A Powerful Paradigm: Predictive, Preventive, and Personalized Medicine with Multiomics of Human Pituitary Adenomas

Dominic M. Desiderio and Xianquan Zhan

Abbreviations

2DGE	Two-dimensional gel electrophoresis
3 PM	Predictive, preventive, personalized medicine
ACTH	Adrenocorticotropin hormone
AKT	Protein kinase B
ARE	Antioxidant response element
BMI	Body mass index
Capzb	F-actin capping protein subunit beta
CAT	Catalase
COX6B1	Cytochrome c oxidase subunit 6b1
CT	Computed tomography
DEP	Differentially expressed protein
EpRE	Electrophile response element
ERK	Extracellular signal regulated kinase
FSH	Follicle-stimulating hormone
GH	Growth hormone
GHP	Growth hormone proteoform
GHRH	Growth hormone-releasing hormone
hGH	Human growth hormone

D. M. Desiderio (✉)
Department of Neurology, The University of Tennessee Health Science Center, Memphis, TN, USA
e-mail: ddesiderio@uthsc.edu

X. Zhan
Medical Science and Technology Innovation Center, and Gastroenterology Research Institute and Clinical Center, Shandong First Medical University, Jinan, Shandong, China

HSP	Heat shock protein
iTRAQ	Isobaric tag for relative and absolute quantification
Jak STAT	Janus kinase signal transducer and activator of transcription proteins
Jnk	c-Jun N-terminal kinase
Keap	Kelch-like ECH associated protein
LC-MS	Liquid chromatography-mass spectrometry
LH	Luteinizing hormone
MAPK	Mitogen-activated protein kinase
M_r	Relative molecular mass
MRI	Magnetic resonance imaging
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry; MS^2
NADH	Nicotinamide adenine dinucleotide
ncRNA	Non-coding RNA
NDUFS8	NADH: ubiquinone oxidoreductase core subunit s8
NF	Non-functional
NFPA	Non-functional pituitary adenoma
Nrf-2	NF-E2-related factor 2 (nuclear factor, erythroid 2)
PET	Positron emission tomography
pI	Isoelectric point
PI3K	Phosphatidylinositol 3-kinase
PitNET	Pituitary neuroendocrine tumor
PPPM	Predictive, preventive, personalized medicine
PRL	Prolactin
PRLP	Prolactin proteoform
PTM	Post-translational modification
PVDF	Polyvinylidene difluoride
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SS	Somatostatin
STAT3	Signal transducer and activator of transcription 3
USD	U.S.A. dollar
UV	Ultraviolet
WHO	World Health Organization

1   Introduction

We introduce a new research paradigm that involves PPPM in conjunction with multiomics to study human pituitary adenomas [1]. The goal is to clarify differences between proteins in controls versus adenomas. This research is performed within the framework of predictive, preventive, and personalized medicine (PPPM; 3 PM). Multiomics collects the variety of new methodologies developed over the past
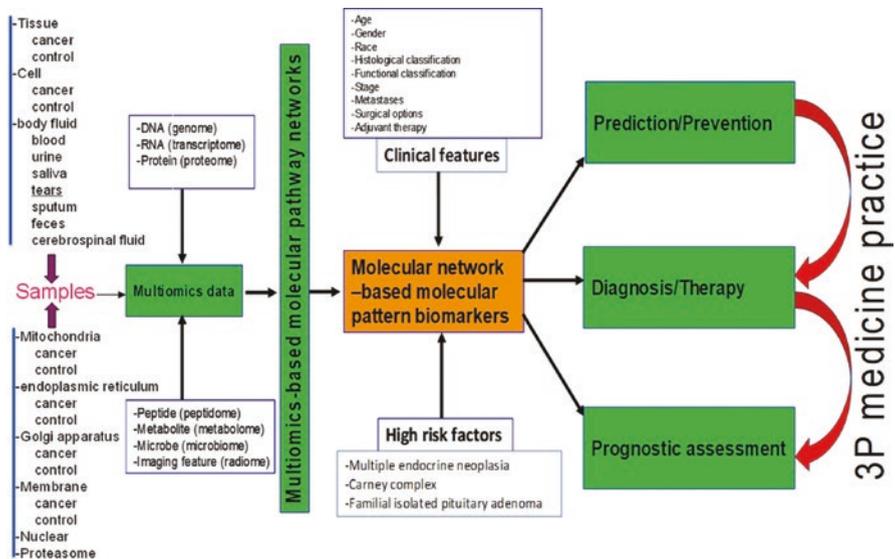
Fig. 1 The basic process of multiomics in pituitary adenomas. (Modified from Li N, Desiderio DM, and Zhan X (2021) [1], copyright permission from Wiley publisher, copyright 2021. Modified from X. Zhan, Zhou, et al. (2019) [2], copyright permission from IntechOpen publisher open-access book chapter, copyright 2019)

several years, and includes transcriptomics, genomics, proteomics, etc. (Fig. 1). We focus here primarily on the proteome and proteoforms of adenomas.

The adenohypophysis (anterior pituitary) contains corticotrophs, gonadotrophs, somatotrophs, thyrotrophs, and lactotrophs [3]. Those anterior pituitary cells are tightly regulated throughout the multiple hypothalamic-pituitary-target axes. Hypothalamic-releasing hormones transit to the pituitary to release the individual hormones from corresponding cells, and the pituitary hormones travel to their target organs. Tumors are a common disease that occurs in the anterior pituitary to affect the hypothalamic-pituitary-target axis systems and affect an individual's health. Many omics studies, especially proteomics and transcriptomics, were performed in this research to reveal the molecular changes and signaling pathway alterations in pituitary adenomas, and to discover effective biomarkers for PPPM practice.

Recently, the concept of proteome has been further developed. The traditional concept was that the canonical protein was the basic unit of a proteome. However, after a protein amino acid sequence is synthetized in a ribosome, it must be translocated to a specific location and form a spatial conformation that interacts with its surrounding molecules to form a complex to exert its final function. In the translocation process, many post-translational modifications (PTMs) form; they are important factors that yield the diversity of a canonical protein. The final structural and functional format of a gene or a canonical protein is termed a proteoform, which is the basic unit of a proteome. The estimate of the number of proteoforms is in the billions. The term "canonical protein" includes multiple proteoforms derived from
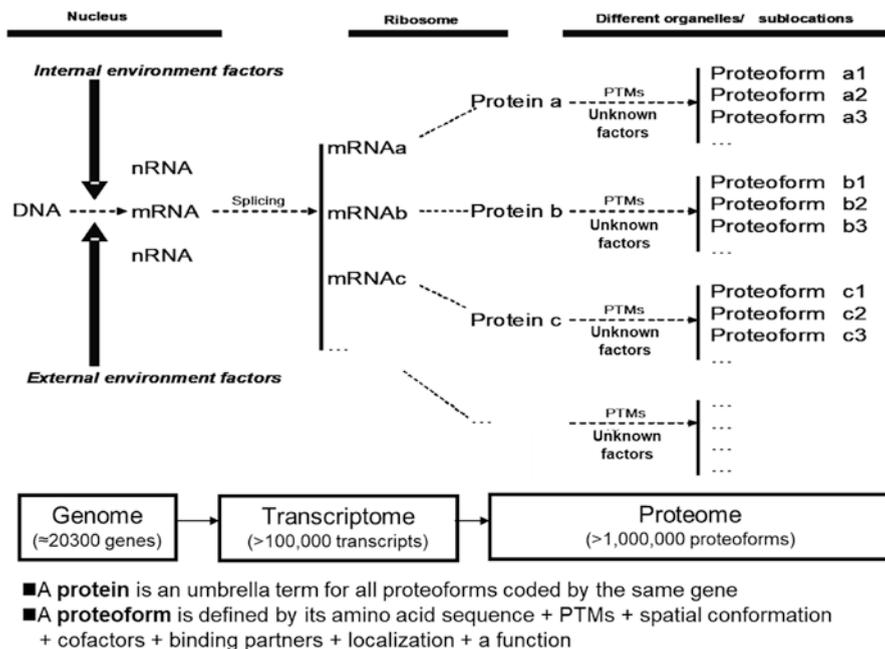
Fig. 2 Relationship of proteoform, protein, and proteome. (Modified from Zhan et al. (2018) [4], with permission from Hapres publisher open access publication, copyright 2018; and reproduced from Zhan, Li et al. (2019) [5], with permission from MDPI publisher open access publication, copyright 2019). *mRNA* messenger RNA, *nRNA* noncoding RNA, *PTM* post-translational modification

