FACULDADE DE MEDICINA
UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

ANA SOFIA FERREIRA DA SILVA

The epigenomics of pituitary tumours: pathogenesis and clinical implications

ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE ENDOCRINOLOGIA

Trabalho realizado sob a orientação de:

DOUTOR LUIS MIGUEL CARDOSO

PROFESSORA DOUTORA MARIA LEONOR VIEGAS GOMES

ABRIL/2021

The epigenomics of pituitary tumours: pathogenesis and clinical implications

Ana Sofia Ferreira da Silva[1]; Maria Leonor Viegas Gomes[1,2]; Luis Miguel Cardoso[1,2]

[1] Faculdade de Medicina da Universidade de Coimbra

[2] Serviço de Endocrinologia, Diabetes e Metabolismo do Centro Hospitalar e Universitário de Coimbra

Contacto: sofiaferreirasilva6@gmail.com

ABRIL/2021

INDEX

TABLE INDEX

FIGURE INDEX

ABBREVIATION LIST

ACTH - Adrenocorticotropic Hormone

ADP - Adenosine Diphosphate

AFAP1-AS1 - Actin Filament-associated Protein 1 Antisense RNA 1

AIP - Aryl Hydrocarbon Receptor-interacting Protein

BCL2 - B-cell Lymphoma 2

BCL-XL - B-cell Lymphoma-extra Large

BMP-4 - Bone Morphogenetic Protein 4

C5orf66-AS1 - C5orf66 Antisense RNA 1

CABLES1 - CDK5 and ABL1 Enzyme Substract 1

CACNA1C - Calcium Voltage-Gated Channel Subunit Alpha1C

CADM1 - Cell Adhesion Molecule 1

cAMP - Cyclic Adenosine Monophosphate

CASC2 - Cancer Susceptibility 2

CASP8 - Caspase 8

CCAT2 - Colon Cancer Associated Transcript 2

CCNA2 - Cyclin-A2

CCNB1 – Cyclin-B1

CDH - Cadherin-1

CDK - Cyclin-dependent Kinase

CDKI - Cyclin-dependent Kinase Inhibitor

CDKN2A - Cyclin-dependent Kinase Inhibitor 2A

cfRNA - Cell-free RNA

CLRN1-AS1 - Clarin 1 antisense RNA 1

CpG - 5'-C-phosphate-G-3'

