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Role of Metformin in Triple-Negative Breast Cancer Obese Patients

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Abstract

Triple Negative Breast Cancer (TNBC) is the most aggressive type of breast cancer. It is a heterogeneous disease that is based on immune histochemistry analyses 1. It is negative for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2.TNBC is significantly observed in young African American women and Hispanic women who carry amutation in the BRCA gene. Obesity has an increased risk for developing TBNC, especially for premenopausal and post-menopausal women. The relation between obesity and TNBC remains difficult to understand. Many studies hypothesized that increased adipose cytokine, Adipokine, mainly Apelin levels due to obesity could be a major factor contributing to both tumor growth and metastasis in TNBC obese patients. Poor prognosis and poor response to treatment are the major characteristics of TNBC. The anti-type II diabetes drug metformin can reduce risk of breast cancer, improves survival of breast cancer patients. It helps to inhibit specific molecular subtypes. Also, Metformin inhibits cell proliferation, colony formation, GM1lipid rafts in TNBC. It activates intrinsic and extrinsic metformin signaling pathways only in TNBC cell lines. These breast cancer cells are extremely dependent on glucose and lipids which are metabolized for the production of energy and proliferation of TNBC cells. So, metformin can induce lipid metabolisms, especially targeting fatty acid synthase, cholesterol biosynthesis. Many researches demonstrated that Metformin can stop several strong enzymes going into glucose metabolism. It has significant role on inhibiting carbohydrate metabolism and lipid metabolism. By increasing key metabolic defect of carbohydrate and lipid metabolism this drug can reduce obesity for TNBC patients. The actual aim of this paper to highlight the partial role of metformin in cellular building blocks and in decreasing a high rate of TNBC cells proliferation, especially against highly aggressive malignant cancer cells for TNBC obese patients.

Keywords: Triple Negative Breast Cancer (TNBC); Malignant cancer; Obesity; Epidemiological studies

Introduction

Triple Negative Breast cancer is aggressive comparatively other type breast cancer. Obesity is known to have a strong linkage with cancer. Many researchers utter that in 2018 2,66,000 and 64,000 patients are diagnosed for new invasive and in situ breast cancer respectively. This led to a study which was conducted in 2008 to examine generally 620 white women and the results showed that out of the 620 white women that were examined, 117 of them had TNBC and that fraction had a strong relation with obesity [1]. The study also revealed that 50% of the patients with TNBC were obese compared to 36% of obese patients with no TNBC [2]. Obesity has a linkage with risk factors for cancer. Nonetheless, Body Mass Index (BMI) is not only measured for adiposity where WHR or Waist – to – Hip ratio has specific measures of central or abdominal obesity. The high risk of breast cancer has been associated with a common corollary of metabolic syndrome and type 2DM. Meta-analysis studies conducted in 2007 for twenty (20) patients estimated a 20% increased risk of breast cancer for women with type 2DM (RR=1.20; 95%Cl, 1.12-1.8) [3].

In the instance where comparison is done between lean patients and breast cancer patients who are also obese, the obese breast cancer patients have more risk of recurrence and a worse prognosis. The outcome of a study where samples of 495,477 US women were taken indicated that increasing Body Mass Index (BMI) was significantly associated with increased death rates for breast cancer patients [4]. As compared to the lowest BMI group (18.5-24.9), there was an increased risk of 34% for BMI of 250-299(RR=1.70; 95%Cl, 1.33-2.21) and for BMI > 40.0 (RR=2.12; 95%Cl, 1.41 – 3.19) for dying breast cancer patients. Physical activities and weight loss are inversely associated with breast cancer dangers and recurrence as suggested by several epidemiological studies [5]. Patients with BMI >25 kg/m2 had significant benefits through post diagnosis exercise. Interestingly, physical activity after diagnosis played a vital role in the reduction of breast cancer deaths by 50% (RR=0.50; 95% Cl, 0.34-74) for tumors with no significant effect on patients with ER- tumors [6-11]. There are lots of recent studies which highly indicated that abdominal obesity improves breast cancer development and outcomes through other mechanism as well and also system shifts in Carbohydrate and fat metabolism up regulation of pro-carcinogenic factors such as cytokines and growth factors (like insulin and insulin like growth factors, modulation of the immune system and macrophage activation have significant effect on obesity and breast cancer as well. Comparatively, breast cancer patients who are obese have more usual recurrence and worse prognosis than lean patients.