the same gene. Each proteoform has its individual isoelectric point (p*I*) and relative molecular mass (M_r). Proteoforms derive from alternate splicing, PTM, proteolysis, etc. The term "protein" collects a number of individual molecular species: precursor to the protein, amino acid sequence, PTMs, conformation, cofactors, binding partners, receptor (which is a protein with its associated proteoforms, PTMs, etc.), localization, and function of that complex (Fig. 2). Complexity increases rather than decreases as we gather more basic structural information.

This book chapter will primarily discuss the proteome changes of adenomas, and proteoforms of two anterior pituitary hormones—growth hormone (GH) from somatotrophs and prolactin (PRL) from lactotrophs.

2   Current Achievements in Pituitary Adenoma Multiomics

Approximately 20% of all intracranial tumors are pituitary adenomas; most are benign (~65%) some are invasive (~35%), and a few (~0.2%) become malignant carcinomas [6]. Some adenomas are macroadenomas (>10 mm) and some are microadenomas (<10 mm). Some are hormone-secreting, and some are non-secreting. The World Health Organization (WHO) developed a new classification

system to include transcription factors in addition to immunohistochemistry and hormone secretion.

We have studied neuropeptidergic systems in human adenomas since 1985 [7, 8]. Those studies include non-functional pituitary adenomas (NFPA), invasive NFPAs, control pituitaries, and secreting adenomas (GH-, PRL-, and ACTH-secreting) [9]. A wide range of methods was used: quantitative transcriptomics (DEGs), quantitative proteomics (DEPs), proteomic mapping, nitroproteomics, phosphoproteomics, proteoformics, and metabolomics.

We have discovered in human pituitary tissues 149 new proteins, 46 GH proteoforms, nine nitroproteins, six prolactin variants, 108 ubiquitinated proteins, 56 differentially expressed proteins (DEPs), 26 phosphoproteins, and two differential peptides. As we delve deeper into the basic molecular chemistry of the pituitary, we continue to uncover more structure-rich information.

We will rationalize in the following sections these experimental data of pituitary adenomas within the PPPM framework.
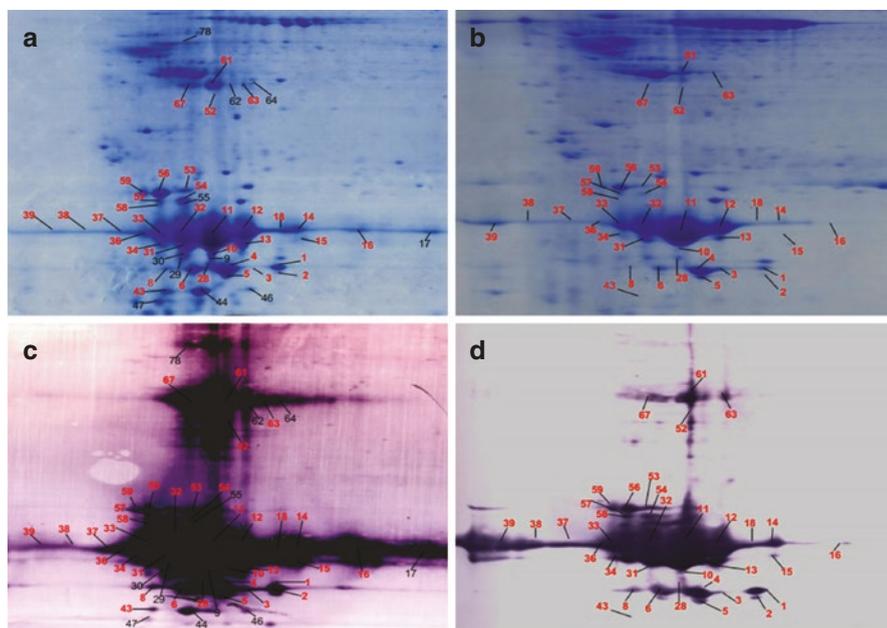
3   Pituitary Hormone Proteoforms of Pituitary Adenomas Within the PPPM Framework

Human pituitary hormones such as GH and PRL are the important message factors in the hypothalamic–pituitary–target axis systems in the human endocrine system, and any changes (structural; quantitative) could significantly affect human health. The traditional opinion is that quantity changes of these hormones (GH and PRL) are the main reasons that cause GH- or PRL-related diseases. However, our recent studies based on two-dimensional gel electrophoresis in combination with mass spectrometry (2DGE-MS) discovered many proteoforms of GH and PRL, which significantly clarified and expanded our knowledge about the structure and functions of hormones, including GH and PRL.

3.1   Human Growth Hormone Proteoforms (GHPs)

GH is biosynthesized in the acidophilic somatotrophs in the anterior pituitary. We discovered 24 GHPs in our 2DGE-MS analysis of pituitaries. GH is released from the pituitary under strict regulation from hypothalamic GH-releasing hormone (GHRH). GH interacts with its receptors in the liver, free fatty acid depot, and, via a long feedback loop, the somatotropin-release inhibiting factor (somatostatin = SS) neuron in the paraventricular nucleus. SS, in turn, downregulates the release of GHRH. Tight regulation of this system is critical. If too much GH is released, then gigantism or acromegaly might occur; too little might lead to stunted growth [10].

We discovered 46 GHPs that were ubiquitinated, acetylated, phosphorylated, and deaminated. It is not known which one (or several, or many) of the GH proteoforms is released from the anterior pituitary in the GH system. Clearly, more data are needed to resolve these important clinical questions.

Fig. 3 2DGE-based western blot of growth hormone proteoforms (GHPs) in GH-secreting pituitary adenoma and control pituitary tissues. (**a**) Coomassie blue-stained 2D gel image (46 GHPs) in the GH-secreting pituitary adenoma tissue. (**b**) Coomassie blue-stained 2D gel image (35 GHPs) in the control pituitary tissue. (**c**) 2DGE-based western blot image of GHPs in the GH-secreting pituitary adenoma tissue. (**d**) 2DGE-based western blot image of GHPs in the control pituitary tissue. (Reproduced from Li B, et al. (2021) [13], copyright permission from Springer open access article, copyright 2021)

The GH receptor exists in an inactive homodimeric, parallel form before GH binding. After GH binding, the receptor shifts to a left-handed crossover configuration [11].