CTCs - Circulating Tumour Cells

ctDNA - Circulating Tumour DNA

CXCR - C-X-C chemokine receptor

DAPK - Death Associated Protein Kinase

DESD protein - Death Effector Domain-containing Protein

DNA - Deoxyribonucleic Acid

DNMTi - DNMT Inhibitors

DNMTs - DNA methyltransferases

E2F1 - E2F Transcription Factor 1

EFEMP1 - EGF-containing Fibulin-like Extracellular Matrix Protein 1

EGF - Epidermal Growth Factor

EML2 - Echinoderm Microtubule Associated Protein Like 2

ESR1 - Oestrogen Receptor Alpha

ESRP2 - Epithelial Splicing Regulatory Protein 2

FDA - Food and Drug Administration

FGF4 - Fibroblast Growth Factor 4

FGFR - Fibroblast Growth Factor Receptor

FOS - Fos proto-oncogene

FOXO1 - Forkhead Box Protein O1

FSH - Follicle-stimulating Hormone

GADD45γ - Growth Arrest and DNA Damage-inducible 45

GALNT9 - Polypeptide N-Acetylgalactosaminyltransferase 9

GH - Growth Hormone

GHRH - GH-releasing hormone

GNAS - Guanine Nucleotide Binding Protein, Alpha Stimulating

HATs - Histone Acetyltransferases

HDAC - Histone Deacetylase Complex

HDACi - HDAC Inhibitors

HDMi - Histone Demethylase Inhibitors

HDMTs - Histone Demethyltases

HMGA - High-mobility Group A

HMTi - Histone Methyltransferase Inhibitors

HOTAIR - Hox Transcript Antisense Intergenic RNA

HOXB1 - Homeobox B1

IFNG-AS1 - IFNG Antisense RNA 1

IGF2 - Insulin-like Growth Factor 2

KCNAB2 - Voltage-Gated Potassium Channel Subunit Beta-2

KCNAB4 - Calcium-Activated Potassium Channel Subunit Beta-4

KMT2A - Lysine methyltransferase 2A

KRAS - Kirsten Rat Sarcoma Viral Oncogene Homolog

LH - Luteinizing Hormone

lncRNA - Long Non-coding RNA

MAGE-A3 - Melanoma-associated Antigen 3

MEG3 - Maternally Expressed 3

MGMT - O6-methylguanine-DNA Methyltransferase

miRNA - MicroRNA

mRNA - Messenger RNA

MSH6 - MutS Homolog 6

MUC1 - Mucin 1

MYC - MYC Proto-Oncogene

NFPAs - Non-functioning Pituitary Adenomas

PIT1 - Pituitary Transcription Factor 1

POMC - Proopiomelanocortin

PRKCD - Protein Kinase C Delta

PRL - Prolactin

PTAG - Pituitary Tumour Apoptosis Gene

PTCH1 - Patched-1 Protein

PTEN - Phosphatase and Tensin Homolog

PTTG1 - Pituitary Tumour Transforming Gene 1

RAS - Rat Sarcoma

RASSF1 - Ras Association Domain Family Member 1

RASSF3 - Ras Association Domain Family Member 3

Rb – Retinoblastoma Protein

RB1 - RB Transcriptional Corepressor 1

RHOD - Rho Related GTP-Binding Protein

RIZ1 - Retinoblastoma Protein-interacting Zinc-finger Gene 1

RNA - Ribonucleic Acid

RPSAP52 - Ribosomal Protein SA Pseudogene 52

SAHA - Suberoylanilide Hydroxamic Acid

SNHG1 - Small Nucleolar RNA Host Gene 1

SOX5 - SRY-Box Transcription Factor 5

SSA - Somatostatin Analogue

SSTR2 - Somatostatin Receptor 2

SSTR5 - Somatostatin Receptor 5

STK26 - serine/threonine kinase 26

TIMP2 - Tissue Inhibitor of Metalloproteinases 2

TSA - Trichostatin A

TSG - Tumour Suppressor Gene

TSH - Thyroid-stimulating Hormone

USP8 - Ubiquitin Specific Peptidase 8

VEGF-R1 - Vascular Endothelial Growth Factor Receptor 1

WHO - World Health Organization

XIST - X-inactive Specific Transcript

YES - YES proto-oncogene

ABSTRACT

Introduction: Pituitary tumours account for ~15% of intracranial tumours and may present with an heterogeneous clinical picture, including manifestations arising from excessive hormone production or compression/invasion of adjacent structures. Although some genetic mutations have been associated with pituitary tumourigenesis, increasing evidence suggests that epigenetic alterations – DNA methylation, histone modifications, miRNAs and lncRNAs – are associated with development of these tumours. The aim of this article is to review the scientific evidence of the epigenomics of pituitary tumours and their biological and clinical implications.

Methods: A literature search was performed in PubMed and Embase in June. MeSH terms ("pituitary tumours", "epigenomics", "DNA methylation", "histone modifications" and "miRNA") and its Emtree synonyms were used with no restrictions on language, publication type, or date.

Results: Several epigenetic alterations have been described in pituitary tumours, and tumour size, invasiveness, treatment response and hormone secretion have been linked to specific epigenetic modifications. DNA methyltransferases 1 and 3A (DNMT1 and DNMT3A) overexpression is associated with larger size and invasive behaviour, as is the upregulation of high-mobility group A (HMGA). Decreased expression of somatostatin receptor 2 (SSTR2) correlates with resistance to somatostatin analogue treatment. Non-functioning pituitary adenomas (NFPAs) overall show higher rates of methylation that could be the explanation for the lack of hormone production. *GNAS* gene is associated with somatotroph tumourigenesis, both through genetic and epigenetic mechanisms. Various studies have suggested the use of these epigenetic alterations in the diagnosis and prognosis of pituitary tumours. New drugs (e.g. Azacytidine, Decitabine, Suberoylanilide Hydroxamic Acid (SAHA), Trichostatin A and Zebularine) have also been developed to reverse epigenetic abnormalities, with promising results.

Conclusion: Specific epigenetic alterations have been associated with clinical characteristics and tumour behaviour. Various alterations have been proposed as tumour markers and new drugs have been developed to reverse these epigenetic modifications, with promising results. This evidence suggests that the incorporation of epigenetic information in tumour workups can assist clinical approach and predict prognosis.

