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Does erroneous differentiation of tendon-derived stem cells contribute to the pathogenesis of calcifying tendinopathy?

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Calcifying tendinopathy is a tendon disorder with calcium deposits in the mid-substance presented with chronic activity-related pain, tenderness, local edema and various degrees of incapacitation. Most of current treatments are neither effective nor evidence-based because its underlying pathogenesis is poorly understood and treatment is usually symptomatic. Understanding the pathogenesis of calcifying tendinopathy is essential for its effective evidence-based management. One of the key histopathological features of calcifying tendinopathy is the presence of chondrocyte phenotype which surrounds the calcific deposits, suggesting that the formation of calcific deposits was cell-mediated. Although the origin of cells participating in the formation of chondrocyte phenotype and ossification is still unknown, many evidences have suggested that erroneous tendon cell differentiation is involved in the process. Recent studies have shown the presence of stem cells with self-renewal and multi-differentiation potential in human, horse, mouse and rat tendon tissues. We hypothesized that the erroneous differentiation of tendon-derived stem cells (TDSCs) to chondrocytes or osteoblasts leads to chondrometaplasia and ossification and hence weaker tendon, failed healing and pain, in calcifying tendinopathy. We present a hypothetical model on the pathogenesis and evidences to support this hypothesis. Understanding the key role of TDSCs in the pathogenesis of calcifying tendinopathy and the mechanisms contributing to their erroneous differentiation would provide new opportunities for the management of calcifying tendinopathy. The re-direction of the differentiation of resident TDSCs to tenogenic or supplementation of MSCs programmed for tenogenic differentiation may be enticing targets for the management of calcifying tendinopathy in the future.

TENDINOPATHY AS A CELL-MEDIATED FAILED HEALING PROCESS

Chronic tendinopathy is a tendon disorder characterized by pain, tenderness, swelling and impaired performance that is extremely common in athletes and individuals whose tendons are subjected to repetitive strain injuries. Calcific deposits can be seen at the late stage of the disease in a subset of tendinopathy called calcifying tendinopathy and is particularly common in rotator cuff and supraspinatus tendon, patellar tendon and Achilles tendon. The presence of calcification worsens the clinical manifestation of tendinopathy with increase in rupture rate, slower recovery times and a higher frequency of post-operative complications. The treatment for patients with calcifying tendinopathy is primarily conservative and symptomatic. If all the conservative treatments fail, surgical excision of the pathological tissue is the last resort. Most of current treatments are neither effective nor evidence-based because of our poor understanding on its underlying pathogenesis. Therefore, better understanding the pathogenesis of calcifying tendinopathy is essential for its effective evidence-based management.

Histopathologically, the tendinopathic tissue shows a failed healing status characterized by increase in cellularity, vascularity, proteoglycan deposition, particularly the oversultated form, collagen matrix degradation, acquisition of chondrocyte phenotypes, matrix metalloproteinase 1 (MMP1), tissue inhibitor of metalloproteinase 1 (TIMP-1) and gelatinolytic activity. Expression of cyclooxygenase-2, prostaglandin E2 and TGF-β has been detected in tendinopathic specimens. These findings suggest that although there are signs of matrix...
Degeneration in the pathological tendon, it is an active cell-mediated process and the healing tendon cells which fail to heal, may play a role in the development of chronic tendinopathy and calcifying tendinopathy.

**ERRONEOUS TENDON CELL DIFFERENTIATION AS THE PATHOGENIC MECHANISM OF CALCIFYING TENDINOPATHY**

Rather than formed by precipitation of inorganic ions, the calcific deposits observed in calcifying tendinopathy are likely to be the results of an active cell-mediated process as marked cellular reactions are observed around them. They are frequently surrounded by chondrocytes in a fibrous, metachromatic and glycosaminoglycan-rich extracellular matrix. In addition to chondrocytes, mesenchymal cells, multinucleated giant cells, macrophages, alkaline phosphate- and tartrate-resistant acid phosphatase (TRAP)-positive cells are frequently observed around and within the calcific deposits. Indeed, the presence of chondrocyte phenotype was not limited to calcifying tendinopathy and they could be observed in both clinical samples and animal model of tendinopathy without calcification, suggesting that they may share common pathological mechanisms.

