Single-Cell and Bulk ICP-MS Investigation of Accumulation Patterns of Pt-based Drugs in Cisplatin–Sensitive and –Resistant Cell Models

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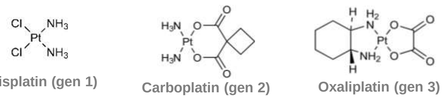
Introduction

What are Pt-based drugs & cisplatin?

- Used to treat various cancers (*e.g.,* ovarian, lung, colorectal, bladder, etc)
- Prominent Pt-based drugs include:



Cisplatin (gen 1) | Carboplatin (gen 2) | Oxaliplatin (gen 3)

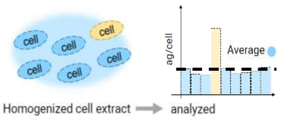
- But, clinical usefulness decreased due to high incidence of *chemoresistance*

Why study accumulation patterns?

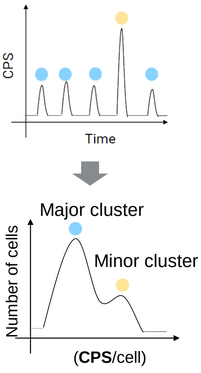
- Resistance mechanisms include:
  1) ↓ Cellular drug accumulation *
  2) Detoxification system (↑ deactivation by thiols)
  3) DNA repair
  4) Apoptosis regulation
  5) Autophagy (self-digestion of drug)

How is ICP-MS relevant?

- Quantification of Pt + other elements
- Agilent ICP-MS modes include:
  1) Conventional bulk analysis
     ⇨ measures Pt mass in overall cell population → limited



2) Single-cell (SC) analysis
   ⇒ measures #cells with Pt + single-cell Pt mass
   ⇒ reveals heterogeneity + intercellular distributions



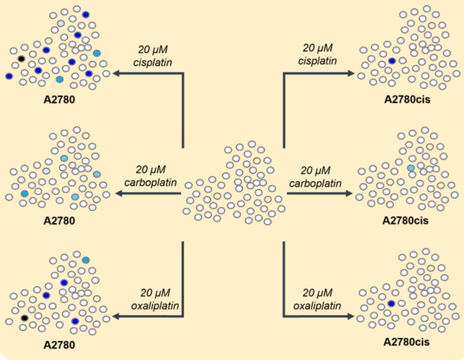
Project-at-a-Glance

Motivation & Significance

Aims

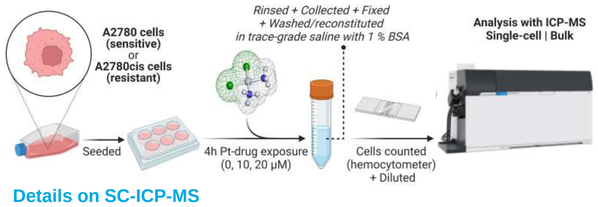
- Investigate accumulation patterns of 3 Pt-drugs in ovarian cancer cell models
- By applying *emerging single-cell ICP-MS technology* *
- Laterally capitalize on quick bulk analysis of other elements
- To ↑ understanding of *(1) Pt-drugs' behavior* & *(2) downstream/ metabolic effects* in cells with/without acquired resistance

Conclusion



Great potential for multi-purpose & logistically efficient technique for cancer drug research

Methods



Details on SC-ICP-MS

- ↓ Interferences ⇨ Use PFA syringe/tubings
- Low, stable 12 μL/min flow rate ⇨ SC nebulization requirements
- ↓ Cell loss (surface adherence) ⇨ Resuspend samples in BSA-containing solution

- ↑ Cell transport efficiency ⇨ Low Ar, high efficiency concentric nebulizer ⇨ Low vol. on-axis spray chamber
- Sample aerosol neb. efficiency ⇨ 1μm SiO_2 reference
- Cell transport efficiency ⇨ Cell count marker (Gd-DTPA)

- Suitable CRC for diff. elements ⇨ Pt & Gd (no gas), Si (H_2)
- Appropriate signal acquisition ⇨ 0.1 ms (for time-resolved single-cell detection)
- Software-enabled data analysis ⇨ MassHunter for 8900 ICP-QQQ (with sNP module)

** Image of spray chamber obtained from Glass Expansion (GE)'s website*

References

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Results & Discussion

(a) Validation of SC-ICP-MS analytical workflow

Table 1. Comparison of normalised Pt masses of cisplatin-treated A2780 and A2780cis cells measured by bulk vs. SC-ICP-MS.