Improvement in insulin resistance or blood glucose may also mediate this effect. According to the Women's Intervention Nutrition Study (WINS), 2437 women were examined with breast cancer [12]. This was a randomized study that engages a dietary intervention group intending to lower the percentage of calories from fat to 15% without impairing the nutrition of these group of people. Another factor that seems to moderate the recurrence and mortality of breast cancer survivors is alcohol consumption. Recent studies conducted on 1,897 individuals revealed that three to four times of alcohol consumption per week was related to 35% (HR=1.35; 95%, 1.00- 1.83) High risk of breast cancer recurrence and 51% (HR=1.5; 95%Cl, 1.00-2.29) increased risk of death as a result of breast cancer [13,14]. A study demonstrated that for all women with both obesity type II diabetes, the risk of breast cancer increases by as much as 20% [15]. Some studies showed that Gestational diabetes, pre-diabetes or family history of diabetes also enhances risk for breast cancer for women [15,16].

Metformin hydrochloride is a diabetes medicine. It is generally used for managing type II diabetes. Because Metformin does not cause weight gain and may help with weight loss, it is generally prescribed for overweight people with type II diabetes. Triple negative breast cancer is one kind of breast cancer whose tumors do not express estrogen receptor, progesterone receptor and HER2 receptor. Approximately 15%-20% among other breast cancer patients are suffering from TNBC. Only chemotherapy can be used for treatment of triple negative breast cancer. Novel targeted therapies would be best for TNBC survives [17-19]. A study of 2012 demonstrated that inhibition of over expression of Fatty acid synthase induces apoptosis of breast cancer cell lines [20]. Another research proved that Metformin decreases fatty acid synthase, cholesterol biosynthesis and GM1 lipid rafts in Triple Negative Breast Cancer cells. There are so many studies which indicate that both obesity and type II highly increase risk of hormone receptor positive breast cancer and also anticipate that obesity plays a vital role to increase breast cancer in young African women (pre-menopausal) and most of the time these type women are diagnosed with Triple Negative Breast cancer. In general, targeted therapeutics would be effective for TNBC [21-26].

Insulin and TNBC and Role of Metformin

Insulin stimulates glucose transport by translocation of GLUT4 proteins from an intracellular vesicular compartment to plasma membrane. Once GLUT4 recruitment occurs. The transpote inserts into plasma membrane allowing uptake of glucose into cell. When cells in our muscles, fat and liver cannot response properly to insulin and abdicate glucose from our blood, insulin resistance occurs. There are significant relationship between obesity and type 2 diabetes. Reducing of insulin stimulated glucose transport, metabolism in adipocytes and skeletal muscle are main cause of insulin resistance for obesity and type 2 diabetic. Insulin is known for its linkage between obesity and breast cancers. Up regulation of insulin has been hypothesized to directly increase the proliferation of breast cancer cells and breast tissue.

Hyperglycemia and hyperinsulinemia are associated with poor prognosis as suggested by data [27]. Functional imbalance also creates the down regulation of the major insulin-responsive glucose transported GLUT4 [28]. In 2007, a case- control study was conducted to examine blood samples in generally premenopausal individuals. The results indicated that high insulin levels and C- peptide were not risks associated with breast cancer. Generally insulin binding IRS-1 and IRS-2 receptor for both muscle and adipocytes. IRS-1 plays a vital role in increasing insulin action, including binding and activation of Phosphotidyplinostio (PI)-3 Kinase and glucose transport [28]. Obese, type 2 diabetic patients' skeletal has normal IRS-1 And IRS-2 protein levels but P13 activity with these [29].

Epidemiologic studies demonstrated that the risk of diabetes and presumably insulin resistance increases according to body fat content (measured by BMI) increases from the very lean to the very obese and body fat has intrinsic roles in insulin resistance [30]. The relationship between insulin resistance and obesity is measured by adiposity and BMI. Only central obesity has significant linkage with insulin resistance, type 2 diabetes and cardiovascular disease [31]. On the other hand for some biochemical structure of intra-abdominal adipocytes may have direct source with insulin sensitivity. A leading hypothesis regarding intra-abdominal reported that adipocytes are active lipolytically for average receptors. It may cause for excessive intraportal FFA level and flux which promotes insulin resistance. So many studies has been investigated the molecular mechanism of TNBC and for the better understanding of these mechanism will help to design novel therapy for TNBC.

