Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i1.2603

METASTASIS REGULATION IN PROSTATE CANCER: THE POSSIBLE ROLE OF IP3- AND cADPR-MEDIATED Ca^{2+} SIGNALING

Tholfiqar Najah Ismeal[1], Leila Sadeghi[1*], Gholamreza Dehghan[1]

[1,2*,3]Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
[1]Email: tholfiqar13288@gmail.com

***Correspondence:** Leila Sadeghi
*Department of Animal Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran, P.O. Box 5166616471, Tabriz, Iran. Tel: (+9841-33392743), Fax: (+9841-33356027), E-mail: l.sadeghi@tabrizu.ac.ir, E-mail: l.sadeghi66@yahoo.com

Abstract

Background: Prostate cancer (PCa) is the second most prevalent cancer among males that are prone to the metastasis. The oxidative imbalance and Ca^{2+} influx are metastasis triggers and also crucial to understanding the pathogenesis of PCa. This study aimed to investigate the reciprocal relationship between Ca^{2+} up-regulation and oxidative stress by evaluation of the inositol-three-phosphate (IP3)- and cADPR-mediated Ca^{2+} influx in PCa tissue and marginal control. The possible correlation between Ca^{2+} up-regulation and matrix metalloproteinase 9 (MMP-9)/CD82 expressions which involve in tissue remodeling, cell proliferation and invasion was also evaluated.

Methods: For this purpose, 50 intermediate- and high-grade of PCa tissue and 50 margins of tumor were used. Western blot was used to evaluate the target proteins. Antioxidant enzymes activity and reactive oxygene species (ROS) content were evaluated in tissue lysate by using standard methods. The case and control groups' data were compared using a two-tailed student t-test using SPSS software.

Results: Our results showed overproduction of the ROS in PCa that accompanied by altered antioxidant enzymes activity that refered to oxidative stress. Immunoblotting analysis approved IP3 receptor (IP3R) and CD38 significantly increased in PCa rather than marginal control that could be interpreted to Ca^{2+} up-regulation. PCa tissue also manifested MMP-9 up-regulation and CD82 down-regulation rather than marginal control that could cause invasion and metastasis.

Conclusion: Our results revealed IP3- and cADPR-mediated Ca^{2+} influx is upstream regulator of oxidative stress and invasion. By considering the leading role of cytosolic free Ca^{2+}, IP3R and CD38 could be considered as effective targets to anti-metastatic drug designe.

Keywords: Prostate cancer; Oxidative imbalance; Antioxidant barrier; Ca^{2+} influx pathways; Metastasis

1. Introduction

Prostate cancer (PCa) is one of the most prevalent diseases in men, which often affects older men over the age of 65 (1). There are various types of PCa, which are separated into two categories based on the disease's type and severity: benign PCa and malignant PCa (2). The malignant types are in medium- and high-grade of Gleason score (3). PCa develops and progresses as a result of numerous environmental and genetic causes (4). Prostate intraepithelial neoplasia (PIN), which progresses slowly and manifests as symptoms only when cancer has spread, is a condition where the structure and size of prostate cells have changed slightly (5). PCa epidemiological and clinical studies suggest that oxidative stress and invasion are the assumed events in the initiation, development, damage, and recurrence of this malignancy (6). The importance of reactive oxygen species (ROS) as a messenger in tumor cell invasion, angiogenesis, and metastasis is supported by recent research (7). ROS can stimulate the early stages and progression of carcinogen-induced cancer via interacting with DNA and genotoxic substances (7). According to the previous experiments, the early cause of ROS overproduction is mitochondrial damages due to free Ca^{2+} up-regulation (8). Redox potensial of cells regulates Ca^{2+} influx also therefore, antioxidant barrier could regulate cytosolic free Ca^{2+}. Intracellular free Ca^{2+} signaling regulates several physiological processes in all cells (8). Allosteric control of voltage dependent and independent Ca^{2+}-chaneels by secondary messeges such as cyclic ADP-ribose (cADPR) and inositol-3-phosphate (IP_3) are main regulators of cytosolic free Ca^{2+} (9, 10). cADPR produced by catalytic activity of cluster of differentiation 38 (CD38) as membrane protein and then bind to the L-type Ca^{2+} channel and activate Ca^{2+} entry (9). IP3 also formed by phospholipase C (PLC) as a result of lysophosphatidic acid (LPA) binding to its membrane receptor (10). To our knowledge, the involvement of described pathways in PCa's Ca^{2+} signaling has not beed studied yet. While it could affect the following process such as proliferation, invasion and metastasis (11).

