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UNDERSTANDING THE ROLE OF PEPTIDYLARGININE DEIMINASES (PADs) IN DISEASES AND THEIR INHIBITORS AS POTENTIAL THERAPEUTIC AGENTS (REVIEW)

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ABSTRACT

Proteins undergo post translational modifications (e.g. methylation, glycosylation, N-acetylation, phosphorylation and citrullination). Among these post-translational modifications is citrullination caused by peptidyl arginine deiminase (EC 3.5.3.15)family of enzymes that play an important role in maintaining homeostasis under normal physiological conditions. When dysregulated, PADs are involved in several diseases. This review describes the types, location, structure, mechanism, role of increased/overexpression of PADs in disease (RA, multiple sclerosis, ulcerative colitis, and CNS disorders and cancer). PADs inhibitors that serve as potential therapeutic agents are also discussed in this article review which include reversible and irreversible inhibitors with TDFA being the most potent selective inhibitor. Besides, some metals are also used as PADs inhibitors like zinc, samarium and manganese. These inhibitors proved to be very beneficial to control the diseases caused by the dysregulation of PADs.

KEYWORDS: Peptidylarginine deiminases (PADs), Rheumatoid arthritis (RA), Central nervous system (CNS) and Threonine-aspartate-F-amidine (TDFA).

1. INTRODUCTION

Many different enzymes are involved in the tempering of proteins once after the proteins are synthesized. Peptidylarginine deiminases (EC 3.5.3.15) are the family of enzymes which are responsible for the modulation of arginine residues of proteins by citrullination as shown in the Figure 1.[1] The function of peptidyl arginine deiminase inside the cell is based on the calcium or other components that carry sulfhydryl group.

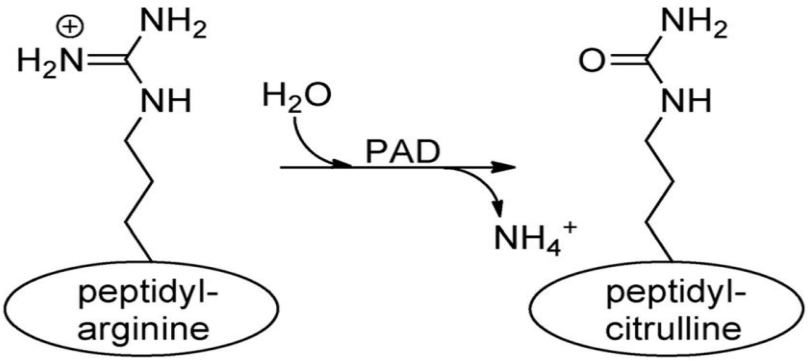

Figure 1. Conversion of peptidylarginine to peptidylcitrulline

As a result of the conversion of the arginine into citrulline, pain in the joints of the wrist and knees can occur which result in the production of antibodies which are anti-citrullinated. The group of these enzymes contain five isozymes which are calcium reliant and they have about fifty percent resemblance in their sequence.[2] Peptidyl arginine deiminases are involved in triggering different types of diseases like multiple sclerosis and various types of cancer. Different types of inhibitors have been used in the treatment and in the controlling of a variety of diseases like infections of the brain, colon and skin. The peptidyl arginine deiminase inhibitor which is extensively used for this purpose is CI-amidine. This inhibitor is involved in the inhibition of peptidyl arginine deiminases which are calcium bounded and they prevent the activity of this enzyme by attacking the

amidine carbon. Some other inhibitors include streptomycin that is likely to be a competitive inhibitor and minocycline as well as chlortetracycline that are mixed inhibitors and have not the ability to act on the active site of the enzyme.[3]

Peptidyl arginine deiminase, are the enzymes that were initially defined in 1977 which were accountable for the post translational modifications and now a days, these are known as cysteine hydrolases.[4]On the active site of the cysteine, citrullination takes place due to the attack by nucleophiles and the whole process occurs on the guanidinium carbon of the substrate. The deposits of citrulline were first discovered in 1960 from hydrolysates which were present in the internal covering of the root cells of hair and brain cells.[5] Inside the cells, citrulline was not found and it was observed that citrulline was synthesized only after the modification of protein synthesis and later on peptidyl arginine deiminases were recognized.[6] Different researches have been carried out on the characteristics of gene, distribution of tissues and substrates required for these enzymes. Calcium is one of the most important component to which PAD4 (a type of peptidyl arginine deaminases) can attach and then result in the development of the active site cleft. The regions which will bind to the calcium were conserved in types 1, 2 and 3 whereas this was not the case for PAD6.[7]

2. Types of PADs and their distribution inside the body: Following are the different types of peptidyl arginine deiminases which highly exist in nature

2.1. PAD1: The normal expression of peptidyl arginine deiminase1 occurs in the epidermis which is the external covering of the organisms as well as in the uterus. This types of PAD is involved in the elimination of the amino group from the keratin K1 and the development of the horny structures of the epidermal tissues.

2.2. PAD2: This type of PAD is largely expressed in a variety of tissues such as uterus, glands which are involved in secretion, skeletal muscles and brain. PAD2 expression is controlled at two points involving the splicing of messenger RNA and at the translation of the proteins.[8] The proteins like myelin present in the central nervous system and vimentin which are present in the skeletal muscles serve as the substrates for PAD2. On the other hand, beta and gamma actins recognized as the substrates for PAD2 in the neutrophils of humans.[10] Peptidyl arginine deiminase can be cytoplasmic as well as nuclear protein. This type of enzyme can bring about the citrullination of histone 3 and histone 4.

