Impact of radiotherapy on PBMCs DNA repair capacity: use of a functional repair assay on support

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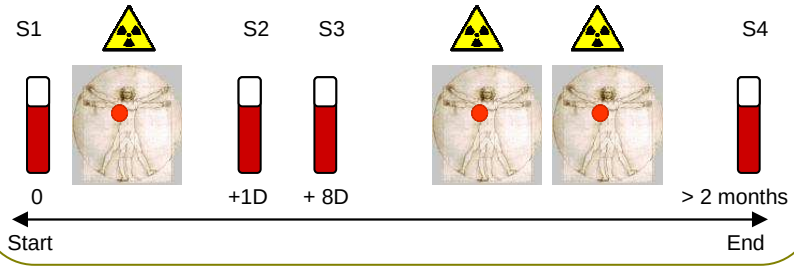
Project

Radiation therapy is an essential part of cancer treatment as about 50% of patients will receive radiations at least once. Significant broad variation in radiosensitivity has been demonstrated in patients. About 5-10% of patients develop acute toxicity after radiotherapy. Therefore there is a need for the identification of markers able to predict the occurrence of adverse effects and thus adapt the radiotherapy regimen for radiosensitive patients. As a first step toward this goal, and considering the DNA repair defects associated with hypersensitivity radiation syndromes, we investigated the DNA repair phenotype of patients receiving radiotherapy. More precisely, we used a functional repair assay on support to follow the evolution of the glycosylases/AP endonuclease activities of PBMCs extracts of a series of patients during the time course of radiotherapy.

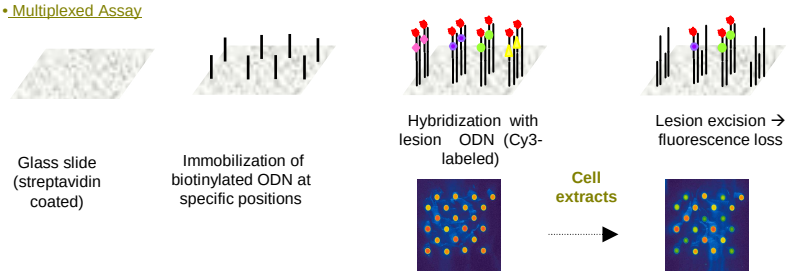
• Radiotherapy regimen and blood sampling



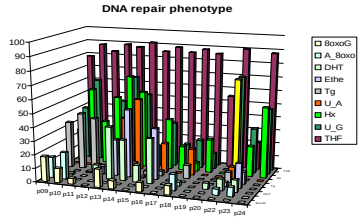
• Patients – Experimental conditions

- Patients with various cancer types were treated by radiotherapy at CHU Grenoble in the Service of Pr J. Balosso. Blood sampling was perfomed under the supervision of Dr M. Rastkhah.
- 7 ml of blood were collected using CPT ™ tubes (BD Vacutainer).
- Nuclear extracts were prepared from the recovered PBMCs.
- Protein content was determined.
- Experiments were performed using 20 µg/ml protein and the excision reaction was conducted on the biochip for 1 h at 30°C.

• Multiplexed Assay

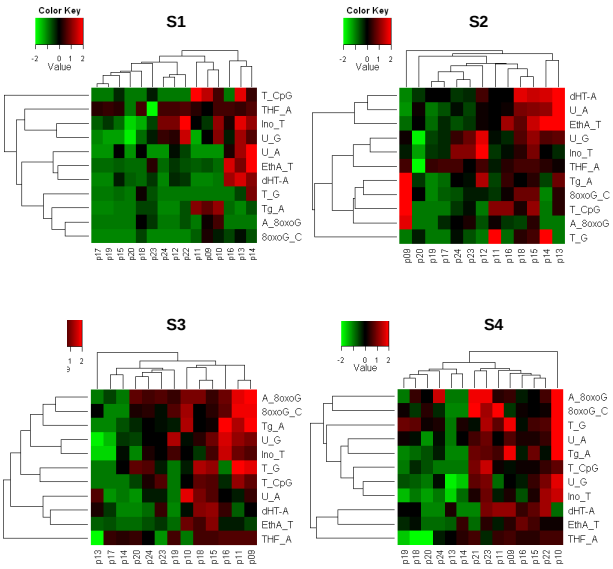


Fluorescence quantification
Percentage of excision of lesions



The Base Excision Repair pathway (Glycosylases and AP endonuclease): a defense against oxidative genotoxic attacks

Substrate	Human Enzyme	Lesions Origin
8-OxoG (opp C)	hOGG1	Oxidation
Thymine Glycol	NTH1, NEIL1	Oxidation
U (opp G)	UNG1,2	Deamination
U (opp A)	SMUG1	Misincorporation
T (opp G)	TDG	Replic Errors
A (opp 8-oxoG)	MUTYH	Replic Errors
Hypoxanthine (Inosine)	MPG	Deamination
EthenoA	MPG	Alkylation
T (opp G in CpG)	MBD4	Deamination
Abasic Site	APE1	Oxidation



• Data treatment

Data (percentage of excision) from the whole set were normalized (mean=0, SD=1) and a hierarchical clustering was perfomed on the data sorted per day. Non transformed data were compared between days using the Wilcoxon test.

Impact of Radiotherapy

• on patients profiles
The number of patient classes tended to decrease with increasing number of irradiations. At the end of the regimen, only 2 classes were found. A patient with an atypical profile was also identified (p10)

• on DNA Repair activities
8oxoG_C (hOGG1): S2 and S3 > S1
A_8oxoG (MUTYH): S3 and S4 > S1
T_G (MBDH): S4 > S1
Tg_A (NTH, NEIL1): S3 >S1

Conclusion

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. Furthermore, we showed that repair of several oxidized bases significantly increased between S1 and S3 or S4 (8oxoG, thymine glycol, A paired with 8oxoG), suggesting an adaptation of patients repair systems to the oxidative stress generated by the ionising radiations.
In conclusion, our preliminary results provided evidence that the DNA repair phenotype was impacted by the radiotherapy regimen.
Further characterization of patients with known repair defects are needed to determine if atypical repair phenotypes could be associated with radiotherapy complications.
Finally, correlation with clinical data would be useful to identify the parameters responsible for the stratification of patients in two sub-classes

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