

Role of PAD Enzymes on Histone Citrullination in Cancer

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Abstract

Citrullination process is a natural pathway which happened at the time of cell dying, but during removal of these products, PAD enzymes may release causing stimuli to the immune system. This can start a consequence of processes and enhancement of cancer disease. In this review, we will focus on this marker and significance of its application for early detection of cancer and for follow-up disease, and the latest approved medications act on this line.

Keywords: Citrullination, histone, PAD-4

INTRODUCTION

DNA inside nuclei is aggregated as chromatin, a standard architecture that is formed of nucleosomes as vital building blocks. Histones are an essential part of the nucleus, acting as a particle containing eight arms or extending, which poses a core with four histone proteins (H2A, H2B, H4, and H3) encased in a 147 bp piece of DNA. Every unique huge histone protein had a temperament side chain that is sluggishly populated with basic lysine and arginine residues. These side chains are attached to strong covalent post-translational modifications that uphold to control the chromatin state.^[1]

Definition of citrullination

Citrulline is a type of amino acid (non-coding one) made by a process of post-translational deimination of peptidyl-arginine. This process is accelerated by peptidyl-arginine deiminase (PAD) enzymes (specifically PAD-4); the step of deimination is considered important for regulating the definition of gene transcription. This allows positively charged transferring of arginine into the neutral citrulline, leading to the formation of hydrogen bond and protein folding, which led to alter lipophilicity and protein reactions.^[2]

Additionally, citrullination stimulated the formation of neutrophil extracellular traps (NETs), a tool to attack and remove bacterial microorganisms in neutrophils.

PAD-4-actions for citrullination of histones provide as a beginning or core point for chromatin de-folding and subsequent NET release, helping encounter microbes attacks.^[3]

Citrullination induced by PAD enzymes had been shown in several normal and abnormal (pathological) processes. Till now, five isoforms of the PAD system are present (PAD-1, PAD-2, PAD-3, PAD-4, and PAD-6).

PAD-4 is the most prominent among PADs. Its location in the nuclear localization signal, whereas others are mainly located in the cytosol, plays an important role in tumor development by facilitating the definition of p53 target genes and is involved at a high rate in the pathogenesis and progression number of inflammatory disorders such as rheumatoid arthritis, Alzheimer's disease, and multiple sclerosis.^[4,5]

In physiological processes, citrullination is defined as a usual cell process that happened at end cell life (apoptosis). So, the immunological cell system is not of interest with these citrullinated proteins. When the process of apoptosis

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occurred, the differential in the somatic characteristic of the dead cell happened after the clearance and engulfment of these cells by phagocytic cells.^[6]

Any defect in the removal of these cells either in ability or latitude may happen due to great cell death and result in the aggregation and drainage of PAD enzyme from the cells necrotized, which may be experienced by the immunological cell system.^[2]

Pathologically, citrullination is present in several diseases and conditions which promote autoimmune activation against these proteins. Anticitrullinated protein antibodies attack the citrullinated proteins/peptides at certain organs or tissues. Citrullination products contain different proteins such as keratin-1, vimentin, myelin basic protein, glial fibrillary acidic protein, fibrin, α -enolase, and collagen II. It can initiate and affect epitopes that induce autoantibody production.^[7]

Citrullination created by a PAD enzyme has an imperative role in many diseases. These diseases include psoriasis, rheumatological conditions such as rheumatoid arthritis (RA), neurodegenerative diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS), systemic lupus erythematosus (SLE), and cancers.^[8]

Citrullination in cancer

Malignant tumors (cancers) had a considerable public health issue worldwide and are the second major cause of death in the USA. This condition is believed to have a hereditary role for initiating its progression. Molecular studies had explained that several mutational events, which enhance cell proliferation and fasten genetic instability, are needed for the development of malignancy.^[9]

Abnormal and irregular cell growth, function, development, and shape are caused by a variety of gene mutations that promote abnormality in the actions for these enzymes encoding them.^[10]