KEYWORDS:

Pituitary Tumours; Epigenomics; DNA Methylation; Histone Modifications; miRNA

RESUMO

Introdução: Os tumores da hipófise representam ~15% dos tumores primários intracranianos com manifestações clinicas heterogéneas , incluindo manifestações resultantes de produção hormonal excessiva ou compressão/invasão de estruturas vizinhas. Apesar de algumas mutações genéticas terem sido associadas com a patogénese dos tumores hipofisários, evidência crescente sugere que alterações epigenéticas - metilação do DNA, modificação das histonas, mRNAs e lncRNAs - estão associadas ao desenvolvimento destes tumores. O objetivo deste artigo é rever a produção científica sobre a epigenómica dos tumores hipofisários em função do seu comportamento biológico e as suas implicações clínicas.

Métodos: Foi realizada uma revisão da literatura existente nos motores de busca PubMed e Embase em Junho de 2020. Foi usada a terminologia MeSH e Emtree sem restrições quanto à lingua, tipo ou data de publicação.

Resultados: Diversas alterações epigenéticas foram descritas em tumores hipofisários e carateristicas tumorais como diâmetro, invasividade, resposta ao tratamento e secreção hormonal foram relacionada com modificações epigenéticas especificas. A sobre-expressão de DNMT1 e DNMT3A está associada a maior diâmetro e comportamento mais invasivo, assim como a sobre-expressão de HMGA. A diminuição da expressão de SSTR2 correlaciona-se com resistência ao tratamento com análogos da somatostatina. Tumores não funcionantes apresentam maiores taxas de metilação que podem explicar a ausência de produção hormonal. O gene GNAS está associado com a tumorigénese dos somatotrofos, tanto por mecanismos genéticos como epigenéticos. Vários estudos têm sugerido o uso destas alterações epigenéticas no diagnóstico e prognóstico de tumores hipofisários. Novos fármacos (e.g. Azacytidine, Decitabine, SAHA, Trichostatin A e Zebularine) têm sido desenvolvidos com o objetivo de reverter estes padrões epigenéticos, com resultados promissores.

Conclusão: Alterações epigenéticas especificas foram têm sido associadas a manifestações clinicas e comportamentos específicos dos tumores hipofisários. Diversas alterações foram propostas como marcadores tumorais e novos fármacos têm vindo a ser desenvolvidos de forma a reverter estas modificações epigenéticas, com resultados promissores. Esta evidência sugere que a incorporação de um rastreio de alterações epigenéticas no estudo dos tumores pode auxiliar a abordagem clínica, a seleção do tratamento mais eficaz e a previsão prognóstica.

PALAVRAS-CHAVE:

Tumores hipofisários; Epigenómica; Metilação do DNA; Modificação das Histonas; miRNA

INTRODUCTION

Pituitary tumours are account for about 15% of all primary intracranial tumours, most of them classified as adenomas.[1, 2] They can be detected at clinical-oriented studies, incidental findings in imaging exams or post-mortem studies. Clinical manifestations may arise from excessive hormone production (e.g., alterations in physical appearance, psychiatric manifestations, reproductive or sexual dysfunction, higher risk of developing cardiovascular or osteoarticular disease, among others) or due to tumour growth and invasion/compression of adjacent structures (e.g., visual disturbances or headaches).[3]

Pituitary tumours can be classified according to hormone production, size and aggressiveness.[4] They can also be classified according to adenohypophyseal cellular lineage and transcription factors (the World Health Organization has recently recommended this approach) in lactotroph, gonadotroph, somatotroph, corticotroph, thyrotroph, and null cell tumours adenomas.[5]

Some genetic mutations have been associated with the pathogenesis of pituitary tumours, particularly in familial syndromes (e.g. multiple endocrine neoplasia types 1 and 4, Carney complex, McCune-Albright syndrome, and DICER1 syndrome).[6] However, growing evidence points to the possibility that epigenetic modifications could be involved in the process of tumourigenesis, including deoxyribonucleic acid (DNA) methylation, histone modifications, miRNAs (micro ribonucleic acid) and lncRNAs (long non-coding RNA).[7, 8]

The aim of this article is to review the scientific evidence on the epigenomics of pituitary tumours and their biological and clinical implications. Diagnostic, therapeutic and prognostic implications will also be discussed.

