leukemia and myelodysplastic syndromes (52-54).

This study attempts, therefore, to improve the knowledge on the differential expression of *BCL2* family genes (*BCL2*, *BAX* and *BCL2L12*) in the articular cartilage of an experimental animal model of OA.

Methods

Animals and tissue collection

For the purpose of this study, a total of 70 New Zealand white rabbits were used (registered supplier Trompetas A., order number EL02BIO03). All animals were kept under conventional housing conditions (single housing, 12 h light/dark, 20–21 ℃ temperature, 45% relative humidity, commercial rabbit food Pezzullo 12C). The operations took place under general anaesthesia of the animal (ketamine, Imalgene 70 mg/kg and xylazine, Rompun 7 mg/kg IM) and included medial patellar incision and anterior cruciate ligament transection (ACLT) to cause OA, followed by

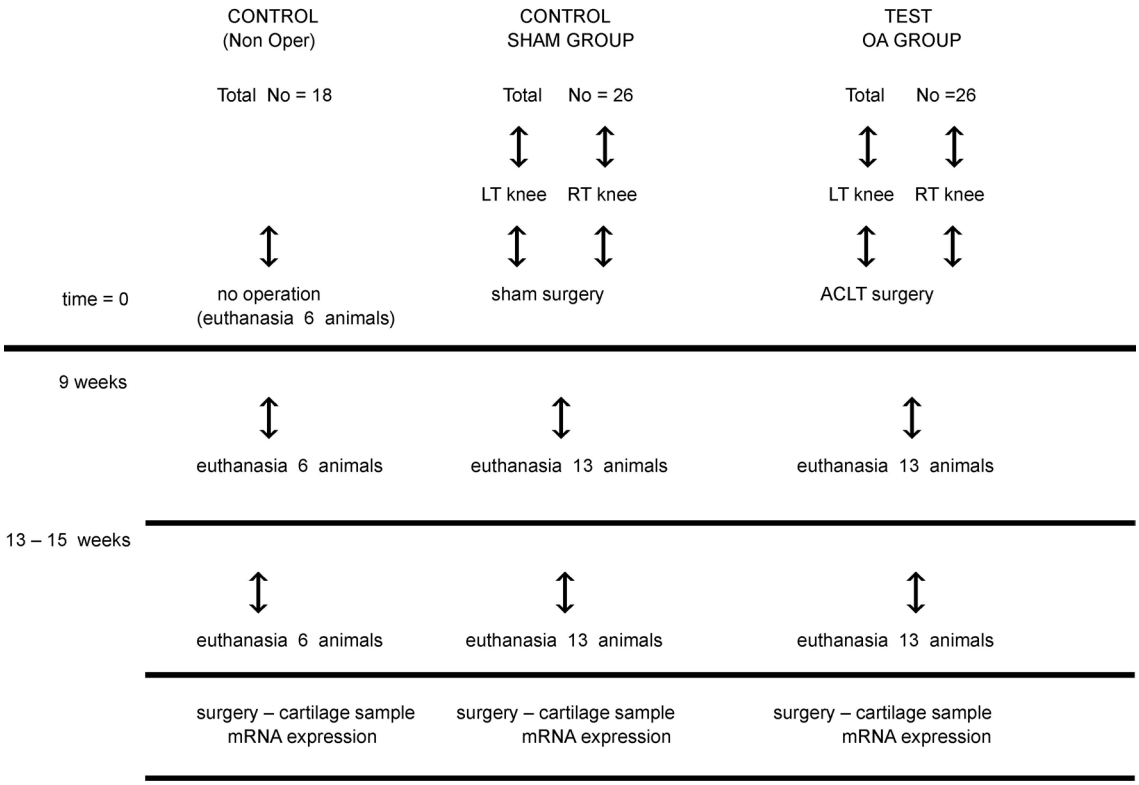

Figure 1 Protocol of operations and the collection of tissues.

wound closure (antimicrobial: enrofloxacin—Baytril 15 mg/kg/24 h s.c./analgesia: carprofen—Rimadyl 5 mg/kg/24 h s.c.). Animals were euthanized with an intracardiac injection of pentobarbital (Dolethal) overdose under general anaesthesia. All procedures were carried out under an approved license (PD 160/1991) and in accordance with the Animals (Scientific Procedures) Act, 1986 (UK).