3.1.1 GHP Pattern Changes in Pituitary Adenomas

2DGE-separation, GH antibodies, mass spectrometry (MS), and MS/MS were used to characterize GHPs. In addition, three selected GHP PTMs were studied: phosphorylated (with 6-plex iTRAQ), ubiquitinated (with an anti-N_e-acetyl-lysine antibody), and acetylated (with an anti-K-GG-antibody) proteomics [12]. MS and MS/MS were used to characterize each GHP and to locate each modification site. The 2D gel, after protein separation and Coomassie staining, contained ~1000 spots. After transfer to a PVDF membrane, GHPs were detected with a GH antibody and characterized with MS (Fig. 3).

3.1.2 Special Note on 2DGE

Many researchers would rather use LC-MS rather than 2DGE because 2DGE is labor-intensive. However, it is crucial that any quantitative LC-MS data demonstrate

a high level of reproducibility (>96%) of LC peak retention times; similar to 2DGE spot volume and spot position data.

2DGE provides several important experimental factors: (1) a "bird's eye view" of a pituitary proteome; (2) a very high level (>96%) of within-gel and between-gel reproducibility of spot volume and spot position; and (3) most importantly, an archival storage for precious human tissue and separated proteins. The latter point is important because, whenever MS improves sufficiently, one can revisit those gels to detect and characterize low-copy number proteins.

Even more important is that significant improvements in 2DGE resolution have been published by Zhan et al. [14], who predict a 2DGE resolution of 500,000 protein species. That quantum improvement in 2DGE will impact significantly onto PPPM, and will allow the separation and characterization of proteins with a very low-copy number.

3.1.3   Discovery of 46 GHPs

We found 46 GHPs in GH-secreting pituitary adenomas and 35 GHPs in control pituitaries. Those 35 GHPs in controls were a subset contained within the 46 GHPs in pituitary adenomas. Eleven GHPs were found only in pituitary adenomas [13]. Such an extensive metabolic re-shuffling of that number of GHPs between pituitary controls and adenomas is quite significant, and leads to an important question: what metabolic dysfunction(s) occurred to create so many extensive alterations? That question requires further research.

3.1.4   Significant PTMs of GHPs

The four types of PTMs found in GHPs [13] include the following:

1. *Phosphorylation* at Ser 77, Ser 132, Ser 134, Thr 174, and Ser 176. Phosphorylation could indicate a potentially new regulatory system. Phosphorylation adds a negative charge at physiological pH. Differential phosphorylation patterns occurred between pituitary controls and adenomas.
2. *Ubiquitination* at Lys 96 was found in pituitary adenomas, but not in controls. Ubiquitination could lead to protein degradation and/or modulate a protein's metabolic activity.
3. *Acetylation* (removes a positive charge) was found at Lys 171.
4. *Deamination* (adds a negative charge) was found at Asn 178.

Again, all of those diverse, multiple PTM data indicate that extensive differential remodeling and PTM formation occur between controls and adenomas. Those differential GHP data lead to a question: What role do these multiple and diverse PTMs play in a wide range of clinically important mechanisms that include GH receptor binding/interactions, signaling pathways, homeostasis, and pathologies?

Removal of a positive charge and addition of a negative charge in GHPs could play a significant role in protein: protein interactions such as in protein: receptor interactions. For example, in the COVID-19 delta variant spike protein, Pro-681 was replaced with Arg near the furin cleavage site. Also, the omicron

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQE

FEEAYIPKEQKYSFLQNPQT-pS-LCFSESIPTPSNREETQQ-ubiK-SNLE

LLRISLLLIQSWLEPVQFLRSVFANSLVYGA-pS-D-pS-

NVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYS–acK-FDpT-N-pS-H-N*-

DDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF

T+S = 31 (5 were phosphorylated. S77; S122, S134, S176, T174).

N+Q = 10 (N*-178 deaminated).

K = 7 (96-ubi; 171-acetyl).

Fig. 4 GH amino acid sequence. 217 amino acids. Signal peptide 1–26 (underlined). Isoform 1 (191 amino acids; MW = 24,847 kDa)

variant has ~50 modifications in several critical binding regions. Profound biologic activity changes might also occur from these charge modifications of pituitary proteins.

Another question: what role do these PTMs play in GH receptor binding, protein interactions, and signaling pathways? Changes in the charge of particular amino acids could increase or decrease the strength of protein: protein binding.

All of these protein data are substantiated with MS/MS amino acid sequence data, which are assembled via tryptic peptide analysis, and incontrovertibly identify each protein. When we use the name of a protein, that means that we know the protein. *No other analytical method provides critical amino acid sequence information of a protein at endogenous biological levels.*

Furthermore, it is quite helpful to illustrate the location of each amino acid modification that we found in GHP. Figure 4 highlights in different colors the modified K, N, T, and S amino acid residues that we discovered during our recent studies. It is clear that the amino acid sequences contained within the region residues 171–178 constitute a highly modified region. GH contains two anti-parallel alpha-helices that, most probably, contribute to the access of those particular residues to modification mechanisms.

3.1.5   Signalomics of GHPs

All GHPs (except for one GHP, T46) found in this study derived from mature GH; that finding means the signal peptide 1–26 was removed from the GH prehormone. These significant data indicate that a potential regulatory system, or a dysregulated system, might participate in the formation of pituitary adenoma.

3.1.6   Splicing Variants of GHPs

Alternative splicing is an important factor to produce protein diversity. Human GH has four splicing variants [15] that include: splicing variant 2 (removal of amino acid sequence 58–72); splicing variant 3 (removal of amino acid sequence 111–148); and splicing variant 4 (removal of amino acid sequence 117–162); all

from the normal GH (GH variant 1) [16]. We found that two GHPs were splicing variant 2, one GHP was splicing variant 3, 43 GHPs were splicing variant 1 (normal GH), and no splicing variant 4, in GH-secreting pituitary adenomas. Three GHPs were splicing variant 2, 32 GHPs were splicing variant 1 (normal GH), and no splicing variants 3 and 4 were found in control pituitaries [13]. Those multiple, diverse, and significant differential splicing variant patterns found between pituitary adenomas and controls reflect differential biosynthetic patterns that were significantly altered between controls and adenomas. More-detailed knowledge of the basic molecular, enzymatic, and PTM mechanisms that produce those differential patterns might help to clarify the pathophysiology of pituitary adenomas.

3.1.7   Rationale for Different GHPs

Several factors that contribute to the different amounts and structures of the GHPs include:

1. *Certain genes*. STAT3 induces GH-secreting pituitary adenoma cell growth. Genes bind specifically to the hGH promoter to induce transcription to further promote GH secretion.
2. *Alterations* in cell-cycle regulation and growth-factor signaling; epigenetic changes (DNA methylome; histone modification) lead to gene mutations for GH hypersecretion.
3. *Mis-translation*: source of great diversity.
4. *PTMs* that impact structure and function of proteins include glycosylation, phosphorylation, acetylation, ubiquitination, deamidation, nitration, plus others. Those PTMs allow for an exponential increase in the number of proteoforms.

3.1.8   Strengths, Weaknesses, and Future GHP Studies. An Important Question for PPPM: Which GHP Interacts with the GH Receptor?

The *strengths* of this GH study include solid experimental protocols and the unambiguous amino acid sequence data that elucidated the molecular basis of differential GHP patterns. The *limits* of this study are the small sample size and the need for more tissue samples. However, it is difficult to obtain pituitary controls (postmortem) and adenomas (post-surgery).

Future studies include several important factors: (1) serum GH proteoform patterns, which could be an effective biomarker for PPPM to treat GH-secreting pituitary adenomas and GH-related diseases; (2) GHP interactions with its GH receptors; and (3) include other PTMs.