2.3. PAD3: Peptidyl arginine deiminase 3 is restricted to the outer covering of the tissues and hair glands. This type is associated with the trichohyalin which is a protein present in the internal layer pf hair follicles. Moreover, it is also associated with the other proteins which are present in the epidermal cells like profilaggrin and filaggrin. Filaggrin is the protein which is involved in the interaction with keratin and control the homeostasis of the epidermis. In addition to its interaction with keratin, it also plays an important role in controlling the normal functions of the epidermis.[11]

2.4. PAD4: PAD4 is normally found in the white blood cells. The main classes involved in the white blood cells are granulocytes as well as monocytes. It is observed that in the case of different types of tissue cancers, this type of PAD is overexpressed.[12]PAD4 have a sequence at N-terminus region and is present in the nucleus. The proteins which get citrullinated by PAD4 are histone 2A (H2A), histone 3 (H3) and histone 4 (H4). In addition to citrullination, PAD4 also plays a vital role in controlling the functions of the nuclei.[13]

2.5. PAD6: PAD6 is known as the egg pad because it was initially isolated from the eggs or fetus of the mouse. Because of the fact that it was originated from the eggs, therefore, it plays an important role in the development of fertility in the females as well as oocyte sheet is also formed.[14] The recent studies indicate that this type of PAD is involved in the regulation of microtubules in the initial stages of embryo development. In the mammalian cells like humans, PAD6 is found in reproductive organs like ovaries, testies as well as blood leucocytes.[15] PAD6 varies from other types of PADs because its calcium residues which are in conserved form are lost fromPAD6 which indicates that it does not perform the function of deamination actively.[16]

3. Distribution: Five different types of PADs have found to be present in the human PAD1, PAD2, PAD3, PAD4 and PAD6 as shown in Table 1. The Table shows that PAD1 is present in epidermis, hair follicles and uterus. PAD2 is found in CNS, spleen, skeletal muscle and WBCs. PAD3 is found in epidermis and hair follicles. PAD4 is found in macrophages, tumors and neutrophils. PAD6 is distributed in eggs ovary and embryo. Out of these types, PAD6 is the type which have no functions inside the cells.[17]

Table 1. Peptidyl arginine deaminases: their types, substrates and distribution.

Types of PADs	Substrate	distribution
PAD1	Keratin K1	Epidermis, hair follicles, uterus
PAD2	Vimentin, glial fibrillary acidic protein	CNS, spleen, skeletal muscle, WBCs
PAD3	Trichohyalin	Epidermis, hair follicles
PAD4	Histones, Vimentin, p300	Macrophages, tumors, neutrophils
PAD6	Unknown	Eggs, ovary, embryo

4. Substrates for PADs: The important substrates of peptidylarginine deiminase are filaggrin and trichohyalin which are involved in the citrullination. When filaggrin in the ratio of 1:1000 and trichohyalin in the ratio of 1:30 is introduced into the PAD, arginines get changed to citrulline in a time period of three hours.[18] Filaggrin consist mainly of beta turns and causes citrullination more often as compared to trichohyalin and it consist of alpha helix. The Arginines which are present afterward the aspartic acid are eighty to ninety percent citrullinated whereas those arginines present near glutamic acid are zero to five percent citrullinated and those which are located near amino acids are poorly citrullinated.[19]

5. Structure, mechanism and Ca^{2+} dependency

Among the PADs, PAD4structure is well understood. PAD4 is about 663 amino acid long with a molecular weight of about 74 kDa.[20, 21] PAD4 has two domains: N-terminal and C-terminal domains. N-terminal domain which is 1-300 amino acid long and a C-terminal domain that comprises 301-663 amino acids. N-terminal domain is further divided into two subdomains. Of two subdomains, one have two calcium binding sites while the other have three calcium binding sites. C-terminal domain contains the active site of enzyme that consists of four residues as: D350, H471, D473 and Cys 645.[22] The enzyme undergoes conformational changes upon binding with calcium ions. These conformational changes help in the movement of active site residues including Cys 645 into such positions that facilitate catalysis. Deimination occurs by nucleophilic attack of active site Cys 645 on guanidinium carbon of arginine to form an intermediate as mentioned in the Figure 2. This intermediate undergo hydrolysis to form citrulline. H471 residue acts as both general acid or base whereas the two aspartic acid residues helps in orientation of guanidinium group for nucleophilic attack by Cys 645 residue.[23]

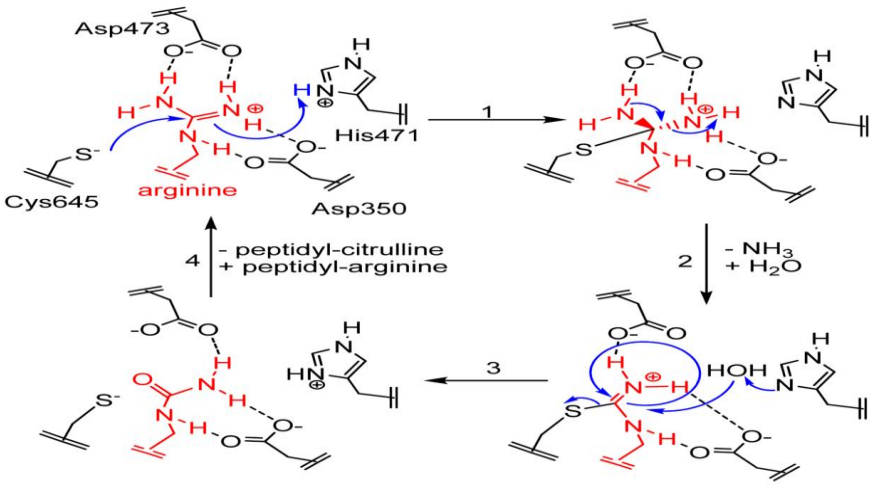

Figure 2. Proposed catalytic mechanism of Peptidylargininedeaminase

6. Role of PADs in Normal Cellular Processes

PADs are usually inactive under normal physiological conditions because the concentration of calcium ions is quite low inside a cell (10^{-8} to 10^{-6}). Calcium ions are required for catalytic activities of PADs.[24] PADs become activated under certain biological events as apoptosis and certain immune responses as calcium ion concentration is increased during these biological events.[25] The processes in which citrullination of proteins play an important role are as follows:

