Significance of Effect of Metformin on Cancer Stem Cells – "Need of an Hour" in oral cancer

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ABSTRACT

Oral cancer is a life-threatening disease. Advanced therapeutic approaches are still insufficient to improve the prognosis. Effect of Metformin on cancer stem cells (CSCs) provides promising future. Research on diverse molecular mechanisms involved in different kinds of cancers showed a definite correlation. The role in oral squamous cell carcinoma(OSCC) is not yet defines. So, it will be fruitful in future if the research on metformin and CSCs would be done in oral cancer. It would be helpful in improving the results of current antitumor treatment in oral cancer.

INTRODUCTION

Oral squamaous cell carcinoma (OSCC) is one of the most prevalent malignancy of head and neck region worldwide and counted as the sixth most common cancer in the world [1]. It is the significant cause of mortality in South Asian countries [2]. Widely used therapeutic interventions include chemotherapy, radiation followed by surgery. Since past decade, metformin is the most preferably used drug of choice for treating OSCC [2,3]. It inhibits proliferation, metastasis and progression of cancer [4]. Moreover, metformin elicits protective effect by decreasing the incidence of different tumors and helps in improving prognosis of patient [4].

Oral cancer stem cells (CSCs) represent biologically distinct subset of cells within the total malignant cell population and possess the qualities of self renewal, tumerogenesis and ability to recapitulate a heterogenous tumor [1]. Cancer stem cells (CSCs) have been reported to be more resistant to chemotherapy and radiation treatment, one of the important cause of tumor recurrence[5,6]. Research on the action of metformin on CSCs has been done on different types of cancers [7-10].It acts via diverse molecular mechanisms on CSCs and found to enhance the effect of targeted drug therapy [4].

In oral cancer, despite the newer advances in technologies and multidisciplinary interventions, survival of the patient has not improved over last 30 years and remains as one of the most lowest among the major cancer type [1]. CSCs has created a new area of research for improving the prognosis and survival of the patient. As metformin found to have effect on cancer cells, recently, research is targeted more toward the implication of metformin on CSCs. The roles of metformin in CSCs have been reviewed in various malignancies; however, its role in oral cancer is still in nascent process. Our aim of the paper is to highlight the array of action of metformin on CSCs so that it will aid in focusing on the importance and requirement of novel targeted therapies in OSCC.

Metformin in oral cancer
Metformin is biguanide derivative and known to prescribe globally as a first line of treatment for diabetes mellitus type -2 patients. It was first describe by Werner and Well in 1922 [10]. Because of its multiple actions like inhibition of

cell proliferation, induction of apoptosis, cell cycle arrest and reduction in occurrence and growth of tumor, metformin is the most common "drug of choice" for cancer patients. Thus, Many "in vitro" studies showed that metformin demonstrated an antitumor activity in variety of cancer e.g. breast, prostate, pancreas etc. [4, 7-10]. Recently, metformin is implicated in the treatment of oral cancer [11]. An "in vivo" study showed that metformin reduces the dose of chemotherapy when given in combination, and at the same time prolong the remission of tumor recurrence [9]. It was observed that head and neck cancer (HNSCC) patients who took metformin showed better and longer disease free survival [2]. Furthermore, various clinical trials have been proved that metformin inhibits EMT genes and thus causes cessation of metastasis [12].

Metformin acts via AMPK dependent, AMPK independent, JNK/p38 MAPK and NF-κB pathways. It was found that metformin has an action on AMPK pathway via cyclin D1, p21, p27, Aktand p53 factors. It causes significant decrease in cyclin D1 protein levels and retinoblastoma protein phosphorylation. It decreases cyclin dependent kinase CDK 4 and CDK6 in cancer cells [13]. Also, it was found that metformin can inhibits mTOR pathway and IGFR pathways. A study demonstrated that metformin exerts inhibitory action on mTOR pathway viaLKB1/AMPK axis [14]. Under hypoxic condition in cancer cells, metformin suppresses proliferation and enhances apoptosis by reducing HIF-1 activity. Importantly, it did not increases the expression of mutant p53 levels [15].

Experiments demonstrated that metformin prevents the development of HNSCC by reducing the size and number of carcinogen induced oral tumoral lesions. It prevents spontaneous conversion of oral premalignant lesion into OSCC. Study observed that metformin specifically acts on basal cell layer of oral squamous cell epithelium by acting as a "mTORpathway inhibitor" [16].

