Dynamics of Degradation: Monitoring Protein Turnover Rates in Response to Chemoresistance

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Problem of drug resistance

• Multi-drug resistance – Failure of Chemotherapy

• Model system – 3 drug resistant HeLa sublines
  – Cisplatin resistant: HeLa:CDDP – 1µM cisplatin (DNA damaging agent)
  – Paclitaxel resistant: HeLa:TXL – 20nM paclitaxel (Mitotic inhibitor)
  – SN-38 resistant: HeLa:SN100 – 100nM SN-38 (topoisomerase I inhibitor)

Overview

• Proteins display altered degradation rates with drug resistance?

• Stable isotope labeling by amino acids in cell culture (SILAC\(^1\)): pulse-chase experiment
  – Monitor protein degradation rates

• Examine changes in degradation rates between drug sensitive and three resistant HeLa cell lines

Protein Turnover

Degradation
1\textsuperscript{st} order
[protein]
Proteasome/
Lysosome activity

Synthesis
0\textsuperscript{th} order
[mRNA]
translation/transcription
Importance of Protein Turnover to Drug Resistance Problem

• Ubiquitin-proteasome system control of cell growth and tumorigenesis

• Proteasome inhibitors – combination anti-cancer therapies
  – Velcade (bortezomib)
  – salinosporamide A, carfilzomib...
Protein Turnover by SILAC

Light HeLa Cells

Lys-$^{13}C_6^{15}N_2$ & Arg-$^{13}C_6$ incorporation

Time

Heavy HeLa Cells

$m/z$

$m/z$

$m/z$

Time-point sampling

RIA = \frac{\text{area light}}{\text{area light} + \text{area heavy}}

LC-MS/MS analysis
Results Output

Peak list (Hardkläör) & Peptide ID (Mascot)

SILACtor

1) Accurate Mass & Retention Time Peptide Database

2) Combine Replicates

3) Target Peptides of Interest

4) Protein Quantitative Analysis

5) Multiple Time Point Analysis

Protein RIA = \frac{1}{n} \sum_{i=1}^{n} \text{peptide}_i \text{ RIA}

Peptide distribution

Peptide

Mass

RT

GNVGFVFTK

967.5127

31.2

IIQLLDDYPK

1216.6703

40.6

Exponential Decay Curve

\[ y = e^{-0.027x} \]

\[ R^2 = 0.9974 \]

\[ k_{\text{loss}} = k_{\text{deg}} + k_{\text{dil}} \]
\[ \lambda = \ln(2)/k_{\text{deg}} \]
Exponential Decay Curve

\[ y = e^{-0.019x} \quad R^2 = 0.9599 \]

\[ y = e^{-0.027x} \quad R^2 = 0.9963 \]

\[ y = e^{-0.075x} \quad R^2 = 0.9131 \]

\[ y = e^{-0.128x} \quad R^2 = 0.9748 \]
Protein overlap Venn diagram

\[ k_{\text{diff}} = k_{\text{deg,Resistant}} - k_{\text{deg,Sensitive}} \]

$k_{\text{diff}}$ Histograms

**TXL**
- Decreased $k_{\text{deg}}$
- Increased $k_{\text{deg}}$

**SN100**
- Increased $k_{\text{deg}}$

**CDDP**
- Decreased $k_{\text{deg}}$

10 common outliers:
- AT1A2_HUMAN
- BAF_HUMAN
- DRG1_HUMAN
- HN1_HUMAN
- TOPK_HUMAN
- CATD_HUMAN
- BUB3_HUMAN
- ITB1_HUMAN
- KINH_HUMAN
- PTPA_HUMAN

Increased $k_{\text{deg}}$

Decreased $k_{\text{deg}}$
TXL resistant altered $k_{\text{deg}}$

**Mitosis/cell cycle**
- Cullin-4a (prostate cancer biomarker) increased levels -> drug resistance
  - Sensitive $\lambda = 15.5$ hr
  - Resistant $\lambda = 21.7$ hr
- Mitotic checkpoint protein BUB3
  - Sensitive $\lambda = 13$ hr
  - Resistant $\lambda = 19$ hr
- 26S proteasome non-ATPase regulatory subunit 6
  - Sensitive $\lambda = 34.3$ hr
  - Resistant $\lambda = 23.4$ hr

