A Novel Assay to Predict Cancer Resistance to Cisplatin

Lacy Brame[1], Vengatesh Ganapathy[1], Ilangovan Ramachandran[1], Lurdes Queimado[1-5]

Departments of [1]Otorhinolaryngology, [2]Cell Biology and [3]Pediatrics; [4]The Oklahoma Tobacco Research Center and [5]The Peggy and Charles Stephenson Cancer Center, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA.

Introduction

- Cisplatin is widely used as chemotherapy drug that induces DNA damage and ultimately triggers apoptosis. However, therapeutic resistance and tumor relapse remains a significant clinical problem.
- Recently, our laboratory developed an assay (Fig. 1) called primer anchored DNA damage detection assay (PADDA) that screens genomic areas for DNA damage[1]. PADDA has been shown to detect a dose-dependent increase in DNA damage caused by genotoxic agent (Fig. 2).
- We hypothesized that PADDA will discriminate the ability of cancer cells to repair damage induced by cisplatin, and therefore predict cancer resistance to cisplatin.

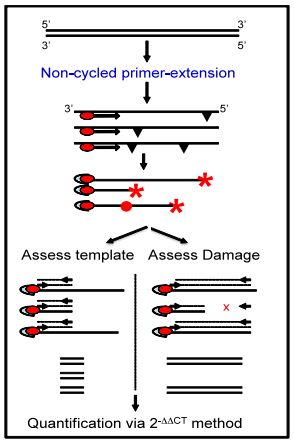

Figure 1. Diagram of PADDA. A single strand-specific non-cycled primer extension performed with a 5'-biotin-tagged primer and Vent exo- DNA polymerase identifies damaged nucleotides (inverted triangles), and generates a pool of highly specific biotin-tagged extended products, each of them derived from one strand of a single DNA molecule. Each extended product has a stop, which represents replicative arrest by a damaged nucleotide or nick. Some extended products will contain misincorporations that represent polymerase lesion by-pass with misincorporation. After several purification steps, the strand-specific, biotin-bound extended products can be used for damage quantification on a high throughput setting q-PADDA).

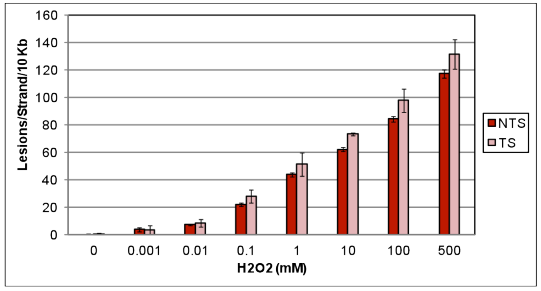

Figure 2. Quantification of induced DNA damage after *in vitro* exposure to a dose escalation of H_2O_2. Strand-specific DNA damage was quantified by q-PADDA. Lesion frequency was estimated via Poisson equation. NTS, non-transcribed strand; TS, transcribed strand; Data represents Mean ± S.E.M

Aim

To define the levels of DNA damage induced at p53 nucleotides by cisplatin treatment and to measure the ability of cancer cells to repair damage induced by cisplatin.

Materials & Methods

PADDA was used on a high-throughput setting to quantify DNA damage in human oral cancer cells (SCC-1) exposed to different doses of cisplatin. Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Data was analyzed by Student's *t*-test.

Results

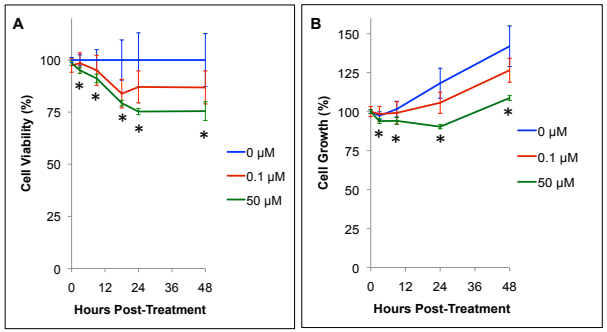

Figure 3. Cell viability assay and cell growth. SCC-1 cells were treated with 0 μM, 0.1 μM, 50 μM concentrations of cisplatin and allowed to repair damage for 0, 3, 9, 18, 24 & 48 hour time intervals. Cell viability (A) and cell growth (B) were determined by MTT assay. Data shown as Mean ± S.D. * $p<0.01$.

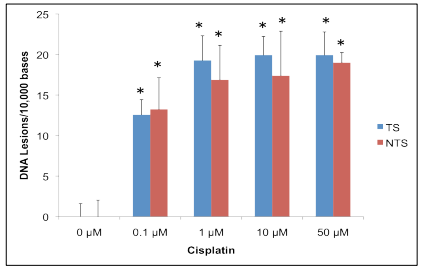

Figure 4. DNA damage measured by q-PADDA in SCC-1 cells exposed to cisplatin for 3 hours. Damage was quantified by q-PADDA in both transcribed (TS) and non-transcribed (NTS) strands. Data shown as Mean ± S.E.M. * $p<0.01$.

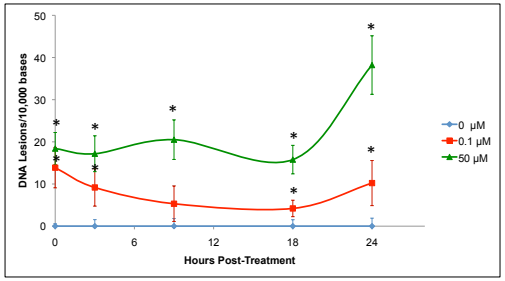

Figure 5. DNA damage measured by q-PADDA in SCC-1 cells after exposure to cisplatin. SCC-1 cells were treated with 0 μM, 0.1 μM, 50 μM concentrations of cisplatin and allowed to repair damage for 0, 3, 9, 18 & 24 hour time intervals. Data shown as Mean ± S.E.M. * $p<0.01$.

Conclusion & Societal Impact

- PADDA was able to quantify DNA damage and repair after cisplatin treatment. This information will allow us to determine if resistance to cisplatin is due to effective damage removal or to damage tolerance. This data would facilitate the development of strategies targeting the mechanism of drug resistance.
- This observation has **significant clinical importance** as it can be used to predict treatment response and direct treatment selection in cancer patients.

Future Directions

- This assay has potential to elucidate the differential efficacy of cisplatin as a chemotherapy drug and act as a preliminary screening method to determine differential cisplatin resistance.
- This project can be extended to determine the genotoxicity and resistance of cisplatin in other head and neck cancer cell lines.
- PADDA can be used to determine if patients will respond or become resistant to not only platinum-based chemotherapy treatments, but also to other treatments that induce DNA damage.

Acknowledgement

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References

[1]Reis AM, Mills WK, Ramachandran I, Friedberg EC, Thompson D and Queimado L. Targeted detection of *in vivo* endogenous DNA base damage reveals preferential base excision repair in the transcribed strand. Nucleic Acids Res. 40(1): 206-219, 2012.