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Abstract

The metastasis-associated protein S100A4 belongs to the large family of S100 calcium-binding proteins that appear to play regulatory roles in diverse biological activities. Moreover, a prognostic role of S100A4 has been suggested for patients with several types of cancer. Cancer promoting properties for S100A4 have been demonstrated, particularly through its regulation of cell motility, proliferation and apoptosis, as well as by stimulation of angiogenesis and remodelling of the extracellular matrix. Increased expression of S100A4 mRNA has been detected in proliferating synovial fibroblasts in rheumatoid arthritis. Furthermore, strong upregulation of the S100A4 protein in rheumatoid arthritis synovial tissue compared with osteoarthritis and control tissues has been demonstrated recently, especially at sites of joint invasion. Several immune and vascular cells were also identified to be producing S100A4 within the synovium. The local upregulation of S100A4 was accompanied by high plasma and synovial fluid concentrations of the S100A4 protein existing in the bioactive oligomeric form in patients with rheumatoid arthritis. Consistent with data from cancer studies, the extracellular S100A4 oligomer appears to be involved in regulation of several matrix-degrading enzymes and modulation of the transcriptional activation function of the tumour suppressor protein p53 in rheumatoid arthritis synovial fibroblasts. Taken together, one can speculate that increased S100A4 protein in circulation and locally at sites of inflammation, particularly at sites of joint destruction, might be linked to the process of aggressive fibroblast behaviour contributing to the pathogenesis of chronic autoinflammatory diseases such as rheumatoid arthritis.

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Abstract

The metastasis-associated protein S100A4 belongs to the large family of calcium-binding proteins that appears to play regulatory roles in diverse biological activities. Moreover, a prognostic role of S100A4 has been suggested for patients with several types of cancer. Cancer promoting properties for S100A4 have been demonstrated, particularly through its regulation of cell motility, proliferation and apoptosis, as well as by stimulation of angiogenesis and remodeling of the extracellular matrix.

Increased expression of S100A4 mRNA was detected in proliferating synovial fibroblasts in rheumatoid arthritis. Furthermore, strong up-regulation of S100A4 protein in rheumatoid arthritis synovial tissue compared with osteoarthritis and control tissues has been demonstrated recently, especially at the sites of joint invasion. Several immune and vascular cells were also identified to produce S100A4 within the synovium. The local up-regulation of S100A4 was accompanied by high plasma and synovial fluid concentrations of the S100A4 protein existing in the bioactive oligomeric form in rheumatoid arthritis patients. Consistent with the data in cancer studies, the extracellular S100A4 oligomer is involved in regulation of several matrix degrading enzymes and modulates the transcriptional activation function of the tumor suppressor protein p53 in rheumatoid arthritis synovial fibroblasts.

Taken together, one can speculate that increased S100A4 protein in circulation and locally at sites of inflammation, particularly at sites of joint destruction, might be implicated in the process of aggressive fibroblast behavior contributing to the pathogenesis of chronic autoinflammatory diseases such as rheumatoid arthritis.

Key words: rheumatoid arthritis, S100A4, apoptosis, matrix degrading enzymes, synovial fibroblasts

Introduction

The S100 proteins are small acidic calcium binding proteins (10-12 kD) that are found exclusively in vertebrates. The name of the whole group "S100" relates to its solubility in 100% ammonium sulphate solution (1). This group consists of 20 members and is considered to be the largest subgroup of the EF-hand calcium binding protein family. S100 proteins share some structural similarities with the well known calcium-binding protein calmodulin and carry two calcium-binding EF-hand motifs with different affinities to bind calcium (2). It results in conformational changes of the proteins, leading to exposure of the hydrophobic surface that enables the S100 proteins to interact with a variety of target molecules (3). However the biological activity of some S100 proteins does not require calcium because it can also be regulated by zinc and copper (4).