One of the affected enzyme functions is PAD-4 isoenzyme, and the citrullinated DNA will result. Plasma of cancer patients showed an increase in the citrullin level, which is a feature of general disease burden. Also metastatic tumors had more serum citrullinated histone H3 than non-invasive cancers. Citrullination accelerates the prognosis of cancer through a variety of methods,^[11] as shown in Figure 1.^[2]

Types of cancers that involve citrullination in their pathogenesis

As mentioned earlier, citrullination is involved in the initiation of cancer disease; we will try to focus on cancer types as citrullination is one of the causative agents and risk factors to develop cancer.^[4]

Gastrointestinal cancer

Protein citrullination is linked with pancreas carcinogenesis. According to Boone *et al.*, who established the autophagy process, the degradation and recycling of damaged organelles and proteins are crucial for NET formation. In order for the cell to survive in the hypoxic, nutrient-poor tumor microenvironment, autophagy is necessary for governing the prognosis of cancer cells in pancreatic cancers, in addition to the presence of citrullinated histone 3 and NET that are highly mentioned in pancreatic cancer murine cells.^[12]

In a study conducted on gastric cancer cell lines, overexpression of PAD-4 and H3 was discovered, and

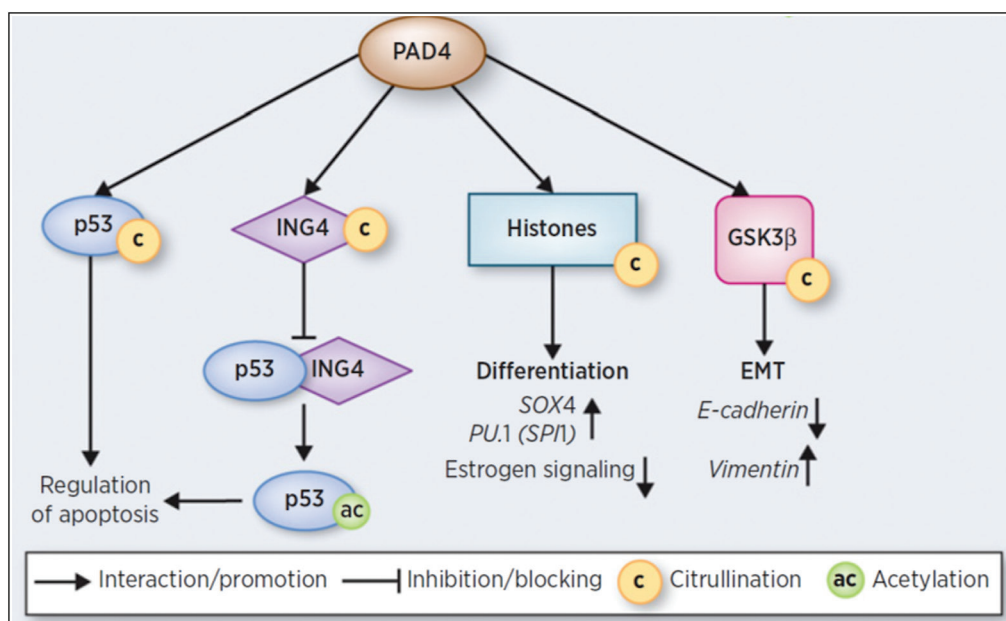


Figure 1: Schematic illustrating how PAD-4 citrullination affects cell signaling to facilitate cancer progression^[2]. ING4 = inhibitor of growth family member 4, P53 = tumor suppressor protein, GSK3 β = glycogen synthase kinase-3 beta, SOX4 = SRY-Box factor 4

this was correlated with a poor prognosis for cancer in patients. This new information advocates the involvement of citrullination process in the development of gastric cancer, and this aids the scientists to develop a reagent with highly sensitive and specific diagnostic features for early detection and diagnosis of tumors.^[13]

