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THE EFFECTS OF ERCC1 EXPRESSION LEVELS ON THE CHEMORESISTANCE 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 was 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.

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, 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 nucleotide excision repair (NER). Interstrand crosslinks (ICL) are repaired through the activation of ICL repair, which involves several repair systems, such as homologous recombination, translesion synthesis, as well as NER.[6]

MATERIAL AND METHODS

It was case control study involving Forty four patients with breast cancer, who underwent platinum base chemotherapy with curative intent between January 2015 to November 2015, were included in the this study was approved by the genetic engineering and biotechnology institute. Thirty one from people apparently healthy regarded as a control. Blood samples were collected in EDTA-containing tubes from cancer patients before or chemotherapy, 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 -23 C°.

Relative quantitative analysis of ERCC1 mRNA using reverse transcription-polymerase chain reaction (RT-PCR)

The amount of total RNA was estimated by nanodrop, absorbance at 260 nm and 280 nm. 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 (600/900nM) 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 (Alpha DNA /Canada).

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 were results a significant difference between patients and control (p-value 0.000) regarding the type level of expression .chi seq was 34.44.

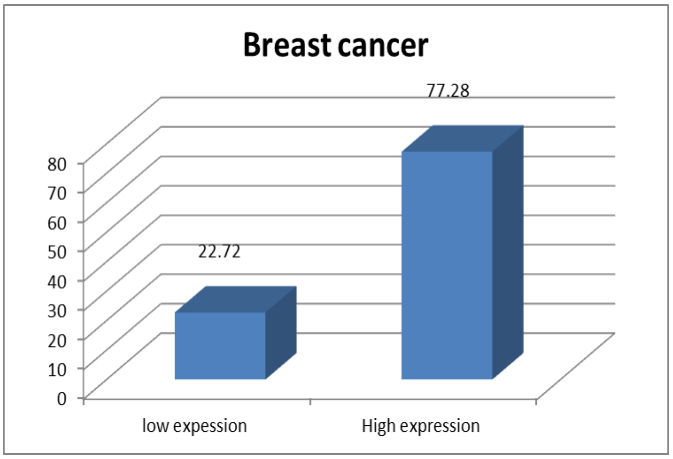

Figure 1: The ERCC1 expression level in breast cancer patients

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 Previously 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

REFERENCES

1. Yong-Gang Lv, Fang Yu, Qing Yao, Jiang-Hao Chen, Ling Wang. The role of survivin in diagnosis, prognosis and treatment of breast cancer. J Thorac Dis 2010; 2: 100-110.
2. Navnath S. Gavandea, Pamela S. VanderVere-Carozzaa, Hilary D. Hinshawb, Shadia I. Jalala, Catherine R. Searsa, Katherine S. Pawelczakc, John J. Turchi. DNA repair targeted therapy: The past or future of cancer treatment?. Pharmacology & Therapeutics 2016; 160: 65–83.
3. Martin, TC Hamilton, RJ Schilder. Platinum resistance: the role of DNA repairs pathways. Clin Cancer Res, 2008; 14: 1291–1295.

4. Chara Papadaki, Maria Sfakianaki, Georgios Ioannidis, Eleni Lagoudaki, Maria Trypaki, Kostas Tryfonidis, Dimitris Mavroudis, Efstathios Stathopoulos, Vassilis Georgoulias, John Souglakos. ERCC1 and BRAC1 mRNA Expression Levels in the Primary Tumor Could Predict the Effectiveness of the Second-Line Cisplatin-Based Chemotherapy in Pretreated Patients with Metastatic Non-small Cell Lung Cancer. Journal of Thoracic Oncology. 2012; 7(4): 663–671.
5. M Cobo, D Isla, B Massuti, *et al*. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. J Clin Oncol, 2007; 25: 2747–2754.
6. Cheryl Clauson, Orlando D. Scha¨rer, and Laura Niedernhofer. (2013). Advances in Understanding the Complex Mechanisms of DNA Interstrand Cross-Link Repair. Cold Spring Harb Perspect Biol; 2013; 5: a012732.
7. Livak, K.J. and Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 2001; 25: 402–408.
8. David Breen, Fabrice Barle´si. The place of excision repair cross complementation 1 (ERCC1)in surgically treated non-small cell lung cancer. European Journal of Cardio-thoracic Surgery 2008; 33: 805—811.
9. Shaloam Dasari and Paul Bernard Tchounwou. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014; 5: 364–378.
10. Rouillon C, White MF. The evolution and mechanisms of nucleotide excision repair proteins. Res Microbiol.; 2011; 162: 19-26.
11. Koberle B, Tomicic MT, Usanova S, Kaina B. Cisplatin resistance: preclinical findings and clinical implications. Biochim Biophys Acta. 2010; 1806: 172-82.
12. Rouillon C, White MF. The evolution and mechanisms of nucleotide excision repair proteins. Res. Microbiol. 2011; 162: 19-26.
13. De Dosso S, Zanellato E, Nucifora M, Boldorini R, et al. ercc1 predicts outcome in patients with gastric cancer treated with adjuvant cisplatin-based chemotherapy. Cancer Chemother. Pharmacol. 2013; 72: 159-165.
14. Yuanming L, Lineng Z, Baorong S, Junjie P, *et al*. BRCA1 and ERCC1 mRNA levels are associated with lymph node metastasis in Chinese patients with colorectal cancer. BMC Cancer 2013; 13: 103.