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Original Research Paper

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EFFECT OF QUERCETIN ON EXPRESSION LEVELS OF MIRNA 210 AND MIRNA 155 IN AGGRESSIVE PANCREATIC CANCER CELLS UNDER HYPOXIC CONDITIONS

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ABSTRACT Pancreatic cancer is the most aggressive malignancy and leading cause of cancer deaths. Tumor hypoxic microenvironment plays an important role in disease progression and therapeutic resistance. Plant flavonoids have a promising effect in the inhibition of cancer cell proliferation. We analyze the effect of Quercetin on expression levels of miRNA210 and miRNA155 under hypoxic conditions. Quercetin treatment was significantly correlated with reduced miRNAs expression levels. Our results suggest that Quercetin treatment significantly influence hypoxia regulated micro RNAs expression levels in aggressive pancreatic cancer cells.

KEYWORDS : Pancreatic cancer, miRNA 210, miRNA 155, quercetin, PC1

INTRODUCTION

Pancreatic cancer is the leading cause of mortality worldwide ranked 7th in incidence. It is one of the most lethal neoplasms in developed countries with varied geographical incidence. According to GLOBOCAN 2020 estimates, pancreatic cancer accounts for 495773 new cases and causes 466003 deaths making it 12th most common cancer worldwide [1]. Pancreatic cancer tumors are mainly two types: pancreatic adenocarcinoma, the most common neoplasm (85% of cases) develops in exocrine glands of the pancreas and pancreatic neuroendocrine tumor (Pan- NET), occurs in the endocrine tissue of the pancreas [2]. Pancreatic adenocarcinoma has a very poor prognosis with 24% of the survival rate after diagnosis. [3] The incidence of pancreatic cancer correlates with several epidemiological factors like age, cigarette smoking and hereditary [4]

Despite having knowledge on potential risk factors and advanced diagnostic techniques for cancer detection, treatment outcome is poor and its incidence is estimated to be increased. This might be due to complex histological structure and tumor microenvironment of pancreas which promote disease progression. Tumor microenvironment consisting with stromal cell, extracellular matrix components and hypoxic regions mediate the development of tumor cell heterogeneity which further lead to aggressive and drug resistance tumor development. Several genes involving in tumor angiogenesis, proliferation and other signaling pathways regulate tumor microenvironment. Among, Hypoxia inducible factor 1 alpha (HIF1α) is master regulator of tumor hypoxic microenvironment mediates patho-physiological functions of the cells. Hypoxic tumor cells exhibit aggressive phenotype with resistance to conventional therapeutic drugs and radio therapy [5].

The aggressive nature and delay in diagnosis of pancreatic tumors along with the limitations of current drug therapies emphasize the need for the development of multidirectional therapeutic strategies. Recent studies have been revealed the crucial role of microRNAs (miRNAs) in disease development, progression, and metastasis [6,7]. Furthermore, aberrant expressions of miRNAs under hypoxic conditions have been correlated with disease development and progression, revealing the potential use of miRNAs in disease management.

Chemo therapeutic agents such as Gemcitabine had extended survival of the pancreatic cancer patients to few weeks which emphasizes the need of alternative therapeutic agents. Natural plant compounds are regarded as potential agents for cancer therapy as they have diversified actions and low toxicity nature. Moreover, combined therapeutic regimens with plant compounds and chemo-therapeutic drugs seem to be an promising strategies for disease management. However, as the hypoxic microenvironment influence therapeutic resistance, knowledge on the effect of plant compounds on new molecular markers under hypoxic conditions may give insights on novel therapeutic strategies. In this study, we aimed to evaluate the effect of Quercetin treatment on miRNAs expression under hypoxic conditions in aggressive pancreatic cell lines.

MATERIAL AND METHODS

We have selected two hypoxia regulated miRNAs (miRNA 210 and miRNA 155) to evaluate the effect of Quercetin on their expression levels under hypoxic conditions. Aggressive pancreatic cell lines AsPC1, were purchased from NCCS, Pune and cultured using MEBM/DMEM+10%FBS medium. Subcultures and passages were performed as per standard protocols [6].

Optimization of CoCl2 and Quercetin treatments on cell lines

Different concentrations of CoCl2 (100 μM, 200μM and 300μM) were prepared. 2000 cells were seeded in each well along with 100 μl of culture media in a 96 well plate. The experiment was carried out in triplicates with three different concentration of CoCl2 at three different time intervals 24 h, 48 h and 72 h to determine the IC50 value of CoCl2 on cell line. Cells without CoCl2 were used as negative control.Cells from different exposures of CoCl2 were subjected to MTT assay to calculate the rate of proliferation. 200μM CoCl2 concentration conveyed significantly reduced cell proliferation rate in comparison with control cells. Three different concentrations of Quercetin (3

μM, 12 μM, 48 μM) were prepared. Each concentration was applied on cell lines at different time intervals (0, 24, 48, 72 hours).

miRNA was isolated using mirVana kit (Thermo Fisher) and subjected to cDNA conversion. Expression analyses of miRNA 210 and miRNA 155 were assessed using Real Time PCR (ABI7500) with Sybr green method. Each experiment was carried out in triplicate and U6 used as an endogenous control.

