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REVIEW

A New Insight Into Metformin Action: Diabetes, Prostate Cancer, and Ion Channels

Ömür KARABULUT-BULAN

ABSTRACT

Diabetes Mellitus is a life-long chronic metabolic disease, requiring continuous follow-up and therapy, it reduces the quality of life of patients with acute and chronic complications, mortality, and its economic burden is high. Cancer is the second cause of death, according to the data from World Health Organization. Prostate cancer is one of the most common cancers in the developed world and the second leading cause of male cancer-related death. As with the other cancer types, metastasis is an essential problem that we are facing and it is not clear whether a tumor will metastasize or not in localized state. It has been reported that there are high levels of voltage-gated sodium channels in metastatic prostate cancer cases. Cancer, ever growing with diabetes, is a major health problem. Studies have shown that diabetic patients have higher cancer rates than those of non-diabetics. Metformin is the drug of choice for the treatment of diabetes. Recently, there are studies in the literature regarding metformin reducing the risk of cancer besides its effect on diabetes This review will explain the possible role of the metformın on the three dimensional relationship of prostate cancer, diabetes and ion channels, and provide a significant contribution to clinical trials.

Keywords: Cancer, experimental diabetes, metformin, voltage-gated sodium channel, MAT-Lylu cells

Ömür KARABULUT-BULAN
Department of Biology, Faculty of Science, Istanbul University, 34134-Vezneciler, Istanbul, Turkey,

Correspondence Address:
Ömür KARABULUT BULAN

E-mail: *bulan@istanbul.edu.tr*
Phone: *90 212 455 57 00*
Fax: *90 212 528 05 27*

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic and an endocrine disorder which occurs due to inadequate production of insulin by pancreas, or ineffective use of bodily insulin produced, progressing with the decrease of insulin-producing cellular counts, characterized by metabolic disorders in carbohydrates, proteins, and lipid metabolism. It has a life-long character, requiring constant monitoring and treatment, causing a dramatic decline in the quality of life, and causing high rates of mortality and economic burden (1). DM affects almost all organs and systems with complications such as retinopathy, nephropathy and neuropathy, cardiovascular complications, and ulceration, *etc.* (2-4). Thus, diabetes includes a wide variety of heterogeneous diseases. The number of patients with DM is expected to increase to ca. 438 million by 2030 and the number was 285 million in 2010 worldwide (5).

Cancer mechanism is a multi-step process, in which the

cells undergo metabolic and behavioral changes, leading to an excessive and untimely proliferation. These changes occur because of the modifications, controlling cellular proliferation, cellular life, relationships with the adjacent cells, and finally, the capacity of escaping the immune system. Cancer has a ranking of two for causes of death, according to the data provided by World Health Organization (WHO) (6, 7). Prostatic adenocarcinoma has a great importance for male morbidity and mortality observed both in our country, and also in the globe. It has a frequency increasing with age, and males in the industrialized countries encounter this disease at the highest frequency. It is at the second rank for cancer-related mortality cases. According to the American Cancer Society, in 2014 there were over 233.000 new cases of prostate cancer diagnosed in the US, resulting in about 29.340 death (8, 9). Prostate cancer can be described as the alteration of the balance between cell proliferation and cell death in the prostate gland which causes a malign increase of the organ volume. Factors leading to prostatic adenocarcinoma can be classified as hormones, dietary measures, environmental factors, and genetic factors (10, 11). To put it simply, as with the other cancer types, the main problem of prostatic adenocarcinoma is metastasis, that is, formation of secondary tumors with the journey of cancer cells to other places in the body and it is almost impossible to determine whether the localized occurrence of the case would behave in a metastatic fashion. Metastatic spread of cancer cells is the mainly reason of death of patients, and elucidation of the molecular mechanisms of this process is a important focal point in cancer research (12).