PCa is more vulnerable to metastasis to different organs with a propensity to skeleton (12). bone metastases are detected in more than 10 % of patients after initial diagnosis of PCa (12). The molecular cascade of metastasis could be categorize in three vital events: invasion, intravasation and extravasation but the exact mechanism has not been understood yet (13). Previous reports confirmed that Ca^{2+} and ROS up-regulations are main triggers of invasion and metastasis in different types of cancer (11, 14). While involvement of some proteins in this casdade is clear such as desmosomes, integrins, matrix metalloproteinases (MMPs) and cluster of differentiation 82 (CD82) (15, 16). Matrix metalloproteinase-9 (MMP-9) is now known to play a role in human malignancies including aggressive and metastatic PCa (16, 17). It has also important role in inflammation process that is one of the main active evens in PCa (17). Previously examined that MMP-9 inhibition could down-regulate metastasis and angiogenesis effectively (18). Another unique protein, CD82, which lowers cancer cell adhesion to their matrix and promotes PCa dissemination, has shown promise in PCa diagnostics (15). CD82 also regulates cellular proliferation through inhibition of the epithelial growth factor (EGF) signaling pathway (19). There is currently no approach that can describe Ca^{2+}- and ROS-mediated metastasis and their relashionship clearly. Therefore, this study aimed to analyse oxidative balance in PCa tumor tissue (medium- and high-grade according to Gleason score) and also in marginal tissue as control by evaluation of the ROS content, reduced and oxidized glutathione and antioxidant enzymes' activities like superoxide dismutase (SOD) and catalase (CAT). Ca^{2+} regulation from the interacellular and extracellular sources was also evaluated by direct mesurment of CD38 and IP3R proteins. Finally accompaniment of oxidative imbalance and Ca^{2+} dysregulation with metastasis ability of tumor was assessed by MMP-9 and CD82 expression, the proteins involved in tissue remodeling and cell death, in order to develop a novel approach to treating PCa.

2. Materials and Methods

2.1 Sample preparation

This study was conducted in Imam Reza Hospital, Mashhad, Iran. All of the process in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later modifications. The Mashhad Branch of Azad

University's Ethics Committee gave its approval to the current study protocol (Ethics number???). The patients who selected in this research were refered to the urology section of hospital between march 1 and September 1, 2021. These patients suffer from lower urinary tract sypmtoms (LUTS) and after physical and labratorry examination by specialists, the patients who were over 45 years of age with high level of PSA that underwent surgery were enrolled in this experiment. After being informed of the study's goals, procedures and purpose, each participant willingly agreed to participate. Written informed consent was given to every patient. After surgery and pathological analysis, 50 prostate tumor tissues and 50 tumor marginal tissues were selected to made up the study groups. The computed tomography scans are used to estimate the average tumor volume, and the tissue that was taken is not more than 5 cm from the tumor's margin. 0.12 cm^3 was produced throughout the operation. To assess the biochemical parameters, each sample was put into a separate tube and kept at -20 $^{\circ}$C until use.

2.2. ROS content analysis

ROS content of tumor and margin tissues was measured according our previous work (20). This method is based on oxidative conversion of Dichloro-dihydro-fluorescein diacetate (DCFH-DA) to dichlorofluorescin (DCFH) as a fluorescent compound which measured by 485 nm excitation and 530 nm emission.

2.3. Evaluation of reduced to oxidased glutathione ratio (GSH/GSSG) in tissue samples

The total amount of gluthation and also its reduced form were measured by using the GSH/GSSG Ratio Detection Assay Kit II (Fluorometric - Green) (ab205811) according to the related protocol. Befor detection procedure tissue homogenite should be deprotenized with trichloroacetic acid (TCA) and then neutralized using sodium hydrogen carbonate (NaHCO3) according to manufacturer's guideline. Data were normalized against the stardard solutions and GSH/GSSG ratio was calculated.

2.4. Superoxide dismutase (SOD) enzyme's activity mesurment

The Marklund technique was used to measure the SOD enzyme's activity by looking for hypoxanthine and superoxide radicals produced by xanthine oxidase using the riboflavin/nitrotrazolium blue (NBT/RF) assay (20). SOD was contrasted with NBT for oxygen created by RF under illumination using 1.5 ml of the reaction mixture (50 mM KH_2PO_4, pH 7.8, 0.1 mM EDTA, 2 mM riboflavin, and 57 M NBT). The reaction was done under four fluorescent tubes (40 W) to generate 199 mol/m^2 photons. After 5 min absorbance was measured at 405 nm. With no activity and 50 % inhibition, regression analysis was done using the linear component of the log-normal curve. The amount of enzyme that inhibits 50 % of the photochemical NBT reductase was determined to be one unit of SOD activity, data showed as units per mg of protein in each sample.

2.5. Catalase (CAT) activity mesurment

Supernatants resulted from tissue homogenizing were used to evaluate CAT activity (EC.1.11.1.9), and normal tissues were homogenized using a modified version of the Aebi kinetic technique (20, 21). In this method hydrolysis of H_2O_2 and the lowering of absorbance at 240 nm was followed in 1 min. The rate of the enzymatic process was determined to be the conversion of H_2O_2 to water and 1/2 oxygen per min at 25°C.

2.6. Western Blot

200 mg of each sample was homogenized in 2 ml of lysis buffer (50 mM Tris-HCL at pH 7.4, 2 mM EDTA, 2 mM EGTA, 10 mM NaF, 1 mM sodium orthovanadate (Na3VO4), 10 mM glycerol-phosphate and 0.2 % W/V sodium deoxycholate) (22). Supernatants' total protein concentration was measured using the Bradford protein assay method. Before being stored at -80°C, protein lysate aliquots were denatured for 5 min at 95°C in SDS-Laemmli sample buffer with 2 mM DTT. In conclusion, 50 g of proteins were transferred to PVDF membranes after separation by 12 % SDS-polyacrylamide gel (SDS-PAGE). To prevent nonspecific protein binding, PVDF membranes were

blocked for 2 hours at room temperature with 5 % skim milk or 1 % BSA in Tris-buffered saline with 0.1 % Tween 20 (TBST).