6.1. Apoptosis

Calcium acts as a signaling molecule in normal apoptotic cells.[26] PADs play an important role in Citrullination of cytoskeletal proteins like vimentin. PADs citrullinate non-alpha helical head domain of vimentin protein which is actually an intermediate filament.[27] Vimentin is 466 amino acids long and its non-alpha helical head domain contains 2-95 amino acids with 9.2% arginine.[27] Consequently, vimentin becomes destabilized and its monomers get separated from each other. As a result of this, vimentin is unable to provide structural support and organelle anchorage.[19, 28, 29]

Citrullination of histones and nucleophosmin by PAD4 results in collapse of nucleosome and nuclear lamina. As a result, apoptosis is initiated. Without citrullination, nucleophosminoligomerization play an important role in preventing apoptosis by preventing the localization of p53 to mitochondria.[30,31] p53 protein localizes to mitochondria during p53 dependent apoptosis and disrupts the mitochondrial membrane potential, resulting in the release of cytochrome c.[32]

6.2. Structural Support

Role of PADs in organization of structural proteins during the epidermal differentiation is very important.[19, 33] The epidermis is stratified squamous epithelium that results in the formation of protective covering on the skin. Only the basal cells of stratified squamous epithelium have DNA and thus mitotic ability. These basal cells undergo morphological and biochemical changes to get transformed into dead squames that are

deposited to the outer skin.[34] The differentiation of epidermal cells is mediated by calcium ions which in high concentration activates PADs which citrullinate structural proteins (keratin, filaggrin and vimentin). Citrullination of these proteins results in partial unfolding of these proteins. As a result of partial unfolding, these proteins become more susceptible to degradation by proteases.[35]

6.3. Participation in Immune Responses

PAD2 and PAD4 are particularly important in inflammatory immune responses.

PAD4 is present in macrophages, neutrophils and eosinophils whereas PAD2 is mainly expressed in macrophages. In macrophages, PAD2 become activated due to high concentration of Calcium.[36] After activation, PAD4 is translocated into the nucleus of neutrophils and citrullinates histones which results in their decondensation. Hypercitrullination of histones results in the formation of Neutrophil Extracellular Traps (NETs) as shown in the Figure 3 that results in the entrapment of bacteria and other pathogens.[37, 38] NETs then stimulate an autoimmune response against NET-associated nuclear and granule proteins that induced programmed cell death called NETosis. NETs trap bacteria and other pathogens and kill them by specific proteases or with histones as they have inherent antimicrobial properties.[39] Citrullination of Histones such as Histone H3 is stimulated by bacterial and signaling molecules like LPS (Lipopolysaccharides), tumor necrosis factor alpha, lipoteichoic acid (LTA) and hydrogen peroxide.[40]

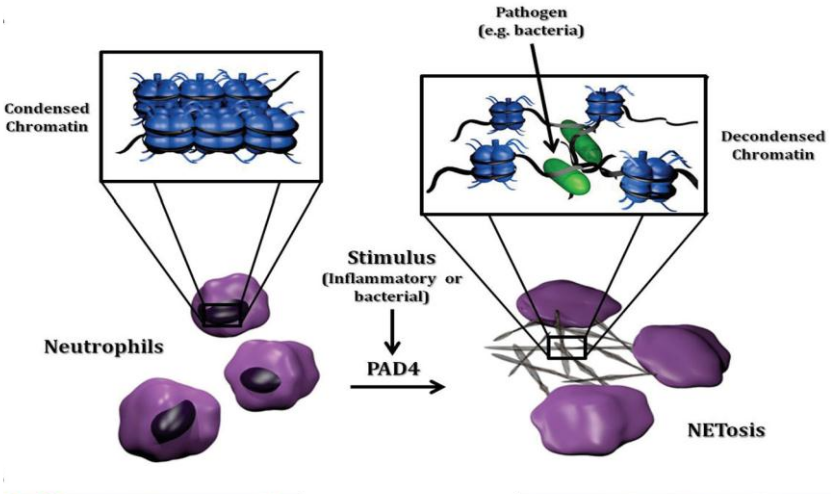

Figure 3.Role of PADs in NETosis.

7. PADs dysregulation and diseases

PADs play an important role in various cellular processes that's why a proper balance is needed in PAD activity. Any disturbance in this balance may lead to abnormal citrullination and various diseases. Specific PADs are related to specific kind of diseases e.g. PAD1 is involved in psoriasis[41] and peptidyl arginine deiminases 2 and 4 are significantly linked with cancer, inflammatory and neurodegenerative diseases.[42,43]

7.1. Causes of dysregulation

Particular reason of PAD dysregulation is not defined but there are some suggested points which may tell about abnormal citrullination such as: high levels of calcium may affect PADs target specificity and their activity, unchecked translation of protein arginine deiminases could cause an increase in citrullination though the actual reason of this increased translation is yet unknown,[44] abnormal TNF alpha signaling is characteristic of Ulcerative colitis (UC) and Rheumatoid arthritis (RA), tumer necrosis factor alpha can induce translocation of PAD4[45] and PADs can citrullinate tumer necrosis factor alpha[46] and autocitrullination of PAD4 may the producer of faulty levels of citrullinated proteins. However, it does not disturb enzyme specificity and activity but it does influences the association with other proteins that are responsible for post translational modification of histones.[47] Besides, sequence of amino acids and confirmation in vicinity of arginine residues can also affect sensitivity to citrullination.[18, 43]

7.2. Diseases

7.2.1. Alzheimer's disease

It is a degenerative disease of brain that starts slowly and becomes severe with the passage of time. During the process of neurodegeneration, PAD2 and PAD4 become stimulated in CNS. Abnormal accumulation of misfolded proteins in hippocampal region of brain leads to Alzheimer disorder. Notably, high levels of citrullinated proteins and PAD2 are found in Alzheimer's patients.[48] Glial fibrillary acidic protein (GFAP) and vimentin are some of structural proteins citrullinated in hippocampus.[49, 50, 51] Similarly, citrullinated proteins and PAD4 high levels were observed at the site of inflammation and neurodegeneration in patients suffering with Alzheimer.[52]

7.2.2. Prion disease

It is also a neurodegenerative disease characterized by misfolded proteins and destruction of different structures

in brain. It is also known as TSE's i.e. Transmissible Spongiform Encephalopathies. Abnormal levels of calcium are probably the cause of increased amounts of citrullinated proteins and active protein arginine deiminases in this disease.