The transport of some molecules through the cellular membrane occurs through the channel proteins. Since they carry inorganic ions, they are also known as ion channels. When open, ion channels make it possible to passively transport through the cellular membrane for ions they are specific to, say, sodium, potassium, calcium, and chloride according to their electrochemical grading. Voltage-gated sodium channels (VGSC), are major ion channel operating in maintaining the electrical stimulation of the depolarization phase of the action potential, in which they are the sole primary responsible entity (13). *In vitro* studies have shown that the expression/activity of VGSC has played a role of an empowering factor in many cellular activities, which constitute the metastatic potential of prostatic adenocarcinoma. Studies have revealed that VGSC, especially the sub-type Nav 1.7, is a complementary factor of the metastatic potential of human and rat prostatic adenocarcinoma (14, 15). It has been observed that the expression/activity of VGSC is also susceptible to growth factors such as "epidermal growth factor" (EGF). Epidermal growth factor (EGF) is a low-molecular weight polypeptide. It binds to the EGF receptor and stimulates proliferation and differentiation. EGF has a regulating role on ion channels, which comprises the VGSCs. Human and rat prostatic cancer (Dunning's model) and breast cancer were investigated for the effects of EGF, and EGF-VGSC upregulation (transcriptional and functional) was shown to increase the metastatic cellular behaviors (16). In rat prostatic adenocarcinoma, EGF was shown to be effective on MAT-Ly1u cells motions. Cellular invasion increases by EGF and thus, it plays an important role in metastatic prostatic cancer (17-19).

A Three-Dimensional Relationship: Cancer - Diabetes - Ion Channels, and Metformin

Along with diabetes, cancer, which is ever growing and expensive, constitutes an important health problem. Association of diabetes and cancer has recently drawn much interest. According to the studies, diabetic patients have higher cancer rates than those of non-diabetics. Especially, diabetic males have a higher tendency to develop prostatic adenocarcinoma, as detailed in the World Health Organization report. The fact that diabetes has not a single form and it is of heterogeneous nature, progressing with abnormal behaviors in many metabolic parameters, people are commenting in diverse manners about the causes of its relation to cancer. The general biological mechanisms for the relation between diabetes mellitus and cancer are hyperglycemia, insulin resistance, insulin growth factor-1 (IGF-1) increase, hyperinsulinemia, increased inflammation, increased oxidative stress, obesity, and damage to the DNA. The hyperinsulinemia, insulin resistance, and oxidative stress, often present in obesity, can be the mechanisms by which obesity induces or promotes tumorigenesis (20). In addition, the decisive factors for the mentioned interrelation are the medications of the diabetic people, dietary applications, and metabolism control levels. Differences in glucose metabolism might initiate cancer formation in all tissues and have a pronounced contribution to its progression (21).

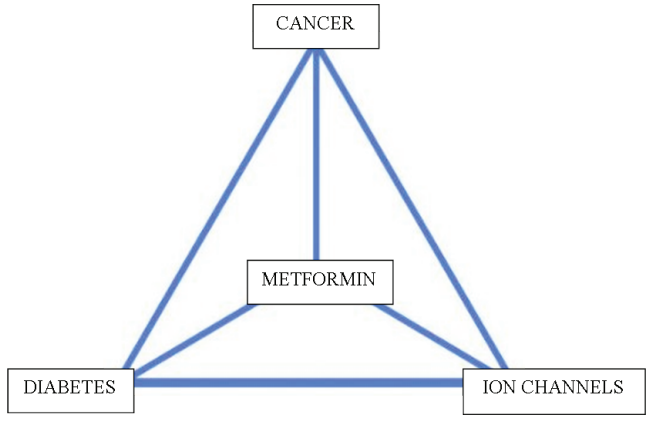
The diagnosis of prostate cancer at the localized stage and its metastatic potential at this period is among the key issues for the clinical treatment today. The metastatic role of voltage-gated sodium channels (VGSC) that is known as to play a reinforcing role in many metastatic behavior and the hypothesis of the inhibition of metastatic spread by blocking the channels have been tested in several cancer models.

Samples taken from prostatic cancer patients have shown,

in a immunohistochemical manner, that voltage-gated sodium channel protein is present in elevated amounts when compared to the normal prostate tissue (22). *In vitro* and *in vivo* studies have indicated a high level of voltage gated sodium channels for metastatic prostatic adenocarcinoma cases. It is known that there is an activity-dependent regulation, which is controlled by a positive feedback mechanism, in the VGSC expression for Mat-LyLu cells, grown *in vitro,* and that prolonged blocking decrease the expression of the channel. Our research group had been running an *in vivo* project, in which we investigated the effect of blocking of VGSCs by tetrodotoxin (TTX) on the metastatic development of prostatic adenocarcinoma for the first time, and we have shown that TTX suppressed the pulmonary metastasis for 44%, and there was a significant prolongation in the experimental animals' lives (23, 24). Prolonged TTX application to the cells has reduced the VGSC flow, Nav 1.7 mRNA levels, and the amount of VGSC proteins in the plasmic membrane. Meanwhile, when the introduction of sodium ions into the cell is increased, the VGSC flow is increased in return. Therefore, VGSCs are considered to be an integral part of prostatic adenocarcinomatous pathology (25, 17).