METHODS

In June 2020, a literature search in PubMed and Embase was conducted using the following MeSH terms (and EMTREE synonyms): "Pituitary Tumours", "Epigenomics", "DNA Methylation", "Histone Modifications" and "miRNA". There were no restrictions on language, publication type, or date. Additionally, reference lists from all major reviews were examined for citations that did not appear in the PubMed or EMBASE search.

RESULTS

1. Introduction

1.1. Epidemiology

Pituitary tumours represent 15% of all intracranial tumours with the majority of them being considered benign.[1, 2, 4] They can be detected during clinical studies precipitated by clinical manifestations caused by hormone production or compression/invasion of adjacent structures, but also as incidental findings in imaging exams or post-mortem.[3] Lactotroph-secreting and NFPAs are the most prevalent pituitary tumours in the adult population, followed by somatotroph, corticotroph and thyrotroph tumours.[6]

Pituitary tumours can be classified according to tumours size (micro or macroadenomas), presence or absence of hormonal secretion (functioning or non-functioning) or according to aggressiveness.[4] A new 2017 WHO classification categorizes these tumours according to their adenohypophyseal cellular lineage, acknowledging the role of transcription factors, in lactotroph, gonadotroph, somatotroph, corticotroph, thyrotroph, and null cell adenomas.[5]

The distribution of the tumour subtypes varies according to age, gender and race. They are generally more prevalent in women between the 3rd and 4th decades, while they seem to appear later in the 5th and 6th decade for men.[9] Pituitary tumours are less common during childhood and adolescence, with only 0,2% being identified in children brain-imaging.[10] The prevalence of each tumour subtypes also varies according to age and sex, in early childhood adrenocorticotropic hormone(ACTH)-secreting tumours are most common, prolactinomas are more prevalent between the second and fourth decades, while NFPAs are more frequent over the age of 40.[11] Regarding the gender variations, prolactinomas, ACTH and TSH-secreting tumours have a higher prevalence in women while men presented more frequently with growth hormone (GH)-secreting or NFPAs, although these discrepancies tend to become more balanced in older age groups.[11] Black people appear to have higher incidence rates in comparison to other races.[9] Studies also show that microadenomas are more frequent in the female population.[12]

1.2. Aetiology

Somatic and germline mutations have been studied in sporadic and hereditary pituitary tumours. Familial syndromes account for less than 5% of all pituitary tumours and have been largely associated with specific gene mutations.[3] The same correlation is not well established in sporadic tumours, with only a small portion of tumours being explained by DNA coding sequence alterations.[4, 8] The association of guanine nucleotide binding protein, alpha stimulating (*GNAS*) gene mutations with somatotrophs and ubiquitin specific peptidase 8

(*USP8*) mutations implicated in corticotrophs are the most studied mutations when it comes to the tumourigenesis of sporadic pituitary tumours.[6]

Epigenetic changes were shown to have better correlation with tumourigenesis in human pituitary tumours than somatic mutations, loss-of-heterozygosity or even rearrangement in genes controlling the cell cycle regulation.[7] This evidence, in addition to the growing understanding of the epigenetic mechanisms in various tumours, led to increased interest in the connection between epigenetic dysregulation and pituitary tumourigenesis. The integration of epigenetics information into tumour classification in other types of cancers (e.g., breast cancer, glioblastoma or meningioma) has enhanced clinical reliability. Even though, the classification of pituitary tumours is complex, incorporating epigenetic alterations could help predicting clinical behaviour and prognosis.[1]

1.3. Physiopathology

Although most pituitary tumours are benign, excess hormone production, hypopituitarism, tumour growth and compression of local structures due to their location within the sella turcica, can lead to increased morbidity and compromised quality of life, even if the tumours are not invasive or metastatic.[7, 12] The first line of treatment for most of these tumours is surgical resection, but for a significant portion of them remission is not achieved. While some cannot be entirely excised due to their location, others are resistant to pharmacological treatment and can recur despite combined therapy, e.g, pharmacological, surgical and radiotherapy.[4]

1.4. Epigenetic Mechanisms

Epigenetic modifications interfere in gene expression without changing the underlying DNA coding sequence and have gained recognition as an important factor in the development of pituitary tumours. The collection of all the epigenetic marks in a cell is called epigenome and it can vary depending on the tissue and even within the same tissue. Although these epigenetic changes are modifiable, they work as marks and can be passed on through cell division and inherited by the following generation.[13]

Considering that these mechanisms are reversible, therapies targeting epigenetic modifications could be of great potential in the treatment of pituitary tumours along with the conventional therapeutic strategies.