Excessive amount of C-peptide/ Insulin increases breast cancer risk factor [32]. IGF1-1 plays a vital role to induce apoptotic activity and to control cell and body size [33]. Many researchers proved that the increased activity and level of compared to normal breast has significant relation with breast cancer risk [34-39]. Our main focus is about TNBC. So we found lots of studies which reported that in TNBC cell lines high IGF-1R receptors has been shown and it helps to develop TNBC and this IGF-1R has strong association with obesity [40,41]. BRCA1 and P53 suppressor gene mutation reduces the activity to resist the increasing level of IGF-1R gene expression [42]. Many studies demonstrated that IGFBP-3 has positive association with BMI and TNBC. This IGFBP-3 has six proteins and they are highly related with poor prognosis for ER, PR negativity, S-phase fraction and tumor size. This IGBP-3 has association with TNBC developing which has bonding with high expression of epidermal growth factor. It also promotes to increase TNBC cells by inducing Sphk-1 mediated EGFR signaling [43-48].

Insulin like growth factor has three ligands, they are IGF-1, IGF-2 and insulin which stimulates signal by paralogous receptor proteins and they are located in plasma membrane. IGF-1, IGF-2 have high relation with type-I receptor and where insulin has high affection for insulin receptor.

The ligands collaborate extracellular domains of receptors which promote the phosphorylation of intracellular adaptor receptors. Increased cell survival, proliferation and migration are promoting by Mitogen-Activated Protein Kinase (MAPK) and AKT by leading of signaling cascades.

Metformin is a biguanide drug is used as a treatment for weight loss, type 2 diabetes, especially in the presence of insulin resistance [49]. By activating of AMP-activated protein kinase, is shown in fig 2 it helps to improve hyperglycemia through concealment of hepatic gluconeogenesis and these biological functions of Metformin plays a significant role in insulin signaling [49]. In contrast Metformin leads AMPK activity which is known as the cause of GLUT4, this GLUT4 develops plasma membrane and as a result it occurs insulin independent glucose uptake. In addition Metformin raises insulin sensitivity, promotes peripheral glucose uptake and fatty acid oxidation [50] **(Figure 1)**.

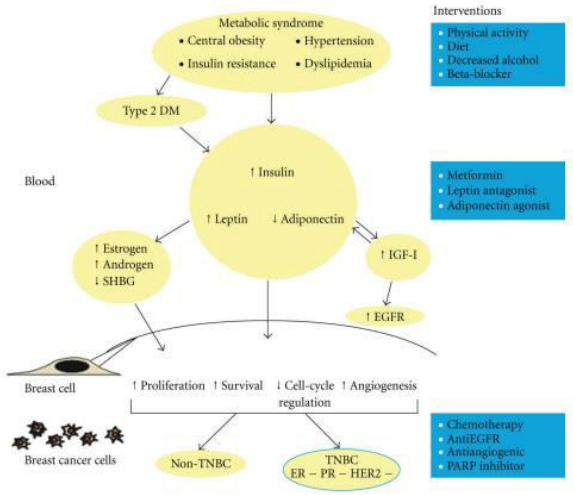

Figure 1: Association between insulin-leptin-adiponectin levels and triple negative breast cancer cell proliferation.

Mechanism of Metformin to Reduce Adiponectin in TNBC

A protein exclusively secreted by the adipose tissue and improves the insulin – sensitivity levels of the entire body is known as adiponectin. Insulin sensitivity levels of adiponectin are inversely correlated with obesity. In a study where 527 patients were sampled and diagnosed with stage I- III breast cancer. They showed adiponectin levels above 15.5 µg/mL. This level justifies improved breast cancer survival rate (HR=0.39; 95%CI, 0.15-0.95) [51,52]. The role of the adiponectin pathway in Single Nucleotide Polymorphism (SNPs) was demonstrated in breast cancer. This was observed through a case-control study on 763 breast cancer patients. The study revealed that two functional polymorphisms of ADIPOQ and one functional polymorphism which has exhibited the ability to change mRNA levels in ADIPORI had significant relation with a high risk of breast cancer. The development of obesity mainly depends on the balance between white adipose tissue and brown adipose tissues. White adipose tissue works for reserving energy and brown tissue works for energy expenditure [53]. Otherwise, brown tissue can affect body metabolism and it can change insulin sensitivity which is responsible to induce obesity [54-56]. Because of obesity not only insulin resistance is increased but also adipose tissue cannot work for energy leading to the reservation of secretory endocrine organs of cytokines, hormones, and proteins that regulate the function of cells and tissues all over the body [57]. Obesity creates a collection of lipids in adipocytes, producing cellular stress and activation of JNK and NF-kB pathways [58,59].

