MINIREVIEW ARTICLE

P2X7 receptor-mediated TG2 externalization: a link to inflammatory arthritis?

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Abstract Transglutaminases have important roles in stabilizing extracellular protein assemblies in tissue repair processes but some reaction products can stimulate immune activation, leading to chronic inflammatory conditions or autoimmunity. Exacerbated disease in models of inflammatory arthritis has been ascribed to sustained extracellular enzyme activity alongside formation of select protein modifications. Here, we review the evidence, with a focus on the link between P2X7R signaling and TG2 export, a pathway that we have recently discovered which ties extracellular protein modifications into the danger signal-mediated innate immune response. These recent insights offer new opportunities for therapeutic intervention.

Keywords Transglutaminase · P2X7 receptor · Purinergic signaling · Arthritis · Cartilage · Inflammation · Autoimmunity

Introduction

A role of transglutaminases (TG) in formation of skeletal tissues was postulated based on functional in vitro studies and by linking the expression of several of the enzymes belonging to this protein family to the developmental program (Aeschlimann and Thomazy 2000). Recent experimental evidence in support of the role of TGs in cartilage development and homeostasis is eloquently outlined in a review by Adamczyk in an accompanying article in this issue of Amino Acids (Adamczyk 2016). While TG2−/− mice had no overt developmental abnormalities (Nanda et al. 2001; De Laurenzi and Melino 2001), deficiencies became apparent once the mice were subjected to injury or challenged in experimental models of disease (Iismaa et al. 2009). This highlighted two points: First, that TG2 is dispensable for skeletal development, a fact that is further reinforced by the absence of overt skeletal abnormalities and grossly normal bone mineral content in TG2 and factor XIIIa double knock-out mice (Cordell et al. 2015). Second, that the inflammatory response in TG2−/− mice is substantially altered, which often results in delayed or compromised tissue repair but may also offer protection in certain circumstances, for example following CNS injury or in neurodegenerative conditions. A detailed discussion of this is beyond the scope of this review, and we will focus here on aspects relevant to joint disease.

TG2 externalization occurs in the context of inflammation

Although having well characterized extracellular functions, TG2 is externalized by cells through an unconventional secretion pathway (Aeschlimann and Paulsson 1994), the details of which remain to be completely deciphered. We recently identified that P2X7 receptor (P2X7R) activation controls active TG2 secretion by cells (Adamczyk et al. 2015). This not only established for the first time a model in which the steps leading to TG2 externalization can now be meaningfully interrogated (P2X7R expressing HEK293

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Our data also suggest that TG2 secretion is independent of the plasma membrane in free form (Adamczyk et al. 2015). We have been able to mechanistically identified direct membrane translocation implicated in IL-1β secretion (Eder 2009). We have been able to mechanistically identify direct membrane translocation implicated in IL-1β secretion (Eder 2009). We have been able to mechanistically identify direct membrane translocation implicated in IL-1β secretion (Eder 2009).