Breast cancer A strong correlation was found between breast cancer and presence of citrullin and PAD isozymes (I and IV). Furthermore, this link is corresponding to the bad progression of breast cancer. Tumor size, histone 3, and PAD level are shown to be reduced when the cell lines are treated with PAD inhibitor, Cl-amidin.^[3,14] Snoderly *et al.* had elucidated the same results about breast cancer and citrullination, in which there is a strong link between PAD-4 and breast cancer through stimulation of NETosis which had a core role in cancer progression.^[15]

Prostate cancer Prostate cancer studies have demonstrated the function of PAD-2, an androgen suppressor gene, in prostate cancer by describing the androgen signaling pathway and citrullination. Prostatic cancer cells must express PAD-2 in order to develop and multiply. Through CitH3, androgen receptor (AR) interaction to target genes is facilitated by cytoplasmic PAD-2, which shields AR from proteasome-mediated degradation. Based on its nuclear localization and enzyme activity, which are linked to elevated CitH3 expression, PAD2 regulates AR. Cl-amidine treatment for prostate cancer slows disease development both *in vitro* and *in vivo* and reduces tumor growth and cellular proliferation in that disease^[16] when given together with PAD inhibitors.

Lung cancer Cancer of the lung is one of the most disastrous neoplasms around the world. Infiltration of neutrophils is usually seen in lung cancerous tissues. NETs, antimicrobial enzymes secreted from neutrophils, and hypercitrullinated histones can enhance the attachment of cancerous cells and segregate lung cancer cells to the circulation. Carcinoma cells readily released protein with high mobility group box 1 (HMGB1). Additionally, HMGB1 in lung cancer cells stimulated and activated the production of NETs.^[9]

Presence of HMGB1 is linked with disease severity, mutagenesis, and bad prognosis as seen in murine cell line study.^[17] NETs have also been shown to have prothrombotic properties, by the activation of more pathways of thrombosis. NETs can attach to red blood cells and platelets, thus scaffolding for thrombosis. NETs have also been detected in human deep venous thrombosis samples as a component of the thrombus matrix, supporting their role in thrombus formation, but more data and studies are needed to confirm it.^[18]

Ovarian cancer Ovarian tumors (malignant one) carry the highest mortality rate among all gynecological cancers.

Delay in tumor diagnosis and patients to available chemical therapy elevate mortality rates. PAD-4 has been found in many cancer types, including ovarian cancer, and its presence in patients is associated with bad pathogenesis of this type of cancer. The critical role in the mechanism of action of PAD-4 is related to ovarian mutagenesis, and this may belong to the p53 gene.^[18] Also, PAD-4 is associated with metastasis of ovarian cancer cell, as found in Cui *et al.*^[19]

Leukemia One type of cancer that involves citrullinated histone, the PAD system, and the pathogenesis of the disease is leukemia, which is also linked to poor prognosis for the condition.

A recent unique study demonstrated that PAD4 concentration is higher along the differentiation of HL-60 leukemia cells enhanced by the all-trans retinoic acid drug administration.^[3]

This change is markedly dependent on the demethylation of the PAD4 promoter. By the citrullination of histones, PAD4 stimulated the presentation of hematopoietic transcription factors SOX4 and PU.1, which in turn stimulate differentiation of HL-60 into granulocytic cells. This explained the significance of arginine deimination in cell differentiation; newly interested research of embryos improved this fact wherein PAD4 and citrullination were established as major promoters of pluripotency. Expression of PAD-4 gradually elevated during the differentiation and proliferation of leukemia cells.^[20]

SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN CITRULLINATED HISTONES

A single nucleotide base change in a DNA sequence is known as an SNP that involves polymorphism. Typically, two distinct nucleotides are changed at a particular locus. This modification took into account the less common allele, which would have a percentage of 1% or higher.^[1]

Human DNA changes can have an impact on how diseases arise and react to substances, infections, medications, vaccinations, and other agents. SNPs are also essential for personalized medicine. Examples include biomedical research, disease causation, pharmacogenetics, and forensics.^[21]