Phosphorylation of proteins, different transcriptional events which help to induce pro- inflammatory molecules, TNF-alpha, IL-6, leptin, resistin, chemokines are promoted by these proliferated signaling pathways and they are significantly responsible for producing monocytes and other inflammatory cells to the adipose tissues. Many cytokines and chemokines are expressed more by the induced inflammatory signal from macrophages which is differentiated from monocyte [60]. In obese patients, T-cell works for producing and promoting pro-inflammatory cytokines and macrophages to the adipose tissue [61]. Adipose tissue can make a connection with each adipocyte, by inflammation signal of inflammatory fat and cells and adipose tissue can keep association with multiple vascular capillaries [62]. Inducing fat microcirculation could promote adipose tissue inflammation.

As our main focus is on TNBC, the question regarding the association between adipocyte and TNBC cannot be left unanswered. High adipogenesis plays a role for worst survival in TNBC is shown in fig 1. High adipogenesis has a strong association with metabolism gene sets; oxidative phosphorylation, fatty acid metabolism, peroxisome, and reactive oxygen species pathway. High adipogenesis TNBC suppresses PDL-1 and PDL-2 and immune checkpoint molecules index, also it is responsible for HRD [63]. As reported by other studies, adiponectin prevents the activities of aromatase and estrogen receptors, a phenomenon that would act on ER tumors [64]. The overexpression of adiponectin lowers mammary tumor size both locally and systemically as shown in studies relating to animals [65]. A study regarding this topic declared that adipocyte has a great impact on cancer progression by raising highly complex cancer cells [66]. Intra-tumoral adipocytes with genes that have an association with inflammation and metastasis, rather than cell proliferation-related gene sets [67]. In addition, intra-tumoral causes inflammation, hypoxia, and angiogenesis [68-70]. Another study revealed the reason behind having strong cell density in proliferated cancer cells where adipocytes cannot move easily in the tumor microenvironment. Also, that study demonstrated that the connection between adipocytes and adipogenesis is not strong in breast cancer and specially adipogenesis in TNBC. Hod lower adipocytes, immune and proliferated pathways, because high adipogenesis TNBC has a significant relation with metabolic-related gene sets, due to this function it is one of the reasons of worst survival. In contrast, TNBC with high adipogenesis and metabolic activity has the worst survival for infiltration of immune cells rather than high cell proliferation. Several studies indicate that adipogenesis is amplified in fat-related pathways rather than the abundance of adipocytes. There is a strong marker for cancer and that is the ratio of leptin to adiponectin in serum [71,72].

With the help of osteogenesis and activation of AMPK in adipocytes, Metformin decreases adipocytes and this is shown in several studies [73]. Another study explained that metformin has poor resistance to adipogenesis in murine C3H10T1/2 MSCs

[74]. For all cell types, different specific effects of Metformin can inhibit adipogenesis by AMPK activation. So many researchers have reported that Metformin has a linkage with differentiated cell lines such as pre-osteoblasts, pre-adipocytes, myoblasts, and neuronal mouse cell lines [75-78], instead of more primitive cell progenitors. For cell differentiation, there is a different time for specific signaling pathways. Early-stage differentiation is regulated by the late stage of the Akt/mTOR signaling pathway's activation. With AMPK assays there is fixed activity of Metformin for the adipogenesis process. Metformin can activate the reduction of PPAR-gamma-Runx2 ratio and mTOR thus, inhibit adipogenesis. The differentiation of MSCs into osteoblasts and adipocytes is regulated by Metformin and it can reduce the mTOR signaling pathways at the early stage. This study also reported that aggressive MEFS is observed to gather lipid and induces the expression of C/EBP- beta to an adipogenic cocktail of IID plus PIO. So, Metformin has a strong ability to inhibit adipogenesis by the activation of AMPK in different cell lines.

Role of Metformin to Control in dysregulation of Carbohydrate and Lipid Metabolism in TNBC

When energy is stored as a triglycerides and obesity is one of criterion which is developed by diet, age, genes, physical activities [79,80]. Then what is the effect of obesity on metabolic change? Adipose tissue which produce adipokines like leptin, adiponectin, apelin etc., which regulate metabolic process in the body [81]. The main mechanism of insulin is to keep glucose level lower in blood from concealment of hepatic glucose production and the increased glucose uptake into muscle and adipose tissue via GLUT4. Adipose tissue express lower glucose level into body and by this lower disposal muscle insulin stimulates glucose uptake in higher level *in vivo*. Various studies have promoted that in systemic glucose homeostasis glucose uptake transform into fat. For obesity GLUT4 become over expressed which causes insulin sensitivity and glucose tolerance [82]. In contrast for obesity this down regulation of GLUT4 occurs and for this case insulin stimulates glucose transport which is decreased in adipocytes [83].

