RESEARCH ARTICLE

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Effects of *ERCC5* rs751402 Polymorphism on Oxidative Stress and the Impact of Curcumin on Catalase Activity in Breast Carcinogenesis

Malinee Pongsavee*

Abstract

Background: Breast cancer is a complex multifactorial disease and polymorphisms in nucleotide excision repair pathway are associated with the potential risk of breast cancer. Pathological processes linked to breast cancer are associated with oxidative stress. Catalase plays an essential role in cell defense against oxidative stress. Curcumin has antioxidant activity that can significantly reduce oxidative stress levels. The aims of this study were to determine *ERCC5* rs751402 polymorphism was associated with oxidative stress in breast carcinogenesis. The impact of curcumin on catalase activity for inhibiting breast cancer progression was also studied. **Methods:** The effect of *ERCC5* rs751402 polymorphism on oxidative stress was studied with different H_2O_2 concentrations in HCC 1937 cell line for 24 h and then analysed by MTT assay. The impact of curcumin on catalase activity was studied in MCF-7 cell line treated with different curcumin concentrations for 24 h and then analysed by trypan blue exclusion assay and catalase activity assay. **Results:** It showed that this polymorphism involved in oxidative stress ($p < 0.05$) and curcumin caused the antiproliferative effect by the catalase activity increase ($p < 0.05$). **Conclusion:** Our study indicated that *ERCC5* rs751402 polymorphism may contribute to the etiology of breast carcinogenesis about the failure of oxidative stress protection and lead to breast carcinogenesis. The antiproliferative effect of curcumin may be associated with catalase activity and protect breast carcinogenesis.

Keywords: *ERCC5* polymorphism - oxidative stress - curcumin

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Introduction

Breast cancer is commonest malignancy among women and foremost cause of cancer related morbidity and mortality throughout the world (Baade, 2017). Underlying mechanism of breast carcinogenesis is still not completely understood. Various genetic polymorphisms among genes responsible for DNA damage responses contribute towards cancer development and linked with proliferated cancer risk. Genes linked with DNA repair mechanisms have been considered as candidate genes for cancer susceptibility because decreased DNA repair efficiency may initiate carcinogenesis (Veronesi et al., 2005).

ERCC5 is a multi-functional gene in NER pathway. It encodes a structure specific endonuclease which catalyses 3′ incision and involves subsequent 5′ incision with the help of ERCC1-ERCC4 heterodimer. DNA excision repair, and DNA repair capacity may be changed by its functional single nucleotide polymorphisms (SNPs), which may contribute to cancer risk (Wood et al., 2005). Single nucleotide polymorphisms in coding region of *ERCC5* results in elusive alteration of *ERCC5* activity which may lead to increase cancer susceptibility. The association between SNPs in the *ERCC5* promoter (rs751402) and development of gastric cancer in a Chinese population was found (Wang et al., 2016). In addition, many reports about *ERCC5* rs751402 polymorphism showed that this polymorphism may be associated with risk of breast cancer in Thai population (Pongsavee et al., 2018) and the other countries (Zhou et al., 2017).

Oxidative stress is a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems. Oxidative stress mechanisms are also involved in the activation of cell signaling pathways, including tumor cell proliferation, increased tumor cell migration, and increased tumor cell proangiogenic factors. It plays a key role in apoptosis, mechanisms that can impact both cancer progression and metastasis. Increased reactive oxygen species (ROS) and the resulting high oxidative stress are key characteristics of malignant tumors (Parri and Chiarugi, 2013).

Cellular constituents of human body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized

*Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Rangsit Campus, Patumthani, Thailand. *For Correspondence: mapongsa@gmail.com*

by enhancing cellular defence in the form of antioxidants. Antioxidants can be categorized in multiple ways. Based on the activity, they can be categorized as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Expression of genes encoding the enzymes such as superoxide dismutase (SOD), catalase (CAT) increases the level of endogenous antioxidants. Non-enzymatic antioxidants such as curcumin, vitamin C and plant polyphenol work by interrupting free radical chain reactions. The dietary antioxidants help in disease prevention (He et al., 2017).

