Biodosimetry for radiation-exposed individuals

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Abstract – Exposure of civil populations to genotoxic chemicals or radioactive hazard is an increased concern. It is not only an industrial risk (chemistry plant accident for example) but also a national security problem due to the threat of radiological and nuclear terrorism. It is important to anticipate the need of a biodosimetry tool aimed at identifying exposed population in the absence of acute syndrome, in order to assure the medical care that would prevent carcinogenic consequences. DNA repair is a biomarker of exposure to genotoxics in individuals. A DNA repair signature can be assessed from Peripheral Blood Mononuclear Cells (PBMCs) that reflects the exposure history of the individuals, using a functional enzymatic assay on biochip. A proof of concept was obtained using PBMCs from patients undergoing radiotherapy regimen. We identified two classes of responses among patients, if we except a very atypical signature in one patient that could reflect defects in DNA repair. Interestingly, repair of the major oxidative lesions increased during the course of the radiotherapy. We propose to implement this fast, quantitative, possibly automatized assay to identify biomarkers of exposure to genotoxics and to validate the exposure biomarkers through *ex vivo* exposure of blood from volunteers.

1. Introduction

Nowadays the possible exposure of populations to dispersed genotoxic chemicals or radioactive material is a real concern for national authorities. Exposed persons might initially present minimal evidence of exposure preventing any medical care that would prevent long term carcinogenic consequences of the exposure. Consequently there is a need for rapid and sensitive diagnostic tool that can 1. Identify exposed persons, 2. Estimate the exposure level, 3. Identify the nature of the genotoxic and the potential associated risk.

Cells have the ability to sense a variety of DNA lesions and elicit a coordinated response that includes activation of transcription, cell cycle arrest, apoptosis and DNA repair processes [1,2]. This global DNA damage response (DDR) is dependent on the nature of the stress and of DNA lesions induced. Genetic factors and in particular individual DNA repair capacities play also a major role in this response. Inter individual sensitivity to genotoxic stress is illustrated by the patient-to-patient variability in normal tissue response to radiotherapy. While about 70% of treated patients respond as expected, 5 to 10% present acute syndrome following radiotherapy treatments. In addition up to 20% of patients present mild sensitivity. Defective DNA repair ability is responsible for this hypersensitivity [3].

In order to gain insights into the biomarkers of exposure and on the inter-individual sensitivity to genotoxic stress, we analyzed the DNA repair signature of Peripheral Blood Mononuclear Cells of a series of patients treated by radiotherapy. For this purpose we used a miniaturized quantitative functional enzymatic DNA repair assay on support [4,5]. Thanks to the multiparametric approach, we could follow the evolution of the signature during the time course of radiotherapy and identify categories of responses. Globally our results demonstrated a specific adaptation of the DNA repair response to the genotoxic stress generated by the treatment and thus the potential value of the signature as biomarker of ionizing radiations.

2. Materials and Methods

2.1 Sample preparation

About 15 patients with various cancer types were recruited at Grenoble University Hospital by the radiotherapy service. At each radiotherapy application, the patients received a total irradiation dose of 2 Gy.

For each patient, we collected one blood sample before the first radiotherapy application (S1) and three samples after (S2 to S4) (one day and one week after application 1, and one at the end of the radiotherapy protocol. These four samples could be analysed for 11 donors.

Blood (7 mL) was collected on CPT tubes (Beckton Dickinson) and the PBMCs were isolated as recommended by the supplier. Nuclear extracts were prepared as described previously. Protein content was determined using micro BCA kit (Interchim).

2.2 Repair assay

We used a multiplexed oligonucleotide (ODN) cleavage assay on support to monitor cleavage efficiency of glycosylases contained in the PBMCs extracts toward several emblematic base lesions. Hence, we focused on initial steps of Base Excision Repair (BER), that is to say the combined action of glycosylase and AP endonuclease or AP endonuclease alone, leading to cleavage of the altered bases.

Each well contained a control-ODN, and 8 lesion-containing ODNs, all in duplicate: 8oxoG paired with C, A paired with 8oxoG, T mispaired with G in a CpG context, hypoxanthine in front of a T, Thymine Glycol paired with A, tetrahydrofuran (THF), as AP site substrate equivalent, paired with A, Uracil paired with G on the one hand and with A on the other hand and EthenoA oppposite T. Each lesion containing ODN was labelled with a Cy3 that was eliminated upon cleavage by the repair enzymes. Measurement of residual fluorescent signal allowed quantifying the percentage of cleavage of each lesion that determined the repair signature for each sample (Figure 1).

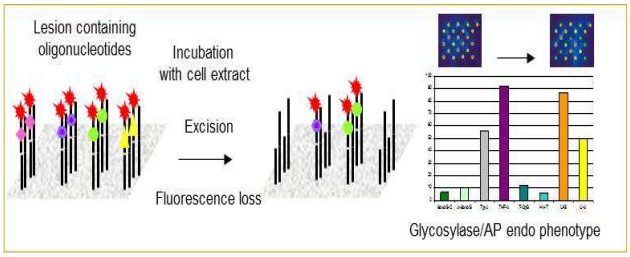

Figure 1 : ODN cleavage assay on support. Cleavage of specific substrates (lesions) reveals associated specific BER enzymes.

Human glycoslases/AP endonuclease with their associated substrates are displayed in Table 1.

