RESEARCH ARTICLE

Influence of *Morinda citrifolia* (Noni) on Expression of DNA Repair Genes in Cervical Cancer Cells

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Abstract

Background: Previous studies have suggested that *Morinda citrifolia* (Noni) has potential to reduce cancer risk. Objective: The purpose of this study was to investigate the effect of Noni, cisplatin, and their combination on DNA repair genes in the SiHa cervical cancer cell line. Materials and Methods: SiHa cells were cultured and treated with 10% Noni, 10 μg/dl cisplatin or their combination for 24 hours. Post culturing, the cells were pelleted, RNA extracted, and processed for investigating DNA repair genes by real time PCR. Results: The expression of nucleotide excision repair genes ERCC1, ERCC2, and ERCC4 and base excision repair gene XRCC1 was increased 4 fold, 8.9 fold, 4 fold, and 5.5 fold, respectively, on treatment with Noni as compared to untreated controls ($p<0.05$). In contrast, expression was found to be decreased 22 fold, 13 fold, 16 fold, and 23 fold on treatment with cisplatin ($p<0.05$). However, the combination of Noni and cisplatin led to an increase of 2 fold, 1.6 fold, 3 fold, 1.2 fold, respectively ($p<0.05$). Conclusions: Noni enhanced the expression of DNA repair genes by itself and in combination with cisplatin. However, high expression of DNA repair genes at mRNA level only signifies efficient DNA transcription of the above mentioned genes; further investigations are needed to evaluate the DNA repair protein expression.

Keywords: *Morinda citrifolia* (Noni) - DNA repair genes - cervical cancer - SiHa cells

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Introduction

Cervical cancer is the second most common malignancy among women worldwide (Gupta et al., 2013). Human papillomavirus (HPV) has been established as an important etiological factor for the development of cervical cancer. However, only a small fraction of women infected with HPV develop cervical cancer, implying the involvement of environmental and genetic cofactors in cervical carcinogenesis (Rosa et al., 2009).

Cisplatin (CP), an antitumor drug, is employed as a first-line chemotherapeutic agent in the treatment of epithelial malignancies, including cancer of cervix, lung, ovarian, testicular, and others (Gupta and Singh, 2013). The destruction of the cancer cells by cisplatin requires binding of the drug to DNA and the formation of platinum-DNA adducts, which may establish inter- and intra-strand DNA crosslinks, thereby inhibiting DNA replication (Su et al., 2014).

DNA repair is a defensive mechanism that copes with ubiquitous DNA damage occurring as a consequence of cellular metabolism or from exogenous exposure (El-Zein et al., 2010). Moreover, a large number of antineoplastic drugs impart their effect by DNA damage. Therefore, an effective DNA damage response is essential for the maintenance of genome stability in normal cells, whereas in malignant cells, the suppression of DNA repair would, presumably, increase the effectiveness of chemotherapy through damage accumulation and consequent apoptosis (Slyskova et al., 2012).

The nucleotide excision repair (NER) genes include ERCC1, ERCC2, and ERCC4; whereas, base excision repair (BER) gene includes XRCC1. The nucleotide excision repair (NER) pathway mainly removes bulky DNA adducts and maintains genomic stability (Min and Pavletich, 2007); whereas the base excision repair (BER) pathway is responsible for removal of oxidized DNA bases that may arise from endogenous or exogenous agents (Zhi et al., 2012).

We selected XRCC1 because deregulation of base excision repair gene expression (XRCC1) has been shown to enhance proliferation in head and neck squamous cell carcinoma (Mahjebeen et al., 2014). Evidence has revealed that expression levels of ERCC4 correlated with risk, progression, response to cisplatin chemotherapy, and clinical outcome of multiple human cancers, including head and neck cancer (Yu et al., 2012), thereby suggesting that altered ERCC4 expression may lead to altered DNA repair capacity (DRC), thereby modulating cancer susceptibility. Earlier studies have linked cisplatin resistance to the expression of ERCC1 mRNA in cell lines of cervical cancers (Bai et al., 2012). Reduced

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expression of ERCC2 has been reported in squamous cell carcinoma of head and neck (Kumar et al., 2012). These reports suggest that DNA repair genes could be involved in cervical cancer, especially as HPV is necessary but not sufficient for cervical cancer formation; and genomic instability/host genetic background often participate in its development and progression as they influence HPV acquisition and persistence (Bajpai et al., 2013).

Morinda citrifolia, commonly known as Noni, is considered to be one of the most important medicinal plants in the Hawaiian Islands (Gupta et al., 2013). Several reports have described health benefits of Noni fruit including immune modulation and antioxidant activities in vitro and in vivo (Gupta and Singh, 2013). Noni has also been suggested to mitigate oxidative damage (Wang et al., 2008); reduce the cancer risk in heavy cigarette smokers (Wang et al., 2009). It does not have a genotoxic potential and that genotoxic anthraquinones do not exist in Noni juice (Westendorf et al., 2007). It also prevents mammary breast cancer at the initiation stage of chemical carcinogenesis (Clafshenkel et al., 2012).