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four iron-containing heme groups that allow the enzyme to react with the hydrogen peroxide. It is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen. Deficiency or malfunction of catalase is postulated to be related to the pathogenesis of many age-associated degenerative diseases like diabetes mellitus, hypertension and anemia (Nishikawa et al., 2009).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the main natural polyphenol found in the rhizome of *Curcuma longa* (turmeric) and in others *Curcuma* spp. Curcumin is a principal curcuminoid of turmeric (*Curcuma longa*), which is commonly used as a spice in cooking and a yellow pigment in the food processing industry. Recent studies have demonstrated that curcumin has a variety of biological activities and pharmacological performances, providing protection and promotion of human health. *Curcuma longa* used in Asian countries as a medical herb due to its antioxidant, anti-inflammatory, antimutagenic, antimicrobial properties (Kocaadam and Sanlier, 2015).

Tumor cells have increased production of ROS, causing oxidative stress and disturbing the redox state, leading to DNA damage, mutations and altered gene expression which contributes to carcinogenesis. At the same time, cancer cells have reduced capacity to remove ROS due to altered antioxidant defense systems. However, ROS also play important roles in inducing apoptosis, implying an anticancer effect. Hence finding the right balance between ROS and antioxidant defense levels in cancer cells is important to ensure that cancer progression can be inhibited while at the same time maintaining apoptosis. For this reason, the investigation about the effect of curcumin on catalase which is the antioxidant enzyme in MCF-7 cell line was done. Therefore, we designed a study to explore the effect of *ERCC5* rs751402 polymorphism on oxidative stress and the impact of curcumin on catalase activity, in breast carcinogenesis.

Materials and Methods

Cell lines

HCC 1937 and MCF-7 cell lines were cultured in DMEM supplemented with 5% fetal bovine serum (Invitrogen, USA.), L-glutamine (5 mmol/l), nonessential amino acids (5 mmol/l), penicillin (100 units/ml) and streptomycin (100 µg/ml). These cell lines were incubated in 5% CO_2 incubator at 37°C.

Study on the effect of ERCC5 rs751402 polymorphism on oxidative stress in breast carcinogenesis

Three HCC 1937 cell culture flasks were culture for two hours in 5% CO_2 incubator at 37°C. and co-transfected with the vectors. The first culture flak was co-transfected with wild type *ERCC5* expression vector (GeneArt® gene synthesis service, Invitrogen, Germany), the second culture flak was co-transfected with *ERCC5* rs751402 expression vector (GeneArt® gene synthesis service, Invitrogen, Germany) and the third culture flask was co-transfected with pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA, USA.). Subconfluent proliferating cells in 96-well dishes were treated with different doses of H_2O_2 (Sigma Chemical Co., St. Louis, MO, USA.) conc. 100, 200, 400, 600 and 800 nM for 24 h incubation time and then assayed for MTT dye reduction, a measure of mitochondrial viability (Gerlier and Thomasset, 1986) for study on *ERCC5* rs751402 polymorphism about oxidative stress in breast carcinogenesis. The experiments were repeated three times for this assay. Cell viability was normalized to 0 dose control cells.

Study on the effect of curcumin on catalase activity in breast carcinogenesis

The MCF-7 proliferating cells were subconfluent in 96-well dishes and treated with 20, 40 and 80 µmol/L curcumin for 24 h. After 24 h incubation time, the death of cancer cells was investigated for curcumin action and catalase activity by trypan blue exclusion assay and catalase activity assay kit (Biovision Incoperated, CA, USA.) provided by the manufacturer. The experiments were repeated three times for each assay.

Statistical analysis

For study the effect of *ERCC5* rs751402 polymorphism on oxidative stress, the percentage of cell viability was calculated as mean ± SD and statistical comparison was made using ANOVA. For study the effect of curcumin on catalase action, the percentage of viable cancer cells and the catalase activity in cancer cells were test by ANOVA. $p < 0.05$ was regarded as statistically significant.

Results

The effect of ERCC5 rs751402 polymorphism on oxidative stress in breast carcinogenesis

The effect of *ERCC5* rs751402 polymorphism about DNA damage repair mechanism due to H_2O_2 which is the oxidizing agent and caused oxidative stress in cells was shown in Figure 1. The result showed that the percentage of cell viability in wild type *ERCC5* expression vector was different from the percentage of cell viability in *ERCC5* rs751402 expression vector at various H_2O_2 concentrations in 24 h incubation time ($p < 0.05$).