7.2.3. Multiple sclerosis

It is an unforeseeable disease in which impairment of CNS damage the connection between body parts and brain. Myelin sheath is necessary for transduction of neuronal signal through CNS and myelin basic protein is responsible for keeping his sheath intact.[49] In signal transduction, myelin sheath serve as an insulator with MBP carrying a positive charge. Any damage in myelin sheath could cause a decrease in neuronal signaling. PAD2 and PAD4 have a significant contributions in disease pathology.[53, 45] It is stated that PAD4 is carried to the CNS through infiltrating the macrophages in disease condition that may citrullinate protein which are not natural target in CNS.[54] A major leading cause in this diseases is increased citrullination levels of MBP.[53,55] In acute forms of disorders, 90% MBP is citrullinated. The outcomes of citrullination are: the partial unfolding of MBP results in increased susceptibility for degradation by proteases such as cathepsin D.and the conversion of positively charged arginine into neutral citrullinate, both results are not acceptable for electrical considerations in signal transduction.[56, 19, 57] MBP is targeted by PAD explained by high frequency of arginine residues (10.3%) in amino acid sequence of MBP.[58] Multiple PAD isozymes are included in disease etiology suggested by a model EAE – Autoimmune Encephalomyelitis.[59]

7.2.4. Rheumatoid Arthritis

Rheumatoid arthritis is a long term inflammatory disorder of joints that is boosted by the impairment of PADs. This inflammation may be due to defected citrullination and overexpression of PAD2 and 4. According to a RA model for protein arginine deiminase infiltration into synovial joints, PAD 4 and 2 are expressed in monocytes and macrophages that are assembled to joints. Model points that macrophages and monocytes having inactive PADs are ultimately destroyed in the joints and face apoptosis when PAD isozymes become activated at high levels of calcium. This activation leads to citrullination of cellular proteins for example vimentin and PADs can leak out to target proteins present outside the cells.[47] PAD2 and PAD4 can induce immune response by citrullinating the fibrin in affected joints. Moreover, PAD4 can citrullinate a thrombin inhibitor named as anti-thrombin and citrullination of thrombin inhibitor may lead to arthritis.[60]

7.2.5. NET osis

Peptidyl arginine deiminase type 4 plays an important role in exclusive category of the death of cell which is called as the NETosis. Neutrophil extracellular traps when released catches the microbes like bacteria and other harmful microorganisms that are responsible for the production of this sort of reaction. The impairment in the neutrophil extracellular traps causes different types of diseases like infection of the skin which is known as psoriasis in which interleukin 17 is released into the body when these traps are synthesized.[61]

7.2.6. Ulcerative Colitis

In ulcerative colitis which is a very infectious disease, type 2 and 4 of peptidyl arginine deiminase are included. The symptoms of this disease include irritation of the colon. The mechanism of this disease is still unknown but some studies have revealed that macrophages produce some of the cytokines in this disease.[62]

7.2.7. Cancer

When some impairments occur in the peptidyl arginine deiminase, then it can lead to cancer. All the five types of PADs are involved in causing different types of cancer like cancer of ovaries and liver. PAD2 is involved in causing breast cancer whereas PAD4 is plays an important role in causing renal and colorectal cancer.[63]

7.2.8. Other diseases

PAD inhibitors play a significant role in the treatment of numerous human diseases. Some other diseases associated with PADs dysregulation are named as: HIV/AIDS, Osteoarthritis, Ankylosing Spondylitis, Scrapie, glaucoma.[64, 48, 65]psoriasis.[43] (Systemic lupus erthmatous (SLE), Atherosclerosis, Thrombosis, Inflammatory bowl diseases (IBD).[66]

8. PADs Inhibitors

When dysregulated, PADs are involved in a number of diseases including Alzheimer's disease, Multiple sclerosis, Rheumatoid arthritis, Lupus, Ulcerative colitis, and even various types of cancers. In order to inhibit the PADs in these diseases, PADs inhibitors play a significant therapeutic role. Inhibitors may be reversible or irreversible. PADs inhibitors are divided into following categories as.

8.1. Reversible Inhibitors

They associate with PADs via non-covalent interactions as hydrogen bonds, ionic bonds and hydrophobic interactions. Enzyme-substrate complex can dissociate rapidly. Taxol is a reversible inhibitor of PAD4. Arginine derivatives like benzoyl-Nω-monomethylarginine (Bz-MMA) and benzoyl-Nω, Nω-dimethylarginine (Bz-ADMA) inhibit the activity of PAD4. They are not very strong but relatively modest inhibitors. Streptomycin is a competitive inhibitor of PAD4 because it has two guanidinium groups and can be taken up as alternative substrate by PAD4. Minocycline and Chlortetracycline are mixed inhibitors of PAD4 but their mechanism of inhibition is currently understood. Chlortetracycline differs from Minocycline by hydroxyl group and methyl group at position 6 and a Chloro group at position 7. These two compounds also inhibit the other enzymes like collagenases, lipoxygenases and cysteine

proteinases.[67] Glucocorticoids may also suppress the activities of PADs enzymes.**[68]**Ruthenium red also serves as inhibitor of apoenzyme for PAD2. It is shown to be competitive with Calcium ions and bind at calcium 3, 4, and 5 sites. They also inhibit the activity of other PADs enzymes.[69]