Diluted (1:1500) primary antibodies were used to treat the membranes at 37 °C for two hours. Anti-MMP9 (ab73734), anti-IP3R (ab108517) and anti-CD82 (ab66400) were prepared from Abcam Company, USA and anti-CD38 (sc-374650) was purchashed from Santa Cruz Biotechnology, USA. The membranes were then exposed to the suitable secondary antibodies coupled with horseradish peroxidase enzyme (HRP) (1:3000) in TBST for 180 min at room temperature after three washes (3–5 min) in the buffer. PVDF membranes were then exposed to Pierce ECL detection kit and protein bands visualized by the Alliance 4.7 Gel Doc (UK) and quantified by using imag J softeware. β-actin was used as the reference protein to normalize bands intensity (22).

2.4 Statistical Analysis

All of the experiments repeated at least three times independently and the data were expressed as the mean ± standard deviation (SD) using the GraphPad InStat version 10.0.2 program (GraphPad Software, San Diego, CA). By computing values with the Student's *t*-test at a 5 % significance level ($p<0.05$), statistical significance was evaluated.

3. Results

3.1 Increased ROS content of tumor tissue accompanied by dysregulation of GSH/GSSG ratio

ROS are chemically reactive molecules produced by mitochondria during normal metabolism, while its content increased during abnormal condition such as cancer and tissue damages (23). Consequently, ROS is informative parameter that provide valuable information about oxidative status of the prostate tumor in comparison with marginal tissue as control. According to the results, DCF fluorescence intensity in tumor tissue lysate is significantly more than marginal tissue which interpreted to increased ROS content in PCa cells ($P<0.0001$) (Fig 1).

Fig 1 also revealed that amount of reduced GSH in comparison with oxidased form reduced significantly. GSH level represented antioxidant barrier in cells while GSH/GSSG ration is more informative in this case (24). Our results confirmed GSH level decreased in tumor tissue while GSSG could not manifested significant changes (data not shown). While, analysis of variance confirmed GSH/GSSH ratio remarkably reduced (more than 6-fold) in tumor tissue in comparion with control marginal tissue ($P<0.0001$). the mean of GSH/GSSG ratio for border control calculated as 85.01 and for tumor tissue estimated as 12.64.

3.2 Antioxidant barrier enzymes affected by PCa

SOD and CAT enzymes are the most popular antioxidant barriers in biological systems which could neutralise the extra produced ROS (20). Therefore, the catalytic activity of these enzymes examined in tumor tissue lysate and control border tissue by using standard methods. Fig 2 revealed, SOD enzyme's activity in tumor tissue lysate estimated as 35.21 U/mg protein that increased rather than marginal control tissue that showed 16.83 U/mg protein catalytic activity ($P<0.0001$). Hyperactivation of the SOD in tumor tissue was estimated more than 2-fold. While our results approved H_2O_2 degradation ability in tumor tissue extract is remarkably more than marginal control tissue lysate ($P<0.0001$) which refers to more activity of CAT enzyme.

3.3 Calcium regulatory proteins overexpressed in PCa

Ca^{2+} is an important ion which regulates molecular events in PCa and could impact prostate tumor cell death, proliferation and metastasis (7, 8). Intracellular Ca^{2+} acculumation could be caused by different pathways (22) that IP3 and CD38 pathways were studied here. Western blotting was performed to evaluate the expression of each IP3R and CD38 proteins in the tumor tissues and marginal control tissues. We found that IP3R expression were significantly higher in tumor tissue than in marginal tissue. IP3R is a 320 kDa protein that its expression increased near to the 4-fold in tumor

tissue lysate rather than control marginal tissue ($P<0.0001$) (Fig 3). Fig 3 also showed the CD38 related bands in both type of samples, quantified intensity of bands confirmed CD38 level in tumor tissue is 3-fold more than control ($P<0.0001$).

3.4 PCa causes dysregulation of metastasis assosiated proteins

Recent research has shown MMP-9 dysfunction encourages invasion in a variety of malignancies (17), we looked into MMP-9 expession in PCa. The MMP-9 content in tumor and border tissue lysates were analyzed by using specific antibody in western blotting method. As a loading control β-actin was employed (Fig 4). Results revealed significant increase in MMP-9 content of prostate tissue after cancinogenesis. Comparison of band intensity in both experimental groups showed MMP-9 expression increased more than 3-fold in tumor tissue rather than marginal control ($P<0.0001$).

Previous experiments approved important role of CD82 in homotypic cell adhesion and metastasis obstruction because, it could inhibit epithelial growth factor (EGF) signaling also that leads cells to the apoptosis (19, 25). Therefore decreased expression of CD82 and its regulators causes cancerus cells immortality (19). Here we measured CD82 protein concentration in tumor tissue and border tissue as control. Fig 4 showed significant ($P<0.001$) reduction of CD82 expression in prostate tissue accompanied by cancerous feature. Mariginal control samples have been showed to contain about 2-fold more expression of CD82 rather than tumor cells. There are no significant changed between intermediat- and high-grade of tumors.

