LC-SRM approach to differentiate phosphorylation sites of biomarker proteins, in a multiplex fashion: case study on TAU protein.

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INTRODUCTION

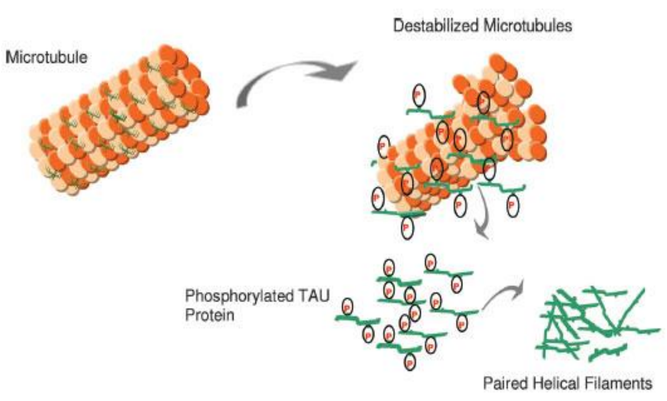

Figure 1 : Tau protein ivolvment in neuronal death

Post-translational modifications (PTM) are known to impact protein activities, and biochemical regulation pathways. Particularly, phosphorylation is one of the most prevalent intracellular protein modifications, involved in numerous cellular processes such as cell differentiation, proliferation, migration, and are concerned in several diseases. As a consequence, pharmacology industry explores many drugs to treat these diseases. Monitoring the treatment effect at specific phosphorylation sites is a crucial information for biologists to a better understanding of ways involved in such diseases.
Classically immuno-based techniques failed in the precise phosphorylation sites location. This study demonstrates LC-MS/MS capabilities to overpass these limits. The present case study deals with Alzheimer Disease and other tauopathies which could be correlated with hyperphosphorylation of tau protein at specific sites.

CASE STUDY ISSUE

- Almost 20% of TAU protein sequence is composed of potential phosphorylation sites (Figure 2).

>sp|P10637|TAU_MOUSE Microtubule-associated protein tau OS=Mus musculus OX=10090 GN=Mapt PE=1 SV=3
MADPRQEFDTMEDHAGDYTLLQDQEGDMDHGLKESPPQPPADDGAEEPGSETSDAKSTPTAEDVTAPLVDERAPDKQAAAQPHTEI
PEGITAEEAGIGDTPNQEDQAAGHVTQGRREGQAPDLGTSDWTRQQVSSMSGAPLLPQGLREATCQPSGTRPEDIEKSHPASELLRR
GPPQKEGWGQDRLGSEEEVDEDLTVDESSQDSPPSQASLTPGRAAPQAGSGSVCGETASVPGLPTEGSVPLPADFFSKVSAETQAS
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VPQLKARVASKDRTGNDEKKAKTSTPSCAKAPSHRPCLSPTRPTLGSSDPLIKPSSPAVSPEPATSPKHVSSVTPRNGSPGTKQMKLK
GADGKTGAKIATPRGAASPAQKGTSNATRIPAKTTPSPKTPPGSGEPPKSGERSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVV
RTPPKSPSASKSRLQTAPVPMPDLKNVRSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKV
TSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPRH
LSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL

Figure 2: potential phosphorylation sites in protein TAU sequence

- Considering biological knowledge, an LC-SRM method was develop to screen 16 mono-phosphorylated and 5 bi-phosphorylated interesting sites. Targeted phosphorylation sites could be located on same proteotypic peptide.

TECHNICAL CAPABILITIES

- With LC-MS/MS method, specific phosphorylation sites were differentiated either by their product ions masses according to high MS specificity (figure 3) or by their chemical properties using chromatographic separation (Figure 4).

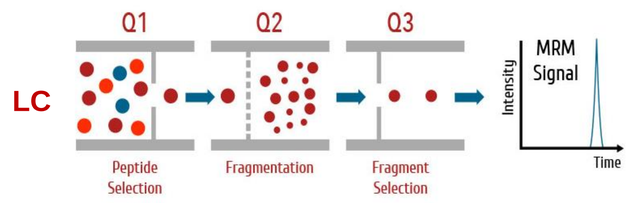

Figure 3: ions speccificity selection in SRM

- Example on the TAU SPVVSGDTSPR sequence containing 4 very close phosphorylation sites ($S^{396}S^{400}T^{403}S^{404}$), specifically differentiated using LC-MS/MS approach. $S^{396}S^{400}S^{404}$ were differentiate regarding their specific transition masses. T^{403} and S^{404} could not be differentiate using their MS properties but their LC properties.

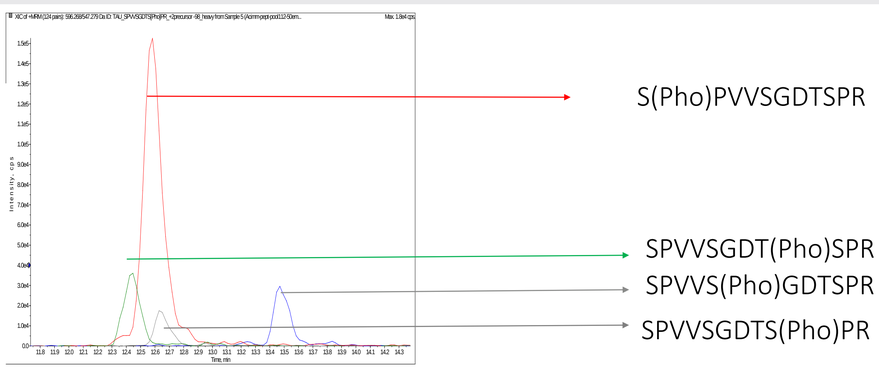

Figure 4 : LC-SRM chromatogram, allowing differentiation of mono-phosphorylated forms of SPVVSGDTSPR tau peptide.