Particularly, 26 of the animals underwent an ACLT (OA group). A second group of 26 rabbits was subjected to a placebo surgery, i.e., access, cross-section of the articular membrane and wound closure including the membrane (SHAM group) and the rest of 18 specimens did not undergo an operation and constituted the control non-operated group (*Figure 1*).

Thirteen weeks later, all of the animals were euthanized and samples of cartilage from the osteoarthritic and non-osteoarthritic knees were collected (from both knees of each animal) (55).

Total RNA extraction and reverse transcription

Tissue specimens were homogenized and then dissolved in TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA). Following the manufacturer's instructions, total RNA was extracted from homogenized samples and was diluted in RNA Storage Solution (Life Technologies Ltd., Carlsbad, CA, USA). The concentration and purity of total RNA samples were assessed spectrophotometrically at 260 and 280 nm. Next, reverse transcription was performed using 1 μg of total RNA, M-MLV Reverse Transcriptase (Life Technologies Ltd.) as the enzyme of the reverse transcription and an oligo-dT sequence as primer. The final reaction volume was 20 μL, as previously described (56).

Quantitative real-time polymerase chain reaction (qPCR)

qPCR was performed using the SYBR Green chemistry in a 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA), as previously described (57,58). For this reason, a pair of gene specific primers were designed for each one of the targeted genes (*Table 1*) using the Pimer-BLAST algorithm. The resulting PCR amplicons for *BCL2*, *BAX* and *BCL2L12* genes were 93, 174 and 107 bp long, respectively. The reaction mixture contained 1 μL

Table 1 List of primers that were used for the mRNA quantification of the targeted genes and the endogenous control gene

Name	Sequence (5'→3')	Length	T_m
B2M F	GGTTTCATCCGCCCCAGAT	19	59.77
BAX F	GCCGACGGCAACTTCAACTG	20	62.44
BCL2 F	GTGGATGACTGAGTACCTGAACCG	24	62.69
BCL2L12 F	GACTTCTACACCCTGGTGGC	20	60.04
B2M R	GTATCCTCAGACCTCCATGCTGTT	24	61.96
BAX R	CCAACCACCCTGGTCTTGGA	20	61.72
BCL2 R	ACGCTGGGGCCGTACAGTT	19	64.14
BCL2L12 R	GCCTCCTTCTCCGTGGCT	18	61.39

The T_m for each primer was calculated using the Primer-BLAST designing tool.

Table 2 Statistical analysis of the differences in the expression of *BAX*, *BCL2* and *BCL2L12* genes between the healthy and OA cartilages (with ACLT, OA group) after Wilcoxon test

BAX, *BCL2* and *BCL2L12* expression in healthy and OA cartilages	N	Mean rank	Sum of ranks
BAX (pathological) *vs. BAX* (normal)			
<	1	1	1
>	9	6	54
=	0		
Total	10		
BCL2 (pathological) *vs. BCL2* (normal)			
<	5	4.7	23.5
>	5	6.3	31.5
=	0		
Total	10		
BCL2L12 (pathological) *vs. BCL2L12* (normal)			
<	1	2	2
>	9	5.89	53
=	0		
Total	10		

OA, osteoarthritis; ACLT, anterior cruciate ligament transaction,

of cDNA, 5 μL KAPA™ SYBR® FAST qPCR Kits (2X) (Kapa Biosystems, Inc., Woburn, MA, USA), and 2 μL of gene-specific primers (final concentration: 200 nM each), in a final reaction volume of 10 μL. The cycling conditions were as follows: a denaturation step at 95 ℃ for 3 min, followed by 40 cycles of 95 ℃ for 3 s, for denaturation of the PCR products, and 60 ℃ for 30 s, for primer annealing and extension (59). Each qPCR reaction was performed in duplicate to evaluate the reproducibility of data. Finally, in the current study, the human beta-2-microglobulin (*B2M*) gene was used as an endogenous control gene so as to normalize PCRs for the RNA amount added to the reverse transcription reactions, as previously described (60,61).