A very difficult, theoretical, but extremely fruitful, goal would be to develop "chrono-pan-omics" experimental strategies to monitor over time all of the ongoing changes in all of the pertinent pituitary proteoforms.

Moreover, when we state that a pituitary "protein" hormone interacts with its receptor, specifically which "protein (GHP)" do we mean out of the dozens of GHPs? That same question exists for any pathology, and is an important question for PPPM studies and clinical practice.
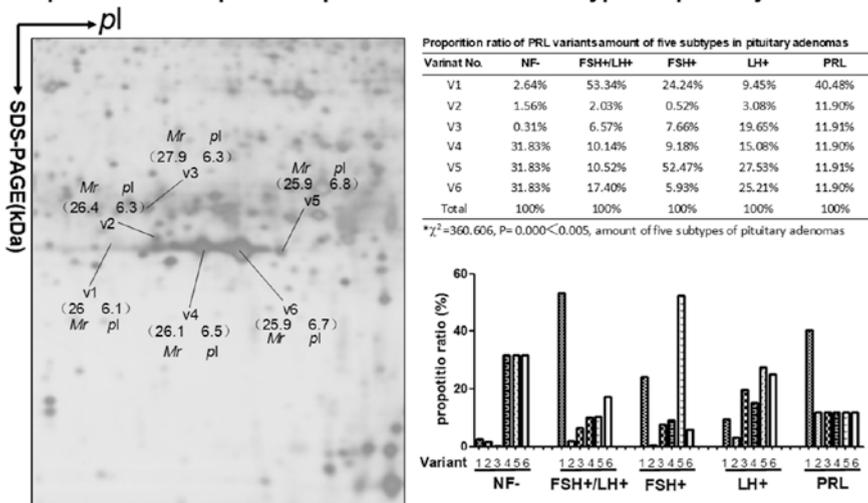
3.2   Human Prolactin Proteoforms (PRLPs)

We discovered six PRLPs in pituitary adenomas [17]. Those six PRLPs had a significantly different distribution pattern among the five groups of pituitary adenomas: NF⁻, FSH⁺/LH⁺, FSH⁺, LH⁺, and PRL⁺ (Fig. 5). The proportional ratio of those PRLPs in the adenoma subtypes demonstrates striking differences that result from a variety of dysfunctional metabolic pathways. Apparently, rich sub-molecular mechanistic pathways occur in PRL-secreting pituitary adenomas, and it is important to elucidate the multiple molecular pathways that participate in those pathways.

Bioinformatic analysis of these PRLPs predicted that all six PRLPs derive from deamidation, phosphorylation, N-glycosylation, and O-glycosylation. Interestingly, in contradistinction to GHPs, all PRLPs derived from the PRL prohormone, and not from the mature PRL [17]. They retain the signal peptide. The GH and PRL data are different and indicate that signalomics is a novel concept that apparently plays a significant role within GH and PRL adenomas, and that could provide an effective diagnostic PPPM tool for those adenomas.

It is again important to know which PRLP interacts with its receptor, and what are the effects of the different PRLPs on the signaling pathways. PRL interacts with either the short PRL receptor or the long PRL receptor. The short PRL receptor activates the PI3K/AKT system, and the long receptor activates the Jak STAT signaling pathway [17]. At this time, we do not know which one of the six PRLPs interacts with which receptor, and thus we do not know the effect on the two separate pathways. Further studies are needed on those crucial molecular pathways.

Fig. 5 2DGE image of prolactin proteoforms (PRLPs) in human pituitary tissues and its proportion ratio among five subtypes of pituitary adenomas. (Modified from Qian S, et al. [17], copyright permission from Frontiersin publisher open access article, copyright 2018)

# 4   Multiomics of Pituitary Adenomas in the Framework of PPPM

4.1   Comparative Proteomics of Pituitary Adenomas

We also studied the detailed comparative proteomics of eight control and 15 macroadenoma pituitary tissue samples [12, 16, 18–22]. We rigorously demonstrated the experimental reproducibility (gel spot volume and spot position) within each gel set, and synthesized a master gel for controls. When we compared each individual adenoma gel to that master gel, we accurately located differentially expressed proteins (DEPs).

Those comparative 2DGE data can be rationalized readily within a 7-dimensional space (five dimensions for protein abundance; one for protein name; one for protein: protein interactions). Protein abundances either increased (10- or 100-fold), did not change, or decreased (10- or 100-fold). Those significant differential abundance data were the first glimpse into the extensive variety of protein changes within a pituitary adenoma, and led to many subsequent studies. Interaction analysis linked how those proteins interacted with each other and yielded a rich interaction network that involved GH, PRL, CAPZB, cytochrome c, Jnk, ERK, among many other proteins. Modified proteins were found in mitochondrial complex 1 (NADH dehydrogenase ubiquitinone Fe-S protein, NDUFS8) and in complex four (cytochrome c oxidase, COX6B1). Those modified proteins might play a role to generate reactive oxygen species (ROS).

Other studies contributed to those comparative proteomics data. Lu, et al. listed several sites of potential pharmacology treatment for pituitary adenomas in the MAPK signaling pathway [23] (Fig. 6). The ERK-MAPK signaling, p38-MAPK signaling, and JNK signaling all play important roles in pituitary adenomas. For the MAPK signaling system in pituitary adenomas, the activation of ERK signaling is generally thought to promote cell proliferation and growth, whereas the activations of p38 and JNK signaling are generally thought to promote cell apoptosis [23]. Some therapeutic drugs exert anti-tumor effects by targeting one of these pathways or all three pathways at the same time. MAPK signaling is a very complex network, and always interacts with other pathways such as the PI3K and cAMP pathways to affect tumor progression. The latest development of MAPK signaling in pituitary adenomas and the related anti-tumor drugs that target MAPK signaling pathways would provide new insights into critical pituitary adenoma pathogenic mechanisms and pre-clinical data for effective treatment [23].

