DNA Repair 11 (2012) 374–380

Contents lists available at SciVerse ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

# DNA repair gene expression level in peripheral blood and tumour tissue from non-small cell lung cancer and head and neck squamous cell cancer patients

Marina Schena[a,1], Simonetta Guarrera[b,1], Lucio Buffoni[a], Angelica Salvadori[a], Floriana Voglino[b], Alessandra Allione[b], Giancarlo Pecorari[c], Enrico Ruffini[d], Paolo Garzino-Demo[a], Sara Bustreo[a], Lorena Consito[a], Paolo Bironzo[a], Giuseppe Matullo[b,e,*]

[a] *SC Oncologia Medica, San Giovanni Battista Hospital, Turin, Italy*
[b] *Human Genetics Foundation - HuGeF, Turin, Italy*
[c] *SCDU Otorinolaringoiatria, San Giovanni Battista Hospital, Turin, Italy*
[d] *SCDU Chirurgia Toracica, San Giovanni Battista Hospital, Turin, Italy*
[e] *Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy*

ARTICLE INFO

*Article history:*
Received 27 July 2011
Received in revised form
28 December 2011
Accepted 5 January 2012
Available online 28 January 2012

*Keywords:*
DNA repair
Lung cancer
Head and neck cancer
*ERCC1*
Gene expression

ABSTRACT

*Background:* The nucleotide excision repair pathway is crucial for cellular DNA integrity and the ERCC1 helicase is also potentially involved in resistance to platinum-based chemotherapy, and high levels of *ERCC1* mRNA in tumours have been associated with cisplatin resistance in different human cancers. The aim of this work was to investigate the correlation between DNA repair gene expression levels in tumour tissue, normal tissue and peripheral blood samples from patients with two common human cancers, non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head and neck (HNSCC), to test if blood gene expression could be a *proxy* for tumour tissue gene expression to predict response to platinum-based chemotherapy.

*Methods:* Using RT-qPCR we determined *ERCC1*, *ERCC2*, *ERCC4*, *XPA*, *XPC*, *XRCC1*, *XRCC3*, *APEX*, *OGG1*, *MGMT* mRNA levels in fresh NSCLC, normal lung and HNSCC tissue, as well as blood, from NSCLC and HNSCC patients who were treated surgically.

*Results:* Target gene expression in NSCLC and HNSCC tissue was higher than in blood. A statistically significant correlation ($p < 0.05$) was found between target gene mRNA expression in tumour tissue and blood, in particular *ERCC1*, *MGMT*, *XPC*, *XRCC1* and *XRCC3* in NSCLC and *APEX*, *ERCC1*, *ERCC2*, *ERCC4*, *XRCC1* and *XRCC3* in HNSCC.

*Conclusions:* The existence of a significant correlation between blood and tumour tissue expression of some genes of clinical interest, such as *ERCC1* in NSCLC and HNSCC, could allow the introduction in clinical practice of a simple test that would measure mRNA levels of DNA repair genes in peripheral blood samples instead of tissue samples to determine prognostic and predictive factors in NSCLC and HNSCC patients.

© 2012 Elsevier B.V. All rights reserved.

## 1. Introduction

The nucleotide excision repair (NER) pathway is crucial for cellular DNA integrity. NER enzymes are able to remove DNA adducts and several types of DNA helix-distorting lesions, including tobacco-related, ultraviolet-induced photolesions, Benzo[a]pyrene Diol Epoxide (BPDE) and cisplatin-induced bulky adducts [1–3].

The NER pathway acts through damage recognition, local opening of the DNA helix, damage excision and gap-filling steps. Several enzymes cooperate in these processes: ERCC1 (endonuclease excision repair cross-complementing 1), together with XPF (*Xeroderma pigmentosum* complementation group F), performs an essential late step in the NER pathway process [4–6]. Associations between genetic polymorphisms in NER pathway genes and lung [7–14], and head and neck [15–23] cancer risk have been reported. The induction of DNA damage by covalent binding of platinum to DNA is responsible for the cytotoxicity of cisplatin [24,25]. Therefore, the NER pathway is also potentially involved in resistance

*Abbreviations:* NSCLC, non-small cell lung cancer; HNSCC, squamous cell carcinoma of the head and neck; NER, nucleotide excision repair; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RQ, relative quantification; Cq, quantification cycle.

* Corresponding author at: Department of Genetics, Biology and Biochemistry, University of Turin, Via Santena 19, I-10126 Turin, Italy. Tel.: +39 0116708450; fax: +39 0112365601.
*E-mail address:* giuseppe.matullo@unito.it (G. Matullo).

[1] Equally contributed.

1568-7864/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2012.01.003

to platinum-based chemotherapy. Indeed, higher levels of *ERCC1* mRNA in tumours have been associated with cisplatin resistance in different human cancers that are usually treated with platinum-based chemotherapy, such as non-small cell lung cancer (NSCLC) [26–28], squamous cell cancer of the head and neck (HNSCC) [29], ovarian [30–32], cervical [33] and gastric [34,35] cancers.