It has been found that in a study of esophageal cancer patients [e.g., esophagus adenocarcinoma and esophageal squamous cell carcinoma (ESCC)], two SNPs that belong to PAD-4 were at high risk to develop esophageal cancer; these are *PADI4* rs10437048 and rs41265997. To ensure the correlation between genetic variant in PAD-4 and squamous cell carcinoma risk,^[22] scientists found the relation of desired SNP strategy in a larger study, in which the researchers studied seven polymorphisms associated with *PADI4* and concluded that the *PADI4* rs2240337

G > A SNP was in high percent associated with lowering risk of ESCC after the Bonferroni correction.^[23]

ANTI-PAD OR PAD INHIBITOR AND THEIR ROLE IN CANCER TREATMENT

In the last years, a novel therapy for inhibiting PAD-4 and for reducing its level leading to a decrease in tumor size is understudy and approval. Highly mutagenic prostatic cell lines were prepared to be treated with PAD inhibitor (PAD-mediated citrullination), chloramidine. It shows a decrease in tumor size and changes in nuclear PAD translocation in concert with PAD inhibition and changes in microvesicle release, which involves cancer progression.^[24]

In addition to the effect of CL-amidine on tumor size, disease progression, there is another study done on cell line to show the effect of F-amidine, another PAD inhibitor, on cancer cells. The results showed that F-amidine may also potentiate TRAIL [tumor necrosis factor (TNF)-related apoptosis-inducing ligand] responses independent of PAD4 inhibition.^[25]

CONCLUSION

PAD4 and citrullinated histone 3 are regarded as risk factors in developing different cancer types and usually associated with bad disease prognosis. Additionally, as the tumor shrank or the patient recovered, the amount of PAD-4 fell. Polymorphism in PAD-4 needs more studies and researches to find its effect on pathogenesis of PAD-4.

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There are no conflicts of interest.

Authors' contribution

NMA: writing and finding the references.

SHA: checking and reviewing the text.

Each author had read and approved the manuscript and completes the requirements for authorship as stated earlier in this document and that each author believes that the manuscript represents honest work.

Ethical consideration

Not applicable.

REFERENCES

1. Audia JE, Campbell RM. Histone modifications and cancer. *Cold Spring Harb Perspect Biol* 2016;8:a019521.
2. Yuzhalin AE. Citrullination in cancer. *Cancer Res* 2019;79:1274-84.
3. Valesini G, Gerardi MC, Iannuccelli C, Pacucci VA, Pendolino M, Shoenfeld Y. Citrullination and autoimmunity. *Autoimmun Rev* 2015;14:490-7.
4. Aliko A, Kaminska M, Falkowski K, Bielecka E, Benedyk-Machaczka M, Malicki S, *et al.* Discovery of novel potential