IL-1β family cytokines and thioredoxin-1 similar to TG2 are leaderless proteins that are not targeted to the classical ER to Golgi pathway for export (Rubartelli et al. 1990, 1992), and their precise mechanism of secretion remains a matter of debate. It is possible that all or part of the mechanism guiding the release of these three proteins is shared, particularly as we have shown that TG2 and thioredoxin are co-secreted (Adamczyk et al. 2015). A common mechanism that enables rapid deployment of these proteins is also consistent with their overlapping functions in innate immunity. Several distinct mechanisms that can support unconventional protein secretion have been identified (for review see Nickel and Rabouille 2009; Rabouille et al. 2012), and microvesicle shedding at the plasma membrane, exocytosis of endo-lysosome-derived vesicles or transporter-facilitated direct membrane translocation implicated in IL-1β secretion (Eder 2009). We have been able to mechanistically separate P2X7R-stimulated vesicle release from TG2 export, and have shown that TG2 is directly secreted across the plasma membrane in free form (Adamczyk et al. 2015). Our data also suggest that TG2 secretion is independent of inflammation assembly but instead relates to the ability of P2X7R to induce ‘membrane pores’ (Adamczyk et al. 2015; discussed below). Interestingly, recent data show that IL-1β secretion can also be de-coupled from NLRP3 or AIM2 inflammasome formation and its maturation by caspase-1 processing, and is mediated by a state of membrane hyperpermeability (Martín-Sánchez et al. 2016). Although single cell analysis supports bulk release of IL-1β in the context of inflammasome activation-driven cell death (Shirasaki et al. 2014), inflammasome-independent P2X7R-driven IL-1β secretion has been demonstrated in cell models (Gudipaty et al. 2003), may have distinct biological functions, notably in non-immune cells, and may relate to P2X7R activation-mediated membrane pore formation that is reversible. Nevertheless, activation of caspase-4/5 can trigger a form of programmed cell death termed pyroptosis. Pyroptosis is part of the innate immune defense to infection and features plasma membrane pore formation that ultimately results in fragmentation of infected cells. Gasdermin D was recently shown to be a critical effector component of the canonical NLRP3, AIM2, and NAIP-NLRC4 inflammasome pathways, substantially impacting on IL-1β secretion without affecting caspase-1 autoprocessing or IL-1β activation (Shi et al. 2015; Kayagaki et al. 2015). Cleavage of gasdermin D by inflammatory caspases-4/5 or -1 leads to dissociation of gasdermin N-domain from its autoinhibitory C-domain and results in formation of large membrane pores (Aglietti et al. 2016; Ding et al. 2016). Whether gasdermin D N-domain pores also support release of TG2 and thioredoxin from cells undergoing pyroptosis remains to be investigated.

Role of the P2X7R-TG2 pathway in rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by specific adaptive immune cell responses, synovial hyperplasia and inflammation-driven cartilage and bone destruction. Citrullination of proteins by members of the peptidyl arginine deiminase (PAD) family of enzymes (primarily PAD4 but PAD2 and PAD3 are also involved) is a characteristic feature of disease (Harris et al. 2008), and the resulting neo-epitopes elicit an immune response via a mechanism that shares some similarity to the pathogenesis of celiac disease (Molberg and Sollid 2006). Antibodies to citrullinated peptides (anti-CCP antibodies or ACPA) signify disease development, and have become an accepted marker in diagnosis (Liao et al. 2013). More recently, a pathogenic loop involving PAD3/PAD4-reactive autoantibodies that activate PAD4 and thereby drive the formation of immune-stimulating epitopes has been implicated in rapid disease progression (Darrah et al. 2013). Current
therapeutic approaches target aspects of immunity (blocking TNF-\(\alpha\) or targeting B cells) but a substantial fraction of patients are nonresponsive to these treatments, highlighting first, the fact that pathogenesis is not uniform and second, the need to identify the implied additional pathways that drive joint destruction.

Activation of P2X7R may drive accumulation of extracellular TG, and ultimately TG-mediated protein modification or crosslinking reactions that have a role in pathogenesis. In support of this, mouse models of disease linked both TG2 and factor XIIIa reaction products mechanistically to an exacerbated inflammatory response that drives disease progression and joint destruction (Dzhambazov et al. 2009; Raghu et al. 2015). Given the role of the NLRP3-dependent inflammasome pathway in proinflammatory cytokine production, unsurprisingly P2X7R−/− mice were protected from inflammatory arthritis as shown using the collagen type II (CIA)-induced arthritis model (Labasi et al. 2002). P2X7R−/− mice also do not develop Freund’s adjuvant (CFA)-induced chronic inflammatory hypersensitivity (Chessell et al. 2005). However, it is noteworthy that TG2 has been shown to modify epitopes targeted by T cells in the CIA model, and to exacerbate incidence, severity, and histopathological features of disease (Dzhambazov et al. 2009). Notably, injection of functional but not inactive enzyme also triggers a B cell response to the enzyme itself, an event that may originate from complex formation of the enzyme with peptides containing T cell epitopes in a process akin of what is seen in celiac disease (Stamnaes et al. 2010b). Interestingly, it has also been shown that P2X7R activation in mice drives PAD2-mediated protein citrullination, an event linked to anti-CCP antibody development in RA (Arandjelovic et al. 2012). Hence, ablation of P2X7R may have effects on the immune response that go beyond suppressing formation of biologically active IL-1 and IL-18, namely, also suppressing the formation of posttranslational protein modifications that are targeted by the adaptive immune system (Fig. 1).

