Basic research

Effects of 744ins20 – ter240 *BRCA1* mutation on breast/ovarian carcinogenesis and the role of curcumin in telomerase inhibition

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Abstract

Introduction: Breast cancer is cancer which develops from breast tissue. It can also begin in the cells of the lobules and in other tissues in the breast. Invasive breast cancer is breast cancer that has spread from where it began in the ducts or lobules to surrounding tissue. Breast cancer risk is correlated with high estrogen level. *BRCA1* gene mutation increases the risk of hereditary breast/ovarian carcinogenesis. Telomerase can protect telomere shortening. It plays a key role in cancer development. Curcumin is a yellow component in *Curcuma longa* Linn. and has antioxidant properties. It has a potential role in inhibition of cellular migration or invasion and even metastasis.
Material and methods: A study on the effect of 744ins20 – ter240 *BRCA1* mutation at exon 10 on DNA repair function by MTT dye reduction and a study on telomerase inhibition caused by curcumin with the telomerase activity assay and trypan blue exclusion assay were conducted.
Results: The percentages of cell viability in mutant cells were lower than the percentages of cell viability in wild type cells at various H_2O_2 concentrations ($p < 0.05$). This mutation caused a DNA repair defect and curcumin could inhibit telomerase function and affected cancer cell progression.
Conclusions: 744ins20 – ter240 *BRCA1* mutation is involved in a DNA repair defect. It drives oxidative stress. Cancer development prevention by enhancing antioxidant defenses may be affected by this mutation, and it causes breast/ovarian carcinogenesis. Curcumin can inhibit telomerase function. Antioxidants need to be explored for the prophylaxis and treatment of hereditary and basal-like breast cancers.

Key words: mutation, *BRCA1* gene, breast/ovarian cancer, telomerase, curcumin.

Introduction

Breast cancer is cancer that develops from breast tissue. The most common type of breast cancer is ductal carcinoma, which begins in the cells of the ducts. Breast cancer can also begin in the cells of the lobules and in other tissues in the breast. Invasive breast cancer is breast cancer that has spread from where it began in the ducts or lobules to surrounding tissue. Risk factors for developing breast cancer include female sex, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first men-

struation, having children late or not at all, older age, and family history. About 5–10% of cases are due to genes inherited from a person's parents, including *BRCA1* and *BRCA2* among others. Genetics plays a more significant role by causing a hereditary breast–ovarian cancer syndrome. This includes those who carry the *BRCA1* and *BRCA2* gene mutations. These mutations account for up to 90% of the total genetic influence with a risk of breast cancer of 60–80% in those affected. Other significant mutations include *p53*, *PTEN*, *STK11*, *CHEK2*, *ATM*, *BRIP1* and *PALB2*. In the presence of BRCA1, cells could sense and repair DNA lesions, which ensures genomic integrity and prevents tumorigenesis, whereas cancer-associated *BRCA1* mutations disrupt the normal DNA damage response [1]. Most *BRCA1* gene mutations prevent BRCA1 protein production. BRCA1-mutated breast cancers are most often aggressive, high grade, aneuploid, triple-negative (for ER, PR and HER2) and are basal-like, as assessed by gene expression and immunohistochemical analysis [2].

Curcumin is a natural, non-toxic compound of a plant, *Curcuma longa* Linn., which has been traditionally used as a seasoning spice in Indian cuisine and also for the treatment of inflammatory conditions and other diseases because of its anti-inflammatory and antioxidant properties. Curcumin appears to be a potent inhibitor of cell growth or proliferation in a variety of tumor cells. It has a potential role in inhibitory cellular migration or invasion and even metastasis. Curcumin acts as a novel inducer of apoptosis or as a potent inhibitor of epidermal growth factor receptor signaling and angiogenesis [3]. Embryonic heart development is a crucial process throughout the whole growth stage, the dysfunction of which may contribute to postnatal cardiomyopathy or congenital heart disease. Curcumin mediates histone acetylation and the expression of cardiac transcription factors, ultimately controlling the progression of normal heart development. It is a protective compound against myocardial ischemia and endothelial injury [4].

Telomerase is a ribonucleoprotein that adds a species-dependent telomere repeat sequence to the 3′ end of telomeres. A telomere is a region of repetitive sequences at each end of a eukaryotic chromatid, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes. The maintenance of telomere integrity protects cells from apoptosis. Telomerase is a reverse transcriptase enzyme. The catalytic core of human telomerase consists of an RNA template and additional telomerase-associated protein. It carries its own RNA molecule (e.g., with the sequence "CCCAAUCCC" in vertebrates) which is used as a template when it elongates telomeres. Telomerase replaces short bits of DNA known as telomeres, which are otherwise shortened when a cell divides via mitosis. Telomerase activation has been observed in ~90% of all human tumors, suggesting that the immortality conferred by telomerase plays a key role in cancer development [5].