Results

The assessment of the articular cartilage condition and its alterations according to the OARSI system confirmed installed osteoarthritic lesions of varying intensity, and of grades 1 to 5, i.e., identified lesions both only in cartilage and in cartilage along with the subchondral bone.

In a first analysis using the Wilcoxon test (Wilcoxon signed ranks test) on 10 pairs of rabbit cartilage samples of the OA group (animals underwent ACLT to cause OA), the difference in the expression of the three genes *BAX*, *BCL2* and *BCL2L12* between healthy and pathological cartilages was tested (*Table 2*) and an overexpression of *BAX* and *BCL2L12* genes was found in ACLT-OA cartilages while the expression of *BCL2* showed a variety among the different cartilages. The increased expression of *BAX* and *BCL2L12* genes was observed in the 9 of the 10 pairs of sample in the OA group, and an increased expression of *BCL2* gene was observed in only 5 of the 10 pairs of OA samples (*Figure 2*).

As shown in *Table 3* the P values for the genes *BAX* (P=0.007) and *BCL2L12* (P=0.009) found smaller than the minimum P value of statistical support (P=0.05), unlike for the *BCL2* gene for which the P value was larger (P=0.683). Therefore, this test supports that the difference in expression of the two genes *BAX* and *BCL2L12* is statistically significant between the healthy and the OA samples of rabbit cartilage. Contrary to these results, the difference in the expression of the *BCL2* gene between the healthy and the OA samples was found statistically insignificant.

A second analysis using the Wilcoxon test on the rabbit cartilage samples of the SHAM group (placebo surgery) showed that the expression of all three genes did not change significantly between the healthy and the operated cartilages (*Table 4*). However, in this case, all the P values obtained for the three genes under study were larger than the statistical threshold P value. More specifically, the P values obtained were P=0.362 for *BAX*, P=0.108 for *BCL2* and P=0.171 for

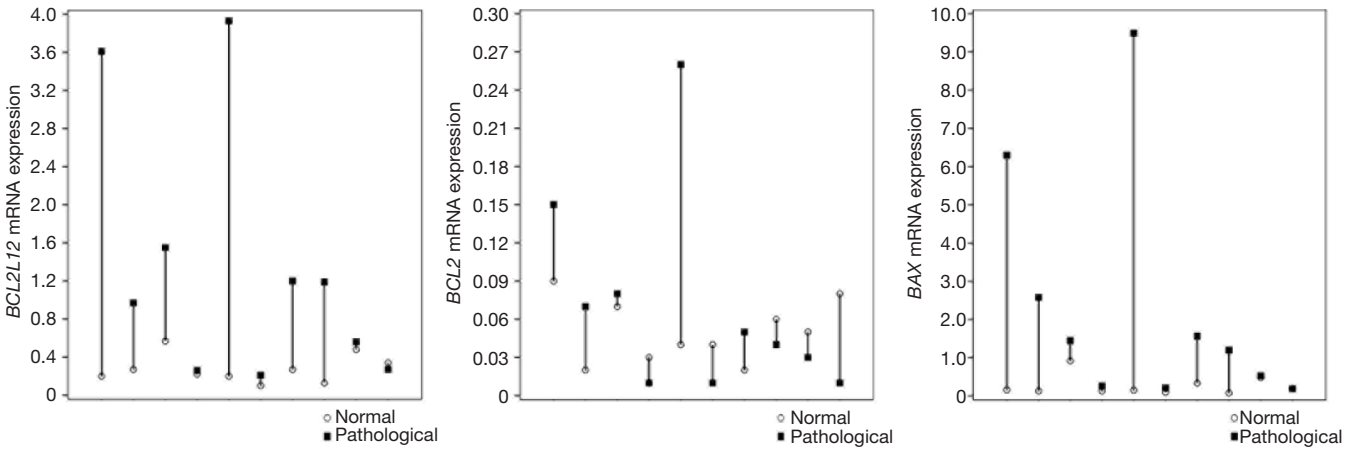

Figure 2 The differential mRNA expression levels of *BAX*, *BCL2* and *BCL2L12* genes between the 10 pairs of healthy and OA samples. An increased expression of *BAX* and *BCL2L12* genes was observed in the 9 of 10 pairs of samples. In addition, *BCL2* was found overexpressed in the 5 of 10 pairs of samples. OA, osteoarthritis.