Obesity has significant effect on lipid metabolism and it is well known that obesity has strong connection with increased basal lipolysis in adipose tissue and promotes circulating FFAS [84]. Several functions play key role to induce basal lipolysis such as acute phase Serum Amyloid A (SAA), alipholytic adipokine in humans. Lipolysis helps to increase SAA production from long adipocytes into circulation which also promotes insulin resistance. Function of SAA circulated through CLA-1 and extra cellular signal regulated kinase signaling pathway and it raises lipolysis directly [85]. Several studies demonstrated that plasma triglyceride concentration is also metabolic variable and for obesity it's affected. Glucose uptake is activated by insulin which promotes Very Low Density Lipoprotein (VLDL), TG production rate and it regulated to endogenous hypertrigly ceridemia [86-88].Because of obesity lipoprotein lipase is started to decrease and it activates lipolysis of chylomicon-TG and inactive inhibition of hormone sensitive lipase mediated lipolysis in adipose tissue [89]. For obesity excess fatty acid increases expression in the prandial period, in normal which is suppressed by insulin and it helps to impact on glucose uptake by as much as 50% [90]. SAA has also significant association with cholesterol metabolism and High Density Lipoprotein (HDL) [91]. Excess obesity regulates SAA in obesity which may be connected between obesity and low HDL.

For the systemic dysregulation of lipid and carbohydrate metabolism causes metabolic syndrome and type II diabetes. These type II diabetes and metabolic syndrome are known as risk factor of breast cancer [92-94]. With these disorder the increased level of insulin resistance and insulin like growth factor are responsible for breast cancer and worst prognosis. A study highlighted that metabolic dysregulation plays a vital role in serum glucose and other energy precursors such as fructose and glucosomine which can be metabolized to adenosine triphosphate which helps to proliferate cancer cell and tumor growth in hypoxic environment [95]. Carbohydrate metabolism dysregulation is used as aerobic glycolysis is well known as hallmark of cancer [96].

A study used a carcinogen-induced rodent model of tumorigenesis and showed that overfed obese animals which is similar to metabolic syndrome which increased 50% glucose uptake by mammary tumor cells and it has strong relation with cancer cell proliferation and it was noticed in human breast cancer cells in vitro [97]. Also that rotent model reported that Metformin has anti cancer effect and this study's epidemiological data showed that patients who are suffering from metabolic syndrome or type II diabetics were able to reduce cancer incidence and improves survival by consuming Metformin [98-100]. Metformin has strong potent against triple negative breast cancer because TNBC is dependent on glucose and glutamine and Metformin inhibits significantly mitochondrial respiration in TNBC cancer cells [101].

Activity of Metformin to inhibit FASN in TNBC

Adipose tissue is now known to play an important role and activate the endocrine organ. It is very well established that adipocytes aids in the storage and release of energy throughout the human body. Adipose tissue may play an important role in Fatty Acid (FA) FLUX and it changes to energize the body in the fasting state. Generally, adipose tissue releases FAs but in the fed state, adipocyte absorbs FAs from circulating triglycerides [102]. When the function will be inversely proportional then obesity, insulin resistance, dyslipemia inflammation, atherosclerosis, hypertension occurs [103,104]. PPAR-gamma or PPARG (Peroxisome Proliferator Activated Receptor Gamma) is known as the glitazone receptor NR1C3 (nuclear receptor subfamily.1 groupc, members) is type II nuclear receptor that is encoded by the PPARG gene PPAR-gamma and plays a significant role in metabolism by regulating many genes [105] and they are involved in fatty acid synthesis. Exogenously derived and endogenously synthesized FA maintains substrates for energy metabolism. The two key enzymes of lipogenesis which are Fatty Acid Synthase and Acetyl- CoA-Carboxylase play an important

role for weight of abdominal adipose tissue [106]. In addition, FASN as a multifunctional enzymatic complex performs a role in the regulation of body weight and increases obesity [106-108]. High intake of carbohydrate diet helps FASN to accelerate endogenous FA biosynthesis in liver and adipose tissue [109]. So many studies have shown higher gene expression of obese *vs* lean separately [110-112]. In contrast a study has demonstrated the role of FASN in obesity by using BMI and metabolic parameters and so many studies have explained association of FASN activity and its expression with obesity, insulin resistance and adipocytokine serum profile.

