Use of multiplexed oligonucleotide cleavage assay on support for selection of Glycosylase/AP endonuclease inhibitors by robotic screening of chemical library

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Introduction – Aim of the project

Many studies link chemo-resistance and over-expression of DNA repair enzymes. Enzymes from Base Excision Repair pathway and in particular apurinic endonuclease-1 (APE1) are attractive targets for anticancer drug development as therapeutic modulation by these enzymes could potentiate effects of genotoxic drugs.

The aim of this project was to bring the proof-of-concept that a multiplexed oligonucleotide (ODN) cleavage assay on support could be used together with commercial HeLa extracts to identify effective DNA *N*-glycosylase/APE1 inhibitors.

Workflow

Multiplexed assay
Selection of immobilized lesions
Selection of HeLa nuclear extract concentration

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Selection of screening positive control
(reference inhibitor: C+)

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Automation of multiplexed assay
Adaptation to screening platform
96-well microplate format

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Training on the platform
Assay performance
Pre-screen (80 compounds)

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Library Screening
1500 compounds (small molecules)
HeLa extract 6 µg/ml + Drug 250 µM

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Confirmation screening
40 pre-selected compounds
HeLa extract 6 µg/ml + Drug 80 and 250 µM

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Validation of hits
Repair activity inhibitory dose/response
9 compounds
Concentration range 15 µM - 250 µM
HeLa 6 µg/ml and 20 µg/ml

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Search for enzymatic target:
4 compounds: C+, I1, I2, I3
Calculation of IC50
(Inhibitory concentration)
hAPE1, hNeil1, hSMUG, hAAG, *E. coli* UDG

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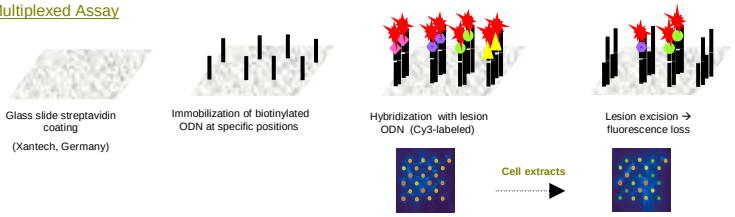
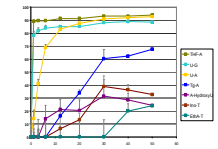
Cell based assays:
Cytotoxicity tests (MTT), sensitization experiments
Cell lines: HeLa, MCF-7, HCT-15, HCT-116
Drugs: CDDP, $KMNO_4$, MMS, 5FU
Inhibitors: C+, I1, I2, I3

• Multiplexed Assay

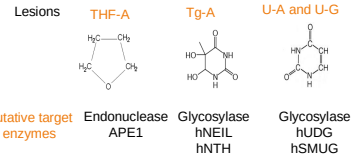


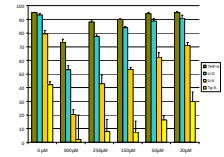
Fluorescence quantification
Percentage of excision of lesions

• Selection of nuclear extract working concentration • Immobilized lesions • Hycanthone as screening positive control



HeLa nuclear extracts (CilBiotech, Belgium)





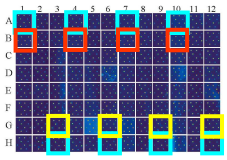
• Adaptation of the assay to the screening platform

Microplate hardware (Arraylt®) compatible with screening platform format

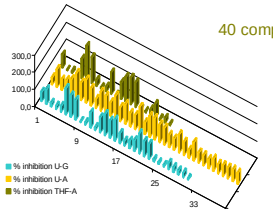
• Assay configuration for the screening

Negative control (DMSO)
Positive control (Hycantone 250 µM)
Positive control (Hycantone 1.5 mM)

Each plate contained 80 molecules and 16 controls

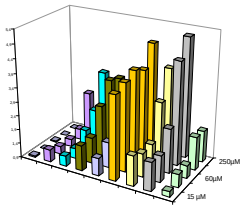


• Pre-selected compounds: inhibitory activity on 3 lesions



40 compounds

• Validation of hits: concentration-dependent inhibition of cleavage acti



9 compounds

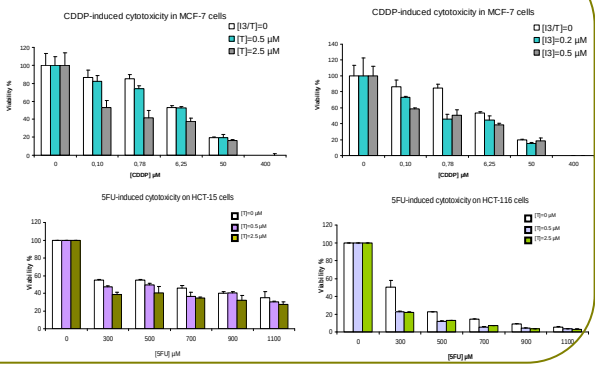
Shown for Tg cleavage

• Determination of IC50 on pure enzymes

Enzyme	C+	I1	I2	I3
hAPE 1	10 µM	60 µM	-	-
NEIL 1	> 100 µM	> 100 µM	> 100 µM	10 µM
hSMUG 1	-	-	> 10 µM	> 60 µM
hAAG	-	-	10 µM	600 µM
UDG *E.coli*	-	60 µM	600 µM	600 µM

Microarray + confirmation PAGE

• Combined effect of genotoxic drugs and inhibitors on cytotoxicity (Partial result



Conclusion

We have adapted a cell-free assay measuring DNA repair activities from Base Excision Repair to a high-throughput screening platform (CMBA, CEA Grenoble). Advantages of cell-free based assays are their simplicity compared to cell-based assays. The multiplexed ODN cleavage assay was easily amenable to automation. Additional advantage of the multiplexed approach was that we gather information on the cleavage activity of DNA repair enzymes toward different lesions simultaneously, resulting in a gain of time. We could identify several potentially interesting hits using complex nuclear extracts as targets containing medium. Among the 4 selected hits, two were already identified as DNA repair enzyme inhibitors (C+ and I1), confirming the interest of the approach. Indeed our findings require further validation on pure putative targets as well as complementary cellular assays to check the effective capacities of the identified molecules to potentialize the cytotoxic effects of genotoxic drugs. Cytotoxicity tests showed that combining the identified 4 hits at low concentration (µM range) with genotoxic drugs was an interesting strategy, although results were highly cell-type dependent, probably because of cell-line genetic background differences and existence of alternative DNA repair pathways.

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