Table 3 P values for the differences in the expression of *BAX*, *BCL12* and *BCL2L12* genes (OA group) after Wilcoxon test

Differences in gene expression	Z	P value
BAX (pathological) *vs. BAX* (normal)	–2.701	0.007
BCL2 (pathological) *vs. BCL2* (normal)	–0.409	0.683
BCL2L12 (pathological) *vs. BCL2L12* (normal)	–2.599	0.009

OA, osteoarthritis.

Table 4 Statistical analysis of the differences in the expression of *BAX*, *BCL2* and *BCL2L12* genes between the healthy and placebo operated knees (without an anterior cruciate ligament transaction, SHAM group) after Wilcoxon test

BAX, *BCL2* and *BCL2L12* expression in healthy and placebo operated knees	N	Mean rank	Sum of ranks
BAX (pathological) *vs.* BAX (normal)			
<	6	4.08	24.5
>	2	5.75	11.5
=	1		
Total	9		
BCL2 (pathological) *vs. BCL2* (normal)			
<	3	3	9
>	6	6	36
=	0		
Total	9		
BCL2L12 (pathological) *vs. BCL2L12* (normal)			
<	7	4.86	34
>	2	5.5	11
=	0		
Total	9		

BCL2L12 gene.

The statistical Mann-Whitney U test, in order to find possible differences in the gene expression between the healthy cartilages of both groups of samples (OA and SHAM groups) but also between the respective pathological, revealed a statistically significant difference in *BAX* and *BCL2L12* gene expression between the operated knees with osteoarthritis and these of placebo surgery (*Figure 3*). More specifically, an overexpression of *BAX* and *BCL2L12* genes was observed in the pathological cartilages with ACLT and OA in relation to pathological cartilages of the animals without ligament transection. However, this pattern was not observed in the healthy knees of the two groups, where no statistically significant differences appeared (*Table 5*).

Discussion

Erlacher *et al.* in their publication aiming to investigate the expression of *BCL2* in healthy and osteoarthritic cartilages in both transcriptional and protein level, found that the *BCL2* gene is expressed in both healthy and osteoarthritic cartilage and that in advanced stage OA cartilages the *BCL2* gene is overexpressed (62). Feng *et al.*, in 1998 were

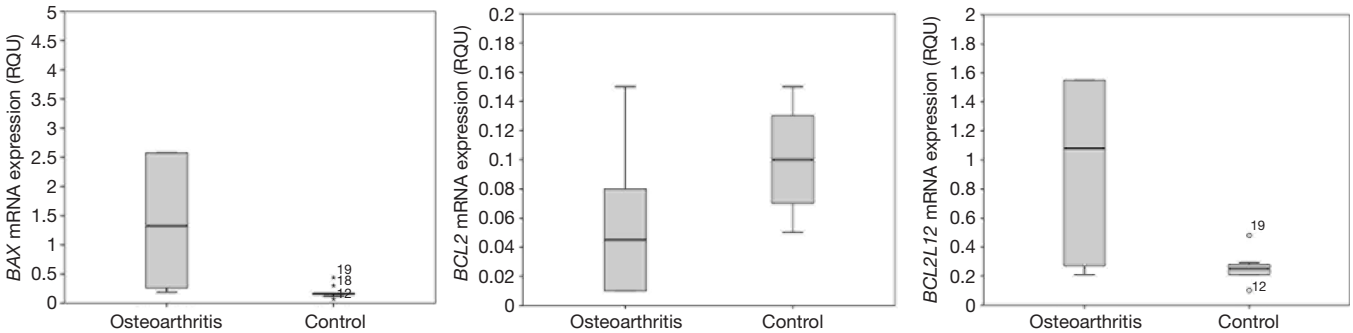

Figure 3 Comparison of the distribution of *BAX*, *BCL2* and *BCL2L12* expression in pathological OA and control samples. *BAX* and *BCL2L12* transcripts are more abundant in the cohort of pathological OA specimens. OA, osteoarthritis.