As accumulation of DNA damage and increased genomic instability have been proposed to play a critical role in several pathophysiological conditions associated with cervical cancer; manipulation of DNA repair mechanisms by Noni/Cisplatin may provide a strategy to prevent cellular dysfunction in the normal cell process. Hence, the aim of this study was to determine the relative expression of DNA repair genes (XRCC1, ERCC1, ERCC2, and ERCC4) at mRNA level by real time PCR, and to examine if these DNA repair genes are altered in HPV-16 positive cervical cancer SiHa cells in response to either Noni, Cisplatin, and their combination, to provide potential therapeutic targets.

Materials and Methods

cell culture and treatments

SiHa (HPV16+) cervical cancer cell line was used in this study and cultured in DMEM media, supplemented with 10% fetal bovine serum (FBS), 100 U/ml Penicillin, and 100 μg/ml Streptomycin.

Noni juice was obtained from Health India Laboratories (a unit of Noni BioTech Pvt. Ltd., Chennai, India). Cisplatin was purchased from Sigma, USA.

SiHa cells were treated with 10% Noni (v/v), 10 μg/dl Cisplatin, and their combination i.e. 10% Noni and 10 μg/dl Cisplatin for 24 hours at 37^0C in 5% CO_2 and 95% humidified atmosphere. Post treatment, the cells were pelleted and stored at -700C for various assays (Gupta and Singh, 2013).

RNA extraction

Total cellular RNA was harvested from the cell pellet using Triazol reagent (Sigma Aldrich, St.Louis USA) according to the manufacturer's instructions.

Reverse Transcription Assay

Synthesis of cDNA was carried out by using cDNA synthesis kit (Fermentas Life Sciences, New Delhi, India) as per manufacturer's protocol (Kumar et al., 2012).

Real time-polymerase chain reaction

Real Time PCR was done using gene specific primers (Table 1). The PCR was carried out in a volume of 20 µl in ABI 7500 Thermocycler using Fast Taq Polymerase (Chromous Biotech, Bengaluru, India) under the following conditions: denaturation at 94°C for 10 sec; annealing (as per primer) for 20 sec; extension at 72°C for 5 sec. The mRNA expression of DNA repair genes was normalized to the housekeeping gene 18S. The comparative CT method was used in order to evaluate the differential DNA repair gene expression in treated cervical cancer SiHa cells with respect to untreatedcontrols. For each target gene, we performed three replicates of qRT-PCR. The relative amount of each target gene to 18S was determined using the following equation:

2-ΔΔCt, where ΔΔCtgene= [Ctgene - Ct18S] Patient - [Ctgene - Ct18S] Control.

Statistical analysis

Results of each experiment represent the mean±standard deviation (SD) of three independent experiments carried out in triplicate. All the data were analysed using Student's t-test. Statistical analysis showing a value of $p<0.05$ was considered significant.

Results

Effect of Noni on the expression level of NER genes and BER gene

The fruit juice of Morinda citrifolia (Noni) is in high demand in alternative medicine for different kinds for illnesses. Several studies have also demonstrated anti-inflammatory, antioxidant, and apoptosis-inducing

Table 1. Primer Sequences of DNA Repair Genes in the Present Study

Gene name	Primers	Sequences	Amplicon size(bp)
XRCC1	Forward	5'-CAGCCCTACAGCAAGGACTC-3'	209
	Reverse	5'-GCTGTGACTGGGGATGTCTT-3'	
ERCC2	Forward	5'-GACCTGGTGTCCAAGGAACTG-3'	165
	Reverse	5'-GATCCTGAGCACCGTCTTCTG-3'	
ERCC4	Forward	5'-TGACTCCAGCACCTCGATG-3'	237
	Reverse	5'-AGTACTTGACCTGGACCACC-3'	
ERCC1	Forward	5'-TTGTCCAGGTGGATGTGAAAGATC-3'	151
	Reverse	5'-GCTGGTTTCTGCTCATAGGC-3'	
18S	Forward	5'-GTAACCCGTTGAACCCCATT-3'	145
	Reverse	5'-CCATCCAATCGGTAGTAGCG-3'	

effects of Noni in various cancers. Our results showed increased expression level of nucleotide excision repair genes (ERCC1, ERCC2, and ERCC4) and base excision repair gene (XRCC1) with Noni treatment as compared to control; and the increase was found to be statistically significant. The expression of ERCC1, ERCC2, ERCC4, and XRCC1 increased by 4 folds, 8.9 folds, 4 folds, and 5.5 folds respectivelyl ($p<0.05$) (Figure 1).

