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A DNA Repair Enzyme Signature to characterize the DNA Damage Response of patients treated for Head and Neck Cancer

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PREUVE DU CONCEPT CANCÉROPÔLE LYON AUVERGNE RHÔNE-ALPES

EACR European Association for Cancer Research

Hôpitaux de Lyon

CHU Grenoble

INTRODUCTION

The therapeutic approach for about half patients afflicted with cancer entails radiation and chemotherapy. These treatments can be associated with tumor resistance over time and about 5-10% of treated patients develop acute toxicity after therapy. Defining sub-group of patients according to their sensitivity/resistance is one of the main developments for increasing therapeutic effects.

Radiotherapy

Chemotherapy

OVER TIME appearance of

RESISTANCE to anticancer treatments

or

TOXICITY to anticancer treatments

Intrinsic tumors resistance and individual sensitivity are determined by both the ability of chemo/radiotherapy to induce DNA damage and cells capability to sense and repair DNA lesions.

DNA Lesions

Signaling → **DNA Repair**

crosstalk – interaction – overlap

DNA Repair : a direct involvement in radiotherapy and chemotherapy failure (toxicity, resistance)

Functional, Multiplexed and Quantitative Assays able to investigate the complexity of DNA repair are needed for identification of markers able to predict the occurrence of adverse effects

OBJECTIVES

- Identify predictive biomarkers of **Radio and Chemo Toxicity**
  Starting from **blood samples** of patients afflicted with Head and Neck Cancers (ChemRad Clinical assay)
- Identify predictive biomarkers of **Radio and Chemo Resistance**
  Starting from **tumor biopsies** of patients afflicted with Head and Neck Cancers (ChemRad Clinical assay)

→ The DNA Damage Response profile characterizes the effective individuals DNA repair capacity

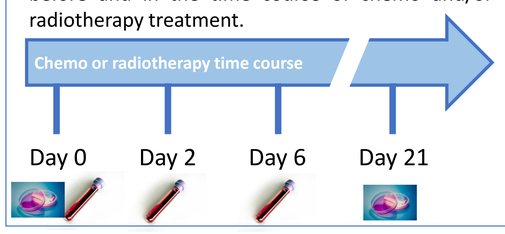
→ Patients stratification → Personalized Medicine

MATERIALS AND METHODS

We proposed innovative biochip-based technologies to investigate the contribution of several DNA repair pathways, simultaneously.

STEP 1: SAMPLES COLLECTION

Blood samples and tumor biopsies of patients afflicted with head and neck cancers were collected before and in the time course of chemo and/or radiotherapy treatment.



STEP 2: PROTEINS EXTRACTION

The cell extract prepared from Peripheral Blood Mononuclear Cells (PBMCs) and tumor biopsies contains all DNA repair enzymes.

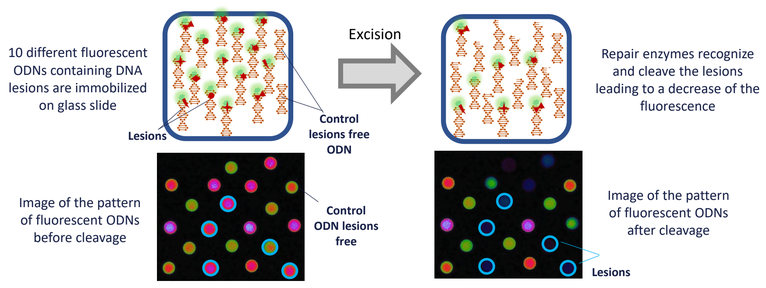
DNA Damage Response
DNA Repair
Dynamic process
> 150 proteins

- NER — Nucleotide Excision Repair
- BER — Base Excision Repair
- ICLR — Intra-Cross Link Repair

STEP 3: FUNCTIONAL ANALYSIS

Multiplexed biochips are functionalized with a panel of specific DNA lesions which are repaired by the enzymes present in the cells extract.

