DNA Repair Enzyme Signature reveals subtypes of responses to targeted therapies in melanoma cell lines

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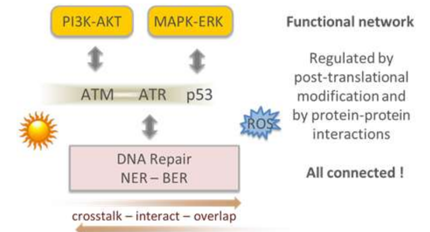
Introduction - Objectives

Melanoma is classified according to mutations in specific genes. About 50% of melanoma carry activating mutation in the BRAF or NRAS genes. BRAF/MEK inhibitors elicit a transient effective response but resistance rapidly develops through various pathway activating mechanisms.

Because kinases belong to a complex network of interacting proteins and enzymes, regulated by post-translational modifications, the functional significance of the mutations remains uncertain. UV irradiation which is the major risk factor for melanoma affects DNA repair pathways. Notably, enzymatic DNA Repair mechanisms are regulated by the MAPK/PI3K/AKT signaling pathway.

We hypothesized that effective inhibition of the MAPK pathway should translate into modifications of DNA Repair capacities. Inversely paradoxical activation of the signaling network could also be reflected at the DNA Repair level. Consequently measurement of DNA Repair capacities could be used to test how cells respond to targeted therapies.



To gain insights into that hypothesis and to further explore the relationship between DNA Repair capacities and melanoma, we monitored simultaneously the activity level of several major DNA Repair pathways before and after treatment of melanoma cell lines with BRAF and MEK inhibitors, alone and in combination.

Materials and Method

- Assay Workflow

Melanoma cells (ATCC)
↓
Vemu (V)
Cobi (C)
Cobi+ Vemu (CV)
↓
Cell extracts
↓
Functional DNA Repair assay on biochip
↓
DNA Repair Enzyme Signature

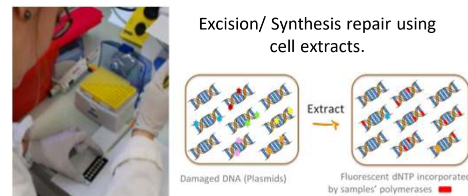
- Cell treatment conditions

Melanoma cell lines	Mutation	Vemurafenib (V)	Cobimetinib (C)	V + C
A7	WT	15 µM	5 µM	15 µM + 5 µM
CHL-1	WT	8 µM	0.03 µM	4 µM + 0.01 µM
M18 ; SK-Mel 2	NRASQ61R	8 µM	0.03 µM	4 µM + 0.01 µM
MZ2	NRASQ61K			
A375 ; Colo829 ; HT-144 ; Malme-3M ; SK-Mel 5; SK-Mel 24 ; SK-Mel 28	BRAFV600E	0.3 µM	0.005 µM	0.1 µM + 0.003 µM

Note that CHL-1 are sensitive to Cobi

Triplicates were prepared for each conditions and tested in duplicate on the microarray

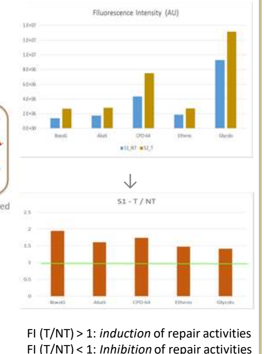
- ExSy-SPOT functional assay



Repair pathway investigated :
Base Excision Repair (BER), Nucleotide Excision Repair (NER)

- UV-induced lesions (NER)
- Oxidative damage (8oxoG, Glycols) (BER)
- Alkylated bases (BER)
- Abasic sites (BER)

- Data Analysis

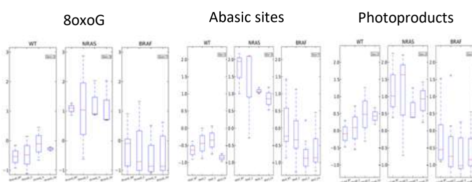


FI (T/NT) > 1: *induction* of repair activities
FI (T/NT) < 1: *Inhibition* of repair activities

Results

- Impact of the treatment by mutation group for each repair pathway

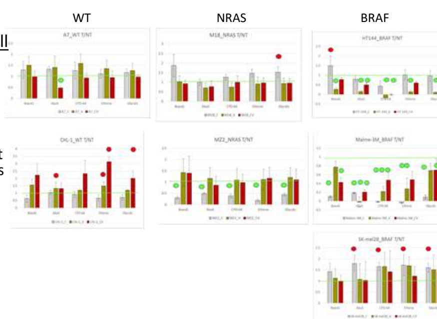
Normalized data (mean:0; SD:1) – Non Treated (NT), C, V, CV



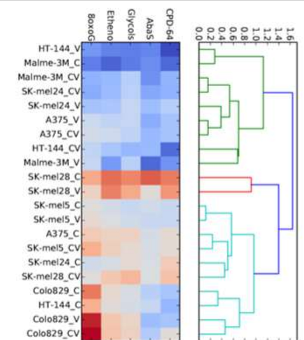
Kruskal-Wallis test – All data

- **NRAS > BRAF**
All repair pathways
$p<0.05$
- **NRAS > WT**
All repair pathways except Photoproducts and Glycols
$p<0.05$
- **WT > BRAF**
Repair of Photoproducts
$p=0.074$

- Results by cell line (T/NT) (some examples)



3 categories of BRAF cells were distinguished

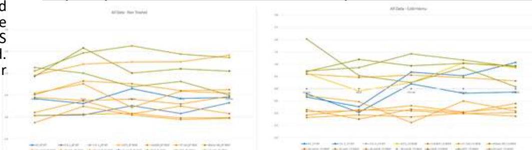


Effect of the treatment on specific repair pathways according to mutation groups (Nemenyi's test)

Repair Pathway	NT		V		C+V	
	Difference	p-value	Difference	p-value	Difference	p-value
8oxoG			NRAS >BRAF	0.026	NRAS >BRAF	0.031
AbaS			NRAS >BRAF	0.022	NRAS >BRAF	0.049
	NRAS >WT	0.035				
CPD-64			NRAS >BRAF	0.056	NRAS >BRAF	0.046
Etheno			NRAS >BRAF	0.038		

Globally, when the cells were treated by Vemu and by Cobi+Vemu, the difference between BRAF and NRAS cells repair pathways increased. V and CV affects essentially repair activities of the BRAF mutated cells.

- Repair profile of each cell line – Impact of CV treatments



Linkage : average
distance : euclidean

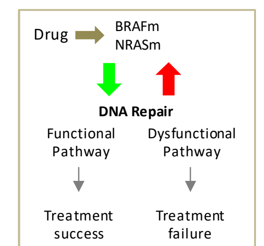
Conclusion

BRAF mutations and NRAS mutations affect the DNA Repair signature (BER-NER) demonstrating a functional link between the signaling pathways and the DNA Repair pathways.

- WT cells: CV induced an unexpected increase of CHL-1 repair activities possibly reflecting a paradoxical activation of the signaling pathways and potential toxicity.
- NRAS cells: C treatment drastically decreased repair activities of one NRAS cell line (MZ2), revealing an effective functional impact on this cell line only.
- BRAF cells: 3 BRAF cells categories were distinguished according to the treatment effects on DNA Repair (down – moderate – up regulation).

Drug efficiency is normally associated with down regulation of the signaling pathways, which we believe is translated at the DNA Repair level. Consequently we propose to use the DNA Repair Signature as a predictive biomarker of drug effect.

This fast assay is adapted to profiling clinical samples (tumors, lymph nodes) and characterizing drug efficiency.



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