1.4.1. **DNA methylation**

The DNA methylation process is the best studied epigenetic mechanism It consists of a transfer of a methyl group from S-adenosylmethionine to 5’ cytosines, typically in CpG islands located within the promoter region of the gene, although it can also occur in CpG sites outside

of CpG islands. The result is chromatin condensation, less access to the methylated regions and consequently gene silencing (Fig.1). This transfer is regulated by DNMTs, DNMT3A and DNMT3B are responsible for *de novo* methylation that is maintained by DNMT1.[14, 15]

Both hypermethylation and hypomethylation could be involved in tumourigenesis by, respectively, promoting gene silencing or facilitating gene expression of tumour suppressor genes (TSGs), oncogenes and other genes implicated in cell-cycle regulation. Despite this data, the association between methylation and gene expression is not always straightforward. Some studies were not successful in correlating methylation to decreased gene expression, while others stated that hypomethylation could also be linked to downregulation, as described regarding the *CASP8* (Caspase 8) and *CADM1* (Cell Adhesion Molecule 1) genes.[16, 17]

Reactivation of TSGs silenced by methylation represents a possible therapeutic strategy in the treatment of sporadic pituitary tumours, either by itself or combined with conventional treatments, with promising results in recent studies.[17]

1.4.2. **Histone modifications**

Chromatin structure regulates which genes are accessible to transcription factors, controlling gene expression.[1] Histone acetylation and methylation of histone lysine residues are two mechanisms by which gene expression can be regulated. Histone modifications by phosphorylation, ubiquitination and ADP-ribosylation have been described, although fewer studies were published regarding these last mechanisms.[18]

Both histone acetylation and deacetylation have been linked to the regulation of transcriptional activity in chromatin.[13] The acetylation of histone tails, especially lysine 9 (K9) and lysine 14 (K14) on histone 3 (H3K9 and H3K14) by histone acetyltransferases (HATs) enables gene transcription by unfolding the DNA structure and allowing access to transcription factors. This mechanism can be reversed by histone deacetylase complex (HDAC) enzymes.[14]

The site and number of methyl groups entailed in the process of histone methylation by histone methyltransferases (HMTs) determines increased or decreased access, resulting in either upregulation or downregulation of gene expression.[2] Like with acetylation, demethylases (HDMTs) can reverse the histone methylation mechanism.[14]

1.4.3. **miRNAs**

MicroRNAs are small noncoding RNAs that regulate gene expression post-transcription by targeting selected mRNAs and binding at the 3'-untranslated regions (3'-UTRs) or at coding regions, resulting in direct mRNA cleavage. In other words, they can induce a faster

degradation of the mRNA before translation into proteins and therefore result in gene silencing.[2, 8]

Deregulation of miRNAs can promote oncogenesis or tumour suppression based on the gene in question, and multiple anomalous miRNAs have been documented in pituitary tumours.

Two thirds of the deregulated miRNAs correlated with pituitary tumourigenesis are responsible for gene downregulation and are recognised as tumour suppressors, and this downregulation is associated with tumour growth by targeting oncogenes (the underexpression of the miRNAs leads to the transcription of the oncogenic proteins).[19] Contrarily, overexpressed miRNAs in pituitary tumours are considered tumour inducers.

1.4.4. lncRNAs

Long non-coding RNAs are transcripts of over 200 nucleotides that are not involved directly in protein coding but regulate molecules associated with cell cycle control (e.g., cyclin-dependent kinase [CDKs] and CDK inhibitors [CDKIs], Rb and p53) and act as regulators of the epigenetic mechanisms and transcription. Dysregulation of lncRNAs has been identified in different cancer types, suggesting their involvement in tumour development.[8]