The Nrf-2-mediated oxidative stress response signaling pathways are important and offer potential targets for personalized medicine of pituitary adenomas [9, 24] (Fig. 7). Oxidative stress is sensed by this Nrf2 system. Oxidative stress derives from a rich variety of sources: heavy metals, drugs, xenobiotics, UV radiation, etc.
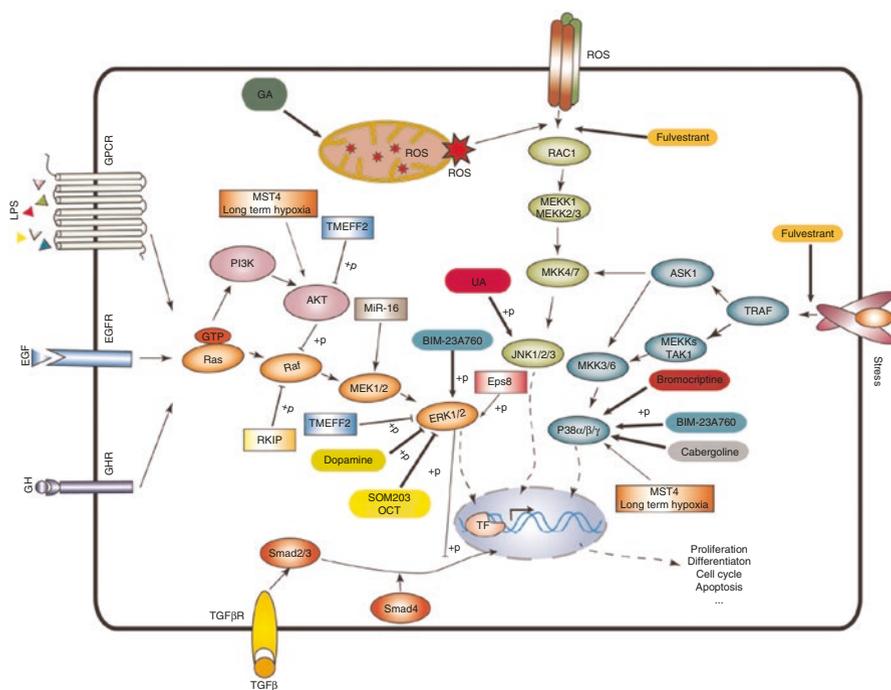
Fig. 6 MAPK signaling pathways and the potential therapeutic targets. In p38 signaling, TRAF activates ASK1, TAK1 or MEKK1, which activates MKK3/6, which subsequently phosphorylates p38 isoforms. In the ERK signaling, Ras activates the serine/threonine protein kinase Raf to activate MEK1/2; then MEK1/2 phosphorylates the ERK1/2. In JNK signaling, RAC1 activates MEKK1 or MEKK2/3 to activate MKK4/7; and then MKK4/7 phosphorylates JNK1/2/3. The ASK1 in the p38 signaling also activates MKK4/7 to crosstalk with JNK signaling. *ROS* reactive oxygen species, *GA* 18β-glycyrrhetinic acid. BIM-23A760 is a dopamine–somatostatin chimeric compound. *OCT* octreotide. SOM230 and OCT are somatostatin analogs. Rectangle denotesmeans the potential drug targets. (Reproduced from Lu et al. (2019) [23], with permission from Frontiersin publisher open-access article, copyright 2019)

That oxidative stress is transferred into the cytoplasm to produce electrophiles [reactive oxygen species (ROS), and reactive nitrogen species (RNS)] that alter multiple cytoplasm pathways and the Nrf2/Keap1 complex. Phosphorylated Nrf2 separates and transfers into the nucleus to interact with the antioxidant-response element (ARE)/electrophile-response element (EpRE). Thereafter, multiple systems are activated: ubiquitination; chaperone and stress response; phase 1 and 2 detoxification and reactive metabolites; phase III detoxifying proteins; and antioxidant proteins. Our comparative proteomics studies found many of the molecules discussed here (HSPs, CAT, SOD, etc.)
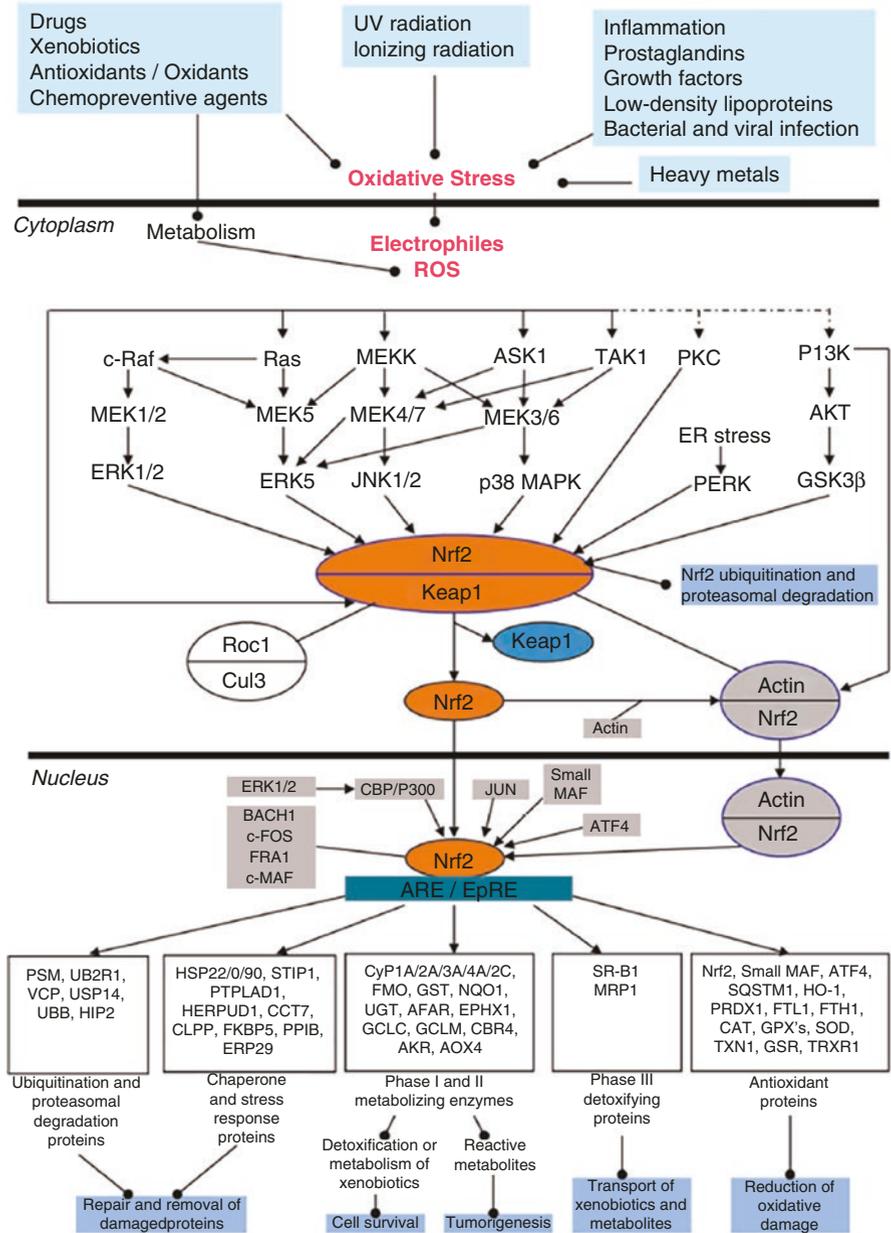
Fig. 7 Nrf2-mediated oxidative stress response signaling pathways in human pituitary adenomas. (Modified from Zhan & Desiderio (2010) [24], with permission from BioMed Central publisher open-access article, copyright 2010; modified from Long et al. (2019) [25], with permission from Frontiersin publisher open-access article, copyright 2019; and reproduced from Zhan et al. (2021) [9], with permission from Frontiersin publisher open-access article, copyright 2021)

4.2   Cellular Systems Significantly Associated with Pituitary Adenomas

Our experimental data demonstrate that mitochondrial dysfunction, oxidative stress (ROS; RNS), cell-cycle dysregulation, and MAPK signaling associate with human pituitary adenomas [24, 25]. Those signaling pathway system changes provide a systematic and in-depth insight into molecular mechanisms, and indicate the effective biomarkers and therapeutic targets for PPPM of pituitary adenomas.

4.3   Quantum Improvements in PPPM of Pituitary Adenomas

The diverse experimental data discussed in this chapter can be readily assembled into a coherent, focused synopsis of their significance and of their roles in PPPM. For example, the pituitary adenoma proteome can be accessed via two separate branches: the tissue proteome and/or the body-fluid proteome/peptidome [26].

For the body-fluid branch, CSF and plasma are used to elucidate pattern variations with protein/antibody microarrays. One can readily and accurately assess the therapeutic response, interventional prevention, and predictive diagnosis for a high-risk population. In this manner, PPPM achieves a very high level of accuracy [26].