RESULTS:

miRNA210 and miRNA155 expression in control and hypoxia induced As PC1 cell line:

Cell lines treated with CoCl2 significantly differed with respect to miRNA210 and miRNA 155 expression levels (1.02±0.02; 1.15±0.01) compared to control (Un treated cell line (0.91±0.005; 0.79±0.002) even before chemical induction of hypoxia. When the both cell lines were exposed to hypoxic condition, miRNAs expression levels were elevated as the duration of hypoxic exposure increased (Table 1). However, the elevation was prominent after 48 hrs of induction. The comparison between treated and control cell lines indicated that response was maximum after 24 hrs in normal cell line but was after 72 hrs in tumor cell line (Table 2).

miRNA210 and miRNA155 expression in Quercetin treated As PC1 cell line:

miRNA210 levels observed to be lowered during 48 hours and 72 hours of quercetin exposure at 3μM concentrations. miRNAs expression levels were not differed at 24 hours of Quercetin treatment (figure 1). However, expression levels were steeply declined at 3μM quercetin concentrations during 48 hours and 72 hours exposure. Other concentrations did not show exert influence with respect to miRNA levels in 24, 48 and 72 hours (Figure 2).

DISCUSSION

MicroRNAs are non-coding RNAs mediate gene expression by mRNA degradation or translation inhibition [8]. About the half of miRNAs are encoded by non-protein-coding sequences and remaining miRNAs are encoded on introns of protein-coding regions. These miRNAs are known as intergenic miRNA and transcribed with host genes and processed to produce the mature miRNA. These miRNAs have several patho-physiological functions and mediates various biological processes. In pancreatic cancers, they influence disease development and progression by regulating several genes involved in apoptosis, cell proliferation and metastasis. As miRNAs play an important role in tumorigenesis, several therapeutic compounds have been studied for their effect on miRNAs regulation including bioflavonoids including Quercetin.

In our study we observed that miRNA210 and miRNA155 expression levels significantly increased under hypoxic conditions. Hypoxic microenvironment influences few crucial transcription factors which regulate several genes involved in tumorigenesis. HIF-1α (Hypoxia Inducible Factor-1 alpha) is master regulator of hypoxic tumor microenvironment and act as a transcription factor, targets hypoxia response element (HRE) of several genes including miRNAs. Upon activation by HIF-1α, miRNAs expression levels may dysregulated results in disease development. Our results were in in accordance with previous study who suggested that miRNAs might have influenced by HIF-1α under hypoxic conditions [9].

Further, we treated pancreatic cancer cell lines with Quercetin a bioflavonoid under hypoxic conditions where miRNA210 and miRNA155 expression levels observed to be decreased. Previous studies have been investigated Quercetin effect on suppression of proliferation, and metastasis [10] in several cancers like pancreatic, lung, and breast cancers [11-13]. There could be two possible underlies mechanisms for Quercetin inhibitory function. Quercetin might be down regulated HIF-1α under hypoxic conditions which results in down regulation of its transcriptional targets miRNA210 and miRNA155. Du G et al showed that Quercetin inhibited intra-tumoral HIF-1α in 4T1 cells and whereas in healthy cells HIF-1α levels increased [14]. It can be attributed that Quercetin might have direct inhibitory effect on miRNAs by influencing its targeted genes. Quercetin deregulated miRNA155 by influencing its transcription factor NF-κB under hypoxic conditions. Previously, it was showed that Quercetin found to be regulated NF-κB transcriptional activity [15]

In conclusion, Quercetin treatment significantly associated with aberrant expression levels of miRNA210 and miRNA155 under hypoxic conditions suggesting that Quercetin might have the potential for down regulation of hypoxia induced miRNAs.

Table 1: miRNA210 expression in control and hypoxia induced As PC1 cell line

Duration of exposer (Hours)	Control cell line X ± S.D	d	Treated cell line X ± S.D	d	t	p-value
0	0.96±0.009		1.11±0.02		5.11	0..009**
24	1.16±0.04	0.13	1.15±0.008	0.15	6.74	0.005**
48	1.10±0.09	0.40	1.32±0.04	0.42	3.40	0.01*
72	1.19±0.007	0.15	1.42±0.008	0.22	3.49	0.02*
F test two way * p<0.05 **p<0.001	Between cell lines** Between durations**					

Table 2: miRNA155 expression in control and hypoxia induced As PC1 cell line

Duration of exposer (Hours)	Control cell line X ± S.D	d	Treated cell line X ± S.D	d	t	p-value
0	1.18±0.03		1.28±0.005		4.52	0.008**
24	1.23±0.002	0.44	1.08±0.006	0.07	3.09	0.006**
48	0.85±0.008	0.28	0.62±0.005	0.35	2.56	0.05*
72	0.71±0.002	0.33	0.38±0.002	0.23	2.17	0.01*
F test two way * p<0.05 **p<0.001	Between cell lines** Between durations**					

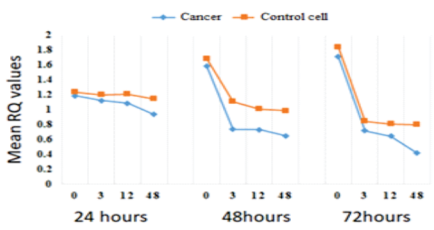

Figure 1: miRNA210 expression in Quercetin treated As PC1 cell line

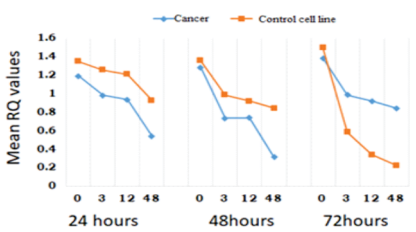

Figure2: miRNA155 expression in Quercetin treated As PC1 cell line