8.2. Irreversible Inhibitors: Irreversible inhibitors have no structural similarity with substrate and bind covalently with the substrate. They form stable non-covalent association with enzyme active site and damage the functional group/groups present in the active site. 2-Chloroacetamidine is a covalent inhibitor of PAD4. It possesses guanidinium like amidinium group that is also present in normal substrate of enzyme (benzoyl-arginine amide). This group makes it potent inhibitor that inhibits the activity of PAD4 in a time dependent manner.[70] F-amidine and Cl-amidine are highly potent PAD4 inhibitors. They modify the C645 residue in the active site of PAD4 enzyme by alkylation. This alkylation of PAD4 occurs through two mechanisms.[71,72] In one mechanism, Cys645 directly displaces the halide ions by an SN2 mechanism. The other mechanism involves several steps to inactivate PAD4 by F-amidine and Cl-amidine. Inactivation of enzyme proceeds via the nucleophilic attack of Cys645 residue on amidinium carbon which results in the formation of tetrahedral intermediate that resembles the intermediate that is formed by hydrolysis of substrate. H471 residue of enzyme active site act as general acid and protonation of above intermediate by H471 stabilizes the intermediate instead of destabilizing it. Then, intermediate undergoes intramolecular halide transfer that results in the formation of sulfonium ring as shown in Figure 4.[73] Three-membered sulfonium ring induces the intermediate to collapse, leading to the formation of thiol-ether linkage.[74]

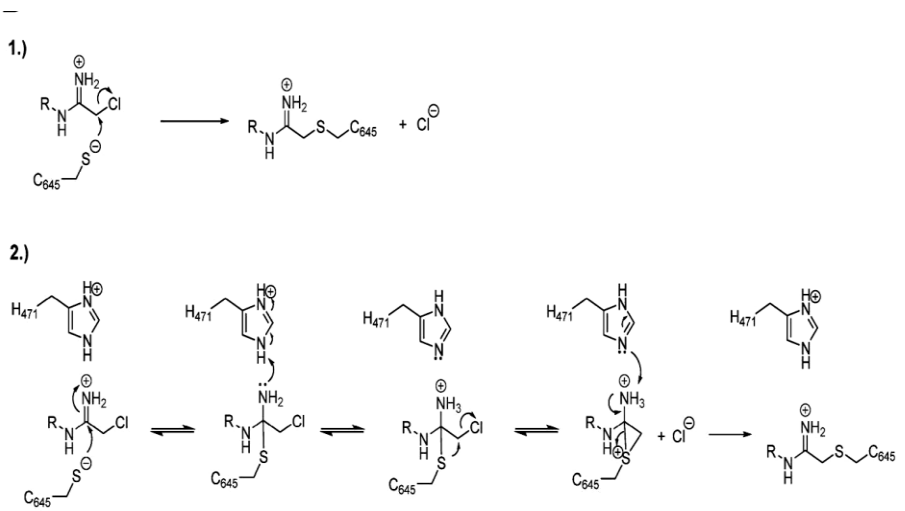

Figure 4.Inactivation of Peptidylargininedeaminase by Chloroacetamidine-base inhibitors.

8.2. Metals as Inhibitors: Some metals like zinc, samarium and manganese inhibit the structural changes in enzyme in the presence of calcium ions that are required to change the enzyme into catalytically active form.[75]

8.3. Other specific PADS inhibitors: o-F-amidine and o-Cl-amidine contain one orthocarboxylate in their parent benzoyl ring. These two compounds have more specificity for PADs than their parent molecules. o-F-amidine inhibits the enzymatic activity of PAD1 whereas o-Cl-amidine cause inhibition of PAD1 as well as PAD4.[76] The most potent selective inhibitor to be reported is TDFA (Threonine-aspartate-F-amidine). TDFA is a tripeptide that consists of threonine, aspartate and F-amidine.[77]

CONCLUSION

PADs are involved in citrullination of arginine residues as described earlier in this article review. Citrullination is a post translational modification and citrullination of proteins by PADs play a significant role in normal cellular processes including apoptosis, epidermal differentiation and immune responses. However, dysregulation of PADs by extreme levels of Ca^{2+} overexpression of PADs /Autocitrullination of PADs results in a number of diseases including RA, Multiple sclerosis, Ulcerative colitis, CNS disorders and Cancer. Involvement of PADs in the development of cancer is an area of intensive research. These diseases can be controlled by PADs inhibitors. PADs isozyme specific inhibitors are continuously being developed to control the diseases caused by PADs dysregulation and three PADs inhibitors are being tested both in-vitro and in-vivo disease models. However, there is a need to develop more potent PADs inhibitors to control diseases caused by PADs dysregulation more efficiently.

REFERENCES

1. Rogers, G.E. and D.H. Simmonds.1958. Content of citrulline and other amino acids in a protein of hair follicles. Nat., 182: 186-187.