For the tissue proteome branch, one detects proteome variations in protein expression, protein modification, and slicing. Those data lead to modality of protein variation and an accurate molecular classification that is used for interventional prevention, personalized treatment, and personalized patient care [26]. Overall, this paradigm can lead to halt the occurrence and progression of tumors.

Thus, the data described in this chapter led to a quantum level of PPPM improvement that has been achieved for pituitary tumor detection, analysis, and treatment. Clearly, the basics of this new paradigm could be readily translated to many other human pathologies within the broad scope of PPPM.

4.4   Omics Biomarkers for Pituitary Tumors

An excellent review demonstrated graphically the omics biomarkers that have been published in the literature of pituitary tumors [27]. The authors correlated 171 different biomarkers with associated pituitary neuroendocrine tumor (PitNET) subtypes (lactotrophs, somatotrophs, corticotrophs, NF-PitNET, null cell, and varied PitNETs). That review is an excellent source for pituitary adenoma biomarkers.

4.5   Differential Patterns and PPPM

Similar to a cell, we can now organize all of the various omics into one coherent differential pattern. For example, all of the patterns obtained from DNA, RNA,

proteins, etc. can be distilled into one "cell-like" "meta-omics" system to accurately reflect the metabolism in normal cells in order to clarify the dysfunctional systems within a pathology [28]. The biomarker patterns that are under study in many laboratories can be assembled into an integrated pattern that, at a "birds-eye view" level, reflects the metabolism and pathology that occurs within a cell [29] (Fig. 8). For example, a DNA marker pattern derives from genomics data (mutation, loss, inserts, fusion); an RNA biomarker pattern from transcriptomics (splicing, ncRNA); protein biomarker patterns from proteomics (PTMs, variants, proteoforms); metabolite biomarker pattern from metabolomics (control and tumor metabolites); and image-texture features from radiomics (PET-CT, MRI, CT) [28–30]. After all of those individual patterns are combined, a clearer, more-accurate picture of the pathology emerges that occurs within an adenoma. The rapid improvements in experimental paradigms, instrumentation, and application in each contributing field provide a dramatically improved ability in our ability to monitor basic molecular events that occur in human control and pathological tissues. PPPM, in turn, improves at a rapid, significant, and more-accurate pace to improve the individual health care of each patient.
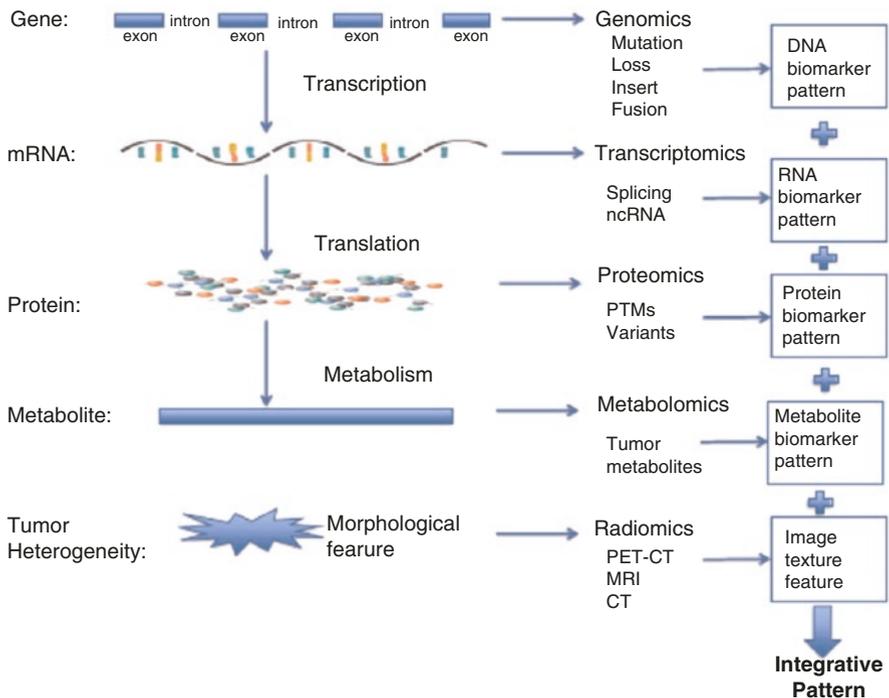
Fig. 8 Multiomics-based integrative pattern biomarkers. (Reproduced from Cheng and Zhan (2017) [29], with permission from Springer publisher open access article, copyright 2017)
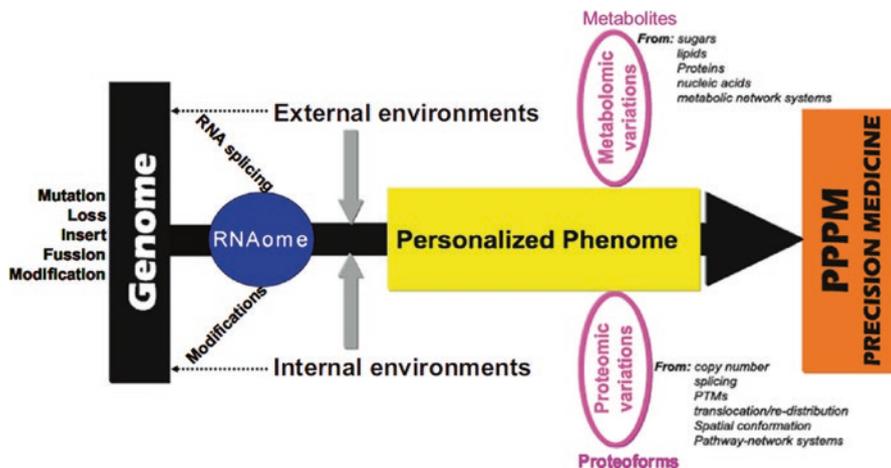
Fig. 9 The contribution of mulltiomics to pituitary adenoma clinical practice. (Modified and upgraded from Zhan et al. (2018) [15], with permission from Elsevier publisher, copyright 2018. Reproduced from Li N, Desiderio DM, and Zhan X (2021) [1], copyright permission from Wiley publisher, copyright 2021)

4.6   Improved PPPM Practice in Pituitary Adenomas

The amount of novel data accumulated in recent pituitary adenoma research provides a quantum leap in our ability to improve accurate PPPM in practice. For example, multiomics data plus biomarker data lead to improved prediction/prevention, diagnosis/therapy, and prognostic assessment [26, 28, 30]. Those improvements derive from several factors. The combination of multiomics data (genome, transcriptome, proteome, peptidome, metabolome, microbiome, and radiome) with molecular network-based pattern biomarkers (age, gender, race, histology classification, functional classification, stage, metastases, surgical options, adjuvant therapy, multiple endocrine neoplasia, Carney complex, familial isolated pituitary adenoma) leads effectively to improved prediction/prevention, diagnosis/therapy, and prognostic assessment [26]. As a result, PPPM improves significantly.

In sum, PPPM is moving from the genome, via the RNAome, to the personalized phenome and PPPM high-precision medicine [1, 15] (Fig. 9).

4.7   PPPM of the Personal Phenome

Along that long route, several components play a significant role. The genome incorporates mutations, losses, inserts, fusion, and modifications [1, 15] (Fig. 9). The RNAome involves RNA splicing and modifications [1]. External and internal

environments impact on the genome, RNAome, and personalized phenome [15]. Variations in the metabolome and proteome impact on the phenome [15]. In sum, the cell (normal; tumor) results from the delicate interplay among a wide variety of molecular components.