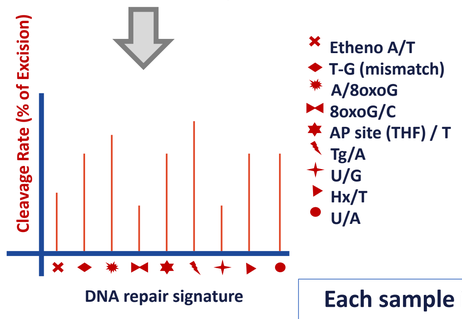
1. Glyco-SPOT: Multiplex Oligonucleotide Cleavage Assay



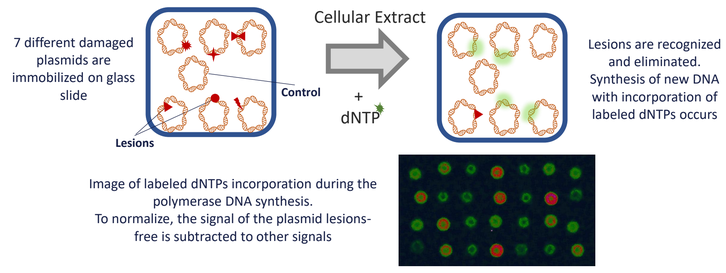
Fluorescence analysis

Glyco-SPOT quantifies the excision activities toward lesions repaired by the glycosylases and AP endonucleases

BER



2. ExSy-SPOT: Multiplex Excision Synthesis Repair Assay



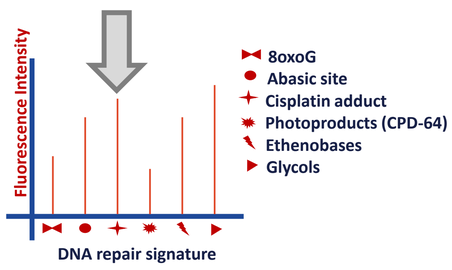
Fluorescence analysis

ExSy-SPOT quantifies the excision/synthesis activities toward lesions repaired by different DNA repair pathways