Table 5 The results of the analysis after Mann-Whitney U test

Gene	Type of tissue	N	Mean rank	Sum of ranks
BAX (normal)	Arthritis	10	9.8	98
	Control	10	11.2	112
	Total	20	–	–
BCL2 (normal)	Arthritis	10	8.4	84
	Control	10	12.6	126
	Total	20	–	–
BCL2L12 (normal)	Arthritis	10	9	90
	Control	10	12	120
	Total	20	–	–
BAX (pathological)	Arthritis	10	14.85	148.5
	Control	10	6.15	61.5
	Total	20	–	–
BCL2 (pathological)	Arthritis	10	8.2	82
	Control	10	12.8	128
	Total	20	—z	–
BCL2L12 (pathological)	Arthritis	10	14.1	141
	Control	10	6.90	69.00
	Total	20	–	–

the first who published the direct evidence that BCL2 regulate apoptosis of articular chondrocytes (63). Two years later, Kim *et al.* in their publication concerning the apoptotic death of chondrocytes in OA showed not only the increased apoptosis during the progress of the disease but also the involvement of BCL2, BAX and Fas. Indeed, similar to Feng *et al.*, this research showed lower levels of BCL2 in OA cartilages compared to the healthy ones but significantly higher expression of BCL2 in OA cartilages of advanced stage than in OA cartilages without serious lesions and constant levels of BAX (64). In 2004, Mistry *et al.* showed that despite the fact that both BCL2 and BAX were detected easily in the chondrocytes of tibial cartilages of mice, there were no changes indicating the activation of an intracellular apoptotic pathway. However, according to the same researchers, it is important to emphasize that, at best, immunohistochemistry is only a semiquantitative technique and in the absence of marked changes in BCL2 relative to BAX, no conclusion as to activation of this pathway is justified (65). Iannone *et al.*, in 2005 published a study aiming to identify the role of p53, BCL2 and Fas/CD95 in controlling the metabolism of cartilage. According to their findings, BCL2 and p53 play a role in apoptosis, but also can help in regulating the growth and differentiation of chondrocytes. The critical conclusion from this study was that the ratio of BCL2/p53 was increased in OA cartilage due to the increase of BCL2 and the decrease of p53 levels in chondrocytes (66). In 2009, Lin *et al.* studied the effects of kneepad on expression of *BCL2* and *TP53* mRNA of chondrocyte in white rabbits with knee OA, so as to explore and treatment mechanism of OA kneepad on apoptosis of chondrocytes of rabbits with knee OA in molecular degree. Their results showed that OA-kneepad can up-regulate the mRNA expression of *BCL2* as well as down-regulate the mRNA expression of *TP53*, thereby to inhibit the apoptosis of cartilage cells and delay the degeneration of articular cartilage changes (67).

In 2015, in the publication of Karaliotas *et al.*, the expression analysis of *BCL2*, *BAX* and *BCL2L12* apoptotic genes, showed that these genes are indeed expressed in cartilaginous tissue and secondly that there are differences

in the expression of these three between osteoarthritic and non-osteoarthritic cartilage. More specifically, in comparison with the non-osteoarthritic cartilages, in the osteoarthritic samples the mRNA levels of both the pro-apoptotic gene *BAX* and the gene *BCL2L12* were increased, while the mRNA levels of the anti-apoptotic gene *BCL2* were downregulated. The results showed that the mRNA expression of *BAX* gene appear to have an increasing trend in the OA cartilages compared with the healthy ones, although without statistical significance. In contrast, the ratio of *BCL2/BAX* gene expression was found to be significantly decreased in the OA cartilages compared with the healthy ones. Furthermore, patients with OA of stage III showed an important overexpression of *BAX* in comparison with the ones of the control group, while the ratio of *BCL2/BAX* gene expression was markedly decreased. On the other hand, both control and OA groups showed a positive correlation between the mRNA levels of *BAX* and *BCL2*. According to the researchers, these results further implicate apoptosis in the pathogenesis of OA, through molecular mechanisms, which include the aberrant expression of the *BCL2* gene family (68), including BCL2L12.

