Monitoring 1432 unique Ras Induced phosphopeptides in dda-PASEF and PRM PASEF

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Elizabeth Gordon[1], Matt Willetts[1], Diego Assis[1], Rodrigo M Ferreira[2], Uma K Aryal[2]
[1]Bruker Daltonik Billerica, MA USA
[2P] Purdue University, West Lafayette, IN, USA

Introduction

Senescence is a tumor suppressive mechanism of cells and acts as a primary layer of protection against the development of cancer. Senescent cells, that lose the ability to divide, are also the underlying mechanism of aging. Therefore, understanding molecular factors and biological processes of cellular senescence provides important insights into the intrinsic cellular mechanisms for cancer prevention, and organismal aging. Herein, we have used trapped ion mobility spectrometry coupled to quadrupole-time of flight mass spectrometry for deep phosphoproteomics analysis to obtain comprehensive data from this complex cell lines and to characterize structural and positional phosphopeptides isomers.

Methods

Human diploid fibroblast strain IMR90 (CCL-186; ATCC, USA) cells were transduced with ER:RAS lentivirus and treated with 100 nM of (Z)-4-Hydroxytamoxifen (4-OHT) for ER:RAS activation and induction of oncogene induced senescence (OIS). Control cells were treated with MeOH. Cells were harvested after 6 days of 4-OHT activation for nuclear extraction, trypsinization, and enrichment of phosphopeptides using Polymer-based Metal-ion Affinity Capture (PolyMAC) spin tips. Pre and post enrichment samples were both run on a nanoElute LC (Bruker Daltonics) using an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) at 400 nl/min with a 70 min gradient 80min run time. LC-TIMS MS/MS data were obtained from a timsTOF Pro instrument operated in PASEF mode. Data were analyzed using PEAKS OnLine (Bioinformatics Solution).

Samples and treatment

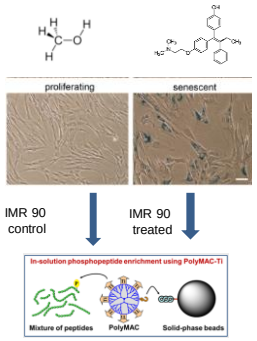
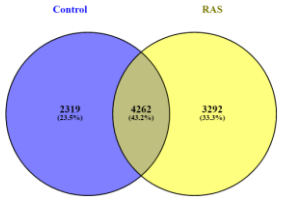


Fig. 1 Unique Phospho sites in IMR90 cells representative of biological triplicates



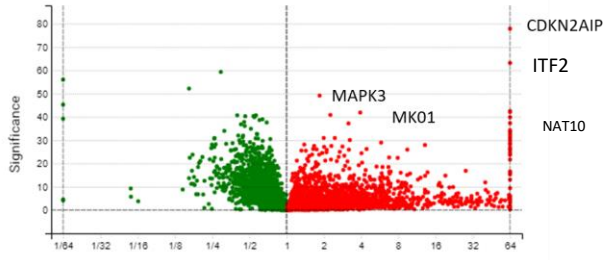
Data Analysis with PEAKS Online

PEAKS

# Protein Groups	#Peptides	# Precursor	#PSM	Phospho Sample name
5,562	14,889	402,589	161,195	Non Treated [MeOH]
2,946	24,483	183,974	49,864	IMR90 Control Phos1 Enriched
2,571	20,425	181,828	43,982	IMR90 Control Phos2 Enriched
2,830	22,718	185,221	47,735	IMR90 Control Phos3 Enriched
5,602	2,346	411,856	164,639	ER Ras Induced IMR90
2,665	21,023	176,094	44,661	IMR90 ER Ras Induced Phos2 Enriched
2,709	23,763	218,496	53,125	IMR90 ER Ras Induced Phos3 Enriched
2,998	24,982	187,177	53,448	IMR90 ER Ras Induced Phos1 Enriched