Effect of Cisplatin on the expression level of NER genes and BER gene

Cisplatin is among the most effective and widely used chemotherapeutic agent employed in the treatment of solid tumors. It is a potent inducer of cell cycle arrest, and apoptosis in most cancer cell types including human cervical cancer cell lines. Our results showed decreased expression level of nucleotide excision repair genes (ERCC1, ERCC2, and ERCC4) and base excision repair gene (XRCC1) on Cisplatin treatment as compared to control. The expression of ERCC1, ERCC2, ERCC4, and XRCC1 decreased by 22 folds, 13 folds, 16 folds, and 23 folds respectivelyl ($p<0.05$) (Figure 2).

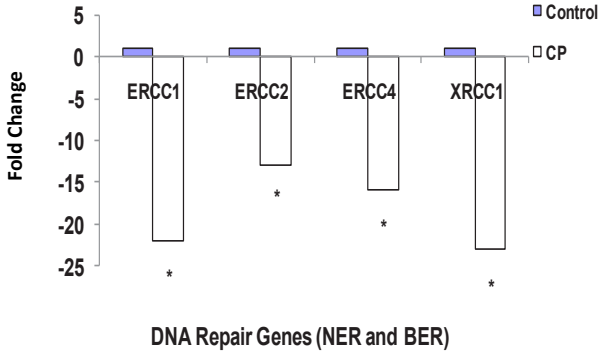

Figure 1. Effect of Cisplatin on the Expression Level of ERCC1, ERCC2, ERCC4, and XRCC1

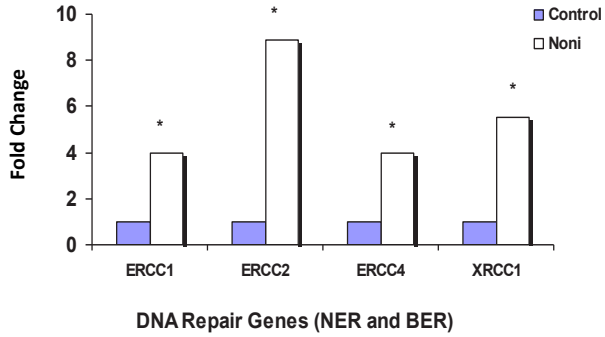

Figure 2. Effect of Noni on the Expression Level of ERCC1, ERCC2, ERCC4, and XRCC1

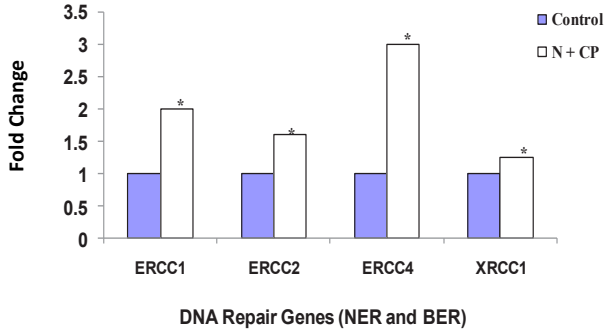

Figure 3. Effect of Noni and Cisplatin on the Expression Level of ERCC1, ERCC2, ERCC4, and XRCC1

Effect of Noni and Cisplatin on NER genes and BER gene

The combination of Noni and Cisplatin has been found to induce apoptosis through the mitochondrial pathway, decrease lipid peroxidation, and enhance Catalase activity in human cervical cancer cell lines. Our results showed increased expression level of nucleotide excision repair genes (ERCC1, ERCC2, and ERCC4) and base excision repair gene (XRCC1) on treatment with the combination of both Noni and Cisplatin, as compared to control. The expression of ERCC1, ERCC2, ERCC4, and XRCC1 increased by 2 folds, 1.6 folds, 3 folds, and 1.24 folds respectively ($p<0.05$) (Figure 3).

Discussion

Exposure to different endogenous and exogenous mutagens and carcinogens can result in various types of DNA damage. These alterations, if not repaired, can cause genetic instability, mutagenesis and cancer. Importantly, to counteract the deleterious consequences of the DNA-damaging agents, evolution has moulded a number of DNA repair systems that as a whole take care of most of the insults inflicted on a cell's vital genetic information. The repair of different types of DNA damage is important for safeguarding genomic integrity. Among the main DNA maintenance mechanisms operating in humans, the NER and BER are the primary defence against DNA damage (Li et al., 2012).