The effect of curcumin on catalase activity in breast

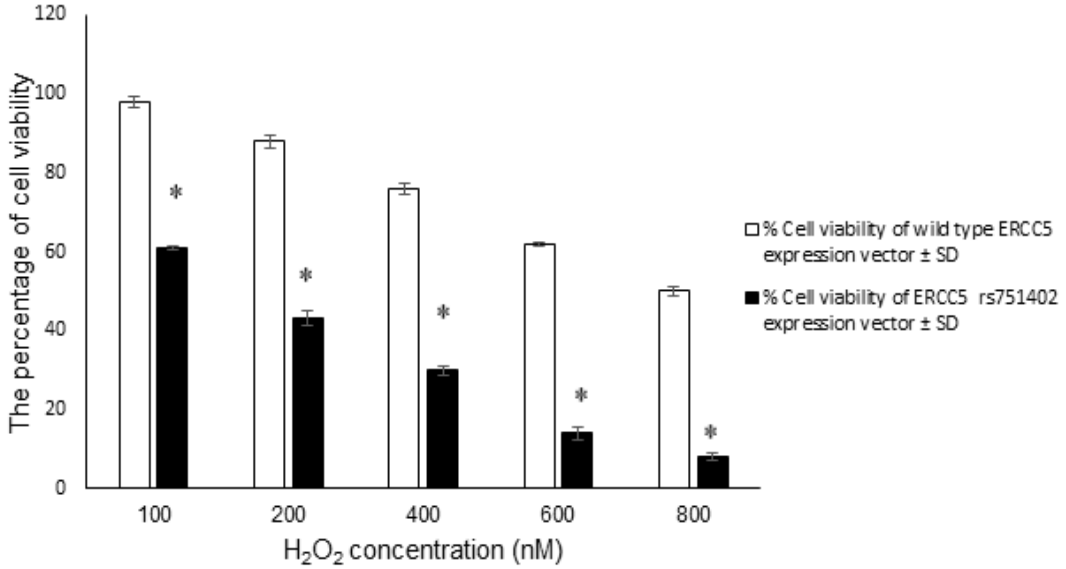

Figure 1. Comparison of Percentage of Viable Cell between Wild Type ERCC5 Expression Vector and ERCC5 rs751402 Expression Vector at Various H_2O_2 Concentrations in 24 h Incubation Time. * The percentage of cell viability in wild type ERCC5 expression vector was different from the percentage of cell viability in ERCC5 rs751402 expression vector in all various H2O2 concentrations ($p < 0.05$).

Table 1. Comparison between the Percentage of Viable MCF-7 Cells and the Various Concentrations of Curcumin in 24 h Incubation Time by Trypan Blue Exclusion Assay

The percentage of viable cancer cells	Curcumin concentrations (μmol/L)			
	0	20	40	80
The percentage of viable MCF-7 cells±SD	98± 0.2	81± 0.1*	67± 0.3*	38± 0.6*

* $p < 0.05$, p value compared with control group

carcinogenesis

The investigation of curcumin induced MCF-7 cells death was shown by trypan blue exclusion assay and catalase activity assay in Table 1 and Figure 2 respectively. The result showed that the percentage of viable MCF-7 cells was reduced when curcumin concentration increased ($p < 0.05$).

For study about the catalase activity, the result showed that the catalase activity of MCF-7 cells treated with various curcumin concentrations was difference from the catalase activity of untreated MCF-7 cells by catalase activity assay. The catalase activity was increased when curcumin concentration increased ($p < 0.05$).