The efficacy of P2X7R antagonists has been extensively examined in rodent models of inflammatory arthritis, with some success (for summary see Table 7 in Bartlett et al. 2014; McInnes et al. 2014). Blocking P2X7R suppresses synovial inflammation substantially and reduces local tissue damage as well as mechanical hyperalgesia, particularly when administered prior to disease onset, with no apparent effect on the systemic acute phase response. Confirmatory clinical studies are underway but have so far not shown the expected efficacy (Keystone et al. 2012; Stock et al. 2012). One reason for this could be the highly polymorphic nature of the P2RX7 gene in the human population. It is becoming increasingly clear that a growing number of amino acid substitutions found in P2X7R have a substantial impact on receptor functionality (Stokes et al. 2010), and some strongly predispose to chronic inflammatory diseases, whereas others offer protection. Indeed, SNP linkage analysis in an RA cohort revealed a positive correlation with the presence of a gain-of-function P2X7R allele (Al-Shukaili et al. 2011) which we have shown to mediate enhanced TG2 release (Adamczyk et al. 2015). Hence, it may be necessary to consider the P2RX7 genotype when evaluating the efficacy of P2X7R antagonists, as antagonist binding affinity or baseline receptor activation state are P2X7R variant-specific and can differ substantially. Indeed, receptor variant-dependent pharmacodynamics has been reported for one of the antagonists in development (McHugh et al. 2012).

The mechanism by which TG2 contributes to RA progression is not completely understood. TG2 is overexpressed in human RA lesions (Weinberg et al. 1991), and the presence of active TG2 substantially increases severity of disease in the CIA model (Dzhambazov et al. 2009) whereas a virally transduced localized knockdown of TG2 appears to alleviate joint destruction (Lauzier et al. 2012). As administration of TG2 alone in the absence of collagen II immunization does not elicit an immune response, and as functional enzyme but not inactive TG2 exacerbates the disease course, this suggests that TG2 does not initiate the autoimmune response but that TG2-catalyzed reactions modify the immune response (Dzhambazov et al. 2009). The fact that the increased disease severity is not localized to the immunization site but systemically affects joints further suggests that the altered disease course is a consequence of exacerbated adaptive immunity (Dzhambazov et al. 2009), and this likely involves targeting of neo-epitopes generated by TG2. However, although Q267 in the immunodominant collagen II T cell epitope (IAGFKGEGQGPK) can be deamidated by TG2, this does not lead to enhanced presentation or T cell stimulation (Dzhambazov et al. 2009). It is possible that other, as yet unidentified epitopes targeted by T cells are generated by TG2. Alternatively, the explanation could also be the development of a B cell response to TG2. With circulating autoantibodies, immune-complex formation at RA lesion sites is likely to occur and promoted by inflammation-driven TG2 overexpression and externalization, and hence could contribute to exacerbated disease. Indeed, a B cell response to TG2 is seen only following administration of functional enzyme (Dzhambazov et al. 2009), and anti-TG autoantibody-driven pathogenesis has been implicated in extraintestinal manifestations of celiac disease (Boscolo et al. 2010; Zone et al. 2011). However, while anti-TG2 antibodies have been reported in RA patients and other immune-mediated forms of arthritis in some studies (Picarello et al. 2003), it is not a prevalent or consistent feature of human RA (Liao et al. 2013).

In contrast to TG2, factor XIIIa does not apparently alter T and B cell responses in the CIA model but plays a role in...
differentiation of myeloid precursor cells into their mature progenies including osteoclasts (Raghu et al. 2015). Nevertheless, factor XIIIa−/− mice display an attenuated proinflammatory response. It remains to be investigated whether this relates to crosstalk between the immune system and the coagulation cascade, leading to enhanced plasma factor XIII zymogen activation and fibrinogen deposition which drives inflammation. Alternatively, this may relate to externalization of the catalytic subunit (α2-form) by myeloid cells which could have direct, coagulation system-independent functions.