Cell line	Cisplatin treatment	Normalised Pt mass (pg / 1000 cells)		Recovery, *SC / bulk* (%)
		SC-ICP-MS	Bulk ICP-MS	
A2780	10 μM	4.69 ± 1.30	7.71 ± 0.01	60.9
	20 μM	10.06 ± 1.96	16.31 ± 0.02	61.7
A2780cis	10 μM	0.43 ± 0.14	0.70 ± 0.00	62.2
	20 μM	1.60 ± 0.23	2.76 ± 0.01	58.0

- Recovery accounted for 2 analytical considerations:
  1) Correction of ionic background in cell samples
     ⇨ To account for any leaked Pt in suspension
  2) Correction of cell count based on CTE
     ⇨ CTE ~15% (via Gd-DTPA-treated cells)
- High SD for SC-ICP-MS is not a limitation, but indicator of tumour cell heterogeneity[1]

(b) Comparison of accumulation patterns of 3 Pt- Drugs (SC)

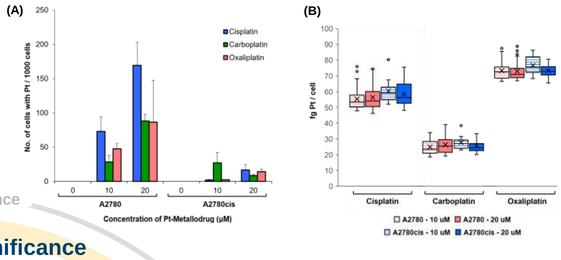

Figure 1. Pt levels accumulated in A2780 and A2780cis cell lines at varying concentrations of Pt-drugs: (A) shows number of cells with Pt, and (B) shows mass of Pt in single cells.

- Comparing number of cells for 3 Pt-drugs in Figure (A):

  ⇨ A2780: cisplatin was 2x of carboplatin or oxaliplatin
  ⇨ More A2780 cells accumulated Pt than resistant A2780cis cells (~10-fold, except at 10μM carboplatin)

- Comparing distribution of Pt levels in Figure (B):

  ⇨ Pt mass was not significantly lowered in A2780cis cells, compared to sensitive cells.
  ⇨ Cisplatin & Oxaliplatin: ~ similar SC Pt masses
  ⇨ Carboplatin: lower single-cell Pt masses

- ⇨ A2780's low sensitivity to carbo/oxaliplatin; exhibited through both (a) # of cells accumulating Pt, & (b) the levels at which Pt were accumulated
- ⇨ Cross-resistance of A2780cis towards carboplatin & oxaliplatin
- ⇨ Different cellular uptake/efflux of 3 Pt-drugs especially for carboplatin[2,3]
- ⇨ Uptake unlikely limited to passive diffusion
- ⇨ Ctr1 & OCT2 transporters with different Pt-drug affinities may be implicated[4,5]

(c) Pt-drugs & diff. endogenous element profiles (bulk)

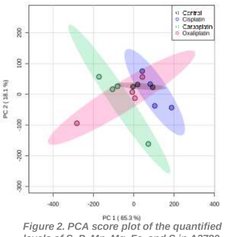

Figure 2. PCA score plot of the quantified levels of S, P, Mn, Mg, Fe, and S in A2780 and A2780cis cells.

- Clustering of carboplatin & cisplatin-treated samples
  ⇨ Attributed mainly to diff. in S & Fe levels (based on PCA loadings plot; not shown here)

- ⇨ Implicates S metabolism (possibly thiols & link with Pt detoxification)
- ⇨ Possibly linked to cisplatin-specific inhibition of Fe regulatory protein[6]

Further SC-ICP-MS work

- Single-nucleus analysis via nuclei extraction ⇨ may study nuclear DNA-platination
- Label metal-tagged antibody biomarkers (imitate flow/mass cytometry) ⇨ *e.g.,* anti-cleaved poly(ADP-ribose) polymerase (PARP) to study level of apoptosis

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