**Apoptosis/cell death**
- Cathepsin D (breast cancer biomarker)
  - Sensitive $\lambda = 18.3$ hr
  - Resistant $\lambda = 9.8$ hr
- Signal Transducer and Activator of Transcription 1 (tumor suppressor activity)
  - Sensitive $\lambda = 17.8$ hr
  - Resistant $\lambda = 27.4$ hr
- DNA damage response/remodeling
  - MCM7
  - PSMD6
  - SSBP
  - ZFR
  - BAF
  - H14
  - ZFR

**Cathepsin D**
- DNA damage response/remodeling
- Sensitive $\lambda = 18.3$ hr
- Resistant $\lambda = 9.8$ hr
- Mitosis/cell cycle
- Apoptosis/cell death
SN38 resistant altered $k_{\text{deg}}$

Apoptosis/cell death

Mitosis/cell cycle

Mitotic checkpoint protein BUB3
Sensitive $\lambda = 13$ hr Resistant $\lambda = 17$ hr

Cathepsin D
(breast cancer biomarker)
Sensitive $\lambda = 18.3$ hr
Resistant $\lambda = 12.4$ hr

Lymphokine-activated killer T-cell-originated protein kinase
(colorectal cancer biomarker)
Sensitive $\lambda = 21.5$ hr
Resistant $\lambda = 16.2$ hr

26S proteasome non-ATPase regulatory subunits
Sensitive $\lambda = 29.5$ hr
Resistant $\lambda = 13.5$ hr

DNA damage response/remodeling

Mitotic checkpoint protein BUB3
Sensitive $\lambda = 13$ hr Resistant $\lambda = 17$ hr

Sensitive $\lambda = 18.3$ hr
Resistant $\lambda = 12.4$ hr

Sensitive $\lambda = 21.5$ hr
Resistant $\lambda = 16.2$ hr

Sensitive $\lambda = 29.5$ hr
Resistant $\lambda = 13.5$ hr

Sensitive $\lambda = 13$ hr Resistant $\lambda = 17$ hr
CDDP resistant altered $k_{\text{deg}}$

Apoptosis/cell death
- MSH6
- NICA
- PTPA
- CATD
- CDK1
- PSMD4
- ITB1
- PAK2

Mitosis/cell cycle
- CDK1
- CDK3
- CDK2
- CDK4
- CDK5
- CD123
- PSMD4
- TYSY
- TOPK
- BUB3
- PAK2

Mitotic checkpoint protein BUB3
Sensitive $\lambda = 13$ hr
Resistant $\lambda = 17$ hr

Cyclin dependent kinases
Sensitive $\lambda = 18$ hr
Resistant $\lambda = 28$ hr

Cathepsin D
(breast cancer biomarker)
Sensitive $\lambda = 18$ hr
Resistant $\lambda = 15$ hr

DNA mismatch repair protein
Sensitive $\lambda = 18$ hr
Resistant $\lambda = 15$ hr

DNA damage response/remodeling
- HAT1
- H14
- PSMD4
- MSH6
- CHD4
- TYSY
- SUMO2
- STAT3
- H13
- DLG1
- CD123
- TOPK
- TYSY
- CD123
Summary

• Dynamic proteome measurements provide insight into mechanism of drug resistance
• Several proteins with altered $k_{deg}$ values related to mechanism of drug action
• Altered protein turnover rates may be effective biomarkers of drug resistance
• Future work: Relate changes in protein half-life to protein abundance level changes and protein-protein interactions
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ASMS presentations
Chad - Poster: MP17 #398
Mike - Poster: MP28 #644
Michelle - Oral: TOD pm - Plant "omics" Time: 2:50
Jake - Poster: TP03 #61
Xia - Poster: WP09 #226
Jim - Oral: ThOE am - Biomolecular Structure Analysis by Covalent Labeling: Future Directions Time: 08:30
Xia - Poster: ThP12 #280

Chemical Elements

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