BCL2L12 is located on the chromosomal region 19q13.3-q13.4, at a distance of 7.5 Mb from the telomeres of the long arm of chromosome 19, close to many cancer-related genes such as the cluster of tissue kallikrein and kallikrein-related peptidases (69,70), a family of peptidases with important properties as biomarkers (71). To date, several miRNAs have been shown to regulate expression of *BCL2L12* and/or other members of the *BCL2* family; most of them constitute important biomarkers (48,72-81).

In the present study, according to the statistical analysis of the expression levels of *BAX*, *BCL2* and *BCL2L12* in healthy and OA cartilages using the Wilcoxon test, *BAX* and *BCL2L12* genes showed an overexpression in OA cartilages, while no conclusion could be exported from the statistically insignificant differences of *BCL2* expression. This result indicates the change of the expression of the *BAX* and *BCL2L12* genes, after the induction of OA and therefore in the apoptosis of the articular chondrocytes. However, it is necessary to underline that the main and significant factor for the control of the viability of human articular chondrocytes are not the absolute values of the expression of each protein but the proportions of the expression of anti- and pro-apoptotic members, following the results of previous researchers such as Feng *et al.* (63).

These differences in the expression of *BAX*, *BCL12* and *BCL2L12* genes between the healthy and OA cartilages clearly demonstrate that the expression of *BAX* and *BCL2L12* is upregulated by signals that induce apoptosis in the articular chondrocytes while changes in the expression of *BCL2* are not supported by statistics. Although Feng *et al.* observed the opposite expression settings, i.e., upregulation of the *BCL2* expression and stable *BAX* expression, in both studies, the ratio *BAX/BCL2* expression is increased in OA and therefore promotes the chondrocyte apoptosis. Of course, the findings of Feng *et al.* confirm a direct implication of BCL2 in the regulation of apoptosis of articular chondrocytes. According to the findings of the present study this implication appeared indirect, but still the differences in the expression of BCL2 between the healthy and OA samples are not supported statistically. Here, a more direct role of BAX and BCL2L12 in the regulation of apoptosis of articular chondrocytes seems to take the place of BCL2.

During the progression of OA, the promotion of the apoptotic process and the predominance of the pro-apoptotic versus the anti-apoptotic BCL2 family members are naturally expected. Trying to interpret the increase in the expression of the classic pro-apoptotic protein BAX no particular problems exist, since all bibliographic data agree that the development of OA is accompanied by the promotion of the apoptotic process and most of them confirm the overexpression of BAX in tissues subjected to the apoptotic death. On the other hand, the absence of statistical support in the differences of expression of the anti-apoptotic member BCL2 does not permit the creation of any hypothesis and of course the export of safe conclusions.

Regarding the overexpression of BCL2L12 in osteoarthritic cartilages, the existence of published results from other tissues, despite their variety and differentiation depending on tissue type, gives the chance to interpret the results of the present study under the premise of two separate scenarios: The first is that the role of BCL2L12, pro- or anti-apoptotic, may present specialization per cell type. Thus, differences in the expression and action of this BCL2 family member, in the way it is presented by different publications, is finally cell type-dependent (13). This argues that in the osteoarthritis cartilage, BCL2L12 role, as shown by its expression levels, is pro-apoptotic. However, the very recent proof of the existence of a lot of different BCL2L12 protein isoforms (1), which because of their different structure (for example isoforms BCL2L12 are characterized as BH3-only) are expected to have different potency, it is not only the second premise for the interpretation of the BCL2L12 overexpression in osteoarthritic cartilage but it could partly interpret the first assumption of cell dependent

action. The increased expression levels of BCL2L12 in the osteoarthritic cartilage, correspond rather to one of the protein isoforms with a pro-apoptotic role.