In developed countries, lung cancer is the most common malignancy (14–24%), and has the highest mortality rate, while squamous cell carcinoma of the head and neck accounts for about 4.5% of all malignancies (http://globocan.iarc.fr/). For both cancers, surgery is the primary intervention in early stages. In locally advanced and metastatic stages, platinum-based chemotherapy represents a fundamental treatment step. However large differences in patient response to chemotherapy highlight the need to develop predictive markers in order to select the best treatment method, be it platinum or non-platinum based chemotherapy. Several clinical trials assessed the feasibility of *ERCC1* expression level to predict response to platinum-based chemotherapy in NSCLC patients with encouraging results [26,27]. *ERCC1* expression level in tumours is usually detected from paraffin-embedded or fresh tumour specimens by reverse transcription quantitative-polymerase chain reaction (RT-qPCR) [26,27]. ERCC1 protein level can be assessed by immunocytochemistry [36,37]. However, in our experience the availability of tumour tissue from the lung is limited, as surgery and core biopsies are only possible in a minority of patients, and most diagnoses in advanced patients rely on cytological specimens obtained from fine needle aspirates. Thus, there is a need to develop affordable and non-invasive methods that can be used to detect prognostic and/or predictive biomarkers, such as mRNA expression levels of DNA repair genes in blood, that can be implemented in clinical practice. If a correlation between mRNA levels in tissues and blood was discovered it would greatly advance this development, and allow for the assessment of cisplatin resistance in NSCLC and HNSCC patients.

Therefore, the aim of the present study was to evaluate the correlation between the expression levels of several DNA repair enzyme coding genes representing the main DNA repair pathways in tumour tissue and peripheral blood.

## 2. Materials and methods

### 2.1. Patients and treatment

Patients treated in the otorhinolaryngoiatric and thoracic surgery departments of the San Giovanni Battista Hospital (Turin, Italy) were screened at NSCLC of HNSCC diagnosis. Inclusion criteria were: (1) histologically confirmed NSCLC or HNSCC; (2) age above 18 years; (3) no previous chemotherapy, radiotherapy or surgical treatment; (4) no previous history of cancer; and (5) planned surgical treatment. A questionnaire including lifestyle factors and clinical history was administered to NSCLC and HNSCC patients who met the inclusion criteria, and an informed consent form was signed by all patients before their inclusion in the study.

### 2.2. Blood and tissue collection, RNA extraction and relative quantification of gene expression levels

Two millilitres of peripheral blood were collected from each patient directly into a PAXgene Blood RNA Tube (PreAnalitiX GmbH, Becton Dickinson, Franklin Lakes, NJ, USA) for lymphocytic RNA extraction. Total RNA was then isolated by a column affinity procedure (PAXgene Blood RNA Kit, QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. A DNA nuclease treatment step was included to prevent genomic DNA carry-over.

After surgical resection, one or more sections (5 mm × 2.5 mm × 2.5 mm) of NSCLC tissue and normal lung tissue for NSCLC cases, and of HNSCC tissue only for HNSCC cases, were selected and cut by a pathologist, and immediately stabilized by embedding the tissue in the appropriate amount of RNAlater Stabilization Reagent (QIAGEN, Valencia, CA, USA) for the protection of RNA in tissue cells. After incubation, according to the manufacturer's RNA stabilization protocol, biopsies were frozen at −20 °C until further processing.

After thawing, two pieces of tissue 2.5 $mm^3$ were cut from each sample and individually processed to obtain two independent RNA samples from each biopsy. After homogenisation, RNA was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. A DNA nuclease treatment step was included.

The random primed reverse transcription of 1 μg total RNA to single stranded cDNA was achieved by using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and adding 1 U/μl final concentration of RNAse inhibitor (Applied Biosystems, Foster City, CA, USA).

Primers and probes for target gene expression analysis (*APEX*, *ERCC1*, *ERCC2*, *ERCC4*, *MGMT*, *OGG1*, *XPA*, *XPC*, *XRCC1*, and *XRCC3*) were purchased as pre-made assays (TaqMan Gene Expression Assay) from Applied Biosystems (Foster City, CA, USA), and the analyses were carried out according to the manufacturer's instructions. As a reference gene, we used β-actin (pre-made Assay Hs99999903_m1, Applied Biosystems), which in a preliminary validation test according to the method described in Applied Biosystems ABI prism 7700 SDS User Bulletin #2, 2001, proved to be suitable for the relative quantification (RQ) of mRNA, showing comparable amplification efficiency with that of the target genes (data not shown). RT-qPCR analyses were carried out on an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Three technical replicates for each sample were analysed, and a no-template control for each assay was included in all the amplification plates. Each individual RNA sample (2 biological replicates for each tissue biopsy) was independently assayed, and the mean value of the two biological replicates was used as the quantification cycle (Cq, formerly known as Ct, threshold cycle).

Real time PCR data were collected and analysed by the ABI Prism 7900 SDS Software (Applied Biosystems, Foster City, CA, USA). A RQ analysis was performed, which measures gene expression levels as: (i) ΔCq, or the difference in Cq between target genes and reference genes; (ii) as RQ ($2^{-\Delta\Delta Cq}$) according to the ΔΔCq method (Applied Biosystems ABI prism 7700 SDS User Bulletin #2, 2001), using blood as the calibrator. Thus, RQ reflects the ratio between the expression of target genes in the target tissues and in the calibrator (blood).

### 2.3. Statistical analysis

The association between gene expression levels and lifestyle factors and/or clinical characteristics was assessed by Mann–Whitney and Kruskal–Wallis tests. Variables included in the analysis were: age (above/below median value), sex, smoking habits (never/ever), alcohol consumption (yes/no), tumour stage and grade. The Wilcoxon non-parametric test was used to compare differences between gene expression levels across different target tissues and blood and the above-mentioned categorical variables. Correlation strength was assessed by the Pearson correlation test. Data analysis was performed using SPSS17 for Windows. All of the values were two-sided, and statistical significance was defined as at least $p \leq 0.05$.

Download English Version:

https://daneshyari.com/en/article/10823463

Download Persian Version:

https://daneshyari.com/article/10823463

Daneshyari.com