Originally, S100 proteins were identified in 1965 by Moore in bovine brain (1). Later it was shown that their biological activity appears to be dependent on the ability to form dimers (5). In the intracellular environment, most of S100 proteins exist as anti-parallel packed homodimers or heterodimers. Certain conditions, particularly the extracellular milieu, contribute to the formation of oligomers and multimers of some S100 proteins. For instance, S100A8/S100A9 forms heterotetramers (6,7), S100A12 hexamers (6,8), and S100A4 tetra- and higher-mers (9). Interaction of S100 proteins with several target proteins might explain their implication in a wide range of cellular events. Most of the S100 proteins are synthesized and localized intracellularly and participate in the regulation of a variety of processes within the cells such as proliferation and differentiation, apoptosis, extracellular matrix remodeling and cell motility (5). Furthermore, several S100 proteins can be released from cells and their extracellular functions have been demonstrated. Once released into the extracellular space, they exert cytokine-like activities. Some of S100 proteins can trigger intracellular signaling pathways through the receptor for advanced glycation end products (RAGE) (10,11). For others, including S100A4, reliable receptor(s) remain to be identified (12).

Numbers of human diseases are associated with an altered expression of S100 proteins. For example, certain S100 proteins have been studied in tumors (S100A1, S100A4, S100A6, S100A7, S100P and S100B), neurodegenerative (S100B, S100A6, S100A12), cardiovascular (S100A8/9, S100A4, S100A1), pulmonary (S100A4) and inflammatory diseases (S100A8/9, S100A12, S100A7, S100A4), diabetes mellitus (S100A12), and allergies (S100A12) (13-22, summarized in 5). Whereas many S100 proteins exist, several, including S100A2, S100A4, S100A6, S100A7, S100P and S100B, have been found to be involved in tumor progression, while others, like S100A8, S100A9, and S100A12 are more likely related to inflammation. Since chronic inflammatory processes perpetuated by the immune system may abet tumor development (23), it can be suggested that certain members of S100 protein family might represent a bridge between cancer and inflammation. Although the association between S100A4 and cancer promoting properties has been established (24-26), its potential role in chronic inflammatory diseases such as rheumatoid arthritis (RA) is a more recent discovery (9,27,28). Here we summarize the current knowledge regarding S100A4 and its relation to the invasive behavior of cells involved in the destructive process of chronic inflammation.

Expression and function of S100A4

S100A4 (also known as metastasin, pEL, p9Ka, FspI, CAPL and calvasculin) is a small 11-kD protein that was originally isolated as a gene differentially expressed in highly metastatic mouse mammary adenocarcinoma cells (29). Subsequently, it was found also in normal tissues (30,31), however the physiological function of S100A4 is not yet understood. In rats, intracellular S100A4 protein was detected in smooth muscle and endothelial cells of blood vessels, epithelial cells, brown adipose and liver tissue, parietal cells of the stomach, neuronal cells and some immune cells within the spleen, thymus, and bone marrow (31,32). There is evidence that the expression of S100A4 is most likely related to different cancerous and immune processes (33). However, S100A4 has been found in normal human and rat tissues in fractions of T-lymphocytes, and neutrophils, while its expression is weak in monocytes, hair follicules and some others. S100A4 protein has also been detected under chronic inflammatory conditions in macrophages, mast cells, neutrophils, certain T-cells, dendritic cells, and pericytes as well as activated synovial fibroblasts (9).

Like other S100 proteins, S100A4 exerts intra- and extracellular effects and is involved in a number of cellular events. Several studies demonstrated that S100A4 may exist as homo- (S100A4/S100A4) or hetero-dimers (S100A4/S100A1) and also has a potential to form oligomers (34,35). Since, no enzymatic activity has been associated with S100A4, it is likely that interactions with other target proteins are critical for S100A4 activity. Intracellularly, S100A4 binds to several target molecules including the heavy chain of non-muscle myosin II (36) and liprin β1 (37), thereby modulating cell motility and adhesion. Furthermore, it interacts with the tumor suppressor protein p53 that may provide a link between S100A4 and apoptosis (30,36,38,39). Extracellular S100A4 has also been documented to stimulate neurite outgrowth of primary hippocampal neurons (40) and the migration of astrocytic tumor cells (41).Moreover, S100A4 is involved in angiogenesis (42) and remodeling of the extracellular matrix by means of up-regulation of proteolytic enzymes (28,43). In this regard Duarte et al. (44) proposed that S100A4 is a novel negative regulator of matrix mineralization that modulates the process of osteoblast differentiation.

Proposed function of S100A4 associated with tumor progression and metastasis

In a complex cascade of events in tumorigenesis and metastatic progression, a variety of regulatory molecules are involved at different stages. Involvement of S100A4, as one of the regulatory elements, has been demonstrated in transgenic mice (26) as well as in humans (24,25). Both, intracellular (interaction with target proteins) and extracellular (cytokine-like triggering of signal transduction) forms of S100A4 contribute to the metastasis-promoting function of the protein.