Metformin and CSCs in oral cancer[Table-1]
The first report of role of metformin on breast cancer CSCs was first demonstrated by Hirsch et al. in 2009 [7]. It is the significant cause of cancer relapse is due to preferential killing of more differentiated cancer cells leaving undifferentiated CSCs [17]. Metformin selectively kills CSCs in various cancers[4,17].It acts as a "CSCs sensitizer" in addition to the current anticancer therapies. It

shows specific effect on CSCs[10]. It inhibits the CSCs mamosphere formation in various types of cancer like breast, lung, pancreas etc. A study also demonstrated that metformin decreases the expression of stem cell signaling marker Notch 1 [14].

Table 1: Study done on metformin and cancer stem cells

	Study done	Cancer
1	Hirsch HA *et al.,* 2009[7]	Breast cancer -*in vivo; in vitro*
2	CuffS *et al.,* 2010[8]	Breast cancer cell lines *in vitro*
3	Iliopoulos *et al.,* 2011[9]	Breast cancer cell lines, lung adenocarcinoma, prostate
4	Bao *et al.,* 2011[4]	Pancreatic cancer

Experimental data proved that metformin acts via inihibition of mTOR signaling pathway. An "in vitro" study on breast cancer cell lines demonstrated that inhibition of mTOR pathway results in reduction in the fraction of CSCs. It selectively kills CD24 /CD44 positive cells in breast cancer cell lines [9,14].

In metastasis, CSCs found to gained mesenchymal phenotype in ectomesenchymal transition (EMT). They are localizes at the invasive front of the HNSCC tumor [1]. Metformin inhibits metastasis by acting on EMT pathways. Research has found out that metformin inhibits the EMT related genes like transcription factors like ZEB1, TWIST, Slug, and TGF-*β*. It blocks the tumor growth by selectively killing of EMT phenotypic cells [4,7,8]. In fibrosarcoma, metformin was found to suppress the migration of cells by acting on CamK-dependent Pathway [14]. An "in vitro" study demonstrated that metformin inhibited the metastasis associated protein (CD24) in triple negative breast cancer cell lines [10]. Also, it ceases the activity of CSCs by acting on NFκB, MMP-9, and MMP-2 factors responsible for migration and by decreasing Akt and Erk1/2 phosphorylation which are responsible for initiating metastasis in cancer cells [8].

Regulations of function of CSCs were found to be occurred by mitochondrial and metabolic reprogramming. Specific metabolites like high energy lactane and ketone encourages the "stemness" of cancer cells by increasing "stemness-associated genes". This process occurs by increase in oxidative phosphorylation in the CSCs. Metformin interfere with mitochondrial process and satisfy the metabolic changes caused

by ketone and lactone [10]. Also, it targets mitochondria and decreases ATP production which is the main source of "energy provider" to CSCs [17].

Array of studies proved that metformin alter the redox effect in CSCs. It inhibits NADH consumption in mitochondria by altering tricarboxylic acid cycle. By doing this, it reduces reactive oxygen species production, thus, reducing the chances of DNA damage and mutation [18]. Moreover, metformin found to modulate the levels of key regulators of stem cell function by altering multiple genes involving miRNA. MiRNA functions as an endogenous posttranscriptional gene regulator by specifically binding to the 30 untranslated regions (30 UTR) of target mRNAs to mediate protein synthesis and mRNA stability. MicroRNA expression is found to be deregulated in cancer. Deregulation of mRNA let-7 is associated with aggressiveness of cancer. It was found that in drug resistant cancer cells, metformin inhibits self renewal capacity of CSCs occurred because of reexpression of let-7 miRNA [19].

Gene EZH2 is responsible for regulation of CSCs and tumor angiogenesis. MiR-26a and miR-101 are tumor suppressor genes which represses EZH2.Study was found out that reexpression of miR-26a results in downregulation of EZH2 as well as EpCAM expression and thus showed an inhibitory role on CSCs [4].

CONCLUSION

Oral cancer is the most deleterious disease of the decade. It exhibits high chances of recurrence inspite of advanced drug therapies. Metformin provides promising effect by sensitizing CSCs and thus by inhibiting tumorosphere formation. Diverse molecular mechanism involving mTOR pathway, miRNA were found to be effective in interaction of metformin and cancer stem cells. It endowed specific response on CSCs in different cancers. The correlation of metformin and its effect on CSCs in oral cancer needs to be explore so as to gain the information about the molecular mechanisms. It would definitely provide a novel strategy for cancer treatment with better therapeutic outcome for the cancer patient.

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