Metformin is an anti-diabetic drug, constituting an example of a sub-biguanide class for insulin-sensitizing drugs. It reduces the occlusion of glucose from the intestines, increases the insulin-mediated glucose usage in peripheral tissues, reduces the fatty acid concentrations, and shows an anti-lipolytic effect by lessening gluconeogenesis. Metformin inhibits hepatic glucose production and it also prevents hyperglycemia.

In the literature, many examples are available about the protective effects of metformin in STZ-induced diabetes (26-28). In a study investigating the protective effect of metformin on hepatocytes, metformin was reported to have damage-protective effect on the biliary acid-dependent apoptosis. Metformin is believed, according to literature reports, to have a therapeutic effect on chronic hepatic disease of inflammatory origin. Metformin reduces the serum ALT and AST levels (29). Also the effect of metformin in diabetes, on efforts that reduce the risk of cancer, have begun to have coverage in the literature. The latter is also known to play a significant role in prostate cancer metastasis. It is suggested that there is the following possible triangular relationship and VGSC could be responsible for the upregulation of the prostate cancer metastases.



Some *in vitro* studies have shown the inhibitory effect of metformin on various cancer types (pulmonary, bladder, ovarian, gastric, hepatic, and prostatic cancers) (30-33). The antihyperglycemic effect of metformin is basically attributed to its AMP-mediated protein kinase activity. Although the exact mechanism of action for metformin has not been elucidated yet, it was reported that it is antiproliferative and of inhibitory essence in the G0/G1 cellular cycle.

Today, bladder cancer is seen as a major problem in elderly male patients. A study about the effect of metformin on bladder cancer has provided the information that metformin has been administered in cases where *in vivo* and *in vitro* bladder cancer has been obtained with different cellular lines. Metformin-administered groups showed an inhibition of proliferation of cancer cells and colony formation has been declined (32).

It has been reported that metformin inhibits the proliferation of human prostate cancer PC-3 cells via the down-regulation of insulin-like growth factor 1 receptor. The anti-tumor mechanisms of metformin include activation of the AMP-activated protein kinase/mTOR pathway. Metformin is related to direct inhibition of insulin/insulin-like growth factor mediated cellular proliferation. In this study, metformin significantly inhibited PC-3 cell proliferation, migration, and invasion. Metformin is a potent inhibitor of the IGF receptor system and may contribute to the treatment of prostate cancer (34).

In vivo and *in vitro* experiments clearly show that the antidiabetic drug metformin exerts an antitumoral effect through a decrease of cyclin D1 level. Cyclin D1 is a key protein implicated in the transition of the G0/G1 phase. Metformin has induced to a strong reduction in cyclin D1 protein level. Metformin, which is given orally or

intraperitoneally, significantly decreases prostate tumor growth in a xenograft model (35).

At the same time, metformin is believed to act as a protecting agent on aging and cancer. In mammals, hyperglycemia and hyperinsulinemia are important geriatric factors. Biguanide compounds like metformin have long been regarded as anti-aging therapeutic agents (36). In a study which investigated the effects of metformin on oral squamous carcinoma, both *in vivo* and *in vitro,* it was shown to inhibit the proliferation of the cellular lines, and most remarkably, to reduce the formation of colonies. The cancer-reducing and antitumoral properties of metformin are the factors leading to the consideration of it as being part of new therapeutic strategies. When the effects of metformin are examined in diabetic patients having colorectal cancer cells, it was shown that metformin-administered patient have higher survival rates of pectoral, pancreatic, and glioblastoma stem cells. In addition, the authors observed a decrease of metastatic rate in the experimental group having metformin (37, 31). However, further *in vivo* studies are needed to elucidate possible cellular and molecular effects of metformin in the triangular concept of diabetes+cancer+ion channels and for detecting whether or not metastatic spread is inhibited by blocking the channels of metformin.

Experimental models

Today, diagnosing several illnesses, shedding light to their pathogeneses, and investigation of their treatment options have been the subject to some studies in which experimental animal models provide advantages. These models allow us to select genetically suitable types to the pathology, work with many samples which are adequate for statistical evaluation, complete the work in a very short time with a suitable type of animal, study more than one risk and pathology, and try diagnostic, protective, and therapeutic approaches (38). In drug investigations, some part of *in vivo* experiments are studied on animal models representing the disease in humans. Although some of these models are similar to the disease in humans in terms of pathological properties, it cannot be said definitely that they represent this disease. Some models are performed by applying different diet to the animal or damaging the targeted organs with toxic substances (39).