It is known that tendon contained cells that could differentiate or transdifferentiate to chondrocytes or osteoblasts under the influence of abnormal environmental cues in pathological conditions. Differentiation of tenocytes into fibrocartilage has been suggested to be the necessary initial stage for mineralization. Calcification was also reported to occur essentially in areas where the tendon had undergone cartilaginous transformation. Ectopic bone formation in Achilles tendon after mid-point tenotomy has also been reported to be due to the direct conversion of tendon tissue to cartilage. We also reported that healing tendon cells have different cellular activities compared to tendon cells isolated from intact tendon. We therefore hypothesized that erroneous differentiation of tendon cells to osteoblasts and/or chondrocytes, rather than to tenocytes as in normal healing, may account for metachondroplasia and ectopic ossification in calcifying tendinopathy. This may then cause aberrant deposition of extracellular matrix, resulting in mucoid degeneration and weaken the tendon. Since ossified tendons will have increased stiffness, ossification can be seen as a localized attempt to compensate for the original decreased stiffness of the tendons. The deposition of calcific deposits in the tendon mid-substance may induce activity-related tendon pain. This hypothesis is supported by the up-regulation of cartilage-associated genes and down regulation of tendon-associated genes in rat supraspinatus tendon and in horse superficial digital flexor tendon after overuse injury. We have also reported the presence of chondrocyte phenotype around the calcific deposits in a collagenase-induced failed healing tendon injury model. In addition, there was expression of chondrogenic markers in some healing tendon cells which preceded their appearance in chondrocyte-like cells in the same animal model.

**TENDON-DERIVED STEM CELLS (TDSCs) AS THE TARGET CELLS UNDERGOING ERRONEOUS DIFFERENTIATION IN CALCIFYING TENDINOPATHY**

Mesenchymal stem cells are cells with high self-renewal and multi-potent differentiation potential to cells of specific lineage. These multi-potent cells have been demonstrated in a variety of mesenchymal tissues including muscle, cartilage and bone. Recent studies have shown that these multi-potential cells were also present in tendon tissues. Our group has also isolated TDSCs from the flexor tendon and patellar tendon of rats, while stem cells play important roles in normal developmental process, increasing evidence suggested that they might also have roles in pathological conditions. We hypothesized that it was the erroneous differentiation of TDSCs due to alteration of mechanical and biological environment that caused the pathogenesis of calcifying tendinopathy.

In this regards, we observed higher osteogenic differentiation potential of TDSCs isolated from our calcifying tendinopathy animal model compared to TDSCs isolated from healthy tendon tissues, suggesting that erroneous differentiation of TDSCs might be involved in the pathogenesis (unpublished result). Tendons of biglycan and fibromodulin double knockout mice (Bgn−/− Fmod−/−) were thinner, disorganized and showed intratendinous ossification. Bi et al reported that the tendon progenitor/stem cells (TPSCs) isolated from this double knockout mice model showed decreased expression of tendon markers (Sex and type I collagen) and higher expression of chondrocyte markers (type II collagen and aggrecan) as compared to cells isolated from wild type (WT) mice, suggesting that the differentiation and functions of TPSCs were altered.

Indeed, aberrant differentiation of stem cells as the pathogenic mechanism of heterotrophic ossification and matrix degeneration seem to be shared by different tissues as it is also seen in other disorders including vascular calcification, skin calcification and skeletal calcification.