TNBC has poor prognosis and there is no targeted therapy available for triple negative breast cancer. A study has shown that FASN expression has association with increasing TNBC in clinico-histopathological means [113]. This study proved this using 100 primary TNBC tumors and it is assessed by immunohistochemistry of FASN, EGFR and C15/6 vimentin expression. One of lipogenic enzyme fatty acid synthase are generally responsible for increasing neoplastic disease and its overexpression are seen in activities of inducing neoplastic disease [113]. The protein acylation, biological membrane, synthesis, DNA synthesis and cell cycle proliferation of cancer cells are promoted by long chain of fatty acid de novo synthesis. Several studies have demonstrated that FASN's over expression could serve as potential biomarkers and therapeutic targets for so many carcinomas. This could be used for breast cancer also. So many studies have shown that reducing FASN increases apoptosis in couple of cancer cells and decreases the growth of human xenografts [114-127]. A study experimented by 29 core – biopsies of TNBC patients and preclinical studies showed that FASN reduction could re- sensitize doxorubicin resistant cell lines. Also, another study which was done using 100 primary TNBC women and were diagnosed between 1990 and 2012 at Hospital Universitri by Dr. Josep Trueta (Giona, Spain). The study showed that FASN expression was positive in almost all TNBC samples (92%). High FASN expression was observed in 45% of TNBC samples. Among the same patients, 22% were observed to have lower FASN expression in non-tumoral tissues. A cohort study analyzed that FASN was positive in 92% of tumor tissue samples and 45% have high FASN levels and this study also reported that FASN expression has relation with positive nod involvement.

Some studies previously showed that identification of tumor cells in lymph nodes will be helpful to predict patient's outcome. So many researches demonstrated that overexpression of FASN is detected as poor prognosis marker in several cancers such as lung, ovarian, gastric or in early breast cancer carcinomas patients. Some preclinical studies reported that FASN expression plays a vital role in drug resistant [128-130].

Metformin kills stem cells, triple negative breast cancer cell lines as FASN has complexity with de novo fatty synthesis and it is essential for TNBC survival. Metformin induces FASN expression and helps to induce apoptosis in TNBC cell lines [131]. According to TNBC structure, the cells of TNBC are sensitive to metformin action. Significantly, cancer stem cells have over expression and dependent on lipogenic enzymes and FASN as well [132-135]. If CSCs are more dependent on FASN, then TNBC are proportionally sensitive to Metformin. This idea has proved why TNBC is sensitive to metformin [136,137]. In Luminal Estrogen Receptor Positive Breast Cancer Cells, FASN is strongly controlled by estrogen and progesterone receptors [138-147]. On FASN and lipogenesis, several kinds of cancer's metastasis, invasion, chemoresistance are dependent [148-150]. These characteristics are also significant in TNBC and that confirms that metformin can be activated to reduce FASN in TNBC. A study experimented and reported that 10 mM metformin effectively reduce FASN in TNBC cells. This study also proved that ten top genes of fatty acid and cholesterol biosynthesis pathways are decreased by metformin. Many studies have shown that several mRNA have been defined as targeting FASN directly or indirectly. Another study promoted that by increasing up regulation of miR- 193b, metformin can reduce apoptosis, reduce FASN and memosphere formation of TNBC [151-173].

THE STAT 3 SIGNALING PATHWAY IN TNBC

More recently, many efforts have been made to identify targetable molecules for treating TNBC through genomic profiling and numerous critical changes have been found, including the overexpression and aberrant activation of Signal Transducer and Activator of Transcription 3 (STAT3) [174,175]. The emerging data suggest that STAT3 may be a potential molecular target and biomarker for TNBC. The STAT family of transcription factors is comprised of seven members with high structural and functional similarity, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 [176,177]. All STAT proteins consist of an amino acid domain (NH2), a Coiled-Coil Domain (CCD) for binding with interactive proteins, a DNA Binding Domain (DBD), a linker domain, a SRC Homology 2 (SH2) domain for phosphorylation and dimerization, and a C-terminal Transactivation Domain (TAD) [177]. Most of these domains are highly conserved among STAT proteins and only TAD is divergent and mainly contributes to their structure diversity [178]. STAT3 was initially discovered to bind to DNA in response to interleukin-6 (IL-6) and Epidermal Growth Factor (EGF) in 1994 [179,180]. Over the past decades, STAT3 has become one of the most investigated oncogenic transcription factors and is highly associated with cancer initiation, progression, metastasis, chemoresistance, and immune evasion [181,182]. The recent evidence from both preclinical and clinical studies have demonstrated that STAT3 plays a critical role in TNBC and STAT3 inhibitors have shown efficacy in inhibiting TNBC tumor growth and metastasis. Considering that there is an unmet medical need for TNBC treatment and innovative therapeutic agents are urgently required, an in-depth understanding of the roles of STAT3 in TNBC will facilitate the development of STAT3 targeted therapeutics and pave the way for a novel TNBC treatment approach.