- reversible peptidyl arginine deiminase inhibitor. *Int J Mol Sci* 2019;20:2174.
5. Witalison EE, Cui X, Causey CP, Thompson PR, Hofseth LJ. Molecular targeting of protein arginine deiminases to suppress colitis and prevent colon cancer. *Oncotarget* 2015;6:36053-62.
6. Alghamdi M, Alasmari D, Assiri A, Mattar E, Aljaddawi AA, Alattas SG, *et al.* An overview of the intrinsic role of citrullination in autoimmune disorders. *J Immunol Res* 2019;2019:7592851.
7. Gazitt T, Lood C, Elkon KB. Citrullination in rheumatoid arthritis—A process promoted by neutrophil lysis? *Rambam Maimonides Med J* 2016;7:e0027.
8. Kaore SN, Amame HS, Kaore NM. Citrulline: Pharmacological perspectives and its role as an emerging biomarker in future. *Fundam Clin Pharmacol* 2013;27:35-50.
9. Ciesielski O, Biesiekierska M, Panthu B, Soszyński M, Pirola L, Aneta Balcerzyk A, *et al.* Citrullination in the pathology of inflammatory and autoimmune disorders: Recent advances and future perspectives. *Cell Mol Life Sci* 2022;79(2):94-.
10. Birner P, Prager G, Streubel B. Molecular pathology of cancer: How to communicate with disease. *ESMO Open* 2016;1:e000085.
11. Kawalkowska J, Quirke AM, Ghari F, Davis S, Subramanian V, Thompson PR, *et al.* Abrogation of collagen-induced arthritis by a peptidyl arginine deiminase inhibitor is associated with modulation of T cell-mediated immune responses. 2016;26430.
12. Boone BA, Orlichenko L, Schapiro NE, Loughran P, Gianfrate GC, Ellis JT, *et al.* The receptor for advanced glycation end products (RAGE) enhances autophagy and neutrophil extracellular traps in pancreatic cancer. *Cancer Gene Ther* 2015;22:326-34.
13. Song S, Xiang Z, Li J, Ji J, Yan R, Zhu Z, *et al.* A novel citrullinated modification of histone 3 and its regulatory mechanisms related to IPO-38 antibody-labeled protein. *Front Oncol* 2019;9:304.
14. Qin H, Liu X, Li F, Miao L, Li T, Xu B, *et al.* PAD1 promotes epithelial-mesenchymal transition and metastasis in triple-negative breast cancer cells by regulating MEK1-ERK1/2-MMP2 signaling. *Cancer Lett* 2017;409:30-41.
15. Snoderly HT, Boone BA, Bennewitz MF. Neutrophil extracellular traps in breast cancer and beyond: Current perspectives on net stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment. *Breast Cancer Res* 2019;21:145.
16. Zhu D, Zhang Y, Wang Sh. Histone citrullination: A new target for tumors. *Mol Cancer* 2021;90.
17. Zhou J, Yang Y, Gan T, Li Y, Hu F, Hao N, *et al.* Lung cancer cells release high mobility group box 1 and promote the formation of neutrophil extracellular traps. *Oncol Lett* 2019;18:181-8.
18. Oklu R, Sheth RA, Wong KHK, Jahromi AH, Albadawi H. Neutrophil extracellular traps are increased in cancer patients but does not associate with venous thrombosis. *Cardiovasc Diagn Ther* 2017;7:140-9.
19. Cui Y, Yan L, Zhou J, Zhao S, Zheng Y-B, Sun B-H, *et al.* The role of peptidylarginine deiminase 4 in ovarian cancer cell tumorigenesis and invasion. *Tumor Biol* 2015;37(4):5375-83.
20. Song G, Shi L, Guo Y, Yu L, Wang L, Zhang X, *et al.* A novel PAD4/SOX4/PU.1 signaling pathway is involved in the committed differentiation of acute promyelocytic leukemia cells into granulocytic cells. *Oncotarget* 2015;7(3):3144-57.
21. The 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* 2015;526:68-74.
22. Chang X, Hou X, Pan J, Fang K, Wang L, Han J. Investigating the pathogenic role of PAD14 in oesophageal cancer. *Int J Biol Sci* 2011;7:769-81.
23. Wang L, Gu H, Long T, Pan H, Lv L, Shi Y, *et al.* PAD14 rs2240337 G>A polymorphism is associated with susceptibility of esophageal squamous cell carcinoma in a Chinese population. *Oncotarget* 2017;8:93655-71.
24. Kholia S, Jorfi S, Thompson PR, Causey CP, Nicholas AP, Inal JM, *et al.* A novel role for peptidylarginine deiminases in microvesicle release reveals therapeutic potential of PAD inhibition in sensitizing prostate cancer cells to chemotherapy. *J Ext Vesicles* 2015;4:26192.
25. Khalid H. PAD4 inhibitors: Potential sensitizers of tumour cells to TRAIL-induced apoptosis. *Biosci Horizons* 2010;9:1-10.