The mechanism leading to the erroneous TDSC differentiation is not clear at the moment. Both biological and mechanical factors might cause erroneous TDSC differentiation. Biomechanically, the initial insult could be due to a series of low subfailure strains. In vitro studies have shown that overloading could induce matrix degeneration. It was also demonstrated that cells isolated from ossified posterior longitudinal ligament (OPLL), but not normal cells from PLL, expressed osteogenic markers in response to mechanical differentiation.
48 Another study reported that injection of cartilage-derived morphogenetic proteins (CDMP-2/BMP-13), in unloaded tendon defects showed bone induction in all animals whereas only 4 out of 10 loaded ones showed cartilage or bone formation.49 The changes in the levels of growth factors, cytokines and extracellular matrix composition as a result of change of mechanical loading as well as modulation of healing process by pharmacological agents might also influence differentiation of TDSCs. Our results showed that there was expression of BMP-2 mRNA and protein in the ectopic chondro-ossification site in our collagenase-induced failed tendon healing animal model and the expression of BMP-2, BMP-4 and BMP-7 was observed in healing tendon cells at earlier time points prior to its expression in chondrocyte-like cells and calcific deposits which appeared at later time points,50,51 suggesting that the expression of these chondro-osteogenic BMPs may be one of the key factors involved in erroneous tendon cell differentiation. Our recent study also showed that repetitive cyclic tensile loading increased the expression of BMP-2 in rat TDSCs and BMP-2 could induce the osteogenic differentiation of TDSCs in vitro.42 Clinically, there was ectopic overexpression of BMPs in the subacromial bursa and was suggested to account for the chondrogenic transformation and ectopic mineralization of rotator cuff tendon in patients.52 Indeed, injection of rhBMP-2 into tendon increased ectopic bone formation, indicating that tendon consisted of cells which are responsive to BMP-2 and could differentiate along the chondro-osseous pathway.53 Bi et al59 also reported that knockdown of biglycan and fibromodulin increased the sensitivity of TPSCs to BMP-2 signaling and impaired tendon formation in vivo. The composition of extracellular matrix may also contribute to the erroneous differentiation of TDSC in calcifying tendinopathy. Our results showed that the effect of TGF-β1 on matrix deposition in tendon cells was influenced by matrix anchorage,54 suggesting that the composition of extracellular matrix can modify or affect the cellular response and may be cellular differentiation of TDSC. The differentiation of tendon progenitor cells into chondrocytes and bone cells was reported to be modulated by the expression of biglycan and fibromodulin.36 Increase in expression of biglycan and aggrecan in clinical samples of tendinopathy has been reported55 and alteration of composition of extracellular matrix was also observed in our collagenase-induced tendon injury model.56 Besides growth factors and extracellular matrix, drugs that are commonly used for the management of tendinopathy may also impact the course of development of tendinopathy. Dexamethasone could induce human spinal ligament derived cells towards osteogenic differentiation.57 Triamcinolone and platelet-rich plasma modulated the chondrogenic gene expression pattern in human tendon explant culture.58

While the current evidence seems to support the erroneous differentiation of TDSCs in the pathogenesis of calcifying tendinopathy, erroneous transdifferentiation of tendon fibroblasts to chondrocytes or osteoblasts cannot be ruled out. Further studies should also look into the possibilities of erroneous transdifferentiation of tendon fibroblasts and the roles of mechanical loading, growth factors, cytokines and extracellular matrix in the erroneous tendon cell differentiation in the pathogenesis of calcifying tendinopathy.

**HYPOTHETICAL MODEL OF THE PATHOGENESIS OF CALCIFYING TENDINOPATHY AND NEW TREATMENT POSSIBILITIES**

A hypothetical model is thus proposed for the pathogenesis of calcifying tendinopathy (Figure). After acute injury, TDSCs would proliferate and differentiate into tenocytes (tenogenesis) in normal tendon healing. However, in the presence of change of mechanical...
loading or the accumulation of micro-injuries as a result of compromised healing capacity of tendon cells to normal daily activities, the TDSCs would differentiate into chondrocytes or osteoblasts (osteochondrogenesis) instead of tenocytes with compromised capacity for tendon healing. The deposition of erroneous extracellular matrix and calcific deposits would weaken the tendon, resulting in failed healing and caused activity-related tendon pain.

If the hypothesis is true, understanding the key role of TDSCs in the pathogenesis of calcifying tendinopathy and the mechanisms which contribute to the erroneous differentiation of TDSCs in calcifying tendinopathy would provide new opportunities for disease management. The re-direction of resident TDSCs to tenogenic differentiation or supplementation of MSCs programmed for tenogenic differentiation may be able to prevent the development of this devastating yet common tendon disorder.

CONCLUSION

There is no effective or evidence-based clinical management of calcifying tendinopathy. As a result, new avenues of treatment are required. Better understanding the pathogenesis of calcifying tendinopathy is essential for the improvement of its clinical outcomes.

Both preclinical and clinical studies have clearly shown that calcification in calcifying tendinopathy is a cell-mediated process. We proposed that erroneous differentiation of TDSCs may contribute to the pathogenesis of calcifying tendinopathy. If the hypothesis is true, understanding the key role of TDSCs in the pathogenesis of calcifying tendinopathy and the mechanisms contributing to their erroneous differentiation would provide new opportunities for disease intervention. The re-direction of resident TDSCs to tenogenic differentiation or supplementation of MSCs programmed for tenogenic differentiation may be enticing targets for the management of calcifying tendinopathy in the future.

REFERENCES


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