4. Discussion

Ca^{2+} ion plays important role in biological system due to its impact on gene expression, cell cycle and cellular development, deferentiation, death and proliferation (7, 8). Therefore, regulation of intracellular free Ca^{2+} is verry important in cellular homeostasis. According to the previous reports transient accumulation of Ca^{2+} in PCa cells induces invasion and potentially favoring bone metastases (8, 11). On the other hand, Ca^{2+} up-regulation causes mitochondrial dysfunction and ROS overproduction that causes inflammation and invasion (22). However, our knowledge about the molecular basis of Ca^{2+} dependent events, blood homeostasis, calcium binding proteins and their role in gene expression regulation is incomplete , so we could not suggest a rational and mechanism based procedure for targeting Ca^{2+} in PCa. According to the our recent report, Ca^{2+} influx could be done from intracellualar (ER) and extracellular sources by using different messengers and channels. Here we evaluated two different Ca^{2+} influx (cADPR- and IP3-dependent) pathways (22). We also examined the possible role of Ca^{2+} up-regulation in ROS overproduction, antioxidant barrier and metastasis ability by measuring the relared enzymes and proteins. Our results revealed increased expression of CD38 and IP3R in tumore cells in comparison with marginal tissue as control. CD38 in a membrane bound enzyme could converts NAD to the cADPR which binds to the calcium channel as a secondary messamger and induces Ca^{2+} influx (9) (Fig 5). IP3 is also a secondary messanger could impose cytosolic free Ca^{2+} from the internal source (10). Our results showed 3-fold more expression of IP3R in ER membrane of the tumor cells which could accept IP3 and cause ER-dependent Ca^{2+} release to the cytosol. Previous experiments also reported up-regulation of the lysophosphatidic acid (LPA) production and its receptor (LPAR) in PCa cells (26). Therefore, LPA and cADPR mediated Ca^{2+} influx (schematicly illustrated in Fig 5) are two main pathways which positively regulate free cytosolic Ca^{2+} in PCa. Icreased cytosolic free Ca^{2+} could translocate to the mitochondria through mitochondrial Ca^{2+} (mCa^{2+}) channel that could cause the mitochondrial DNA abnormalities and electron transport chain failure so, lead to more endogenous ROS production in PCa (27). Our additional analysis also approved more than 95 % of tumor tissues that showed up-regulation of IP3R and CD38 also manifested ROS overproduction simultaneously that refer to the accompaniment of cytosolic free Ca^{2+} and ROS. Increased ROS and also Ca^{2+} up-regulation cause some changes in gene expression of cancer cell lines and tumor tissues (28, 29). However the molecular events that take place in the presence of extra Ca^{2+} and ROS which cause gene expression regulation have not been understood yet. While accompaniment of increased ROS and Ca^{2+} with

metastasis, imortality and high rate of proliferation has been observed in PCa which done by up-regulation of cell cycle proteins and down-regulation of apoptisis inducers (8, 11). This fact approved that free Ca^{2+} and those bond to the specific proteins and also ROS molecules have potensial to gene expression regulation.

Prostate tumors have more potential to invasion and the most favorable goal is skeleton (12). Development of secondary malignant growths at the skeleton far from a primary site of cancer named metastasis that need some additional molecular process and gene expression regulation (13). CD82 also termed Kangai 1 (KAI1) has important negative role in invasion and metastasis of PCa through angiogenesis inhibition (15, 19). Previous experiments reported reduced expression of CD82 in aggressive tumor development (19). Mesurment of CD82 with specific antibody revealed its decreased expression in tumor tissue which could lead to EGF-mediated cell invasion, migration, and endocytosis (15). Comparison of Ca^{2+} regulators level with CD82 expression in each tumor samples and same border control revealed up-regulation of IP3R and CD38 shows more than 83 % correlation with CD82 down-expression. We also observed a significant change in the MMP-9 expression that could be caused by Ca^{2+} or ROS up-regulation. As our previous knowledge, extra Ca^{2+} in cells causes inflammatory condition through cyclooxygenase pathway or matrix metalloproteinases (MMPs) (30). Additionally, clinical studies have validated the link between MMP-9 expression and disease development in patients with various tumor types (31, 32). Our analysis also confirmed about 85 % of tumor samples which showed upexpression of IP3R and/or CD38 also manifested significant increase in MMP-9 level. Therefore, Extreme content of Ca^{2+} and ROS are molecular signals which rise MMP-9 expression that has been observed to be main event in metastasis. According to the previous experiments, ROS molecules also reduce cell adhesion and increase vascular leakage that help to metastasis promotion (33) . This reactive molecules are neutralized by different type of reactions mainly done by SOD and CAT that previous studies described their important role in cancer initiation and development (34, 35). CAT is a multifuctional enzyme that weakens MMP-9 catalytic function, in addition to cellular redox homeostasis, which may affect its anti-metastatic efficacy (36). Our results revealed significant reduction of CAT's activity in tumore tissue in comparison with border control tissue. Additionally, breast, lung, cervix, ovary, stomach, colon, leukemia, bladder, and liver cancers have reduced CAT's activity and expression (37). The raised SOD activity was observed in tumor tissue could be an adaptation process in ROS overproduction condition. However SOD and CAT play various functions in cancer developing which need to further investigation.

