PASEF-DDA enables deep coverage single-shot phosphoproteomics and ion mobility-based elucidation of phosphosite isomers

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Introduction

To elucidate cellular signaling mechanisms, a detailed and highly resolved analysis of phosphorylation sites is crucial. Although LC-MS/MS proved as a powerful tool for in-depth phosphoproteome analysis, challenges remain in the correct determination of the phosphorylation site. Coeluting and isobaric phosphopeptide isomers, harbouring the phosphogroup on different residues, are often impossible to resolve in classical MS/MS analyses. Ion mobility spectrometry (IMS) enables their separation based on their collision cross section (CCS), as the position of the phosphogroup affects the ion geometry in the gas phase. Parallel accumulation serial fragmentation data-dependent acquisition (PASEF-DDA) on the timsTOF Pro mass spectrometer allows the application of IMS on large scale phosphoproteomic studies. Here, we present a high-coverage phosphopeptide dataset from patient-derived osteosarcoma samples [1].

Methods

Tryptic phosphopeptides from osteosarcoma samples before and after treatment were enriched in three replicates from 1 mg lysate each by TiO_2 from GL Science. Enriched phosphopeptide samples were separated within 100 min (2 to 35 % B, B: 0.1 % FA in ACN, 400 nL/min flow rate) on a reversed-phase C18 column with an integrated CaptiveSpray Emitter (25 cm x 75 µm, 1.6 µm, IonOpticks, Australia). After ESI ionization, peptides were analyzed using timsTOF Pro with PASEF enabled at 120 Hz. Trapped ion mobility accumulation and elution times were synchronized at 166 ms. In addition to high resolution (40,000) accurate mass (<10 ppm) the mass spectrometer records mobility (1/K0), and with charge state and m/z deciphers CCS. The data was processed using PEAKS X + (BSI) and MaxQuant v1.6.10.43 (MPI of Biochemistry). The resulting phosphopeptide information was submitted for Integrated Kinase Activity Score analysis (InKA).

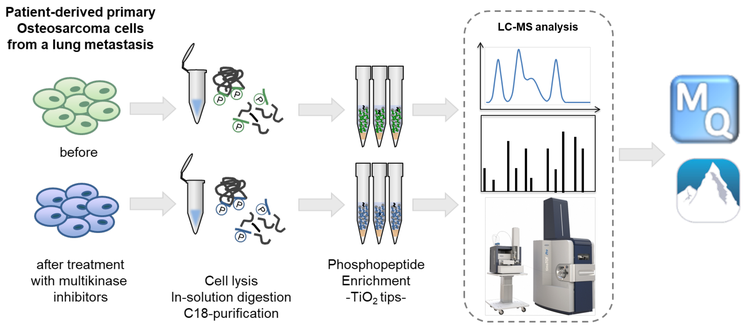

Figure 1

IMS enables separation of isobaric phosphopeptide isomers

The presented sample preparation procedure and subsequent database search with Peaks X+ resulted in 27,768 identified phosphopeptides without any sequential enrichment or fractionation (fig. 2 / 3). Of these, 11,247 can be classified as *Class I* phosphopeptides.

4,672 phosphopeptide pairs were isobaric positional isomers with p = 0.75 propability of correct phospo-site determination (i.e. AScore > 6) of which a major part was coleuting during RP-LC (Δ RT < 0.5 min). Of those, 457 phosphopeptide pairs could be separated by IMS (Δ1/k0 > 2 %, fig. 3).

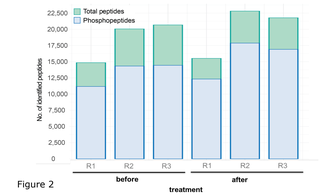

Figure 2

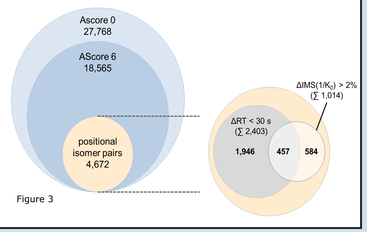

Figure 3

Hyperactive kinase scoring in cancer research requires accurate phosphosite information

timsTOF Pro raw data was analyzed using MaxQuant to facilitate submission to the online interface for InKA score calculation [4]. This score combines information from different sources to obtain a comprehensive picture of hyperactive kinases (fig. 8).