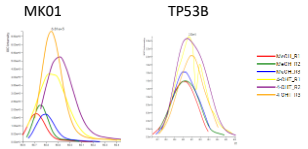
DDA LFQ

Figure 2. The volcano plot for proteins.
Top 3 peptides control vs ER RAS activated

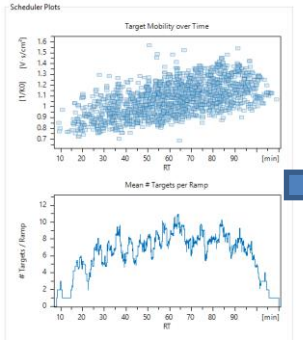


Results

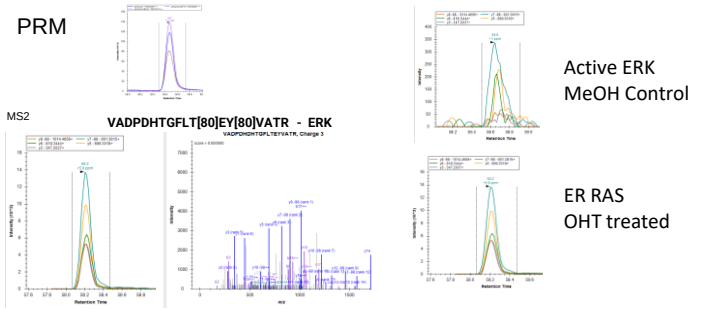
DDA LFQ



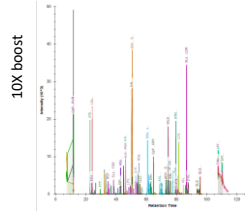
1505 PRM scheduled



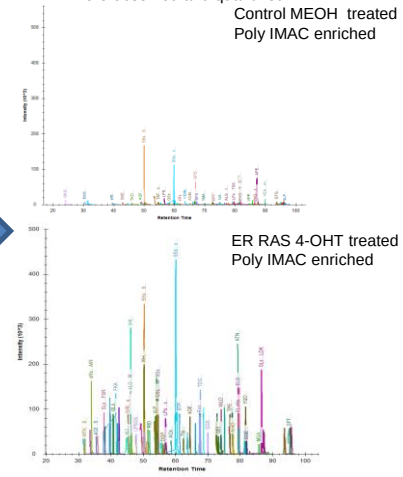
PRM allows clear identification & Quantification in Polymac Enriched & Unenriched Samples



793 phosphopeptides are observed
in targeted in PRM in the unenriched ER RAS activated IMR90 cells



920 Peptides and 1,200 Precursors were observed and quantified



Summary

- In the ER RAS activated Senescent cells roughly 3,767 protein groups were detected, after enrichment there were 3,401 of which had one or more phospho-peptides observed.
- Control cells 2,319 phospho-peptides were identified in PRM by monitoring 1,200 precursors representing 920 phosphopeptides, the majority of which showed reproducible quantification.
- DDA PASEF and Label free quantification of 4,171 Protein groups.
- Utilizing the PRM PASEF smart scheduling 1,505 targets with 2 minute retention time window were scheduled which equate to less the 10 targets per ramp.
- Control cells were run in triplicate and 920 phosphopeptides and 1,200 precursors and covering 433 proteins were observed in at least 2 of the 3 samples and not in a blank sample.

Conclusions

- Phospho enriched IMR90 control cells we see 6,581 distinct phosphosites with a A score greater than 20.
- 4-OHT treated ER RAS induced IMR90 cells had 7,554 unique phosphosites.
- 43% of the sites identified overlapped between the control treated IMR90 cells and the ER RAS activated cells, an additional 33% of the sites were only observed in the ER RAS activated samples.
- This allowed for an impressive 920 phosphopeptides to be identified and quantified in a poly IMAC phospho enriched ER RAS activated sample and 793 still were able to be detected and quantified in the unenriched samples.
- timsTOF ion mobility allows for many phospho peptides to be both identified and quantified in DDA PASEF and PRM PASEF.

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