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The Effects of ERCC1 Expression Levels on the Chemo-resistance of Breast Cancer Patients Treated with Platinum-based Adjuvant Chemotherapy

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Abstract : Excision repair cross-complementing 1 (*ercc1*) is reported to be involved in the sensitivity of cancer patients to platinum-based chemotherapy. The present study to evaluate the effects of ERCC1 expression on the chemosensitivity of platinum agents in breast cancer. *ercc1* expression levels were measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The results demonstrated patients with low *ercc1* levels had chemosensitive than those with high *ercc1* levels. These results suggest that overexpression of *ercc1* is correlated with platinum drug resistance in breast cancer patients .The patients with low levels of *ercc1* expression demonstrate a benefit from platinum-based adjuvant chemotherapy.

Key words: *ercc1* ,breast cancer ,platinum chemotherapy , qRT-PCR.

Introduction

Breast cancer is by far the leading cause of cancer death in women throughout the world and its incidence continues to rise (1).

The function of ercc1 thus impacts on the DNA damage response, particularly in antitumor therapy when DNA damaging agents are employed.*ercc1* excision repair cross-complementation group 1) plays essential roles in the removal of DNA intrastrand crosslinks by nucleotide excision repair (NER), and that of DNA interstrand crosslinks by the NER pathway (2). *ercc1* expression has been proposed as a predictive biomarker of the response to platinum-based therapy (3).

Platinum-based therapy is the corner stone in treatment of cancer, and the development of tumor resistance to platinum compounds is a major clinical problem in the treatment of cancer (4). Although, the molecular mechanism of platinum resistance is complex and multifactorial, DNA repair is essential to clinical drug resistance.

The identification of molecular markers that can help guide treatment decisions in cancer is very useful to improving the therapeutic index of the current arsenal of chemotherapeutic drugs. Platinum chemotherapy, such as cisplatin and carboplatin, are part of standard chemotherapy regimens in several cancer types, including non-small cell lung cancer and colorectal cancer. Therefore, to improve upon patient survival and quality of life, the identification of a predictive biomarker profile for platinum-based

chemotherapy is essential, so that only patients that are likely to respond receive platinum chemotherapy. Platinum compounds inhibit tumor cell proliferation and induce cell death due to the formation of intracellular platinum-DNA adducts(5). These adducts consist of platinum-DNA monoadducts, platinum-DNA intra- and interstrand crosslinks, as well as DNA-protein crosslinks. Platinum-DNA monoadducts and intrastrand crosslinks can be processed and repaired by NER. Interstrand crosslinks (ICL) are repaired through the activation of ICL repair, which involves several repair systems, such as homologous recombination, translation synthesis, as well as NER (6).

Material and Methods

It was case control study involving Forty four patients with breast cancer, Patients received at least two cycles of adjuvant chemotherapy. The curative intent between January 2015 to November 2015, were included in this study and approved by the institute of genetic engineering and biotechnology. Thirty one from healthy people regarded as a control. Blood samples were collected in EDTA-containing tubes from cancer patients before or chemo-therapy, and stored in TRIzol® LS (Life Technologies,Invitrogen) until preparation of RNA extracts. RNA can be extracted with TRIzol® LS. It is always better to extract RNA from fresh sample and store it at -80 C°.Primer pairs (forward and reverse) sequence for the qRT-PCR reactions were: Primers of *ercc1* gene Forward`5 GGG AAT TTG GCG ACG TAA TTC 3´ Reverse`5 GCG GAG GCT GAG GAA CAG 3´Reference gene primer *b-actin* Forward `5 TGA GCG CGG CTA CAG CTT 3´ Reverse `5 TCC TTA ATG TCA CGC ACG ATTT 3´ They were purchased from Alpha DNA/Canada Company.

Relative quantitative analysis of *ercc1* mRNA using reverse transcription-polymerase chain reaction (RT-PCR)

The amount of total RNA was estimated by nanodrop. Complementary DNA (cDNA) was prepared by reverse transcription (The Goscript™ reverse transcription system-promega) of RNA and amplified with *ercc1* primer by using KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal /KAPA company.

ercc1 and an internal reference gene (β-actin) cDNA fragments were amplified separately by PCR in triplicates. The PCRs were carried out in a total volume of 20 µL including 2 µL cDNA, the primer concentrations were 10 µM/µL (600nM Reverse /900nM Forward) and the polymerase chain reaction conditions were with two hold steps (95°C for 3 min,) followed by 40 cycles of 95°C for 5 s and 57°C for 1 min. Reactions were set up in duplicate for each sample, and *ercc1* expressions were normalized to human *b-actin* expression.

Relative quantification was used to compare and evaluate the gene expression: The ΔCT and ΔΔ CT and $2^{-\Delta\Delta Ct}$ were calculated according to their equations ΔCt(test) = Ct(target, test) – Ct(ref, test) ΔCt(calibrator) = Ct(target, calibrator) – Ct(ref, calibrator). Second, normalize the ΔCT of the test sample to the ΔCT of the calibrator: ΔΔCt = ΔCt(test) – ΔCt(calibrator).