Animal Models of Diabetes Mellitus

Experimental diabetes is one of the models utilized for this purpose, and it can be produced with chemicals like alloxane and streptozotocin (STZ), and surgical methods, diet, genetic modifications, and application of anti-insulin hormones in high doses can produce experimental diabetes as well. The STZ and alloxan models of chemically induced diabetes are commonly used. STZ and alloxane selectively damage beta cells, inhibits glucose-stimulated insulin release, and its high doses cause cellular necrosis.

Alloxan is generally used in the insulin-dependent type I diabetes mellitus model. Due to its short half life, its intravenous (iv) use is preferred (40, 41). Although the diabetogenic dose of alloxan to create type I diabetes mellitus is accepted to be 40-45 mg/kg, Golfaman *et al.* reported that they injected a single dose 65 mg/kg alloxan through iv to rats, and 100% diabetes mellitus has started (42, 43). In the literature, alloxan is seen to be used in a broad range like 40-100 mg/kg and through iv route (44, 45). Intraperitoneal (ip) and subcutaneous (sc) applications of alloxan required that the 2-3-fold of the dose applied through iv route must be applied (46, 47).

STZ is preferred more in experimental studies, due to its more specific beta cell cytotoxicity. STZ is frequently used to create insulin-dependent type I and non-insulin-dependent type II diabetes models. STZ impairs the oxidation of glucose and reduces the biosynthesis of insulin and its release. STZ is taken into the metabolic cycle with glucose carrier (GLUT2) by pancreatic beta cells. The decrease of expression for GLUT2 is determined to prevent the diabetogenic effect of STZ. STZ first causes the loss of glucose response of beta cells. STZ has been documented to have DNA damage in pancreatic beta cells. STZ-induced diabetes indicates that the main cause of the deaths of beta cells is the alkylation of DNA itself (40,1). When a diabetes model is constructed with STZ, a significant degenerative damage occurs in tissues like liver, kidneys, and testicles, according to biochemical and morphological studies (48-52, 26, 2, 53, 54).

The diabetogenic dose range of STZ is not as narrow as alloxane. To create type I diabetes mellitus, generally the application of single-dose 40-60 mg/kg STZ through iv in mature rats is preferred, but broader doses in the range of 35-80 mg/kg can also be used (40, 55, 56). For STZ, intraperitoneal and subcutaneous uses are also mentioned with similar doses through iv (57, 58). STZ is used to create type I diabetes in mice with a dose similar for rats (59-61). To create type II diabetes, STZ is applied to neonatal animals

within the first week of birth, especially in the first and second days. It was found that much of the damage of pancreatic β cells due to STZ application is regenerated and cause a similar condition with type II diabetes mellitus. This method was first demonstrated in 1974 by Portha *et al.* to the neonatal rats by applying 100 mg/kg STZ (62). Dağıstanlı *et al.* (63) have used STZ in the 100 mg/kg concentration through i.p while Sinzato *et al.* (64) have used STZ in the same concentration, but through sc This method has been the mostly preferred chemical diabetes mellitus model to create type II diabetes because it offers operational ease. Similarly, it is considered to be the best model to reflect type II diabetes mellitus clinic among the other models (40) . With STZ application, type II diabetes mellitus could be created in an alternative way, which includes a high-fat (41, 65) or high-fructose (66, 67) diet followed by low-dose STZ injection. Srinivasan *et al.* (68) have applied 35 mg/kg STZ through i.p. after having the rats eat a high-fat diet for two weeks (58% fat, 25% protein and 17% carbohydrate as the percentages of total calories) (69).

In order to determine whether the animals have been rendered diabetic after application of STZ, after 72 hour of STZ administration, tail vein blood is collected to determine blood glucose levels. Rats with blood glucose ≥200 mg/dL are considered diabetic. In addition, for determination of diabetes, low blood insulin level (<0.04 μg), daily elevated urine output (>25 mL/day), and glycoseurea (>2%) are also considered (38, 70).

Animal Models of Prostate cancer

The use of experimental model systems in cancer research have provided an important contribution in the understanding of cancer illness, the biological aspects of metastasis, as well as prevention of cancer and development of new therapeutic methods.