It is the goal of this research program to elucidate (as many as possible) the molecular components in those systems to improve significantly PPPM.

5   PPPM-Related Aspects in Pituitary Adenomas

5.1   Literature Analyses of BMI, Pituitary, Adenomas, Carcinomas, and Neoplasias

The recent EPMA position paper 2021 that focused on "normal" BMI is very important (via a serendipitous circuitous route) to our study of pituitary adenomas [31]. The section on prostate cancer was significant to DMD, and circuitously prompted the question: "what effect does BMI have on the pituitary." A literature search found two pertinent papers that expanded on that question [32, 33]. One paper discussed malignant transformation in a non-functional pituitary adenoma—NFPA (pituitary carcinoma) [32], and a second paper discussed increased incidence of neoplasia in patients with pituitary adenomas [33]. Those papers are striking, and suggest that one must consider *all* of the molecular mechanisms and dysfunctional systems that lead from a control, to an adenoma, and on to a neoplasia. It is quite clear that something (that we do not yet know- but is very important) happens in those special cases, and warrants further study. All of these intertwined pieces of information point to potential future studies on pituitary carcinomas.

5.2   Economics of Acromegaly Care

It is quite helpful to place this research into proper perspective, and therefore to also analyze the economic aspects of pituitary adenomas.

The U.S.A. population is $\sim 3.3 \times 10^8$, and acromegaly patients total $\sim$ 4/million (330 million $\times$ 4/million = 1320 acromegaly patients.). It has been estimated that 25 years of acute care costs for an acromegaly patient are ~1000,000 USD. Therefore, the estimated total cost to the U.S.A. healthcare system is 1320 $\times$ 1000,000 USD, or 1.3 billion USD over 25 years.

Correspondingly, for a world population of 7.9 billion, costs for acromegaly patients (total ~ 31,300) equal 31,300 $\times$ 10^6, or 31.3 billion USD over 25 years.

All of those total costs increase further when all of the other pituitary hormones and associated adenomas are included.

5.3   Guiding Principle of this Research Program

The greatest level of understanding of any human disease always derives from an in-depth and accurate analysis of the molecules that are involved in that disease. That concept means that the structural elucidation of a molecule- and the amino acid sequence determination of each peptide, protein, PTM, proteoform, etc. must always be rigorously established in order to secure the highest confidence and the most-accurate PPPM care of a pituitary adenoma patient. These concepts apply to all human pathologies.

6   Conclusions and Expert Recommendations for PPPM in Pituitary Adenomas

Multiomics is an effective approach to resolve the complex pituitary adenomas in the framework of 3P medicine (PPPM) [1, 15]. However, an individual phenome (especially proteome and metabolome) is a bridge to link the genome to PPPM practice [15]. Phenome-centered multiomics is a trend for PPPM practice in pituitary adenomas [1]. Metabolomics and proteomics are equally important to provide insight into phenomic variations to discover effective biomarkers and therapeutic targets for pituitary adenomas [1, 15]. Moreover, biomolecular modifications, including DNA modifications, RNA post-transcriptional modifications, protein PTMs, and RNA alternative splicing are important factors that produce proteoforms [34]. A proteoform is the basic unit of a proteome, which is the final structure and functional format of a canonical protein or a gene [5]. Therefore, proteoformics is the future direction of next-generation proteomics. In this study, significant multiomics-based signaling pathways, biomarkers, and therapeutic targets [13, 17, 23–25, 27] were found for pituitary adenomas in the framework of PPPM.

We recommend an emphasis on multiomics-driven PPPM for pituitary adenomas in the following three aspects:

1. Predictive approach. Pituitary adenomas are an intracranial, very complex, chronic, and whole-body disease, with high-level heterogeneity, and involve a series of molecular changes in the levels of DNA, RNA, protein, and metabolite. Multiomics-based body-fluid biomarkers (for example, from CSF and serum) are an effective approach to predict high-risk person who might develop pituitary adenomas, or prognostically assess the therapeutic effects [26]. In this aspect, serum peptidomics and metabolomics might offer important roles.
2. Targeted prevention. For targeted prevention, multiomics based on pituitary adenoma tissues can offer significant signaling pathway changes to discover important hub molecules. Those important signaling pathway changes, and the corresponding hub molecules, would elucidate the molecular mechanisms of

pituitary adenomas and discover novel therapeutic targets for targeted prevention [1, 26]. Another aspect, invasive behavior, is a very challenging clinical problem. Multiomics analysis of invasive vs. noninvasive pituitary adenomas can uncover invasiveness-related molecules for targeted prevention of invasive behavior.

3. Personalized medical services. Pituitary adenomas are highly heterogenous, and include different subtypes and invasive vs. noninvasive subtypes. Multiomics analysis can discover effective biomarkers to stratify different subtypes of pituitary adenoma patients for personalized treatments [35, 36]. For example, we can establish serum growth hormone proteoform pattern biomarkers to discriminate the invasive vs. noninvasive pituitary adenoma patients for personalized therapy [13]. If the patient is stratified into invasive group, then this patient will receive radiation therapy and even drug therapy in addition to neurosurgery. If the patient is stratified into the noninvasive group, then this patient will only receive neurosurgery, which would be fine.

References

1. Li N, Desiderio DM, Zhan X (2021) The use of mass spectrometry in a proteome-centered multiomics study of human pituitary adenomas. Mass Spectrom Rev 41:1–50. https://doi.org/10.1002/mas.21710

2. Zhan X, Zhou T, Cheng T, Lu M (2019) Recognition of multiomics based molecule-pattern biomarker for precise prediction, diagnosis and prognostic assessment in cancer. In: Samadikuchaksaraei A (ed) Bioinformatics tools for detection and clinical interpretation of genomic variations. InTech-Open Science Publisher, London

3. Batt J, Asa S, Fladd C, Rotin D (2002) Pituitary, pancreatic and gut neuroendocrine defects in protein tyrosine phosphatase-sigma-deficient mice. Mol Endocrinol 16(1):155–169. https://doi.org/10.1210/mend.16.1.0756

4. Zhan X, Li N, Zhan X, Qian S (2018) Revival of 2DE-LC/MS in proteomics and its potential for large-scale study of human proteoforms. Med One 3:e180008

5. Zhan X, Li B, Zhan X, Schlüter H, Jungblut PR, Coorssen JR (2019) Innovating the concept and practice of two-dimensional gel electrophoresis in the analysis of proteomes at the proteoform level. Proteomes 7(4):36. https://doi.org/10.3390/proteomes7040036

6. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE (2004) The prevalence of pituitary adenomas: a systematic review. Cancer 101(3):613–619. https://doi.org/10.1002/cncr.20412

7. Desiderio DM, Cezayirli RC, Fridland G, Robertson JT, Sacks H (1985) Metabolic profiling of radioreceptor-assayable opioid peptides in a human pituitary ACTH-secreting tumor. Life Sci 37(19):1823–1828. https://doi.org/10.1016/0024-3205(85)90225-5

8. Kusmierz JJ, Sumrada R, Desiderio DM (1990) Fast atom bombardment mass spectrometric quantitative analysis of methionine-enkephalin in human pituitary tissues. Anal Chem 62(21):2395–2400. https://doi.org/10.1021/ac00220a026

9. Zhan X, Li J, Zhou T (2021) Targeting Nrf2-mediated oxidative stress response signaling pathways as new therapeutic strategy for pituitary adenomas. Front Pharmacol 12:565748. https://doi.org/10.3389/fphar.2021.565748