Unlike the comparison between the healthy and osteoarthritis cartilages, according to the statistical analysis of the difference in the expression of *BAX*, *BCL12* and *BCL2L12* genes between healthy and pathological (placebo operated and not osteoarthritic) cartilages, showed that the expression of all three genes did not significantly vary. This result gives value to the findings of the previous comparison and partly supports any hypothesis about the differential gene expression between osteoarthritic and healthy samples. Furthermore, the statistically significant difference in *BAX* and *BCL2L12* gene expression between the OA and SHAM cartilages, while this is not observed in healthy samples, also supports the validity of the first analysis.

According to Iannone *et al.* (66), the differentiation of the results among the different publications on the role of BCL2 family members in chondrocyte apoptosis, can be attributed to the different age of patients and donors, because the expression of these proteins by chondrocytes depends on age (82), the degree and the progression of the disease and the different quantification methodologies. So, the study of the expression of anti-and pro-apoptotic proteins into active chondrocytes of an osteoarthritic cartilage will lead to conflicting results, depending on the relative prevalence of proliferation or apoptosis in this particular stage of the disease and in such specified area of cartilage (66).

Since apoptosis is the main feature of the degeneration of the cartilage in OA, the effective inhibition of apoptosis of chondrocytes could provide new and interesting ideas in a therapeutic strategy for the treatment of the disease. The results of this study indicate the pro-apoptotic action of BAX and BCL2L12 but not the generally acceptable anti-apoptotic effect of BCL2. Although the therapeutic targets in several types of cancer are the anti-apoptotic activities that maintain the survival of cancer cells, in the case of OA, the survival of the chondrocytes is crucial for the inhibition of progression of the disease but also for healing. In OA, the treatment goals are changed completely and now are not the anti- but the pro-apoptotic actions that promote cell death. So the results of this study highlight the BCL2 family members BAX and BCL2L12 as potential therapeutic targets in OA. However, a restriction is the fact of non-identification of the particular protein isoform or isoforms of BCL2L12 which in this case showed a pro-apoptotic action. On the other hand, this restriction is also a candidate question for a future research.

Conclusions

While the chondrocyte apoptosis is one of the most important factors in the pathogenesis of OA the focus of the research interest on molecular signals that mediate apoptosis of the articular chondrocytes represents progress in the efforts to find new therapeutic targets. The assessment of articular cartilage condition and lesions with the OARSI system confirmed installed osteoarthritic lesions of varying intensity, grades 1 to 5, indicating that the method of ACLT is an effective mean of causing OA in white New Zealand rabbits.

In addition, the differences between the expression of *BAX*, *BCL12* and *BCL2L12* between the healthy and OA cartilages show that the expression of *BAX* and *BCL2L12* is upregulated by signals that induce apoptosis in chondrocytes while changes in the expression of *BCL2* are not statistically supported. During the progression of OA, the promotion of the apoptotic process and the predominance of pro- versus anti-apoptotic BCL2 family members are naturally expected. In trying to interpret the increase in the expression of pro-apoptotic protein BAX no particular problems are present. Furthermore, the absence of statistical support in the differences of expression of the anti-apoptotic member BCL2 does not permit the creation of any hypothesis and of course the export of safe conclusions. The very recent proof of the existence of many different protein isoforms of BCL2L12 which, because of their different structure are expected to have different action, could interpret partly the assumption of cell type-dependent action of this member. Certainly, there is a need for further investigation for new transcripts of *BCL2L12* that is necessary for our understanding about the exact role of distinct protein isoforms of BCL2L12 in apoptosis and the induction or progression of OA. Since apoptosis is the main feature of the degeneration of the cartilage in OA, the effective inhibition of apoptosis of chondrocytes can provide new and interesting ideas in a therapeutic strategy for the treatment of OA. In the case of OA, the survival of the cells is crucial for inhibition of progression of the disease but also for healing. The therapeutic targets need to be the pro-apoptotic actions that promote cell death. Thus, BAX and BCL2L12 are highlighted as potential therapeutic targets in OA.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: All procedures were carried out under an approved license (PD 160/1991) and in accordance with the Animals (Scientific Procedures) Act, 1986 (UK).

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