**Role of P2X7R and TG2 in inflammation associated with gout**

Enhanced TG2 expression by synovial mononuclear cells from patients with gouty arthritis is associated with increased production of bioactive TGF-β (Yen et al. 2015). TG2 has also been implicated in the clearance of apoptotic cells by phagocytes in acute inflammation models (Szondy et al. 2003), including a mouse model of gout-like inflammation where it is thought to facilitate clearance of apoptotic neutrophils by macrophages (Rose et al. 2006).
mechanism for this appears to involve interactions of extracellular TG2 with β3-integrin and MFG-E8 but is independent of catalytic activity (Rose et al. 2006; Tóth et al. 2009). TG2 secretion normally brings about its activation through Ca$^{2+}$-induced conformational changes (Pinkas et al. 2007). However, it is possible that the high concentrations of extracellular nucleotides present at sites of inflammation or an interaction with heparan sulfate-bearing cell surface proteins (Lortat-Jacob et al. 2012) stabilizes the nucleotide-bound conformation and thereby prevents Ca$^{2+}$-binding and activation. The importance of TG2 in regulating inflammation in this context was further substantiated by the fact that TG2+/− mice exhibited an exacerbated inflammatory response in the acute gout-like peritoneal inflammation model (Yen et al. 2015). Hyperuricemia and gout are metabolic diseases caused by purine metabolism disorder. Gout has many manifestations including chronic inflammatory arthritis, treatment of which remains a challenge. Mechanistically, hyperuricemia, i.e., uric acid, the end product of purine metabolism, drives monosodium urate crystal (MSU) formation (Marillo et al. 2014). MSU crystals activate the immune system via toll-like receptor activation and inflammasome signaling. An acute episode may be brought about by stimulation of synovial macrophages and monocytes to release large amounts of proinflammatory IL-1β and IL-18 (Rock et al. 2013), a view that is supported by IL-1 antagonism providing clinical benefit in patients with gout-associated arthritis (Schlesinger et al. 2012). Epidemiological studies have shown that only about 10 % of patients (range 2–36 % depending on study) with hyperuricemia will develop gout suggesting that other factors play an overriding role. While genetic variations in P2X7R are suspected of contributing to disease (Gong and Chen 2015), no such link has been made for TG2. Attention has switched to immune activation as a cause because immunoglobulins from the synovial fluid of patients with gout but not other forms of arthritis promote MSU crystal formation. Decoration of crystals with immunoglobulins drives inflammation through activation of Fc-receptor bearing cells. Interestingly, MSU immunized B cell-deficient mice displayed reduced effector T cell function, and uric acid-induced immune activation could be restored by antibody transfer supporting that MSU crystals evoke a danger signal response (Kanevets et al. 2009).

**P2X7R-TG2 pathway in osteoarthritis: a link to inflammation-driven pain?**

P2X7R expression is not restricted to the hematopoietic lineage but it is widely expressed in many tissues (Bartlett et al. 2014) including the musculoskeletal system where ATP release in response to mechanical loading has been postulated to have a regulatory role in tissue homeostasis (Garcia and Knight 2010). P2X7R is expressed by chondrocytes and, hence, exposure of cells to excessive mechanical stress in osteoarthritis (OA) may lead to ATP release, which in turn may trigger TG2 secretion through activation of P2X7R. Hence, both tissue intrinsic TG2 released by chondrocytes themselves as well as associated with the inflammatory response could contribute to the elevated levels of γ-glutamyl-ε-lysine crosslinks present in OA tissue (Huebner et al. 2009).

P2X7R has several activation states; ATP stimulation initially causes ion channel opening, that besides K$^{+}$ efflux supports Ca$^{2+}$ and Na$^{+}$ influx, leading to membrane depolarization and activation of intracellular signaling cascades (Coddou et al. 2011; Bartlett et al. 2014). This is functionally linked to a disintegrin and metalloproteinase (ADAM)-10 activation, which leads to chemoattractant release that supports lymphocyte homing (Fig. 1) (Garbers et al. 2011). Prolonged ATP exposure leads to formation of a ‘membrane pore’ that enables membrane permeability to larger organic cations (Virginio et al. 1999; Browne et al. 2013). The identity of this pore remains controversial as there is conflicting evidence suggesting either dilation of the P2X7R channel itself or an interaction of P2X7R with another plasma membrane channel, potentially identified as pannexin-1. However, recent studies demonstrate that inflammasome activation is pannexin-1 independent (Qu et al. 2011; Fowler et al. 2014). Interestingly, mutations in P2X7R that interfere with membrane pore formation have been associated with reduced chronic pain in OA patients (Sorge et al. 2012). Studies in animal models highlighted the role of microglia cell-produced proinflammatory cytokines in hypersensitivity to pain, and demonstrated that P2X7R pore formation is responsible for neuropathic pain sensing (Sorge et al. 2012; Nieto et al. 2016). As TG2 externalization is also controlled specifically by P2X7R membrane pore activity (Fig. 1) (Adamczyk et al. 2015) and extracellular active TG2 is therefore likely to be present in this context, it would be interesting to test whether it has a role that affects pain signaling.