Experimental model systems are used in the research on prostate cancer, which is a quite complicated disease. Among the preclinic models used in prostate cancer research, PC-3, LNCaP, and DU-145, all of which uses *in vitro* cell lines and the xenograft models created by using these lines, as well as animal models like rodents such as mouse and rat and dogs and model systems developed with transgenic techniques (71-73). Animal models, particularly rat models, play an important role in the study of the etiology, prevention and treatment of prostate cancer. Four model systems for prostate cancer research in rats were established. These are Dunning, Noble, ACI and Pollard tumor models. The prostatic adenocarcinoma model in Copenhagen-type rats is of powerful metastatic nature, obtained with subcutaneous injection of rat Mat-LyLu cells, which is classified as a Dunning adenocarcinoma model. The designation MATLyLu is an abbreviation for Metastatic Anaplastic Tumor Metastasizing to Lymph node and Lungs (74). Dunning's model is one of the experimental prostatic adenocarcinoma model, arising first in 1961 by W. F. Dunning, appearing as a spontaneously occurring in the dorsal prostate of an old Copenhagen type rat, having good distinctive properties, growing slowly, and is androgen-selective (72). In the beginning, this model was used in the investigations of carcinogenesis from a hormonal perspective, but now it is still practiced as a useful model for investigations of androgen-independent growth of prostatic adenocarcinoma cells and the molecular basis of metastases.

Though Dunning's rat prostatic cancer model is one of the first ones that was accepted, it is still widely used, having many subcellular strains, grown both *in vivo* and *in vitro* (75-78). Prostatic cancer is formed by the inoculation of highly metastatic Mat-LyLu cells from these strains into Copenhagen rats and metastasizes to the lungs and the lymphatic system (76, 79, 80).

CONCLUSION

The role metformin in diabetes has been studied in detail and additionally there are some studies linking diabetes-metformin-insulin resistance-aging. On the other hand, effects of metformin in experimental cancer and diabetes+cancer models *in vivo* have not been investigated. This review highlights the potential therapeutic effect of metformin in prostate cancer and diabetes model. We designed a study with regard to this issue. Our planned study and the novel *in vivo* model should be important for improving clinical insight. The study we are planning is constructed upon meeting the requirement of providing early diagnosis and determining the metastatic potential of prostatic adenocarcinoma, and best exemplified by the phrase "VGSCs have an empowering effect of the metastatic potential of prostatic adenocarcinoma and metastatic spreading can be suppressed by blocking the channels". In our study, the triangular concept of diabetes, prostatic adenocarcinoma, and ion channels have the interconnections first investigated using *in vivo* methods and the overall effect and therapeutic potential of metformin will be described.

Metformin Etkisine Yeni Bir Anlayış: Diyabet, Prostat Kanseri, ve İyon Kanalları

ÖZ

Diabetes Mellitus yaşam boyu süren, sürekli izlem ve tedavi gerektiren, akut ve kronik komplikasyonları nedeniyle hastanın yaşam kalitesini azaltan, mortalitesi ve ekonomik yükü yüksek, kronik bir metabolik hastalıktır. Kanser ise, ölüm nedenlerinde dünya sağlık örgütü verilerine göre ikinci sıradadır. Prostat kanseri gelişmiş ülkelerdeki erkeklerde en sık rastlanan kanserlerden biridir ve kanserle ilişkili ölümlerde ikinci sırada yer almaktadır. Diğer kanserlerde olduğu gibi prostat kanserinin tedavisinde de metastaz temel problem olarak karşımıza çıkmakta ve lokalize evrede iken tümörün metastatik yayılım gösterip göstermeyeceği belirlenememektedir. Metastatik prostat kanseri vakalarında voltaj kapılı sodyum kanallarının yüksek seviyede olduğu bildirilmiştir. Diyabetle birlikte daha da büyüyen bir hastalık olan kanser önemli bir sağlık sorunudur. Yapılan çalışmalar sonucunda diyabetik hastalarda kanser oranının diyabetik olmayanlara göre daha yüksek olduğu saptanmıştır. Metformin diyabet tedavisinde kullanılan bir ilaç seçeneğidir. Son yıllarda metforminin diyabetteki etkisinin yanı sıra kanser riskini azalttığına yönelik çalışmalar literatürde yerini almaktadır. Bu çalışma prostat kanseri, diyabet ve iyon kanallarının üç boyutlu ilişkisi üzerinde metforminin muhtemel rolünü açıklayacak ve klinik çalışmalara önemli bir katkı sağlayacaktır.

Anahtar kelimeler: Kanser, deneysel diyabet, metformin, voltaj kapılı sodyum kanalları, MAT-Lylu hücreleri.

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