2. Fujisaki, M. and K. Sugawara.1981. Properties of peptidylarginine deiminase from the epidermis of newborn rats. J. Biochem., 89(1): 257-263.
3. Knuckley, B., Y. Luo and P.R. Thompson. 2008. Profiling Protein Arginine Deiminase 4 (PAD4): a novel screen to identify PAD4 inhibitors. Bioorg Med Chem., 16(2): 739-745.
4. Rogers, G. E., H. W. Harding and I. J. Llewellyn-Smith. 1977. The origin of citrulline-containing proteins in the hair follicle and the chemical nature of trichohyalin, an intracellular precursor. Biochim Biophys Acta., 495(1): 159-175.
5. Rogers, G.E. 1962. Occurrence of Citrulline in Proteins. Nat., 194: 1149-1151.
6. Vossenaar, E.R., A.J.W. Zendman, W.J. van Venrooij and G.J.M. Pruijn.2003. PADa, growing family of citrullinating enzymes: genes, features and involvement in disease. Bio Essays., 25: 1106-1118.
7. Liu, Y.L., I.C. Tsai, C.W. Chang, Y.F. Liao, G.Y. Liu and H.C. Hung. 2013. Functional Roles of the NonCatalytic Calcium-Binding Sites in the N-Terminal Domain of Human Peptidylarginine Deiminase 4. PloSone., 8: 51660.
8. Ishida Yamamoto, A., T. Senshu, R.A.J. Eady, H. Takahashi, H. Shimizu, M. Akiyama and H. Iizuka. 2002. Sequential Reorganization of Cornified Cell Keratin Filaments Involving Filaggrin-Mediated Compaction and Keratin 1 Deimination., 118: 282-287.
9. Vossenaar, E.R., S. Nijenhuis, M.M.A. Helsen, A. van der Heijden, T. Senshu, W.B. van den Berg, W.J. van Venrooij and L.A.B. Joosten. 2003. Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis & Rheumatism., 48: 2489-2500.
10. Darrah, E., A. Rosen, J.T. Giles and F. Andrade. 2012. Peptidylarginine deiminase 2, 3 and 4 have distinct specificities against cellular substrates: novel insights into autoantigen selection in rheumatoid arthritis. Annals of the Rheumatic Diseases., 71: 92-98.
11. Nachat, R., M.C. Mechin, M. Charveron, G. Serre, J. Constans and M. Simon. 2005. Peptidylarginine Deiminase Isoforms Are Differentially Expressed in the Anagen Hair Follicles and Other Human Skin Appendages. J Investig Dermatol., 125: 34-41.
12. Chang, X. and J. Han. 2006. Expression of peptidylarginine deiminase type 4 (PAD4) in various tumors. Mol Car., 45: 183-196.
13. Lee, J., J. Sayegh, J. Daniel, S. Clarke and M.T. Bedford. 2005. PRMT8, a new membrane-bound tissue-specific member of the protein arginine methyltransferase family. The Journal of biological chemistry., 280: 32890-32896.
14. Esposito, G., A.M. Vitale, F.P.J. Leijten, A.M. Strik, A.M.C.B. Koonen-Reemst, P. Yurttas, T.J.A.A. Robben, S. Coonrod and J.A. Gossen. 2007. Peptidylarginine deiminase (PAD) 6 is essential for oocyte cytoskeletal sheet formation and female fertility. Molecular and Cell Endo., 273: 25-31.
15. Chavanas, S., M.C. Méchin, H. Takahara, A. Kawada, R. Nachat, G. Serre and M. Simon. 2004. Comparative analysis of the mouse and human peptidylarginine deiminase gene clusters reveals highly conserved non-coding segments and a new human gene. PADI6, Gene., 330: 19-27.
16. Arita, K., H. Hashimoto, T. Shimizu, K. Nakashima, M. Yamada and M. Sato. 2004. Structural basis for Ca (2+)-induced activation of human PAD4. Nat StructMol Biol., 11(8): 777-783.
17. Raijmakers, R., A.J. Zendman, W.V. Egberts, et al. 2007. Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases in vitro. J Mol Biol., 367(4): 1118-1129.
18. Tarcsa, E., L.N. Marekov, G. Mei, G. Melino, S.C. Lee and P.M. Steinert. 1996. Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. J Biol Chem., 271(48): 30709-30716.
19. Gyorgy, B., E. Toth, E. Tarcsa, A. Falus and E.I. Buzas. 2006. Citrullination: a posttranslational modification in health and disease. Int J Biochem Cell Biol., 38(10): 1662-1677.
20. Arita, K., H. Hashimoto, T. Shimizu, M. Yamada and M. Sato. 2003a. Crystallization and preliminary X-ray crystallographic analysis of human peptidylarginine deiminase V. ActaCrystallogr D BiolCrystallogr., 59: 2332-2333.
21. Arita, K., T. Shimizu, H. Hashimoto, Y. Hidaka, M. Yamada and M. Sato. 2006b. Structural basis for histone N-terminal recognition by human peptidylarginine deiminase 4. Proc. Natl. Acad. Sci., 103 (14): 5291-5296.
22. Wang, S. and Y. Wang. 2013. Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. Biochim Biophys Acta., 1829(10): 1126-1135.
23. Jones, J., C. Causey, B. Knuckley, J. L. Slack-Noyes, and P.R. Thompson. 2009. Protein arginine deiminase 4 (PAD4): current understanding and future therapeutic potential. Curr Opin Drug Discov Devel., 12(5): 616-627
24. Takahara, H., H. Okamoto and K. Sugawara. 1986. Calcium-dependent Properties of Peptidylarginine Deiminase from Rabbit Skeletal Muscle. Agric Biol Chem., 50(11): 2899-2904.
25. Asaga, H., M. Yamada and T. Senshu. 1998. Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun., 243(3): 641-646.
26. Mattson, M.P. and S.L. Chan. 2003. Calcium orchestrates apoptosis. Nat Cell Biol., 5(12): 1041-1043.
27. Pruitt, K.D., G.R. Brown, S.M. Hiatt and et al. 2014. RefSeq: an update on mammalian reference sequences. Nucleic Acids Res., 42: 756-763.
28. Inagaki, M., H. Takahara, Y. Nishi, K. Sugawara and C. Sato. 1989. Ca2+-dependent deimination-