Intracellular S100A4 is known to interact with cytoskeletal components including the heavy chain of non-muscle myosin, non-muscle tropomyosin and F-actin (30,36,39), thereby affecting the motility of cancer cells. Interaction of S100A4 with the heavy chain of non-muscle myosin (MHC) results in inhibition of protein kinase C (PKC) and casein kinase (CK)-2 dependent phosphorylation of MHC (45,46). This interaction increases the solubility of myosin and regulates cytoskeletal dynamics. Similarly, binding of S100A4 to non-muscle tropomyosin is also thought to be responsible for the disassembly of actin filaments (30). These data suggest that S100A4 can modulate the invasiveness of tumor cells. While these interactions have been documented to be calcium-dependent, there are some reports on calcium-independent interactions of S100A4 (for review see ref. 3).

Besides cell motility, cell-cell adhesions are considered to be other properties of metastatic cells. Cooperation between S100A4 and E-cadherin (transmembrane glycoprotein that mediates Ca^{2+} dependent cell-cell adhesion) has been studied in mouse tumor cells as well as in humans. In both cases, an inverse correlation in the expression of E-cadherin and S100A4 was demonstrated, suggesting that the invasiveness of tumors expressing S100A4 could be induced by the abrogation of E-cadherin expression (47,48). Moreover, it has been proven that S100A4 contributes to cell adhesion via binding to liprin β1 (transmembrane tyrosine phosphatase-interacting protein), thus modulating LAR (transmembrane phosphotyrosine phosphatase)-dependent signaling directly involved in cell adhesion (37).

S100A4 has also been reported to regulate proliferation and apoptosis. Recently, it was shown that the expression of S100A4 is associated with an increased amount of p53, however the conformational form of p53 was not studied (49). Moreover, physical interaction of S100A4 with the C-terminal regulatory domain of tumor suppressor protein p53 has been shown earlier (38). Induction of S100A4 in cell lines expressing wild type p53 modulated the expression of p53 downstream target genes including p21/WAF and bax (38). It has been suggested that S100A4 can enhance p53-dependent apoptosis and thereby accelerate the loss of wild type p53 functions, and consequently contribute to the development of a more aggressive phenotype during early tumor progression. Moreover, a reduced frequency of apoptosis was observed in the spleen of S100A4-/- animals after whole-body gamma-irradiation compared to wild-type animals (50). On the other hand, the extracellular S100A4 has been found to down-regulate bax in mouse adenocarcinoma cells, which might increase tumor cell survival. Both oligomeric and dimeric forms of S100A4 exerted equal inhibitory effect on the transcription of bax, however, no influence on the expression of p21/waf was observed (51).

Another important hallmark of invasive tumor growth and cancer metastasis is angiogenesis**.** S100A4 has been detected to exert its role in angiogenesis particularly via the modulation of the expression of thrombospondin 1 and matrix metalloproteinases (MMPs). Thrombospondin 1 belongs to the class of extracellular matrix glycoproteins with anti-angiogenic effects that can repress the progression of tumors (52). Thus, the treatment of tumor and endothelial cells with S100A4 oligomer induced down-regulation of thrombospondin 1 gene expression (38,51). Moreover, the S100A4 oligomer was capable of stimulating angiogenesis by promoting the chemotactic motility of endothelial cells *in vitro*, and of inducing corneal neovascularization *in vivo* (51). Dysregulation of MMPs participates in remodeling of the extracellular matrix, tumor cell migration and invasion (53). An association between S100A4 and the production of MMPs has also been reported (38,43,51). Extracellular S100A4 strongly stimulated the proteolytic activity in endothelial and tumor cells, particularly by up-regulating MMP-13. In a highly metastatic osteosarcoma cell line transfected with a specific ribozyme against the S100A4 gene transcript, the down-regulation of S100A4 expression resulted in a reduction of the mRNA levels of MMP-2, MMP-14, and the endogenous tissue inhibitor TIMP-1 (54). In addition, the suppression of S100A4 has been shown to reduce significantly the expression and proteolytic activity of MMP-9 (55).