**Conclusions**

Based on our recent work and this literature review, we conclude that there is potential for the pathological role of TG2 contributing to chronic inflammation and autoimmunity to be targeted with P2X7R antagonists. Importantly, P2X7R inhibition blocks acute release of large amounts of soluble TG2 by macrophages but has no apparent effect on the level of cell surface-associated enzyme (Adamczyk et al. 2015) that has a critical function in the phagocytic activity of these cells. Recent evidence suggests that an
aspect of P2X7R functionality known as ‘membrane pore formation’ is more important than the ion channel activity of this receptor in inflammation. As TG2 externalization is likewise mediated by the P2X7R membrane pore functionality, selectively targeting this activity of the receptor is likely to be more effective therapeutically and this also reduces the risk of undesired side effects. Development of suitable pharmacological inhibitors is an area currently under intense investigation. Unexpectedly, nucleoside reverse transcriptase inhibitors currently used as anti-viral agents have been shown to selectively block large membrane pore activity (Fowler et al. 2014), and hence, may offer for the first time an opportunity to test the efficacy of selective therapeutic intervention.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This is a review article and as such does not contain any primary data pertaining to clinical studies or animal work.

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References

Adamczyk M (2016) Transglutaminase 2 in cartilage homeostasis: novel links with inflammatory osteoarthritis. Amino Acids. doi:10.1007/s00726-016-2305-1

Adamczyk M, Griffiths R, Dewitt S, Knäuper V, Aeschlimann D (2015) P2X7 receptor activation regulates rapid unconventional export of transglutaminase-2. J Cell Sci 128(24):4615–4628

Aeschlimann D, Paulsson M (1994) Transglutaminases: protein crosslinking enzymes in tissues and body fluids. Thromb Haemostasis 71:402–415

Aeschlimann D, Thomazy V (2000) Protein crosslinking in assembly and signaling of extracellular matrices: the role of transglutaminases. Connect Tissue Res 41:41–27

Aglietti RA, Estevez A, Gupta A, Ramirez MG, Liu PS, Kayagaki N, Ciferri C, Dixit VM, Dueber EC (2016) GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. Proc Natl Acad Sci USA 113(28):7858–7863

Al-Shukali A, Al-Kaabi J, Hassan B, Al-Araimi T, Al-Tobi M, Al-Kindi M, Al-Maniri A, Al-Gheilani A, Al-Ansari A (2011) P2X7 receptor gene polymorphism analysis in rheumatoid arthritis. Int J Immunogenet 38(5):389–396

Arandjelovic S, Mckenney KR, Leming SS, Mowen KA (2012) ATP induces protein arginine deiminase 2-dependent citrullination in mast cells through the P2X7 purinergic receptor. J Immunol 189(8):4112–4122

Bartlett R, Stokes L, Sloyter R (2014) The P2X7 receptor channel: recent developments and the use of P2X7 antagonists in models of disease. Pharmacol Rev 66:638–675

Blachère NE, Parveen S, Fak J, Frank MO, Orange DE (2015) Inflammatory but not apoptotic death of granulocytes citrullinates fibrinogen. Arthritis Res Ther 17(1):369

Boscolo S, Lorenzon A, Sbattero D, Florian F, Stebel M, Marzari R, Not T, Aeschlimann D, Ventura A, Hadijvassiliou M, Tongiorgi E (2010) Anti-transglutaminase antibodies cause ataxia in mice. PLoS One 2010:e9698