Given that cervical carcinogenesis is usually a multistep, multigenic process, it is unlikely that one individual DNA repair gene would have a significant effect on cancer risk. Therefore, single-gene studies are likely to provide limited value in evaluating cervical cancer risk. Evaluation of the combined effect of a panel of DNA repair genes that interact in different DNA repair pathways may amplify the effects of individual variation in expression level and enhance predictive power. Although reduced expression of DNA repair genes has been shown to be associated with squamous cell carcinoma of head and neck (Kumar et al., 2012) and smoking-related cancers such as lung cancer (Wei et al., 1996); few studies have evaluated the expression level of DNA repair genes in the etiology of cervical cancer. Hence, we measured the relative expression levels of NER genes (ERCC1, ERCC2, and ERCC4) and BER gene (XRCC1) in SiHa cells on treatment with Noni alone, Cisplatin alone, and combination of both Noni and Cisplatin for 24 hours. We found that Noni enhanced the expression level of ERCC1, ERCC2, ERCC4, and XRCC1 by itself and in combination with Cisplatin, suggesting that Noni might influence and enhance the expression level of these DNA repair genes. However, their expression was found to be decreased with Cisplatin as compared to control. On Cisplatin treatment, the mRNA expression level of both NER and BER decreased significantly as compared to control. This is due to the fact that SiHa cells are Cisplatin sensitive cells (Bergs et al., 2007); and hence Cisplatin forms DNA-adducts, primarily intra-and inter strand DNA adducts (Tanida et al., 2012), and activates several

signaling pathways such as p53, leading to activation of apoptosis (Gupta et al., 2013), and thereby adversely affecting DNA repair genes (Rabik and Dolan, 2007).

Some studies have suggested that low ERCC1 expression is associated with increased chemotherapeutic sensitivity and thus considered a predictive marker for patients with colorectal cancer (Ni et al., 2014). Our findings also corroborates with earlier studies which have linked cisplatin resistance to the expression of ERCC1 mRNA in cell lines of cervical cancers (Bai et al., 2012). Notably, recent clinical evidence suggests that ERCC1 levels predict response to platinum-based therapies in non-small cells lung cancer (Olaussen et al., 2007) and bladder cancer (Bellmunt et al., 2007). Reduced expression of ERCC2 has been reported in squamous cell carcinoma of head and neck (Kumar et al., 2012). Chinese hamster cell lines defective in ERCC4 were found to be hypersensitive not only to UV but also to DNA interstrand cross-linking agents (Yu et al., 2012). This could also explain why reduced expression of above mentioned DNA repair genes were seen on cisplatin treatment.

The expression of above mentioned NER and BER genes increased with Noni alone as compared to control. Phytochemicals contained in vegetables and fruits, including flavonoids and other types of polyphenolic compounds, show complementary and overlapping mechanisms of action, such as antioxidant effects, stimulation of the immune system, modulation of hormone, and regulation of gene expression in cell proliferation and apoptosis (Guarrera et al., 2007). In recent times, plant polyphenols have been the attraction as effective antioxidants (from diet or supplementation) in prevention and treatment of several diseases, including cancer (Choi et al., 2012). It has also been reported that most natural antioxidant compounds often work synergistically with each other to produce a broad spectrum of effects against free radical attack (Gupta and Singh, 2013).

In agreement with our findings, a moderate and significant upregulation of DNA repair capacity in lymphocytes has been reported after a 3-week intervention with cooked carrots (Astley et al., 2004); and several DNA repair genes were upregulated following a flavonoid-rich diet for 4 weeks (Guarera et al., 2007). Another study showed that drinking 1 to 4 oz of TNJ (Tahitian Noni Juice) daily may reduce the cancer risk in heavy cigarette smokers by blocking carcinogen-DNA binding or excising DNA adducts from genomic DNA (Wang et al., 2009).

Similarly, green tea polyphenols (GTPs) have been shown to prevent UV-induced immunosuppression by rapid repair of UV-induced DNA damage and enhancement of ncleotide excision repair genes. The DNA repair by GTPs is mediated through the induction of interleukin (IL)-12 which have been shown to have DNA repair ability (Katiyar, 2011). Thus, we suggest that upregulation of above studied genes involved in different DNA repair pathways may explain, atleast partly, the observed increase in expression of DNA repair genes induced by Morinda citriflia (Noni). Nevertheless, the few studies on the DNA repair gene, ERCC1, reported no changes in gene expression levels *in vivo* after a fruit and vegetable or antioxidant-rich diet (Møller et al., 2003).

Based on the above results, present findings also demonstrates the presence of possible interrelationship and crosstalk between Noni and Cisplatin which may be related, at least, partly to the expression of DNA repair genes in cervical cancer SiHa cell line.

In conclusion, these results showed the ability of Noni by itself and in combination with Cisplatin to increase the expression level of ERCC1, ERCC2, ERCC4, and XRCC1; and this might assist in treatment of cervical cancer patients. However, high mRNA expression of above genes only signifies efficient DNA transcription of above mentioned genes; and does not necessarily reflect the production of their functional protein. The protein expression of above mentioned DNA repair genes needs to be evaluated to determine whether these findings can be used for selection of cervical cancer patients for treatment.

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