BER

NER

ICLR

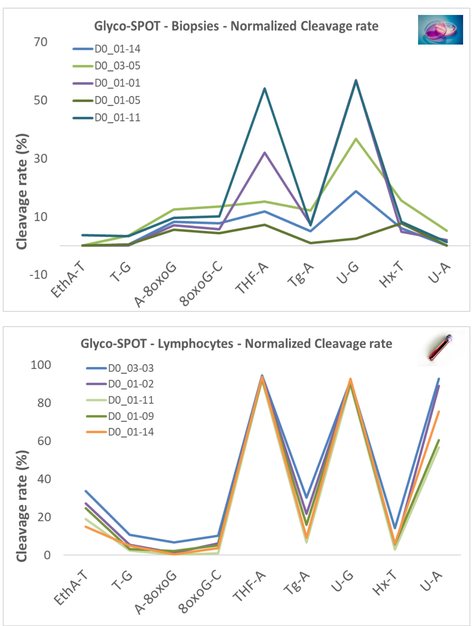


Each sample is characterized by a highly specific DNA Repair Signature

RESULTS

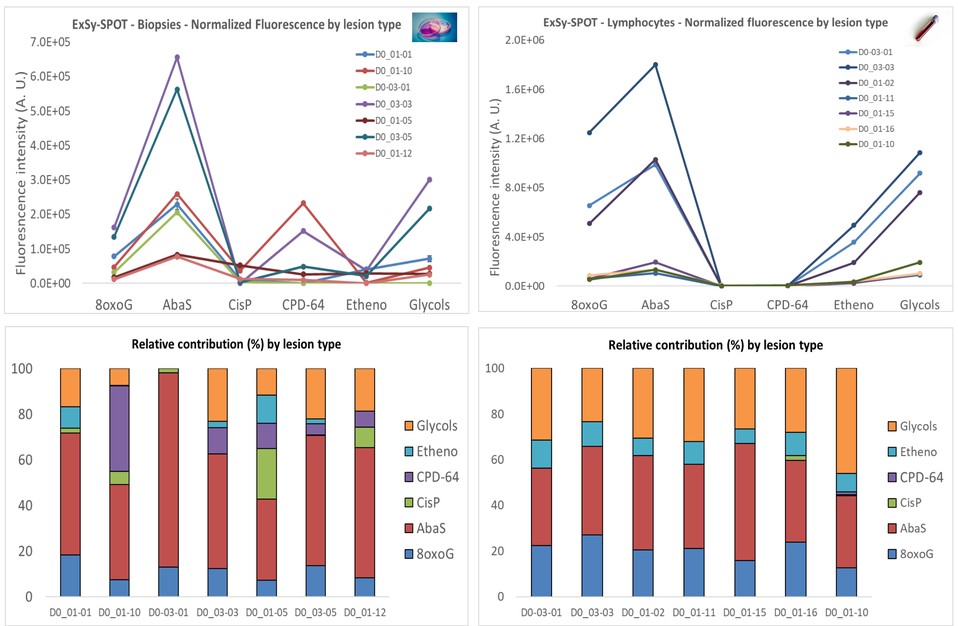
The preliminary results of this approach show an inter-individual variability in DNA repair capacities at basal level in both PBMCs and tumor cells.

1. Glyco-SPOT

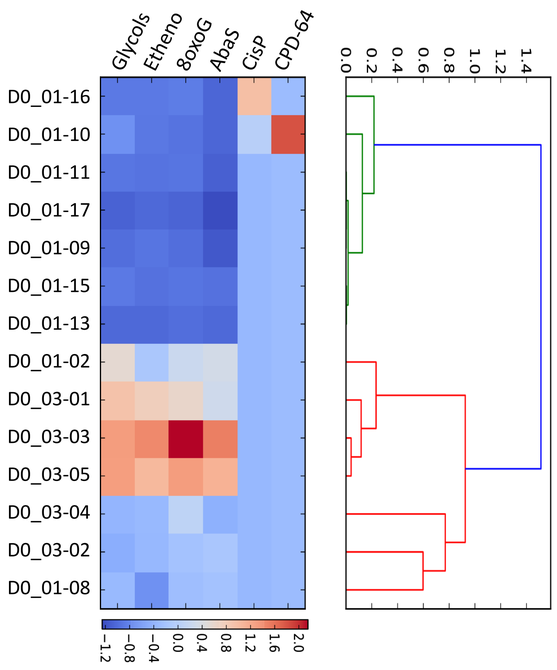


The cleavage rate for each type of lesion present on the biochip is shown. Several cell extracts obtained from biopsies and lymphocytes at the Day 0 of treatment are analyzed.
The AP endonuclease and glycosylase activities are specific for each sample with pronounced differences in the cleavage of U-G and U-A lesions.

2. ExSy-SPOT



An high inter-individual variability is shown by the enzymes Excision/Synthesis activity of samples obtained from biopsies and lymphocytes at the basal level (Day 0 of the treatment). This high variability is more evident for the re-synthesis of abasic sites, photoproducts, etheno adducts and glycols.
The relative contribution of re-synthesis of different lesions is unstable in the case of samples obtained from biopsies whereas is almost constant in the case of samples obtained from lymphocytes.



Hierarchical clustering algorithm with correlation dissimilarity measure groups in a color-coded grid the different patients at Day 0 as a function of their repair profile. Patients are clustered by similarity of their DNA repair response profile in two main groups with various subgroups. These preliminary data have to be completed with data obtained 2, 6 et 21 days after treatment.

PERSPECTIVES

The preliminary results show an inter-individual variability in DNA repair capacities at basal level in both PBMCs and tumor cells.
To analyze any change in the DNA repair ability of tumors or patients each DNA repair signature will be compared with data obtained after 2, 6 and 21 days after treatment.
We want to correlate these experimental data with the clinical data at the end of the study in order to

- Classify tumours based on their real DNA repair capacities
- Identify patients at risk of adverse effects
- Refine mutation-based classification to define personalized treatments