Browne LE, Compan V, Bragg L, North RA (2013) P2X7 receptor channels allow direct permeation of nanometer-sized dyes. J Neurosci 33:3557–3566

Chellisp J, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL, Grahames CB, Casula MA, Yioungou Y, Birch R, Anand P, Buell GN (2005) Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114(3):386–396

Coddou C, Yan Z, Obsil T, Huidobro-Toro JP, Stojilkovic SS (2011) Activation and regulation of purinergic P2X receptor channels. Pharmacol Rev 63:641–683

Cordell PA, Newell LM, Standeven KE, Adamson P, Smith KA, Jackson CL, Grant PJ, Pease RJ (2015) Normal bone deposition occurs in mice deficient in factor XIII-A and transglutaminase 2. Matrix Biol 43:85–96

Darrah E, Giles JT, Ols ML, Bull HG, Andrade F, Rosen A (2013) Erosive rheumatoid arthritis is associated with antibodies that activate PAD4 by increasing calcium sensitivity. Sci Transl Med 5(186):186ra65

De Laurenzi V, Melino G (2001) Gene disruption of tissue transglutaminase. Mol Cell Biol 21:148–155

Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, Sun H, Wang DC, Shao F (2016) Pore-forming activity and structural autoinhibition of the g asdermin family. Nature 535(7610):111–116

Dzhambazov B, Lindh I, Engström A, Holmdahl R (2009) Tissue transglutaminase enhances collagen type II-induced arthritis and modifies the immunodominant T-cell epitope CI260–270. Eur J Immunol 39:2412–2423

Eder C (2009) Mechanisms of interleukin-1beta release. Immunobiology 214:543–553

Fowler BJ, Gelfand BD, Kim Y, Kerur N, Talavolo V, Hirano Y, Amarnath S, Fowler DH, Radwan M, Young MT, Pittman K, Kubes P, Agarwal HK, Parang K, Hinton DR, Bastos-Carvalho A, Li S, Yasuma T, Mizutani T, Yasuma R, Wright C, Ambati J (2014) Nucleoside reverse transcriptase inhibitors possess intrinsic anti-inflammatory activity. Science 346(6212):1000–1003

Garbers C, Jänner N, Chalaris A, Moss ML, Floss DM, Meyer D, Koch-Nolte F, Rose-John S, Scheller J (2011) Species specificity of ADAM10 and ADAM17 proteins in interleukin-6 (IL-6)-transsignaling and novel role of ADAM10 in inducible IL-6 receptor shedding. J Biol Chem 286(17):14804–14811

Garcia M, Knight MM (2010) Cyclic loading opens hemichannels to release ATP as part of a chondrocyte mechanotransduction pathway. J Orthop Res 28:510–515

Gong QY, Chen Y (2015) Correlation between P2X7 receptor gene polymorphisms and gout. Rheumatol Int 35(8):1307–1310

Gudipaty L, Munetz J, Verhoef PA, Dubyak GR (2003) Essential role of P2X7 receptor gene polymorphism analysis in rheumatoid arthritis. Int J Immunogenet 30(186):186ra65