The presented experimental setup enabled the statistical evaluation by t-test to compare before and after treatment status (fig. 9 / 10).

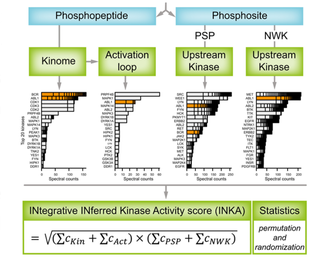

Figure 8 from [4]

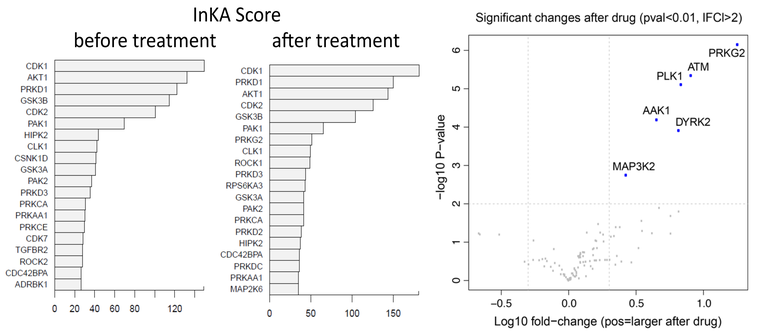

Figure 9

Figure 10

Example 1 - SRRM2

Serine/arginine repetitive matrix protein 2. Aberrant phosphorylation in liver cancer on position S1691 [2]. Multiple coeluting isobaric phosphopeptide isomers were separated by IMS.

Table 1

#	Peptides	-10lgP	Mass	Obs. *m/z*	RT
1	RS(+79.97)SRSS(+79.97)PELTR	55.08	1434.60	718.31	8.14
2	RSS(+79.97)RSS(+79.97)PELTR	57.48	1434.60	718.31	8.15
3	RSS(+79.97)RS(+79.97)SPELTR	59.34	1434.60	479.21	8.63

Figure 4

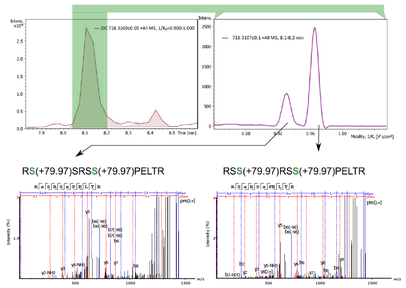

Figure 5

Example 2 - AKAP-12

A-kinase anchor protein 12. Scaffold protein for many key signalling factors, such as protein kinase C (PKC), PKA, cyclin as well as F-actin. S286, which is involved in EGF response [3], was identified as significantly decreased after treatment.

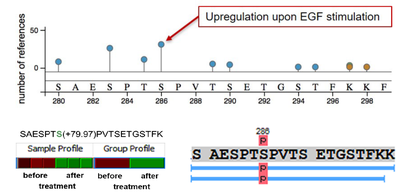

Figure 6

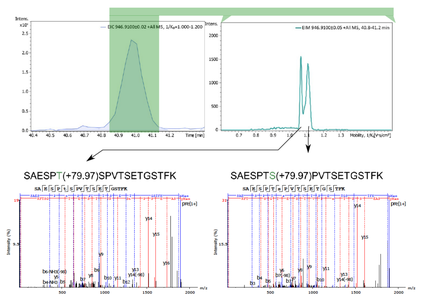

Figure 7

Outlook

In combination with a label-free quantitative analysis of the cellular proteome, the identification of hyperactive kinases enables the elucidation of the mode of action of the applied chemotherapy and the molecular basis for the acquired resistance phenotype of the osteosarcoma metastasis. In the future, we will apply additional complementary phosphopeptide enrichment workflows, including phospho-tyrosine immunoprecipitation (pTyr-IP) and immobilized metal ion affinitychromatography (IMAC) to further enhance the phosphoproteome coverage.

References

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