induced disassembly of intermediate filaments involves specific modification of the amino-terminal head domain. J Biol Chem., 264(30): 18119-27.
29. Katsumoto, T., A. Mitsushima and T. Kurimura. 1990. The role of the vimentin intermediate filaments in rat 3Y1 cells elucidated by immunoelectron microscopy and computer-graphic reconstruction. Biol Cell., 68(2): 139-146.
30. Dhar, S.K. and D.K. St Clair. 2009. Nucleophosmin blocks mitochondrial localization of p53 and apoptosis. J Biol Chem., 284(24): 16409-16418.
31. Jain, Y., Z. Gao, J. Sun and et al. 2009. RNA aptamers interfering with nucleophosminoligomerization induce apoptosis of cancer cells. Oncogene., 28(47): 4201-4211.
32. Marchenko, N. D. 2000. Death Signal-induced Localization of p53 Protein to Mitochondria. A potential role in apoptotic signalling. Bio Chem., 275(21): 16202-16212.
33. Senshu, T., S. Kan, H. Ogawa, M. Manabe and H. Asaga. 1996. Preferential deimination of keratin K1 and filaggrin during the terminal differentiation of human epidermis. Biochem Biophys Res Commun., 225(3): 712-719.
34. Fuchs, E. 1990. Epidermal differentiation: The bare essentials. J. of Cell Bio., 111(6): 2807-2814.
35. Lee, H.J., M. Joo, R. Abdolrasulnia and et al. 2010. Peptidylarginine deiminase 2 suppresses inhibitory {kappa} B kinase activity in lipopolysaccharide-stimulated RAW 264. 7 macrophages. J Biol Chem., 285(51): 39655-39662.
36. Pearton, D. J., B. A. Dale and R. B. Presland. 2002. Functional analysis of the profilaggrin N-terminal peptide: identification of domains that regulate nuclear and cytoplasmic distribution. J Invest Dermatol., 119(3): 661–669.
37. Nauseef, W.M. and N. Borregaard. 2014. Neutrophils at work. Nat Immunol., 15(7): 602-611.
38. Zawrotniak, M. and M. Rapala-Kozik. 2013. Neutrophil extracellular traps (NETs) - formation and implications. Acta Biochim Pol., 60(3): 277-284.
39. Kan, R., M. Jin, V. Subramanian, C.P. Causey, P.R. Thompson and S.A. Coon-rod. 2012. Potential role for PADI-mediated histone citrullination in preimplantation development. BMC Dev Biol., 12: 19.
40. Bicker, K.L. and P.R. Thompson. 2013. The protein arginine deiminases: Structure, function, inhibition, and disease. Biopolymers., 99(2): 155-163.
41. Ishida-Yamamoto, A., T. Senshu, H. Takahashi, K. Akiyama, K. Nomura and H. Iizuka. 2000. Decreased deiminated keratin K1 in psoriatic hyperproliferative epidermis. J Invest Dermatol., 114(4): 701-705.
42. Chumanevich, A.A., C.P. Causey, B.A. Knuckley, et al. 2011. Suppression of colitis in mice by Cl-amidine: a novel peptidylarginine deiminase inhibitor. Am J Physiol Gastrointest Liver Physiol., 300(6): 929-938.
43. Witalison, E.E., P.R. Thompson and L.J. Hofseth. 2015. Protein Arginine Deiminases and Associated Citrullination: Physiological Functions and Diseases Associated with DysregulationCurr Drug Target., 16(7): 700-710.
44. Suzuki, A., R. Yamada, X. Chang, et al. 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet., 34(4): 395-402.
45. Mastronardi, F.G., D.D. Wood, J. Mei, et al. 2006. Increased citrullination of histone H3 in multiple sclerosis brain and animal models of demyelination: a role for tumor necrosis factor-induced peptidylarginine deiminase 4 translocation. J. Neurosci., 26(44): 11387-1196.
46. Moelants, E.A., A. Mortier, K. Grauwen, I. Ronsse, J. Van Damme and P. Proost. 2013. Citrullination of TNF-α by peptidylarginine deiminases reduces its capacity to stimulate the production of inflammatory chemokines. Cytokine., 61(1): 161-167.
47. Slack, J.L., Jr.L.E. Jones, M.M. Bhatia and P.R. Thompson. 2011. Autodeimination of protein arginine deiminase 4 alters protein-protein interactions but not activity. Biochem., 50(19): 3997–4010.
48. Ishigami, A., T. Ohsawa, M. Hiratsuka, H. Taguchi, S. Kobayashi, Y. Saito, S. Murayama, H. Asaga, T. Toda, N. Kimura and N. Maruyama. 2005. Abnormal accumulation of citrullinated proteins catalyzed by peptidylcholinedeaminase in hippocampal extracts from patients with Alzheimer's disease. J. Neurosci Res., 80(1): 120-128.
49. Méchin, M.C., M. Sebbag, J. Arnaud, et al. 2007. Update on peptidylarginine deiminases and deimination in skin physiology and severe human diseases. Int J Cosmet Sci., 29(3): 147-168.
50. Nicholas, A.P., J.L. King, T. Sambandam, et al. 2003. Immunohistochemical localization of citrullinated proteins in adult rat brain. J Comp Neurol. 459(3): 251-266.
51. Acharya, N.K., E.P. Nagele, M. Han, et al. 2012. Neuronal PAD4 expression and protein citrullination: possible role in production of autoantibodies associated with neurodegenerative disease. J Autoimmun., 38(4): 369-80.
52. Jang, B., E. Kim, J.K. Choi, J.K. Jin, J.I. Kim, A. Ishigami, N. Maruyama, R.I. Carp, Y.S. Kim, E.K. Choi. 2008. Accumulation of citrullinated proteins by up-regulated peptidylarginine deiminase 2 in brains of scrapie-infected mice: a possible role in pathogenesis. Am J Pathol., 173(4): 1129-1142.
53. Lamensa, J.W. and M.A. Moscarello. 1993. Deimination of human myelin basic protein by a peptidylarginine deiminase from bovine brain. J. Neurochem., 61(3): 987-996.
54. Raijmakers, R., J. Vogelzangs, J. Raats, et al. 2006. Experimental autoimmune encephalomyelitis