Established functional roles for *BRCA1* include the regulation of cell cycle progression, DNA damage signaling and repair, maintenance of genomic integrity, and the regulation of various transcriptional pathways, but the specific functions of the *BRCA1* gene that contribute to tumor suppression are unclear. 744ins20 – ter240 frameshift mutation at exon 10 of *BRCA1* caused breast/ovarian carcinogenesis [6]. The defect function caused by this mutation type remains unknown. Oxidative stress affects DNA repair function and high levels of oxidative stress are associated with aggressiveness in cancer [7]. Our study investigated the effect of 744ins20 – ter240 *BRCA1* frameshift mutation on oxidative stress for DNA repair function defect. Curcumin has immense therapeutic potential in a variety of diseases via anti-oxidative and anti-inflammatory pathways. Telomerase is a target not only for cancer diagnosis but also for the development of novel anti-cancer therapeutic agents. The role of curcumin in telomerase inhibition was also investigated for anti-cancer therapy in this study.

Material and methods

Study of the effect of 744ins20 – ter240 *BRCA1* frameshift mutation on DNA repair function

The *BRCA1* DNA repair defect causing failure to protect against oxidative stress was studied.

Cell lines and culture

The breast cancer cell line (HCC1937) was grown in DMEM supplemented with 5% fetal calf serum, L-glutamine (5 mmol/l), nonessential amino acids (5 mmol/l), penicillin (100 units/ml) and streptomycin (100 µg/ml).

BRCA1 expression vector, transfection and MTT dye reduction

Cells of two cell culture flasks were transfected with *BRCA1* expression vectors. Wild-type *BRCA1* expression vector (wt.*BRCA1*) consisting of the full-length *BRCA1* cDNA within the pcDNA3 mammalian expression vector (GeneArt gene synthesis service, Invitrogen, Germany) was transfected in a cell culture flask. The other cell culture flask was transfected with 744ins20 – ter240 *BRCA1* expression vector (GeneArt gene synthesis service, Invi-

Table I. Percentage of cell viability in wild type cells compared with the percentage of cell viability in mutant cells at different H_2O_2 concentrations

% Cell viability (% relative to 0 dose control)	H_2O_2 concentration [nM]			
	100	200	300	400
% Cell viability in wild type *BRCA1* expression vector ± SD	80 ±0.5	60 ±1.3	35 ±1.2	18 ±1.4
% Cell viability in mutant 744ins20 – ter240 *BRCA1* expression vector ± SD	45 ±1.5	25 ±0.3	15 ±1.2	5 ±0.7

trogen, Germany). Both flasks further underwent MTT dye reduction for *BRCA1* DNA repair defect causing failure to protect against oxidative stress. 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, 300 and 400 nM for 24 h incubation time and then assayed for MTT dye reduction, a measure of mitochondrial viability. The experiment was repeated three times for this assay. Cell viability was normalized to 0 dose control cells. The percentages of cell viability were calculated as means ± SD. Statistical comparisons were made using the *t* test.

Study of the role of curcumin in telomerase inhibition

Cell lines and culture

The breast cancer cell line (MCF-7) was grown in DMEM supplemented with 5% fetal calf serum, L-glutamine (5 mmol/l), nonessential amino acids (5 mmol/l), penicillin (100 units/ml) and streptomycin (100 µg/ml).

Trypan blue exclusion assay and telomerase assay

Subconfluent proliferating cells in 96-well dishes were treated with 40 µmol/l curcumin for 24 h incubation time. After 24 h incubation time, cells were investigated for curcumin's role in telomerase inhibition by trypan blue exclusion assay and the telomerase assay kit provided by the manufacturer. The experiments were repeated three times for each assay.

Results

Study of the effect of 744ins20 – ter240 *BRCA1* frameshift mutation on DNA repair function

The results showed that 744ins20 – ter240 *BRCA1* frameshift mutation affected DNA damage repair. The wt.BRCA1 cells were significantly more resistant to H_2O_2 but the mutant cells did not show resistance. The percentages of cell viability in mutant cells were lower than the percentages of cell viability in wild type cells at different H_2O_2 concentrations (Table I) ($p < 0.05$). It showed that wt.BRCA1 mediated protection against H_2O_2 for DNA repair.

Study of the role of curcumin in telomerase inhibition

The results showed that curcumin could inhibit MCF-7 cell progression. The numbers of living cells in curcumin-treated MCF-7 cells was lower than the numbers of living cells in untreated MCF-7 cells ($p < 0.05$). The mean numbers of living cells ± SD in this experiment are shown in Figure 1. The absorbance value of telomerase activity in curcumin-treated MCF-7 cells was lower than the absorbance value of telomerase activity in untreated MCF-7 cell (Table II).