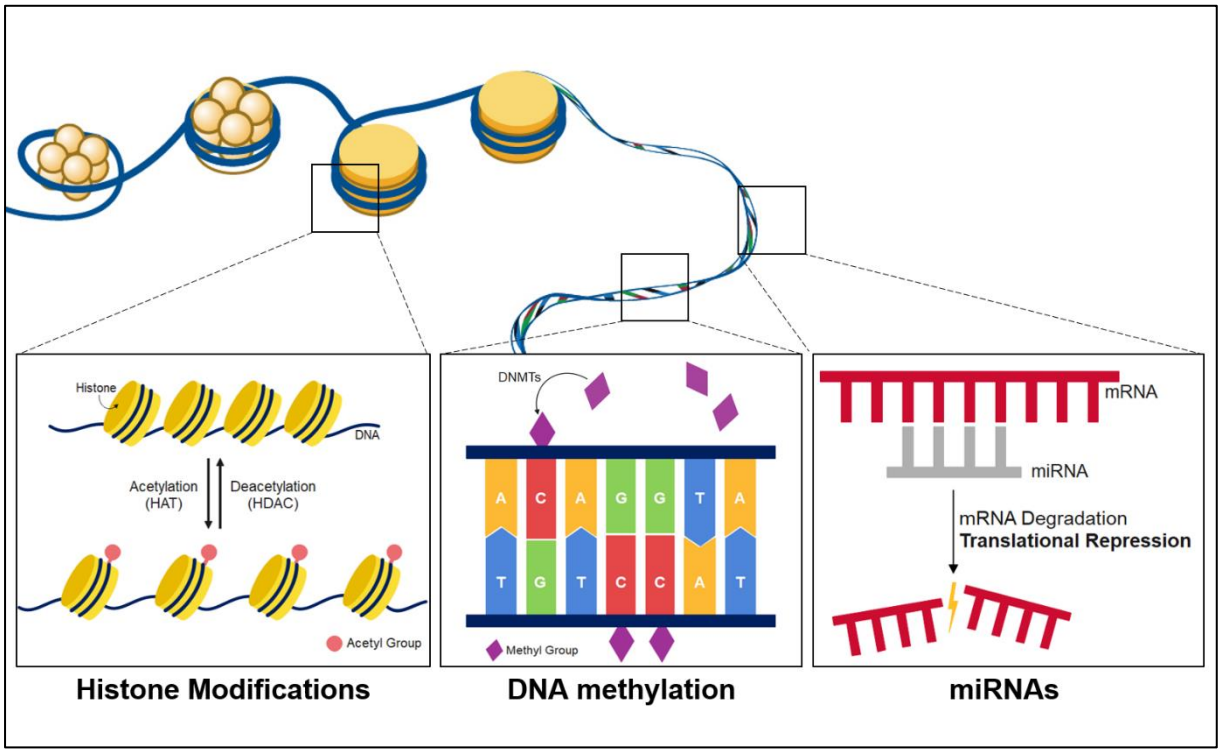

Figure 1. Schematic representation of some of the epigenetic mechanisms involved in pituitary tumourigenesis including DNA methylation, histone modification and miRNAs. Adapted from https://www.genome.gov/genetics-glossary/histone

DNMT: DNA Methyltransferases; HAT: Histone Acetyltransferases; HDAC: Histone Deacetylase Complex

2. Epigenetics in pituitary tumours

Several epigenetic changes have been described in pituitary tumours when compared to normal pituitary glands, some of them were found to upregulate or downregulate pituitary gene expression in various tumour subtypes, while others were correlated to certain characteristics of the tumour such as size, invasion, hormone production, recurrence or resistance to treatment.[4]

DNMT1, DNMT3a and DNMT3b expression was increased in pituitary tumours in comparison to normal pituitary glands, emphasizing the role of *de novo* methylation in the pathogenesis of these tumours.[14] However, no correlation was shown between DNMT overexpression and patient age, sex or KI-67 proliferation rate.[20] In general, it was found that tumours with upregulation of various DNMTs were linked to high-methylation status. It was also demonstrated that DNMT3b has the capacity to modulate the expression of other elements involved in cell cycle regulation like Rb, p21 and p27, and TSGs important in the pituitary tumourigenesis. DNMT3b downregulation promoted their expression and resulted in decreased cell proliferation. This information provides the rational to use of DNMT inhibitors in the treatment of pituitary tumours.[21]