10. Marks DL, Butler AA, Turner R, Brookhart G, Cone RD (2003) Differential role of melanocortin receptor subtypes in cachexia. Endocrinology 144(4):1513–1123. https://doi.org/10.1210/en.2002-221099

11. Dehkhoda F, Lee CMM, Medina J, Brooks AJ (2018) The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. Front Endocrinol 9:35. https://doi.org/10.3389/fendo.2018.00035

12. Desiderio DM, Zhan X (2003) The human pituitary proteome: the characterization of differentially expressed proteins in an adenoma compared to a control. Cell Mol Biol (Noisy-le-Grand) 49(5):689–712

13. Li B, Wang X, Yang C, Wen S, Li J, Li N, Long Y, Mu Y, Liu J, Liu Q, Li X, Desiderio DM, Zhan X (2021) Human growth hormone proteoform pattern changes in pituitary adenomas: potential biomarkers for 3P medical approaches. EPMA J 12(1):67–89. https://doi.org/10.1007/s13167-021-00232-7

14. Zhan X, Yang H, Peng F, Li J, Mu Y, Long Y, Cheng T, Huang Y, Li Z, Lu M, Li N, Li M, Liu J, Jungblut PR (2018) How many proteins can be identified in a 2DE gel spot within an analysis of a complex human cancer tissue proteome? Electrophoresis 39(7):965–980. https://doi.org/10.1002/elps.201700330

15. Zhan X, Long Y, Lu M (2018) Exploration of variations in proteome and metabolome for predictive diagnostics and personalized treatment algorithms: innovative approach and examples for potential clinical application. J Proteome 188:30–40. https://doi.org/10.1016/j.jprot.2017.08.020

16. Zhan X, Giorgianni F, Desiderio DM (2005) Proteomics analysis of growth hormone isoforms in the human pituitary. Proteomics 5(5):1228–1241. https://doi.org/10.1002/pmic.200400987

17. Qian S, Yang Y, Li N, Cheng T, Wang X, Liu J, Li X, Desiderio DM, Zhan X (2018) Prolactin variants in human pituitaries and pituitary adenomas identified with two-dimensional gel electrophoresis and mass spectrometry. Front Endocrinol 9:468. https://doi.org/10.3389/fendo.2018.00468

18. Zhan X, Desiderio DM (2003) Spot volume vs. amount of protein loaded onto a gel: a detailed, statistical comparison of two gel electrophoresis systems. Electrophoresis 24(11):1818–1833. https://doi.org/10.1002/elps.200305375

19. Zhan X, Desiderio DM (2003) Differences in the spatial and quantitative reproducibility between two second-dimensional gel electrophoresis systems. Electrophoresis 24(11):1834–1846. https://doi.org/10.1002/elps.200305389

20. Zhan X, Desiderio DM (2003) Heterogeneity analysis of the human pituitary proteome. Clin Chem 49(10):1740–1751. https://doi.org/10.1373/49.10.1740

21. Zhan X, Desiderio DM (2005) Comparative proteomics analysis of human pituitary adenomas: current status and future perspectives. Mass Spectrom Rev 24(6):783–813. https://doi.org/10.1002/mas.20039

22. Zhan X, Evans CO, Oyesiku NM, Desiderio DM (2003) Proteomics and transcriptomics analyses of secretagogin down-regulation in human non-functional pituitary adenomas. Pituitary 6(4):189–202. https://doi.org/10.1023/b:pitu.0000023426.99808.40

23. Lu M, Wang Y, Zhan X (2019) The MAPK pathway-based drug therapeutic targets in pituitary adenomas. Front Endocrinol (Lausanne) 10:330. https://doi.org/10.3389/fendo.2019.00330

24. Zhan X, Desiderio DM (2010) Signaling pathway networks mined from human pituitary adenoma proteomics data. BMC Med Genet 3:13. https://doi.org/10.1186/1755-8794-3-13

25. Long Y, Lu M, Cheng T, Zhan X, Zhan X (2019) Multiomics-based signaling pathway network alterations in human non-functional pituitary adenomas. Front Endocrinol 10:835. https://doi.org/10.3389/fendo.2019.00835

26. Zhan X, Desiderio DM (2010) The use of variations in proteomes to predict, prevent, and personalize treatment for clinically nonfunctional pituitary adenomas. EPMA J 1(3):439–459. https://doi.org/10.1007/s13167-010-0028-z
27. Aydin B, Caliskan A, Arga KY (2021) Overview of omics biomarkers in pituitary neuroendocrine tumors to design future diagnosis and treatment strategies. EPMA J 12(3):383–401. https://doi.org/10.1007/s13167-021-00246-1
28. Hu R, Wang X, Zhan X (2013) Multi-parameter systematic strategies for predictive, preventive and personalised medicine in cancer. EPMA J 4(1):2. https://doi.org/10.1186/1878-5085-4-2
29. Cheng T, Zhan X (2017) Pattern recognition for predictive, preventive, and personalized medicine in cancer. EPMA J 8(1):51–60. https://doi.org/10.1007/s13167-017-0083-9
30. Lu M, Zhan X (2018) The crucial role of multiomic approach in cancer research and clinically relevant outcomes. EPMA J 9(1):77–102. https://doi.org/10.1007/s13167-018-0128-8
31. Golubnitschaja O, Liskova A, Koklesova L, Samec M, Biringer K, Büsselberg D, Podbielska H, Kunin AA, Evsevyeva ME, Shapira N, Paul F, Erb C, Dietrich DE, Felbel D, Karabatsiakis A, Bubnov R, Polivka J, Polivka J Jr, Birkenbihl C, Fröhlich H, Hofmann-Apitius M, Kubatka P (2021) Caution, "normal" BMI: health risks associated with potentially masked individual underweight-EPMA position paper 2021. EPMA J 12(3):1–22. https://doi.org/10.1007/s13167-021-00251-4
32. Lenders N, McCormack A (2018) Malignant transformation in non-functioning pituitary adenomas (pituitary carcinoma). Pituitary 21(2):217–229. https://doi.org/10.1007/s11102-017-0857-z
33. Popovic V, Damjanovic S, Micic D, Nesovic M, Djurovic M, Petakov M, Obradovic S, Zoric S, Simic M, Penezic Z, Marinkovic J (1998) Increased incidence of neoplasia in patients with pituitary adenomas. The pituitary study group. Clin Endocrinol (Oxf) 49(4):441–445. https://doi.org/10.1046/j.1365-2265.1998.00536.x
34. Zhan X, Li J, Guo Y, Golubnitschaja O (2021) Mass spectrometry analysis of human tear fluid biomarkers specific for ocular and systemic diseases in the context of 3P medicine. EPMA J 12(4):1–27. https://doi.org/10.1007/s13167-021-00265-y
35. Liu D, Li J, Li N, Lu M, Wen S, Zhan X (2020) Integration of quantitative phosphoproteomics and transcriptomics revealed phosphorylation-mediated molecular events as useful tools for a potential patient stratification and personalized treatment of human nonfunctional pituitary adenomas. EPMA J 11(3):419–467. https://doi.org/10.1007/s13167-020-00215-0
36. Wang Y, Cheng T, Lu M, Mu Y, Li B, Li X, Zhan X (2019) TMT-based quantitative proteomics revealed follicle-stimulating hormone (FSH)-related molecular characterizations for potentially prognostic assessment and personalized treatment of FSH-positive non-functional pituitary adenomas. EPMA J 10(4):395–414. https://doi.org/10.1007/s13167-019-00187-w