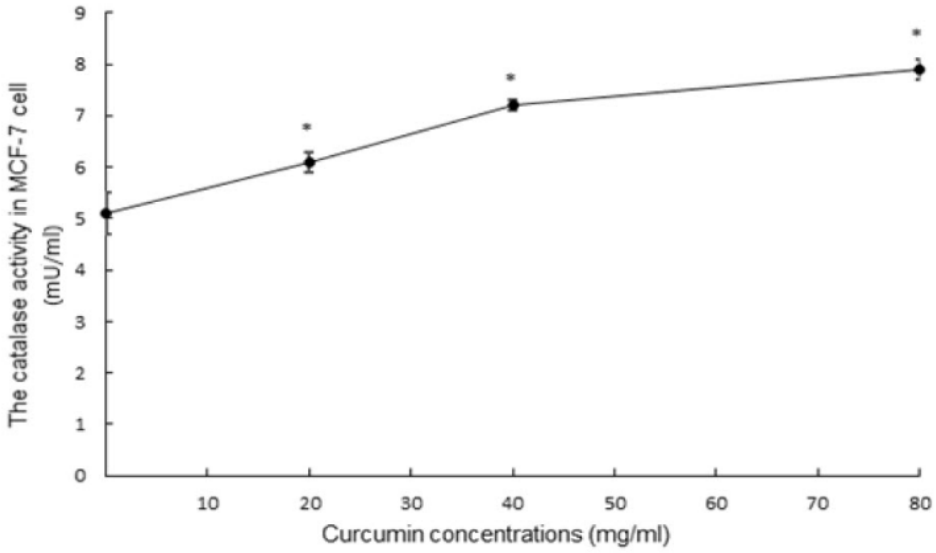

Figure 2. Comparison of Activity of Catalase in MCF-7 Cells Treated with Various Curcumin Concentrations in 24 h Incubation Time by Catalase Activity Assay. * $p < 0.05$, p value compared with control group.

Discussion

Breast cancer is the leading cause of death among female cancers. Genetic polymorphisms of genes (SNPs) involved in multiple biological pathways, including DNA excision repair, and DNA repair capacity have been identified as potential risk factors for breast cancer. There are early studies have found *ERCC5* polymorphisms to be a potential marker for a variety of cancers (Yoon et al., 2011).

Common variants in genes related to the stress pathway and DNA damage repair genes have been good candidates for cancer susceptibility and prognosis. Genetic polymorphisms of genes (SNPs) involved in multiple biological pathways, including DNA repair, have been identified as potential risk factors for breast carcinogenesis (Vurusaner et al., 2012 ; Yi et al., 2014). The association between genetic variants and the progression of breast cancer has been found (Smith et al., 2003). Certain study has additionally reported the association between *ERCC5* polymorphisms and risk of breast cancer (Pongsavee et al., 2018). Excess oxidative stress as a consequence of the alteration of the balance between reactive oxygen species (ROS) and antioxidant enzymes may lead to cellular apoptosis, proliferation and/or tumor promotion (Calaf et al., 2018). The impaired balance between prooxidants and antioxidants are suggested to be involved in induction and progression of cancer. This study showed about the relationship between *ERCC5* rs751402 polymorphism and the oxidative stress in cells. The percentage of cell viability in *ERCC5* rs751402 expression vector at various H_2O_2 concentrations in 24 h incubation time was lower than the percentage of cell viability in wild type *ERCC5* expression vector. The percentage of cell viability was correlated with the various H_2O_2 concentrations and the impaired antioxidant system balance in cells. Induction of oxidative stress in cells affected the induction and progression of breast carcinogenesis. Cells could not remove H_2O_2 and reduced oxidative stress due to *ERCC5* DNA repair function defect. *ERCC5* rs751402 polymorphism effected DNA repair function. *ERCC5* rs751402 polymorphism may involve in the induction and progression of breast carcinogenesis. *ERCC5* rs751402 polymorphism caused DNA repair function defect and lead to breast carcinogenesis in human.

The anticancer properties of curcumin were reported that it has been associated with arrest of cancer cells in S, G2/M cell cycle phase and apoptosis activation (Chauhan, 2002; Zhang et al., 2016). Curcumin was found to suppress inflammatory cytokines such as TNF-α, IKKβ kinase, IL-6, and IL-8. It also suppresses the activity of protein kinases, including protein kinase A, phosphorylase kinase, the mammalian target of rapamycin (mTOR), and mitogen-activated protein kinases (MAPKs) which play essential roles in various cellular responses, including regulation of cell growth, proliferation, division, survival and death (Unlu et al., 2016). The investigation of curcumin effect on triple negative breast cancer (TNBC), known to have poor prognosis was studied. The administration of curcumin to TNBC cell cultures was found to inhibit TNBC cell proliferation. The inhibition of EGFR (epidermal growth factor receptor) pathway was thought to be the underlying mechanism of this result (Sun et al., 2012). In another study, curcumin also showed an antimetastatic effect on breast cancer and prostate cancer (Bachmeier et al., 2007). It inhibited metastasis of prostate cancer cells by reducing CXCL1 and CXCL2 expression which play an important role in metastasis (Killian et al., 2012). The antiproliferative effect of curcumin on breast cancer cells were investigated in this study. Our study showed that the percentage of viable MCF-7 cells was reduced when the curcumin concentration increased.