5. Conclusions

By considering the vital role of Ca^{2+} signaling in ROS overproduction and PCa metastasis, this study was designed to invertigate the active molecular mechanism of Ca^{2+} influx and its possible effects on invasion-related proteins expression. In-depth review of the results and comparison with related literature revealed a positive feekback loop between Ca^{2+} influx, oxidative stress and metastasis propensity in intermediate- and high-grade of PCa. This loop approved upstream role of Ca^{2+} signaling which could affect the other process so could be considered as a risk factor in initiation and developing of PCa. Our results also demonstrated a significant accompaniment between Ca^{2+}-induced ROS and up-regulation of MMP-9 that could be caused inflammation process in PCa. Ca^{2+}-induced oxidative imbalance also was negatively correlated with CD82 level that could limit aggressive capability of prostate tumor. Therefore, given the studied Ca^{2+} influx pathways and their role in inflammation and metastasis, LPAR, IP3R and CD38 could be considered as therapeutic targets to drug design.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Reference

1. Zhang Z, Zhanghuang C, Wang J, Mi T, Liu J, Tian X, et al. A Web-Based Prediction Model for Cancer-Specific Survival of Elderly Patients Undergoing Surgery With Prostate Cancer: A Population-Based Study. Front Public Health. 2022;10:935521.
2. Xing P, Chen L, Yang Q, Song T, Ma C, Grimm R, et al. Differentiating prostate cancer from benign prostatic hyperplasia using whole-lesion histogram and texture analysis of diffusion- and T2-weighted imaging. Cancer Imaging. 2021;21(1):54.
3. Jones J, Feger J, Bell D, et al. Gleason score. Reference article, Radiopaedia.org (Accessed on 11 Mar 2023) https://doi.org/10.53347/rID-9054.
4. Rawla P. Epidemiology of Prostate Cancer. World J Oncol. 2019;10(2):63-89.
5. Brawer MK. Prostatic intraepithelial neoplasia: an overview. Rev Urol. 2005;7 Suppl 3(Suppl 3):S11-8.
6. Minciullo PL, Inferrera A, Navarra M, Calapai G, Magno C, Gangemi S. Oxidative Stress in Benign Prostatic Hyperplasia: A Systematic Review. Urol Int 2015;94:249-254. doi: 10.1159/000366210
7. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biol. 2019;25:101084.
8. Rossi A, Pizzo P, Filadi R. Calcium, mitochondria and cell metabolism: A functional triangle in bioenergetics. Biochimica et biophysica acta Molecular cell research. 2019;1866(7):1068-78
9. Wei W, Graeff R, Yue J. Roles and mechanisms of the CD38/cyclic adenosine diphosphate ribose/Ca(2+) signaling pathway. World J Biol Chem. 2014 Feb 26;5(1):58-67. doi: 10.4331/wjbc.v5.i1.58. PMID: 24600514; PMCID: PMC3942542.
10. Courjaret, R., Machaca, K. Mid-range Ca^{2+} signalling mediated by functional coupling between store-operated Ca^{2+} entry and IP_3-dependent Ca^{2+} release. *Nat Commun* **5**, 3916 (2014). https://doi.org/10.1038/ncomms4916.
11. Ardura JA, Álvarez-Carrión L, Gutiérrez-Rojas I, Alonso V. Role of Calcium Signaling in Prostate Cancer Progression: Effects on Cancer Hallmarks and Bone Metastatic Mechanisms. Cancers (Basel). 2020 Apr 25;12(5):1071. doi: 10.3390/cancers12051071.
12. Manna F, Karkampouna S, Zoni E, De Menna M, Hensel J, Thalmann GN, Kruithof-de Julio M. Metastases in Prostate Cancer. Cold Spring Harb Perspect Med. 2019 Mar 1;9(3):a033688. doi: 10.1101/cshperspect.a033688.
13. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011 Oct 14;147(2):275-92. doi: 10.1016/j.cell.2011.09.024.
14. Liao, Z., Chua, D. & Tan, N.S. Reactive oxygen species: a volatile driver of field cancerization and metastasis. *Mol Cancer* 18, 65 (2019). https://doi.org/10.1186/s12943-019-0961-y
15. Yan W, Huang J, Zhang Q, Zhang J. Role of Metastasis Suppressor KAI1/e in Different Cancers. J Oncol. 2021 Jul 9;2021:9924473. doi: 10.1155/2021/9924473.
16. Reunanen N, Kähäri VM. Matrix Metalloproteinases in Cancer Cell Invasion. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6598/
17. Aalinkeel R, Nair BB, Reynolds JL, Sykes DE, Mahajan SD, Chadha KC, et al. Overexpression of MMP-9 contributes to invasiveness of prostate cancer cell line LNCaP. Immunol Invest. 2011;40(5):447-64.
18. Webb AH, Gao BT, Goldsmith ZK, Irvine AS, Saleh N, Lee RP, Lendermon JB, Bheemreddy R, Zhang Q, Brennan RC, Johnson D, Steinle JJ, Wilson MW, Morales-Tirado VM. Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. BMC Cancer. 2017 Jun 20;17(1):434. doi: 10.1186/s12885-017-3418-y.
19. Xiaohua Huang, Ying Li , Xin He , Yang Chen , Wei Wei , Xuesong Yang , Keli Ma. Gangliosides and CD82 inhibit the motility of colon cancer by downregulating the phosphorylation of EGFR at different tyrosine sites and signaling pathways. 2020. 22(5): 3994-4002. https://doi.org/10.3892/mmr.2020.11467.