Summarizing the abovementioned data, one can support the idea that the metastasis-inducing S100A4 plays a pivotal role in modulating the tumor stroma (51), because it has been shown that S100A4 is involved in the regulation of cancer invasiveness and metastasis (summarized in 56). Both intracellular as well as extracellular functions of S100A4 have been proposed to be involved in this process, and the protein was suggested as a prognostic marker for several tumors (33).

Proposed function of S100A4 in rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by bone and cartilage destruction, chronic synovial inflammation and hyperplasia. In recent years, synovial fibroblasts (SF) have been suggested to play an active role in the pathogenesis of RA (57,58). Masuda et al. (59) found certain genes, including S100A4, to be up-regulated in proliferating compared with non-proliferating RA-SF. Moreover, S100A4 mRNA has been detected both in the lining as well as sublining layer of RA synovial tissues, while its expression was not observed in healthy synovial tissues. Recently, we have demonstrated a strong up-regulation of S100A4 protein in RA compared with osteoarthritis (OA) and control synovial tissues (9,28). Most importantly, the expression of S100A4 protein has been detected at sites of cartilage and bone destruction. Synovial fibroblasts, as well as several immune and vascular cells, were identified to produce S100A4 (9). In comparison with other S100 proteins such as S100A7, S100A8, S100A9 and S100A12, the majority of cells in RA synovial tissue have been found to express S100A4. The up-regulation of S100A4 in RA synovial tissue was consistent with the finding of rather high concentrations of the protein in RA compared with OA plasma (1100 vs. 211 ng/ml) and synovial fluid (1980 vs. 247 ng/ml) (Fig.1). In plasma and synovial fluid of RA patients, S100A4 exists in a bioactive oligomeric conformation whereas in OA the majority of extracellular S100A4 was detected in the less active, dimeric form. Consistent with observations in tumor models, extracellular S100A4 induced the up-regulation of several MMPs such as MMP-1, MMP-3, MMP-9, and MMP-13 (28) and stabilized the tumor suppressor protein p53 in RA-SF (9). The active oligomeric S100A4 protein also revealed notable effects on the transcriptional regulation of p53 target genes including Bcl-2, p21/WAF and HDM2 that are involved in proliferation and apoptosis.

Taken together, S100A4 is increased in patients with RA both locally at sites of inflammation, as well as systemically in circulation. Moreover expression of S100A4 is localized specifically at sites of joint destruction and was shown to modulate *in vitro* proliferation, apoptosis and the production of several MMPs in RA synovial fibroblasts (Fig.2).

Recently, the potential implication of S100A4 has also been studied in the pathogenesis of OA (60). Enhanced expression of S100A4 compared with normal tissue was detected by immunohistochemistry and western blot in cartilage from OA patients and in a human articular chondrocyte cell line. Richard Loeser's group demonstrated that S100A4 binds to multiligand RAGE and thereby stimulates the expression of MMP-13 in human articular chondrocytes. Activation of RAGE by S100A4 in chondrocytes triggers signal transduction pathways stimulating phosphorylation of Pyk-2 and MAP kinases (ERK-1/2, p38, and JNK), the activation of NF-κB and the production of reactive oxygen species (ROS). These data suggest that S100A4-RAGE signaling might be involved in the process of cartilage degeneration in OA (60). It appears that S100A4 is involved in numerous processes leading to joint destruction.

Proposed function of other S100 proteins in rheumatoid arthritis

Within the S100 protein family a subgroup of phagocyte-specific proteins (calgranulins) has been identified. The three members of this subgroup, S100A8, S100A9 and S100A12 are predominantly expressed in cells of myeloid origin and exert proinflammatory functions in the extracellular milieu via interaction with RAGE (11,61-63). Their elevated levels have been associated with a number of inflammatory diseases, particularly with rheumatoid arthritis.

S100A12 (calgranulin C or EN-RAGE) is mainly detected in granulocytes (64) and in lower levels in monocytes (3). However, increased levels of S100A12 in inflamed tissues have also been documented in a number of disorders including psoriasis (65,66), inflammatory bowel diseases (11,67), Kawasaki disease (68,69), giant cell arteritis (70), type 2 diabetes (71) and Alzheimer disease (72).