Cancer cells are characterized by an increased production of reactive oxygen species (ROS) compared to normal cells and a rather altered expression of antioxidant enzymes. These characteristics represent an advantage in terms of cell proliferation. However, increased antioxidant defense which balances the oxidative status within the cancer cells suggests that high ROS levels prevention may do via various mechanisms (Scharstuhl et al., 2009). Antioxidants may inhibit carcinogenesis through other nonantioxidant action such as by modulating signaling pathways involved in cellular functions such as proliferation, cell growth and differentiation, by influencing activities of cancer-related enzymes such as cyclooxygenase-2 and phase I or II metabolizing enzymes (Aggarwal et al., 2005; Sen et al., 2012). Catalase is a key enzyme in the metabolism of H_2O_2 and reactive nitrogen species. It plays an essential role in cell defense against oxidative stress. Its expression and localization are markedly altered in tumors and there were some reports about the severe decrease of catalase activity in MCF-7 cells (Glorieux et al., 2016). Curcumin could induce the activities of superoxide dismutase, catalase and glutathione peroxidase for wound healing (Panchatcharam et al., 2006). Curcumin could elevate the activity of catalase, superoxide dismutase and glutathione peroxidase in the study about the protective effects of curcumin on macrophages under oxidative stress in vitro. It improved the capacity of cells to eliminate ROS. Low- and middle-dose curcumin could eliminate ROS, either by elevating the activity of catalase, superoxide dismutase and glutathione peroxidase or by increasing the protein level of Nrf2 (the nuclear factor erythroid 2-related factor 2) and helping Nrf2 migrate to the nucleus to regulate the expression of haemoxygenase-1 and glutamate-cysteine ligase, a catalytic subunit (GCLC). The increased ability of cells to eliminate ROS helps cells maintain resistance to oxidative stress and potentially reduces apoptosis and increases macrophage survival (Lin et al., 2019). Experimental animal studies provide precedent for curcumin stimulation of increases in antioxidant enzyme activities (Iqbal et al., 2003). Increased activity of catalase in this study implied the ability of curcumin to remove ROS and protect against oxidative damage while at the same time inhibiting cell proliferation. Curcumin may cause the antiproliferative effects through increased activity of catalase (antioxidant enzyme) which helped in maintaining the balance between ROS production and removal.

Many studies have reported positive correlation between antioxidant activities of plants and their

antiproliferative effects, suggesting the potential action of antioxidants in inhibiting cancer cell growth (Abrahim et al., 2012). Curcumin may be used as adjuvant therapy to enhance doxorubicin in human colorectal cancer cell line (Khameneh et al., 2019). Curcumin in combination therapy with 5- Flouracil may induce lower toxicity in non-malignant fibroblast cells and reduce possible side effects (Sarkhosh et al., 2018). It was observed that the use of curcumin in conjunction with other agents intended for cancer treatment. Dietary manipulations might have an important role in the prevention of numerous human cancers (Chauhan, 2002). There is a great potential to develop curcumin as chemotherapeutic agents in breast cancer treatment.

ERCC5 rs751402 polymorphism effects on oxidative stress and may lead to breast carcinogenesis. Curcumin may cause the antiproliferative effect through increased activity of catalase.

Author Contribution Statement

Malinee Pongsavee : Study conception and design, Methodology, Data collection, Analysis and interpretation of results, Manuscript preparation and approved the manuscript before submission, Funding acquisition.

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Data Availability Statement

Data are provided within the article.

Disclosure of potential conflicts of interest

The author declares that I have no competing interests.

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