ACKNOWLEDGMENT

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MATERIALS AND METHODS

- Samples were brain mouse lysates (cortex or hippocampal)
- Sample preparation : *in-solution* digestion protocol (denaturation with DTT; alkylation of cysteine residues with IAA; trypsin digestion) followed by HLB SPE to remove salts and to concentrate sample.
- Several proteotypic and specific peptides (phosphorylated and unphosphorylated) are selected for SRM analyses.

SCREENING OF DIFFERENT MOUSE MODEL

Cortex samples from treated or untreated mice revealed different TAU peptide signals. A huge phosphorylated sites screening allows us to compare 12 mono-phosphorylated and 2 bi-phosphorylated peptides signals in different mouse models. This kind of screening could help to select mouse models for testing treatment.

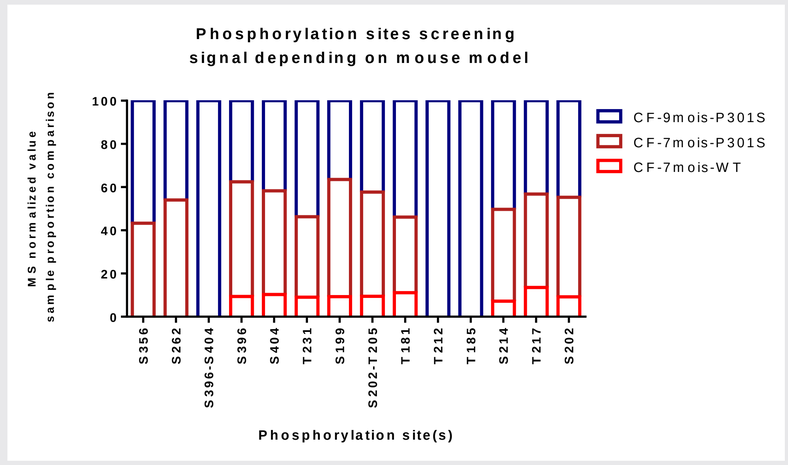

Figure 5: Screening of several phosphorylation site by LC-SRM, in different mouse model brain cortex frontal samples.

EFFICACY STUDY ON PHOSPHORILATION SITES

After treatment, hippocampal samples from treated and untreated WT and P301S mice were analyzed using LC-SRM method. MS results revealed an increase of global sequence phosphorylation after treatment, demonstrated by the decrease of unphosphorylated SPVVSGDTSPR signal (Figure 6 Left). Focus on pS404 reveals this specific phosphorylation as the main responsible of the total phosphorylation increase (Figure 6 Right).

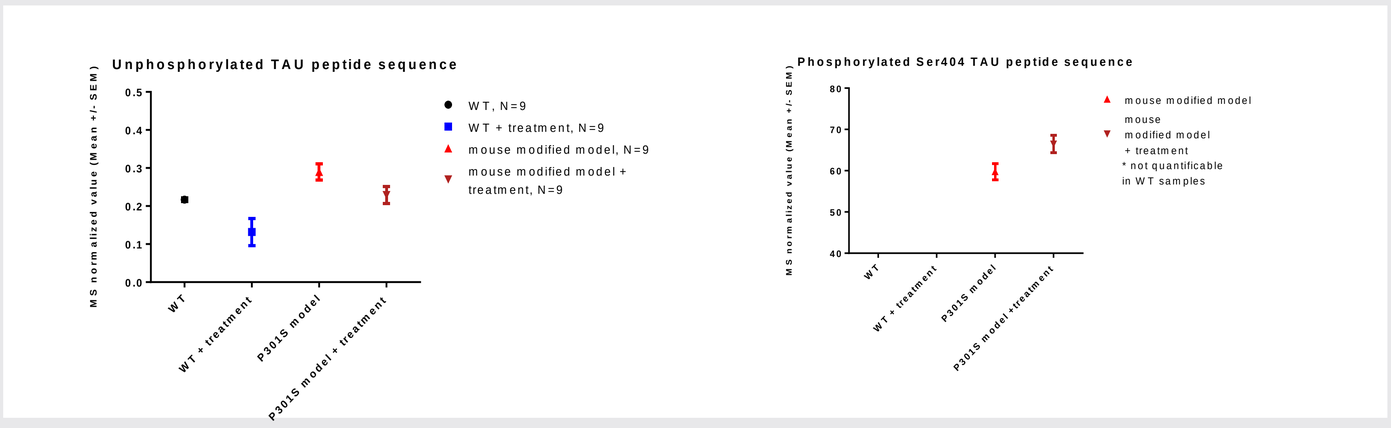

Figure 6 : Phosphorylation of TAU S404 observed on P301S mice with or without treatment

CONCLUSION

LC-SRM approach allows a specific phosphorylation site differentiation, which is not always possible with immuno-based assays. This approach bring additional information, particularly at quantitation level, and demonstrates high multiplexing capabilities. This large scale screening could be useful either for animals model selection but also for treatment efficacy assessment.