The results from *in vitro* studies and animal models have revealed the impact of S100A12 in the pathogenesis of chronic arthritis. S100A12 has been immuno-detected within the synovial-sublining layer, the perivascular region and the synovial-lining layer in RA. Staining of sequential sections of the synovial lining-layer by CD68 confirmed occasional S100A12 positive macrophages (73). S100A12 may stimulate the accumulation of neutrophils by inducing their release from the bone marrow as well as by activating their adhesion and migration toward inflammatory sites (74). In early stages of inflammation, binding of S100A12 to RAGE may lead to stimulation of endothelial cells by increasing surface expression of adhesion molecules (VCAM-1, ICAM-1) and to promotion of transendothelial migration of phagocytes (75). Furthermore, S100A12 up-regulates NF-κB driven transcription of some inflammatory cytokines such as tumor necrosis factor (TNF) -α in inflammatory cells (11). Increased levels of S100A12 were found in synovial fluid and plasma from patients with RA, gout and psoriatic arthritis, however, they were undetectable in patients with non-inflammatory disorders such as OA (74). Foell et al. (76) found in juvenile idiopathic arthritis (JIA) that the S100A12 serum concentration correlates with disease activity and it decreases in response to different anti-inflammatory therapies. Moreover elevated serum S100A12 could be detected weeks before clinically apparent relapses in patients with previously well controlled disease.

The other calgranulins, S100A8 (MRP8 or calgranulin A) and S100A9 (MRP14 or calgranulin B) exist in general as a heterodimer and are actively secreted by human monocytes and activated granulocytes (77). The expression of S100A8 and S100A9 without concomitant formation of their complex has been rarely found, e.g. in a chronic type of inflammatory reaction in glomerulonephritis or in chronic renal allograft rejection (78,79). Up-regulation of the S100A8/9 complex, known as calprotectin, however, has been well described in inflammatory disorders such as cystic fibrosis (80-83), psoriasis (84), tuberculosis (85), Crohn´s disease and ulcerative colitis (67). The presence of S100A8 and S100A9 in infiltrated macrophages in RA was detected in 1987 (86). The authors demonstrated that resting normal tissue macrophages did not express both MRP-8 and MRP-14, macrophages in acutely inflamed tissues expressed MRP-14 but not MRP-8, and in chronic inflammation, infiltrated macrophages expressed both proteins.

Additionally, Youssef P et al. found predominant expression of MRP-8, MRP-14, and their heterodimer at the site of cartilage destruction in RA (87). Analysis of blood and synovial fluid showed significantly elevated levels of S100A8/9 in plasma and synovial fluid of patients with RA compared with OA (74,88). Moreover, concentrations of the dimer were significantly higher in synovial fluid than in serum, and they correlated with each other showing a strong association with disease activity, and it was suggested that MRP8/14 could be a parameter of prognostic value for further disease flare in patients with JIA (89,90). *In vitro*, the S100A8/A9 heterodimer may enhance inflammation through increased production of proinflammatory cytokines including TNF-α (91). In addition to chronic inflammation, most recent data point to the fact that the MRP8/14 dimer is associated with the development of acute coronary syndrome. Because its blood levels increase before the levels of markers of myocardial necrosis, the MRP8/14 dimer might serve as a novel sensitive predictor of the syndrome (14). Based on the abovementioned data, one can suggest that both S100A12 and S100A8/A9 proteins are valuable markers of inflammation as well as potential targets for future therapies.

Conclusions

Over the past decade, many data have produced great strides in understanding the role of S100A4 in tumor cell invasion and the subsequent metastasis. As shown by several research groups, the interaction of the protein with its several effectors can activate signaling pathways and modulate detachment of the extracellular matrix, adhesion, cell motility, angiogenesis, cell proliferation and apoptosis. Interestingly, hallmarks of metastatic activity of tumor cells and the invasive behavior of synovial fibroblasts in RA appear similar. Analogous to the environment of tumors, S100A4 has been found to be increased in inflamed synovial tissue and body fluids in patients with RA. S100A4 is proposed to be up-regulated upon synovial fibroblast activation/proliferation (59) as well as by several inflammatory cells that accumulate in RA synovial tissue (9,28). Thereby, we suggest that the S100A4 properties are not clearly RA-specific, however, can be related to systemic inflammation or/and activation of synovial fibroblasts. Based on *in vitro* studies, S100A4 can up-regulate the production of matrix degrading enzymes and stabilize the tumor suppressor protein p53 in synovial fibroblasts (9,28). Thereby, it can be suggested that S100A4 takes part in stimulating cell proliferation, modulating apoptosis, and tissue remodeling and thereby contributes to the process of inflammation and tissue destruction. Further analysis and characterization of the molecular mechanisms of S100A4 regulating pathways are needed to clarify the contribution of S100A4 to the immune/inflammatory processes leading to synovial hyperplasia and inflammation.