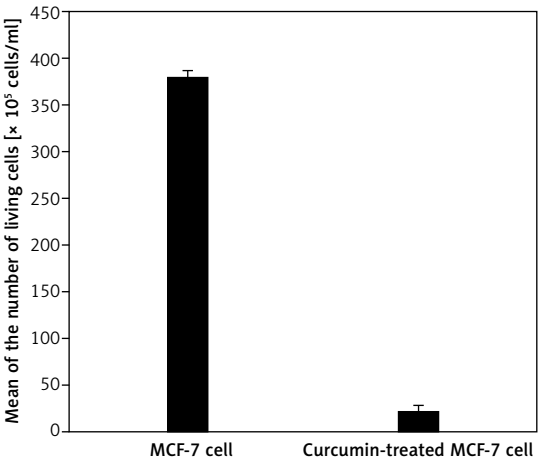

Figure 1. Effect of curcumin on MCF-7 cell death

Table II. Absorbance values compared between untreated MCF-7 cells and curcumin-treated MCF-7 cells

Type of cell	Absorbance value at 450 nm, (units) ± SD
MCF-7 cell	2.5 ±0.1
Curcumin-treated MCF-7 cell	1.3 ±0.2

Discussion

BRCA1 could be one of the key proteins in the DNA damage response. Double-stranded DNA inside the cell nucleus constantly encounters damage induced by both external and internal hazards, such as IR, UV and oxidative stress. Accumulated DNA damage will cause genomic instability and finally lead to tumorigenesis. In the presence of BRCA1, cells could sense and repair DNA lesions, which ensures genomic integrity and prevents tumorigenesis, whereas cancer-associated *BRCA1* mutations disrupt the normal DNA damage response. BRCA1 relocates to DNA damage sites and forms nuclear foci following DNA double-strand breaks (DSBs) [8]. BRCA1-deficient cells were hypersensitive to DNA damage agents and impaired DNA damage repair, further suggesting that BRCA1 plays an important role in DNA repair [9, 10]. BRCA1 can suppress the nuclease activity of MRE11 and BRCA1 is required for ATM-dependent phosphorylation of NBS1 following DNA damage [11–13]. Although the molecular mechanisms underlying BRCA1's roles in the DNA damage response are emerging, they are far from clear, and many discrepancies still exist. Oxidative stress is known to be important in the development of aging, degenerative diseases and carcinogenesis. The ability of BRCA1 to protect against oxidant toxicity may be due, in part, to stimulation of antioxidant defenses. The ability of BRCA1 to protect against oxidative stress may contribute to its caretaker function because reactive oxygen species (e.g. H_2O_2 and hydroxyl radicals) generated endogenously in mitochondria and other organelles can cause DNA damage (oxidation). *BRCA1* may prevent cancer development by enhancing antioxidant defenses (e.g. increased expression of antioxidant genes), thereby protecting cells against damage caused by exogenous and/or endogenous reactive oxygen species. Apart from established roles in the repair of DNA damage, BRCA1 may prevent DNA damage due to ionizing radiation and other sources through the detoxification of reactive oxygen species. 744ins20 – ter240 *BRCA1* frameshift mutation affected DNA damage repair. Cancer development prevention by enhancing antioxidant defenses may be affected by this mutation, and it causes breast/ovarian carcinogenesis.

Curcumin inhibits human colon cancer cell growth [14]. Curcumin modulates numerous molecular targets and exerts antioxidant, anti-inflammatory, anticancer, and neuroprotective activities. Curcumin promotes chromatin condensation, pro-survival kinase phosphorylation, PARP degradation and caspase 3 activation [15]. Telomere biology is important in human cancer. Cancer cells need the mechanism to maintain telomeres if they are going to divide indefinitely, and telomerase solves this problem [16]. Curcumin could inhibit MCF-7 cell progression. MCF-7 cells could not proliferate because curcumin inhibits telomerase function. The growth arrest induced by short telomeres may be a potent anti-cancer mechanism [17]. Curcumin might affect telomerase function.

Our study revealed that 744ins20 – ter240 *BRCA1* frameshift mutation drives oxidative stress. Antioxidants need to be explored for the prophylaxis and treatment of hereditary and basal-like breast cancers. Antioxidant therapy should be considered for future cancer prevention trials.

In conclusion, 744ins20 – ter240 *BRCA1* mutation involved in DNA repair defect and curcumin could inhibit telomerase function.

Acknowledgments

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Conflict of interest

The author declares no conflict of interest.

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