Human enzymes		Substrates
Uracil DNA Glycosylases	UNG, SMUG1	U/A, U/G
Alkylbase Gycosylase	MPG	Methyladenine, Hypoxanthine
Adenine Specific Mismatch Glycosylase	MYH	A in 8oxoG/A
DNA glycosylases for oxidized bases	NTH1 OGG1	Thymine Glycol, 8oxoGuanine
AP Endonuclease	APE1	Abasic site (THF)

Table 1: Human glycosylases/AP endonucleases and associated substrates

2.3 Result analysis

Data set (percentage of cleavage) were first standardized per day of sampling. Then unsupervised hierarchical clustering was used to explore the structure of the dataset, to describe and visualize the relationship between the different treatments and the different cell lines. The analysis was performed using the free software environment for statistical computing and graphics R (http://r-project.org/). The hierarchical average linkage clustering algorithm was run with the Euclidean distance, which aggregates profiles with both similar intensity levels and covariation. Results were displayed as heat map.

In the first dimension, samples were clustered by similarity of their DNA repair signature covariation. In the second dimension, lesions' repair was clustered by similarity of their pattern covariation across the samples (Figure 2).

The Wilcoxon test was used to compare inter day repair levels and the p-value was adjusted by a Bonferroni correction.

3. Results

Clustering analyses of the results demonstrated a great heterogeneity of responses among the patients. Interestingly, this heterogeneity decreased between S1 and S4 where only 2 classes of patients remained, if we except one patient that exhibited an atypical DNA repair phenotype.

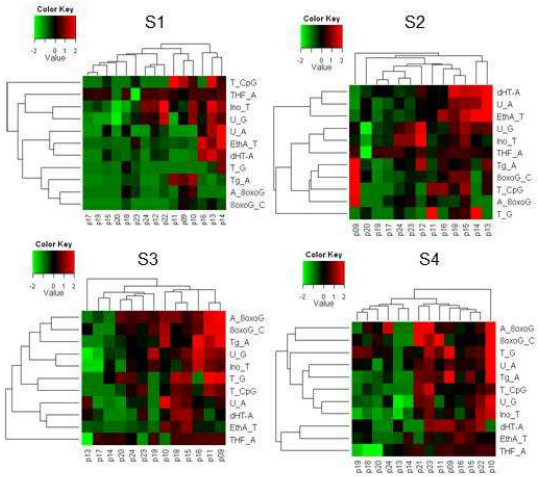

Figure 2 : Analysis of the DNA repair signature of the patients by sampling day using hierarchical clustering, displayed as a heat map.

Statistical analysis revealed that repair of hallmarks of oxidative damage, that is to say, 8oxoguanine (presumably by OGG1) was significantly higher for S2 and S3 compared to S1. Similarly, repair of A opposite 8oxoguanine (presumably by hMYH) was higher for S3

and S4 than for S1. The same feature was observed for repair of Thymine Glycol (S3>S1) and for the T/G mispair (S4>S1).

4. Discussion

Ionizing radiations generate Reactive Oxygen Species (ROS) responsible for the majority of the base lesions formed. BER is the predominant pathway that eliminates this damage [6]. Cells respond to radiation by inducing specific glycosylases/AP endonucleases. OGG1 recognizes predominantly the oxidation products of purines, of which 8-oxoguanine. MYH that removes A opposite 8-oxoguanine is considered as a back-up system. Another important enzyme is NTH1 that recognizes a wide spectrum of oxidation products of pyrimidine such as thymine glycol.

Nevertheless, this response is affected by genetic factors and by complex gene-environment interactions [7]. For example, age and life style have marked effects on the regulation of BER enzymes [8]. BER is also regulated through post-translational modifications. Therefore investigating BER enzymes at functional level is particularly relevant to gain insight into the individuals' organism defense. We showed here that patients receiving radiotherapy could be classified according to their repair response. A large DNA repair signature heterogeneity probably initially reflected a high inter-individual variability in DNA repair capacities [9]. After three treatments, DNA repair signatures were classified in only two major groups. In addition after one to two treatments, globally the glycosylases, effectors of antioxidant defenses, were up-regulated showing the consistency between the nature of the stress and the cell response.

We have already shown that ultra-violet radiation specifically impact the repair pathway that takes in charges photoproducts. Therefore the DNA repair signature properly reflects the nature of the genotoxic stress and could be considered as a specific biomarker of exposure [10].

Among the patients tested, we identified an atypical profile. This feature could be associated with a particular sensitivity of this patient to ionising radiations. Indeed, further studies conducted on large cohorts are needed to determine if DNA repair signature is an effective predictor of toxicity.

5. Conclusion

We showed that a minimally invasive assay can be used to establish the radiation-induced functional DNA repair signature of individuals. Repair of oxidative lesions increases either due to the direct effect of the treatment or possibly to the inflammatory consequences of irradiation. The DNA repair signature reveals combined effects of both extrinsic and intrinsic factors in which genetic heterogeneity of patients plays also a role.

We propose to implement this fast, quantitative, possibly automatized assay to identify biomarkers of exposure to genotoxics and to validate the exposure biomarkers through *ex vivo* exposure of blood from volunteers. The feasibility of this approach has already been successfully demonstrated using gene expression endpoints [11].

Adverse reactions after chemotherapy could rely on the same genetic bases than adverse effects of radiotherapy as chemotherapeutic drugs are DNA-damaging agents [12]. Specific DNA repair signatures relying on the mechanisms of action (MOA) of drugs can be obtained for drug-treated cells [13]. Then we can assume that a specific repair pattern could also be obtained for genotoxic compounds treated samples that would give an indication of the MOA of the compounds.

Consequently our versatile approach, under the form of a biochip functionalized by a variety of DNA lesions, might prove useful to identify biomarkers of exposure to different sorts of genotoxics and might possibly be applied to detected exposed persons and susceptible individuals in case of genotoxic hazard. In the future, it might become a general biodosimetry tool.

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