Finally, calculate the fold expression:

$2^{-\Delta\Delta Ct}$ = Normalized expression ratio(7).

Statistical analysis

Data analysis was performed using SPSS 13.0 for Windows. *ercc1* levels were categorized into low and high value using cutoff 1. The relationship between the mRNA, levels and clinical characteristics were assessed by Mean ±SD and χ^2.

Results

Demographic distribution

Forty four patients were included in the study. Mean age ±SD was 49.5±11.08 .Median age at diagnosis was 48.5 years with a range of 26 to 80 years.

Excision repair cross complement-1 (*ercc1*) expression

For *ercc1* gene a comparison of expression levels between patients cDNA and control yielded significantly higher expression levels in patients , 13.02±14.09 fold P = 0.000, than control 1.56±1.76 fold P = 0.0005, respectively. (Table 1) shows the level of expression either high or low, according to $2^{-\Delta\Delta Ct}$ values. It can be observed that patients have a higher fold change than control.

In this study, high *ercc1* expression was detected in (34) 77.28% patients with breast cancer. Patients with low, expression of *ercc1* were (10) 22.72%(Figure 1)

There was a significant difference between patients and control (p-value = 0.000) regarding the type level of expression (X^2 = 34.44).

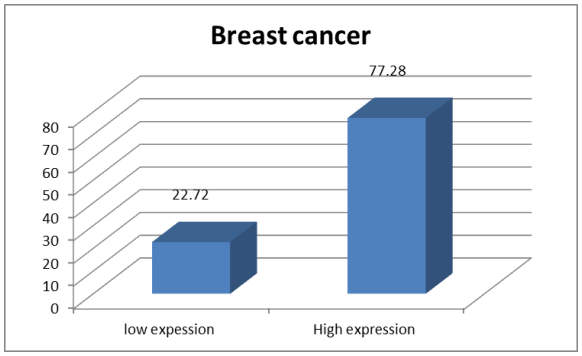

Figure (1): The *ercc1* expression level in breast cancer patients.

Discussion

The *ercc1* the major gene whose elevated expression is associated with poor response to chemotherapy (8). The main activities have been identified as potential responses that modulate the resistance. These include changes in intracellular accumulation of the drug, increased production of intracellular thiols to modulate toxicity, and increased capability of cells to repair cisplatin-DNA damage (9). Nucleotide excision repair (NER) is a major DNA repair mechanism that removes mainly DNA lesions that distort the DNA helix or form bulky injuries to the genome. Among the most affected drugs with NER activity are platinum compounds such as cisplatin, the backbone for many chemotherapy treatments of solid tumors including testicular, bladder, ovarian, head and neck, cervical, lung and colorectal cancer (10). It has been demonstrated that NER is the major DNA repair mechanism that removes cisplatin-induced DNA damage, and that resistance to platinum-based therapy correlates with high expression of *ercc1*, a major element of the NER machinery (11). Therefore, one way to improve such drugs and reduce their acquired resistance is by developing inhibitors that would regulate the NER machinery(12).Clinical studies have found that high *ercc1* expression is associated with resistance to platinum-based chemotherapy and worse prognosis in patients with advanced NSCLC. Some studies suggested that impaired DNA repair within the tumor could lead to the decreased removal of

platinum-DNA adducts and, therefore, increased clinical response to platinum chemotherapy.

The results indicate that *ercc1* was associated with response to chemotherapy. The high expression correlated with resistance to chemotherapy whereas low expression of correlated to a better response to therapy in patients with breast cancer.

Previous studies have indicated a close relationship between the expression of *ercc1* and response to platinum base chemotherapy in various types cancers, including stomach cancer and colorectal cancer (13, 14).

In the present study, a real-time PCR method was applied to target *ercc1* repair gene involved in the responsiveness to platinum base chemotherapy. Among *ercc1* transcripts have been extensively investigated in cancer by different authors and its expression inversely associated with survival of patients treated with platinum based regimens. Similarly, some investigators showed that *ercc1* mRNA high levels is associated with chemo resistance and low level is correlated with a good response to chemotherapy .

In conclusion, this study reports that low expression of *ercc1* can be used as a predictor of response to platinum-based chemotherapy in patients with cancer. This observation suggests that *ercc1* may substantially contribute to individualized cancer treatment in cancer patients.

Table (1): Compression between cases and control in Ct, ΔCt, ΔΔCt and $2^{-\Delta\Delta Ct}$

Group	No	Mean ± SD of Ct	Mean ± SD of ΔCt	Mean ± SD ΔΔCt	$2^{-\Delta\Delta Ct}$
Breast cancer	44	26.33±2.21	1.94±2.22	-2.36±2.22	13.02±4.09
Control	31	31.22±2.43	7.54±1.85	0.34±1.85	1.56±1.76

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