20. Sadeghi L, Tanwir F, Yousefi Babadi V. Physiological and Biochemical Effects of Echium Amoenum Extract on Mn^{2+}-Imposed Parkinson Like Disorder in Rats. Adv Pharm Bull. 2018 Nov;8(4):705-713. doi: 10.15171/apb.2018.079. Epub 2018 Nov 29.
21. Mittal M, Flora SJ. Vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice. Drug Chem Toxicol 2007;30(3):263-81. doi:10.1080/01480540701380075.
22. Sadeghi, L., Rizvanov, A.A., Dabirmanesh, B. *et al.* Proteomic profiling of the rat hippocampus from the kindling and pilocarpine models of epilepsy: potential targets in calcium regulatory network. *Sci Rep* **11**, 8252 (2021). https://doi.org/10.1038/s41598-021-87555-7
23. Zhou D, Shao L, Spitz DR. Reactive oxygen species in normal and tumor stem cells. Adv Cancer Res. 2014;122:1-67. doi: 10.1016/B978-0-12-420117-0.00001-3.
24. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J, Trnkova L, Kruseova J, Eckschlager T, Kizek R. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncol Lett. 2012 Dec;4(6):1247-1253. doi: 10.3892/ol.2012.931.
25. Lee MS, Byun HJ, Lee J, Jeoung DI, Kim YM, Lee H. Tetraspanin CD82 represses Sp1-mediated Snail expression and the resultant E-cadherin expression interrupts nuclear signaling of β-catenin by increasing its membrane localization. Cell Signal. 2018 Dec;52:83-94. doi: 10.1016/j.cellsig.2018.09.001.
26. Geraldo, L.H.M., Spohr, T.C.L.d.S., Amaral, R.F.d. *et al.* Role of lysophosphatidic acid and its receptors in health and disease: novel therapeutic strategies. *Sig Transduct Target Ther* **6**, 45 (2021). https://doi.org/10.1038/s41392-020-00367-5
27. Matuz-Mares D, González-Andrade M, Araiza-Villanueva MG, Vilchis-Landeros MM, Vázquez-Meza H. Mitochondrial Calcium: Effects of Its Imbalance in Disease. *Antioxidants*. 2022; 11(5):801. https://doi.org/10.3390/antiox11050801
28. Thiel G, Schmidt T, Rössler OG. Ca^{2+} Microdomains, Calcineurin and the Regulation of Gene Transcription. Cells. 2021 Apr 12;10(4):875. doi: 10.3390/cells10040875.
29. Morel Y, Barouki R. Repression of gene expression by oxidative stress. Biochem J. 1999 Sep 15;342 Pt 3(Pt 3):481-96.
30. Seabra Rodrigues Dias Ivo R., Mok Simon W. F., Gordillo-Martínez Flora, Khan Imran, Hsiao Wendy W. L., Law Betty Y. K., Wong Vincent K. W., Liu Liang. The Calcium-Induced Regulation in the Molecular and Transcriptional Circuitry of Human Inflammatory Response and Autoimmunity.Frontiers in Pharmacology. 2018; 8 https://doi.org/10.3389/fphar.2017.00962
31. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science. 2002;295(5564):2387-92.
32. Rhee JS, Coussens LM. RECKing MMP function: implications for cancer development. Trends Cell Biol. 2002;12(5):209-11.
33. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res. 2010 May;44(5):479-96. doi: 10.3109/10715761003667554. PMID: 20370557; PMCID: PMC3880197.
34. Marilisa Galasso, Simona Gambino, Maria Grazia Romanelli, Massimo Donadelli, Maria Teresa Scupoli, Browsing the oldest antioxidant enzyme: catalase and its multiple regulation in cancer, Free Radical Biology and Medicine, 2012; 172 (264-272).
35. Che M, Wang R, Li X, Wang HY, Zheng XFS. Expanding roles of superoxide dismutases in cell regulation and cancer. Drug Discov Today. 2016 Jan;21(1):143-149. doi: 10.1016/j.drudis.2015.10.001. Epub 2015 Oct 19.
36. Hyoudou K, Nishikawa M, Ikemura M, Kobayashi Y, Mendelsohn A, Miyazaki N, et al. Prevention of pulmonary metastasis from subcutaneous tumors by binary system-based sustained delivery of catalase. J Control Release. 2009;137(2):110-5.
37. Aalinkeel R, Nair BB, Reynolds JL, Sykes DE, Mahajan SD, Chadha KC, et al. Overexpression of MMP-9 contributes to invasiveness of prostate cancer cell line LNCaP. Immunol Invest. 2011;40(5):447-64.

Figure legends

Fig 1. Comparison of GSH/GSSG ratio and ROS content level in prostate tumor and marginal tissues. Analysis of variance showed increased ROS content is accompanied by reduced GSH/GSSG ratio in prostate cancer. All data were showed as mean ± SD. Significant differences indicated by star symbol (****, P=0.0001).

