Mass Spectrometry for the Classification of Pituitary Neuroendocrine Tumors

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Introduction

Existing clinical tools to detect & measure proteins in solid organ biopsy sections (e.g., immuno-histochemistry, IHC) suffer technical limitations. Mass spectrometry offers improved standardization, multiplexing and quantification. The classification of pituitary neuroendocrine tumors (PitNETs, formerly "pituitary adenomas") is an application for which MS may be better suited than standard IHC alone, due to limited tissue quantity and other challenges.

Methods

We utilized liquid chromatography-tandem mass spectrometry to measure pituitary hormone and other markers in formalin-fixed paraffin-embedded (FFPE) PitNET samples. FFPE PitNET tissue specimens (prolactin, ACTH and growth hormone-producing tumors) were microdissected and processed using heat-denaturation and trypsin digest (**Figure 1**). MS was performed using data-dependent acquisition (DDA) and parallel reaction monitoring (PRM) on a Thermo EASY-nLC 1000 and QE+ MS.

Results

Untargeted LC-MS/MS analyses differentiated tumor hormone expression in all cases (n = 6), based on spectral counting for the key protein hormone markers as determined using anti-pituitary hormone IHC (**Fig. 2, left**). For all 3 PitNET subtypes evaluated (somatroph, lactotroph, and corticotroph tumors), at least two novel and high-quality surrogate peptides were identified (**Fig. 2, right**). MS appeared to be sensitivity-limited for measuring transcription factors.

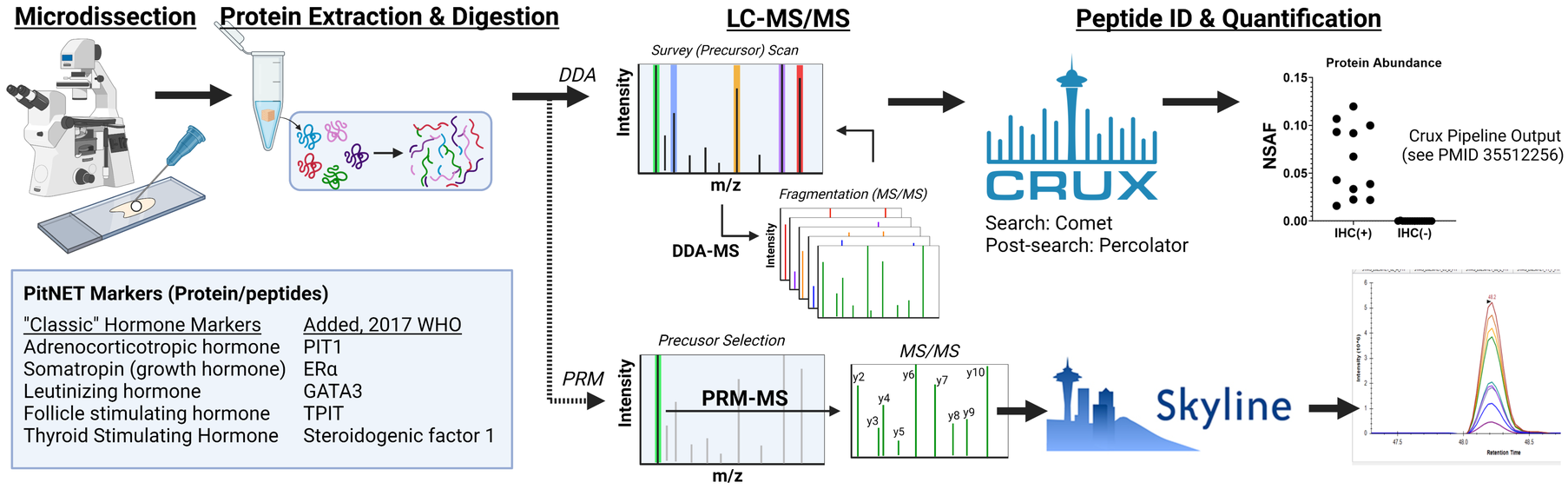
Conclusions

The application of LC-MSMS to solid tissues represents a complementary pathway to IHC, allowing for more objective and highly parallel quantification of hormone expression in tissue samples. Sensitivity continues to be a challenge for certain targets (e.g., transcription factors), though implementation of additional measures (e.g., peptide immunoaffinity enrichment) could help overcome this.

Acknowledgements

Figure 1 was created in BioRender (middle portion inspired by Krasny & Huang in PMID 33034323). Thank you to UW Lab Med for funding this work and Drs. Bill Noble and Priska Von Haller for technical support.

Figure 1 – Workflow



PitNET Markers (Protein/peptides)

"Classic" Hormone Markers	Added, 2017 WHO
Adrenocorticotropic hormone	PIT1
Somatropin (growth hormone)	ERα
Leutinizing hormone	GATA3
Follicle stimulating hormone	TPIT
Thyroid Stimulating Hormone	Steroidogenic factor 1

Figure 2 – Representative Results (DDA ➔ PRM)