induction in peptidylarginine deiminase 2 knockout mice. J Comp Neurol., 498 (2): 717-726.
55. Moscarello, M.A., F.G. Mastronardi and D.D. Wood. 2007. The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple sclerosis. Neurochem Res., 32(2): 251-256.
56. Wood, D.D., J.M. Bilbao, P. O'Connors and M.A. Moscarello. 1996. Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. Ann Neurol., 40(1): 18-24.
57. Cao, L., R. Goodin, D. Wood, M.A. Moscarello and J.N. Whitaker. 1999. Rapid release and unusual stability of immunodominant peptide 45–89 from citrullinated myelin basic protein. Biochem., 38(19): 6157-6163.
58. Pritzker, L.B. and M.A. Moscarello, 1998. A novel microtubule independent effect of paclitaxel: the inhibition of peptidylarginine deiminase from bovine brain. Biochim Biophys Acta., 1388(1): 154-160.
59. Musse, A.A., Z. Li, C.A. Ackerley, et al. 2008. Peptidylarginine deiminase 2 (PAD2) overexpression in transgenic mice leads to myelin loss in the central nervous system. Dis Model Mech., 1: 229-240.
60. Chang, X., R. Yamada, T. Sawada, A. Suzuki, Y. Kochi and K. Yamamoto. 2005. The inhibition of antithrombin by peptidylarginine deiminase 4 may contribute to pathogenesis of rheumatoid arthritis. Rheumatol., 44(3): 293-298.
61. Chen, C.C., H. Isomoto, Y. Narumi and et al. 2008. Haplotypes of PADI4 susceptible to rheumatoid arthritis are also associated with ulcerative colitis in the Japanese population. Clin Immunol., 126(2): 165-171.
62. Ordás, I., L. Eckmann, M. Talamini, D.C. Baumgart and W.J. Sandborn. 2012. Ulcerative colitis. Lancet., 380: 1606-1619.
63. Chang, X., J. Han, L. Pang, Y. Zhao, Y. Yang and Z. Shen. 2009. Increased PADI4 expression in blood and tissues of patients with malignant tumors. BMC Cancer., 9: 40.
64. Bhattacharya, S.K. 2009. Retinal deamination in aging and disease. IUBMB Life., 61(5): 504-509.
65. Kinolch, A., K. Lundberg, R. Wait, N. Wegner, N.H. Lim, A.J. Zendman, Y. Saxne, V. Malmstrom and P.J. Venables. 2008. Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. Arthritis Rheum., 58(80): 2287-2295.
66. Lewis, H.D. and N. Mariana. 2016. iPAD or PADi — 'tablets' with therapeutic disease potential? CurrOpi in Chem Biol., 33: 169-178.
67. Fuhrmann, J., A. Schmidt, S. Spiess, A. Lehner, K. Turgay, K. Mechtler, E. Charpentier and T. Clausen. 2009. McsB is a Protein Arginine Kinase that Phosphorylates and Inhibits the Heat-Shock Regulator CtsR. Science., 324 (5932): 1323−132.
68. AC Fisher, B and P. K. Venables. 2012. Inhibiting citrullination in rheumatoid arthritis: taking fuel from the fire. Arthr Res and therap., 14 (108): 1-2.
69. Lewallen, D. M., K.L. Bicker, F. Madoux, P. Chase, L. Anguish, S. Coonrod, P. Hodder and P.R. Thompson. 2014. A FluoPol-ABPP PAD2 High-Throughput Screen Identifies the First Calcium Site InhibitorTargeting the PADs. ACS Chem. Biol., 9(4): 913-921.
70. Stone, E. M., T.H. Schaller, H. Bianchi and M.D. Person. 2005. Fast, W. Inactivation of two diverse enzymes in the amidinotransferasesuperfamily by 2-chloroacetamidine: dimethylargininase and peptidylarginine deiminase. Biochem., 44(42): 13744-13752.
71. Knuckley, B., C.P. Causey and J.E. Jones, et al. 2010.Substrate specificity and kinetic studies of PADs 1, 3, and 4 identify potent and selective inhibitors of protein arginine deiminase 3. Biochem., 49(23): 4852-4863.
72. Knuckley, B., C.P. Causey, P.J. Pellechia, P.F. Cook and P.R. Thompson. 2010. Haloacetamidine-Based Inactivators of Protein Arginine Deiminase 4 (PAD4): Evidence that General Acid Catalysis Promotes Efficient Inactivation. Bio Chem., 11(2): 161-165.
73. Wu, C.Y. H.J. Lee, S.H. Wu, S.T. Chen, S.H. Chiou and G.G. Chang. 1998. Chemical mechanism of the endogenous arginine succinatelyase activity of duck lens delta2-crystallin. Biochem. J., 333: 327-334.
74. Luo, Y., K. Arita, M. Bhatia, B. Knuckley, Y.H. Lee, M.R. Stallcup, M. Sato and P.R. Thompson. 2006. Inhibitors and inactivators of protein arginine deiminase 4: functional and structural characterization. Biochem., 45(39): 11727-11736.
75. Kearney, P.L., M. Bhatia, N.G. Jones and et al. 2005. Kinetic characterization of protein arginine deiminase 4: a transcriptional corepressor implicated in the onset and progression of rheumatoid arthritis. Biochem., 44(31): 10570-10582.
76. Causey, C.P., J.E. Jones, J.L. Slack and et al. 2011. The development of N-α-(2-carboxyl)benzoyl-N(5)-(2-fluoro-1-iminoethyl)-l-ornithine amide (o-F-amidine) and N-α-(2-carboxyl)benzoyl-N(5)-(2-chloro-1-iminoethyl)-l-ornithine amide (o-Cl-amidine) as second generation protein arginine deiminase (PAD) inhibitors. J.Med.Chem., 54(19): 6919-6935.
77. Jones, J.E., J.L. Slack, P. Fang and et al. 2012. Synthesis and screening of a haloacetamidine containing library to identify PAD4 selective inhibitors. ACS Chem Biol., 7(1): 160-165.