Fig 2. Comparison of antioxidant enzymes' activity in prostate tumor and marginal tissues. Results confirmed increased activity of the SOD enzyme and reduced catalytic function of the CAT during tumorigenesis in prostate gland. Data were represented as mean ± SD and star symbol on columns manifested significant difference in comparison with control (****, P=0.0001).

Fig 3. Western blot results display increased expression of CD38 and IP3R in PCa. The first three lanes are related to tumor tissue and 4, 5 and 6 lans are related to marginal tissue. As a loading control, β-actin was utilized. Quantification of immunoblotting bands were represented in plot after normalization. Difference in both proteins expression is significant in comparison with marginal control (****, P< 0.001).

Fig 4. Western blot analysis of MMP-9 and CD82 expressions in PCa tissue. Quantified data were represented in column plot that revealed expression of both proteins is significantly different between two experimental groups (P<0.0001 for MMP-9 and P<0.001 for CD82 epressions). β-actin was used to control of protein concentration in lanes. Lane 1, 2 and 3 is biological replicates of prostate tumor tissue while lane 4, 5 and 6 related to border tissue.

Fig 5. Schematic overview of intracellular Ca^{2+} influx through IP3 and cADPR signaling in PCa.

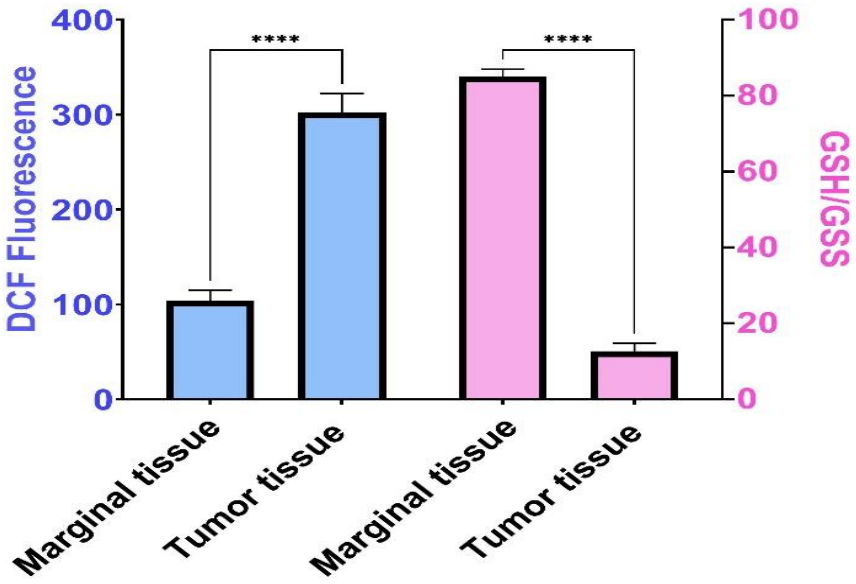

Fig 1

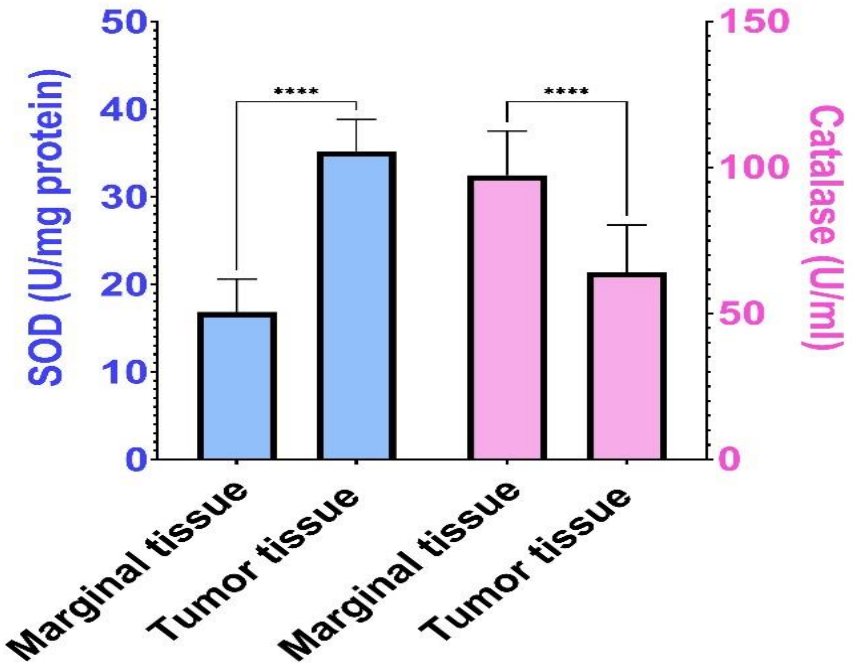

Fig 2

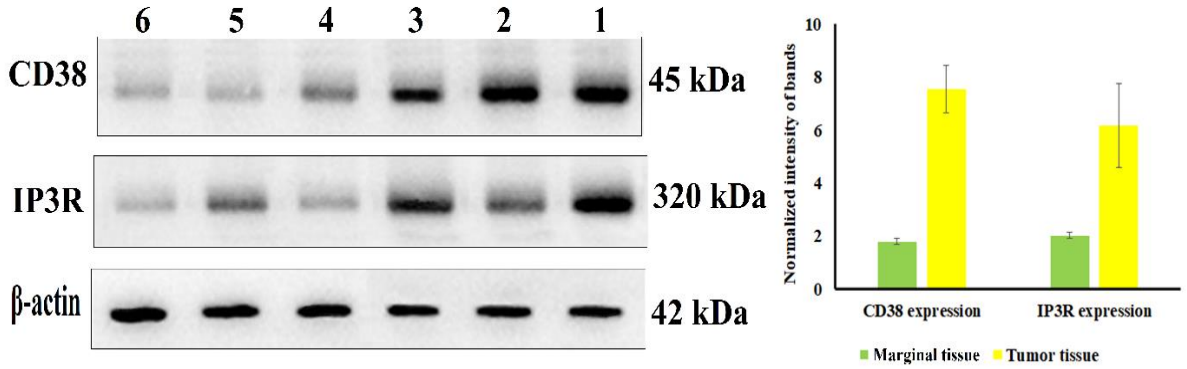

Fig 3

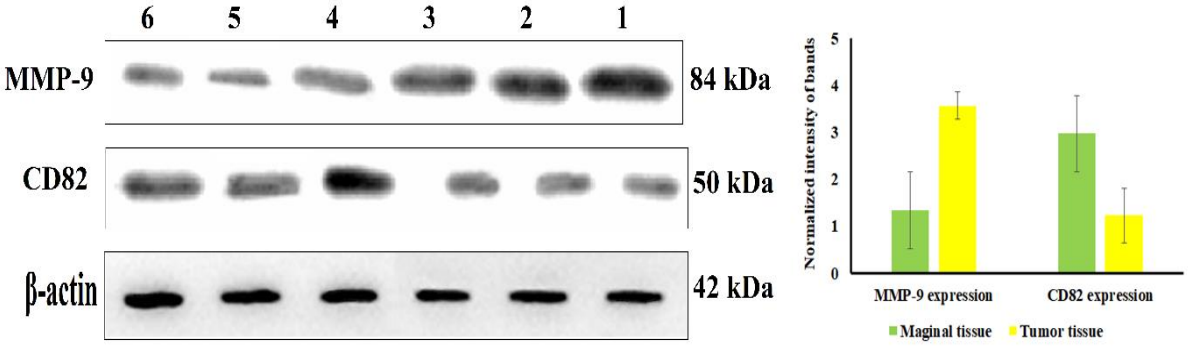

Fig 4

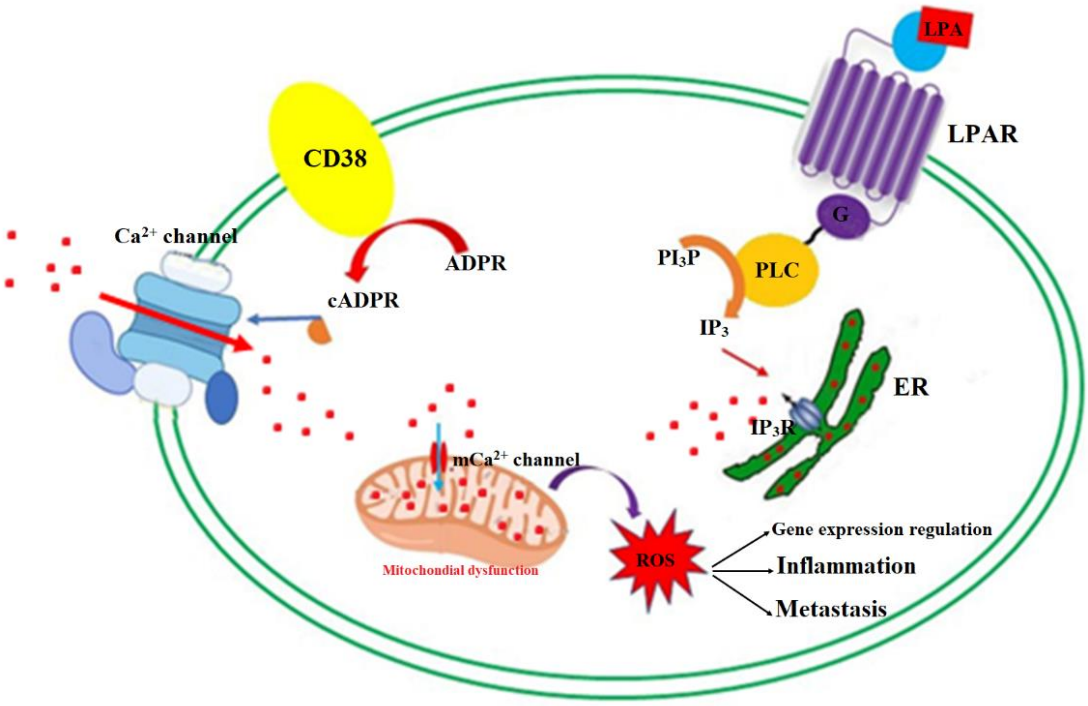
LPA
LPAR
CD38
G
Ca^{2+} channel
ADPR
cADPR
PI_3P
PLC
IP_3
ER
IP_3R
mCa^{2+} channel
Mitochondial dysfunction
ROS
Gene expression regulation
Inflammation
Metastasis

Fig 5