The oncogenic prospects of Stat3 have been noticed generally through its engagement in modulating the expression of genes associated with proliferation of cancer cells, self-renewal of stem cells and maintenance and autophagy [183,184]. Most especially, the overexpression of Stat 3 and activation in TNBC which is most often related to the initiation of TNBC,

progression, metastasis and resistance to chemotherapy and abysmal survival results. Stat 3 does not only play a role of eliciting the expression of cancer related genes, but have contact interaction and functionally ally with other oncogenic transcription factors. Example of which is the GLT1 which enhances the aggressiveness of TNBC. A recently conducted study has also realized a reduction of the Gene related to the Retinoic – Interferon- Induced Mortality 19 (GRIM-19) an integral inhibitor of Stat 3 transcription escorted by the overexpression of Stat 3 in TNBC. TCPTP plus two splice variants TC45 and TC48 have shown down- regulation in the cells of TNBC in vivo and in vitro which also plays a role in the stat 3 signaling activation [185]. A recent study revealed that acetylated Stat 3 is heightened in TNBC, leading to the methylation and inactivation of tumor- suppressor gene promoters [186]. Indeed, STAT3 has also been found to localize in the mitochondria, where it is termed mitoSTAT3 and regulates the mitochondrial functions, including electron transport chain, ATP synthesis, calcium homeostasis, and Reactive Oxygen Species (ROS) accumulation [187,188]. Moreover, mitoSTAT3 has been shown to promote breast cancer cell growth, in which the phosphorylation of Serine 727 plays a critical role [189]. Of note, several approved drugs have shown potent inhibitory effects on pSTAT3 and may be repositioned as anticancer drugs. Niclosamide, an FDA-approved anthelmintic drug was identified as a potent STAT3 inhibitor. A recent study demonstrated that niclosamide not only inhibits TNBC cell viability but also sensitizes TNBC cells to Ionizing Irradiation (IR) by blocking IR-induced STAT3 phosphorylation and activation [190]. Flubendazole, another wildly used anthelmintic agent and disulfiram, a clinical drug for treating chronic alcoholism were found to eradicate TNBC stem cells-like cells that express high levels of pSTAT3 [191,192]. Further studies showed that both drugs were able to cause TNBC cell growth arrest and apoptosis in vitro and suppress TNBC tumor growth, angiogenesis, and metastasis in vivo by inhibiting STAT3 [191,192]. Moreover, salinomycin, an antibacterial and coccidiostat ionophore therapeutic drug and metformin, an antidiabetic drug has exhibited potent inhibitory effects on STAT3 phosphorylation and TNBC cell growth in vitro [193,194]. However, further evaluation of their anti-TNBC efficacy in in vivo models is critically needed. Recent studies have disclosed that targeting STAT3 acetylation may be a potential therapeutic approach for treating cancer. SH-I-14, a newly synthesized carbazole was shown to inhibit STAT3 phosphorylation through increasing SHP-1 expression [195]. A follow-up study reported that SH-I-14 also inhibited STAT3 acetylation and disrupted DNMT1-STAT3 interaction, resulting in DNA demethylation and re-expression of tumor suppressor genes [196]. It's in vitro and in vivo activity has also been demonstrated in TNBC model, suggesting the effectiveness of inhibiting STAT3 acetylation in TNBC therapy **(Figure 2)**.

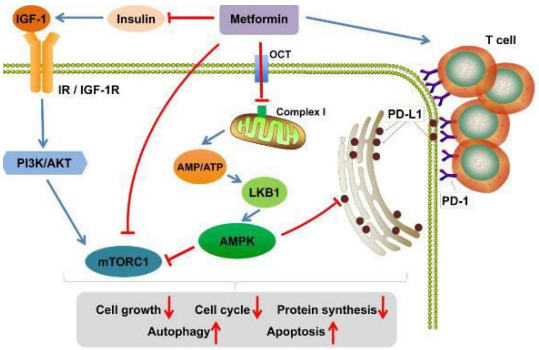

Figure 2: Mechanism of metformin to activate adenosine monophosphate activated protein kinase pathway (AMPK).