Harris ML, Darrah E, Lim G, Bartlett SJ, Giles JT, Grant AV, Gao P, Scott WW Jr, El-Gabalawy H, Casciola-Rosen L, Barnes KC, among others.
Bathon JM, Rosen A (2008) Association of autoimmunity to pep-
tidyl arginine dimethylase 4 with genotype and disease sever-
ity in rheumatoid arthritis. Arthritis Rheum 58(7):1958–1967
Hattori M, Gouaux E (2012) Molecular mechanism of ATP binding
and ion channel activation in P2X receptors. Nature 485:207–212
Horiuchi K, Le Gall S, Schlute M, Yamaguchi T, Reiss K, Murphy
G, Toyama Y, Hartmann D, Saftig P, Blobel CP (2007) Substrate
selectivity of epidermal growth factor-receptor ligand sheddases
and their regulation by phosphol esters and calcium influx. Mol
Biol Cell 18:176–188
Huebner JL, Johnson KA, Kraus VB, Terkeltaub RA (2009) Transglu-
taminase-2 is a marker of chondrocyte hypertrophy and osteo-
arthritis severity in the Hartley guinea pig model of knee OA.
Osteoarthr Cartil 17:1056–1064
Islam SE, Mearns BM, Lorand L, Graham RM (2009) Transglutami-
nases and disease: lessons from genetically engineered mouse
models and inherited disorders. Physiol Rev 89:991–1023
Jin X, Stummae J, Klöck C, DiRaimondo TR, Solliod LM, Khosla C
(2011) Activation of extracellular transglutaminase 2 by thio-
doxin. J Biol Chem 286:37866–37873
Kanevets U, Sharma K, Dresser K, Shi Y (2009) A role of IgM anti-
bodies in monosodium urate crystal formation and associated
adjuvanticity. J Immunol 182(4):1912–1918
Kayagaki N, Stowe IB, Lee BL, O’Rourke K, Anderson K, Warm-
ing S, Cuellar T, Haley B, Roose-Birgers M, Phung QT, Liu PS,
Lill JR, Li H, Wu J, Kummerfeld S, Zhang J, Lee WP, Snipas
SJ, Salvesen GS, Morris LX, Fitzgerald L, Zheng Y, Bertram
EM, Goodnow CC, Dixit VM (2015) Caspase-11 cleaves gas-
dermin D for non-canonical inflammasome signalling. Nature
526(7575):666–671
Keystone EC, Wang MM, Layton M, Hollis S, McInnes IB,
Lill JR, Li H, Wu J, Kummerfeld S, Zhang J, Lee WP, Snipas
SJ, Salvesen GS, Morris LX, Fitzgerald L, Zheng Y, Bertram
EM, Goodnow CC, Dixit VM (2015) Caspase-11 cleaves gas-
dermin D for non-canonical inflammasome signalling. Nature
526(7575):666–671
Nickel W, Rabouille C (2009) Mechanisms of regulated unconven-
tional protein secretion. Nat Rev Mol Cell Biol 10:148–155
Nieto FR, Clark AK, Grif J, Hathway GJ, Chapman V, Malcangio M
(2016) Neuro-immune mechanisms contribute to pain in early
stages of arthritis. J Neuroinflamm 13(1):96
Normanska MV, Belkin AM (2012) Cellular functions of tissue
transglutaminase. Int Rev Cell Mol Biol 294:1–97
Picarello A, Di Tola M, Sabbatella L, Vetrano S, Anania MC, Spadaro
A, Sorgi M, Taccari E (2003) Anti-tissue transglutaminase anti-
bodies in arthritic patients: a disease-specific finding? Clin Chem
49:2091–2094
Pinkas DM, Strop P, Brunger AT, Khosla C (2007) Transglutaminase
2 undergoes a large conformational change upon activation.
PLoS Biol 5:e327
Qu Y, Misaghi S, Newton K, Gilmour LL, Louie S, Cupp JE, Dubyak
GR, Hackos D, Dixit VM (2011) Pannexin-1 is required for ATP
release during apoptosis but not for inflammasome activation. J
Immunol 186(11):6533–6561
Rabouille C, Malhotra V, Nickel W (2012) Diversity in unconven-
tional protein secretion. J Cell Sci 125:5251–5255
Raghu H, Cruz C, Rewerts CL, Frederick MD, Thornton S, Mullins
ES, Schoenecker JG, Degen JL, Flick MJ (2015) Transglutami-
nase factor XIII promotes arthritis through mechanisms linked
to inflammation and bone erosion. Blood 125(3):427–437
Rock KL, Kataoka H, Lai JH (2013) Uric acid as a danger signal in
gout and its comorbidities. Nat Rev Rheumatol 9(1):13–23
Rose DM, Sydlausk AD, Agha-Babakhani A, Johnson K, Terkeltaub
R (2006) Transglutaminase 2 limits murine peritoneal acute
gout-like inflammation by regulating macrophage clearance of
apoptotic neutrophils. Arthritis Rheum 54(10):3363–3371
Rothmeier AS, Marchese P, Petrich BG, Furlan-Fregua C, Ginsberg
MH, Ruggeri ZM, Ruf W (2015) Caspase-1-mediated pathway
promotes generation of thrombinflammatory microparticles. J
Clin Invest 125(4):1471–1484
Rubartelli A, Cozzolino F, Tallo M, Sitia R (1992) A novel secre-
tory pathway for interleukin-1 beta, a protein lacking a signal
sequence. EMBO J 9(5):1503–1510
Rubartelli A, Bajetto A, Allavena G, Wollman E, Sitia R (1990) A novel secre-
tory pathway. J Biol Chem 265:24161–24164
Schlesinger N, Alten RE, Bardin T, Schumacher HR, Bloch M,
Gimona A, Krammer G, Murphy V, Richard D, So AK (2012)