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Legends to figures:

Figure 1
Comparison between the S100A4 protein (Mts1) levels in plasma and synovial fluid from patients with rheumatoid arthritis and individuals with non-inflammatory knee osteoarthritis.

Figure 2
Postulated implications of S100A4 protein (Mts1) in the process of inflammation and joint destruction in rheumatoid arthritis. Increased amounts of the protein in both synovial tissue and synovial fluid may interact with resident cells and modulate the function of the tumor-suppressor protein p53 as well as the production of several matrix degrading enzymes.

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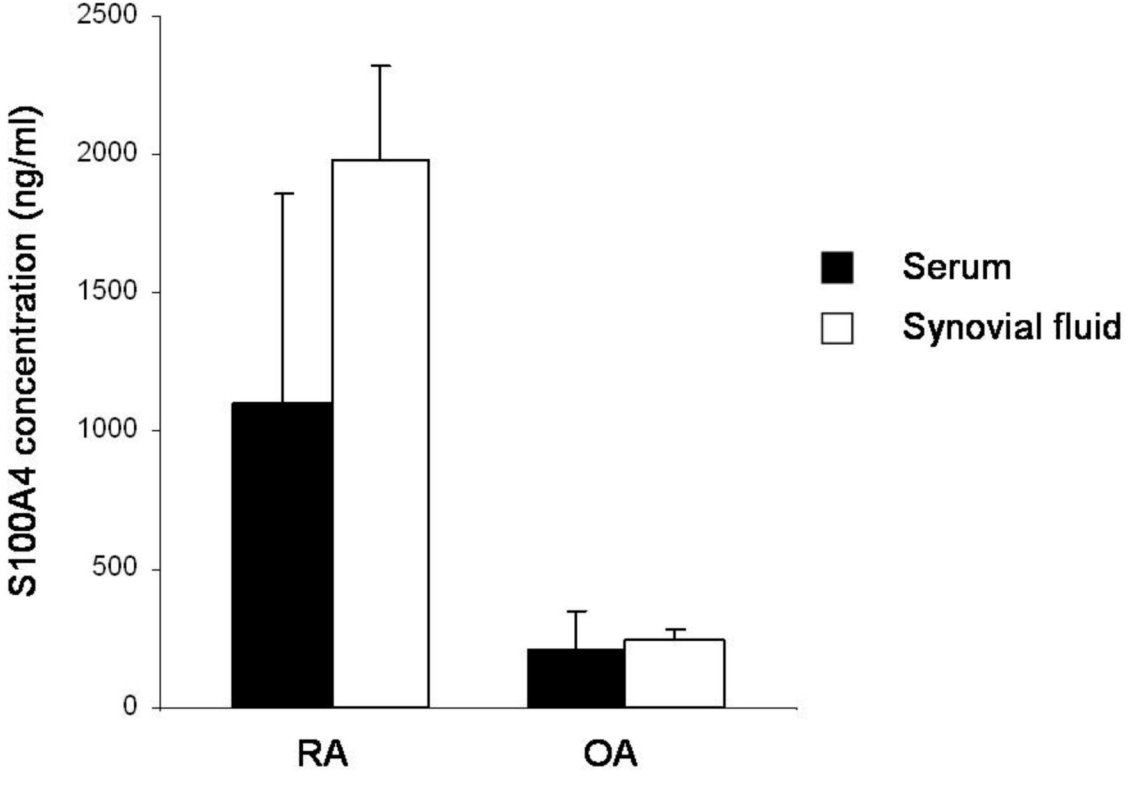
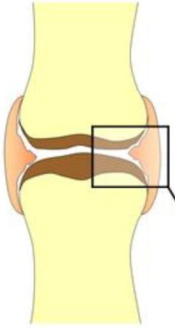

2500
2000
1500
1000
500
0
S100A4 concentration (ng/ml)
Serum
Synovial fluid
RA
OA



inflammatory cells → **S100A4** ↔ synovial fibroblasts → matrix destruction (MMPs)

synovial fibroblasts → apoptosis (p53, p21, etc.)