METFORMIN as inhibitor of STAT3 signaling pathway in TNBC

Metformin (1,1-dimethylbiguanide hydrochloride), the most frequently used first-line drug for type 2 diabetes worldwide, has recently been appreciated to have anticancer properties. It is widely reported to act through up regulation of Adenosine Monophosphate-activated Protein Kinase (AMPK) [197,198] the mammalian Target of Rapamycin (mTOR), the ribosomal protein S6 kinase and the eIF4E-binding protein 1.22 Metformin has been shown to decrease breast cancer risk [199-202] and improve survival in patients with breast cancer [203-206]. A retrospective, non-randomized study has recently shown that the addition of metformin to neoadjuvant chemotherapy results in a significantly higher rate of pathologic complete response [206]. Metformin has been shown to inhibit mammary carcinogenesis, growth, migration and invasion in vivo and in vitro in animal and cell line model systems [207]. On the basis of these data, several randomized trials of metformin (typically in combination with other agents) have been initiated in breast cancer patients. We have shown that metformin preferentially affects TN breast cancer cells, inducing partial S-phase arrest and apoptosis. In contrast, in other breast cancer subtypes (luminal A, B and HER2-expressing cells), it induces a partial G1 cell cycle arrest without apoptosis induction. Others have reported that metformin may selectively target breast cancer stem cells, weaken TGFβ-induced EMT and modulate cancer-associated inflammation and an immune response [207]. Given the known overexpression and activation of Stat3 in TNBC, we conducted studies to determine whether metformin might have a previously unrecognized role in down regulating Stat3 expression and/or activity in this subtype of breast cancer.

In a study conducted by Deng was revealed that metformin inhibits growth and cell signaling where they initially studied metformin's anti-proliferation /anti-survival activity against six basal breast cancers cell lines MDA-MB-468, HCC70, HCC1806, MDA231, BT20 and HCC1937 and established IC50s for each line. The result revealed that Metformin induced growth inhibition in each of the six TN cell lines with MDA 468 and HCC70 showing the greatest sensitivity.

Further studies were conducted using four representative lines treated with metformin at corresponding IC50s. Western

Blot was employed to analyze for signaling changes and it showed that Metformin impressively lowered both tyrosine and serine phosphorylation of STAT3 (P Stat 3 at tyr705 or Ser727) with modest to limited changes in Stat 3 protein expression.

Conclusion

Triple negative breast cancer has few potent targeted therapeutic options, because of its aggressive phenotype feature and its molecularly diversity. It also has strong chemo resistance. Usually, TNBC patients have worst survival rate and cancer cells have two types of effects for carbohydrate and lipid metabolism, these are "Warburg effect'' and "Lipid switch", respectively. When women are suffering from metabolic dysregulation, often they are connected significantly with cancer, especially breast cancer and TNBC. In this paper we have demonstrated that anti type II diabetic drug metformin has potent feature to reduce triple negative breast cancer risk factor, especially for TNBC patients who are obese. Usually metformin works with two main functions. One is insulin-dependent and another is insulin independent. We also mentioned so many researches where it was proved that metformin induces biological responses and it plays a vital role to reduce TNBC. Other studies demonstrated that it's mechanism to induce biological and molecular responses are responsible for reducing breast cancer as well. For inhibiting TNBC, metformin plays so many functions such as targets STAT3 signaling pathway, FASN, decreases lipid and carbohydrate dysregulation, which have significant association with breast cancer and TNBC which are highlighted in our study. Another recent study reported that metformin attenuates over twenty genes and enzymes that are responsible for cholesterol biosynthesis in TNBC. An important matter we want to highlight is that triple negative tumors are more expressive in younger and in black women. There are several studies that anticipated that African American women are affected more by TNBC. Their data demonstrated about a 27% diagnosed breast cancer patients and they were premenopausal African American. Among them, 27% were African and 25% were younger black British women. Undoubtedly, obesity has significant relation with breast cancer, but obese African American women are affected more rather than others. There are recent studies which have done clinical experiment with metformin for non-diabetic breast cancer patients. A review study reported that metformin not only works for diabetic breast cancer patients but also works for non-diabetic lung cancer, prostate cancer, endometroid endometrial cancer. A study experimented with obese non-diabetic breast cancer patients. In that experiment, 1000 mg/day metformin was more effective than placebo 500 mg/day metformin. Other randomized control clinical trial showed by giving 50 mg/day metformin for six months reduced the number of metastatic cases of hormonal therapy. For all of these studies, we are proposing that maybe metformin could be effective therapeutic drug for non-diabetic African American triple negative obese breast cancer patients.

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