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Canakinumab for acute gouty arthritis in patients with limited treatment options: results from two randomised, multicentre, active-controlled, double-blind trials and their initial extensions. Ann Rheum Dis 71(11):1839–1848

Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526(7575):660–665

Shirasaki Y, Yamagishi M, Suzuki N, Izawa K, Nakahara A, Mizuno J, Shoji S, Heike T, Harada Y, Nishikomori R, Ohara O (2014) Real-time single-cell imaging of protein secretion. Sci Rep 4:4736

Sorge RE, Trang T, Dorfman R, Smith SB, Beggs S, Ritchie J, Austin JS, Zaykin DV, Vander Meulen H, Costigan M, Herbert TA, Yarkoni-Abitbul M, Tischauer D, Livneh J, Gershon E, Zheng M, Tan K, John SL, Slade GD, Jordan J, Woolf CJ, Peltz G, Maixner W, Diatchenko L, Seltzer Z, Salter MW, Mogil JS (2012) Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. Nat Med 18(4):595–599

Stock TC, Bloom BJ, Wei N, Ishaq S, Park W, Wang X, Gupta P, Mebus CA (2012) Efficacy and safety of CE-224,535, an antagonist of P2X7 receptor, in treatment of patients with rheumatoid arthritis inadequately controlled by methotrexate. J Rheumatol 39(4):720–727

Stokes L, Fuller SJ, Sluyter R, Skarratt KK, Gu BJ, Wiley JS (2010) Two haplotypes of the P2X7 receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function effect and enhanced interleukin-1β secretion. FASEB J 24:2916–2927

Strowig T, Henao-Mejia J, Elinav E, Flavell R (2012) Inflammasomes in health and disease. Nature 481:278–286

Szondy Z, Sarang Z, Molnar P, Nemeth T, Piacentini M, Mastroberardino PG, Falasca L, Aeschlimann D, Kovacs J, Kiss I, Szegedi E, Lakos G, Rajnavolgyi E, Birckbihler PJ, Melino G, Fesus L (2003) Transglutaminase 2−/− mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. Proc Natl Acad Sci USA 100(13):7812–7817

Tóth B, Garabuzzi E, Sarang Z, Vereb G, Vámossy G, Aeschlimann D, Blaskó B, Bécsi B, Erdődi F, Lacy-Hulbert A, Zhang A, Falasca L, Birge RB, Balajthy Z, Melino G, Fésüs L, Szondy Z (2009) Transglutaminase 2 is needed for the formation of an efficient phagocyte portal in macrophages engulfing apoptotic cells. J Immunol 182(4):2084–2092

Virginio C, MacKenzie A, North RA, Surprenant A (1999) Kinetics of cell lysis, dye uptake and permeability changes in cells expressing the rat P2X7 receptor. J Physiol 519(Pt 2):335–346

Weinberg JB, Pippen AM, Greenberg CS (1991) Extravascular fibrin formation and dissolution in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 34:996–1005

Yen JH, Lin LC, Chen MC, Sarang Z, Leong PY, Chang IC, Hsu JD, Chen JH, Hsieh YF, Pailai A, Köröskényi K, Szondy Z, Tsay GJ (2015) The metastatic tumor antigen 1-transglutaminase-2 pathway is involved in self-limitation of monosodium urate crystal-induced inflammation by upregulating TGF-β1. Arthritis Res Ther 17:65

Zone JJ, Schmidt LA, Taylor TB, Hull CM, Sotiriou MC, Jaskowski TD, Hill HR, Meyer LJ (2011) Dermatitis herpetiformis sera or goat anti-transglutaminase-3 transferred to human skin-grafted mice mimics dermatitis herpetiformis immunopathology. J Immunol 186(7):4474–4480