Most human pituitary tumours exhibited evidence of deregulation through CpG island methylation in at least one of the cell cycle regulatory genes, the most common alterations are in genes involved in the Rb pathway. The hypermethylation of tumour suppressor $p16^{INK4a}$ gene was found to be the most frequent dysregulation and was, therefore, suggested as a major target for epigenetic therapies.[22]

Promoter hypermethylation of TSGs growth arrest and DNA-damage-inducible-g (*GADD45*γ) (particularly in NFPAs), Ras association domain family 1 isoform A (*RASSF1A*) and maternally expressed 3 (*MEG3*) leading to decreased expression was found to be present in pituitary tumours. Inactivation of the pituitary tumour apoptosis gene (*PTAG*) was also described due to promoter hypermethylation.[14]

The *RB1* gene encodes the Rb nucleoprotein and is involved in the expression of genes that are necessary for the progression of the cell cycle. Methylation of the promoter region of *RB1* has been identified in various tumours, including pituitary tumours, leading to decreased expression.[3, 8]

Studies have shown TSGs fibroblast growth factor receptor (*FGFR2*), cyclin-dependent kinase inhibitor 2A (*CDKN2A)*, also referred to as p16, and *GADD45*γ to be silenced due to promoter hypermethylation in pituitary tumours.[3] *FGFR2* has been implicated in the regulation of p21, p27 and p53 expression and regulation of the Melanoma-associated antigen

A3 (MAGE-A3) complex. In contrast, *FGFR4* was shown to be overexpressed in pituitary tumours.[10]

CDKI showed reduced expression due to epigenetic mechanisms such as downregulation of histone methyltransferase lysine methyltransferase 2A (KMT2A) and $p27^{Kip1}$ at the mRNA level.[14]

HDAC11 was associated with lower p53 expression. Sirtuins, a family of HDACs, are differentially expressed in various pituitary tumour subtypes, which could mean they can be used as markers for pituitary tumours. Knockdown of HDAC11 resulted in increased p53 expression, pointing to the therapeutic potential of the HDAC11 inhibition in pituitary tumours.[23]

Decreased expression of the EGF containing fibulin-like extracellular matrix protein 1 (*EFEMP1*) gene was associated with higher methylation levels [15], but also with histone modifications and chromatin condensation, demonstrating that different epigenetic mechanisms, and even somatic mutations, can participate in the regulation of the same gene in different tumours.[17]

Dysregulation of miR-196a-2 and miR-212 has been identified in all tumour subtypes. While miR-196a-2 expression was found to be decreased, miR-212 was upregulated, targeting death effector domain-containing protein (DESD), involved in apoptosis signalling, and patched-1 protein (PTCH1), a TSG, stimulating tumour growth and invasion. MiR-15a and miR-16-1 were found to be downregulated in pituitary tumours targeting B-cell lymphoma 2 (BCL2), an oncogenic protein that promotes cell death inhibition.[19]

MiR-107 overexpression, targeting and inhibiting the aryl hydrocarbon receptor-interacting protein (AIP), was documented in pituitary tumours, controlling cell proliferation through the cAMP pathway. Studies have suggested that miR-107 could work as a tumour suppressor-miR by inhibiting AIP expression.[14, 24] Vascular endothelial growth factor receptor 1 (VEGF-R1) is downregulated in pituitary tumours, targeted by miR-24-1. MiR-26a overexpression targeting *ZAC1* gene (intertwined with AIP regulation) and miR-128a, miR-155, miR-516-3p overexpression targeting *WEE1* gene were also described in pituitary tumours.[8]

Ikaros is a hematopoietic stem cell chromatin remodeler that is involved in the regulation of hypothalamic neuroendocrine and adenohypophysial development. It functions as a transcriptional activator or repressor, depending on the isoform created by alternative splicing. On one hand, Ikaros limits access to Pit-1 activator by deacetylation of histone 3 residues in the *GH* promoter, and, on the other hand, it promotes Prolactin gene (*PRL*) expression by the acetylation of histone 3 on the gene promoter.[14] Fibroblast growth factor 4 (FGF4) is

overexpressed in pituitary tumours and has been shown to be connected to Ikaros isoforms. Ikaros isoform 6 (Ik6), in particular, is overexpressed through DNA and histone modifications, leading to anti-apoptotic gene B-cell lymphoma-extra large (*BCL-XL*) overexpression, increased tumour cell survival and proliferation.[3, 8]

MAGE-A3 expression is significantly increased in pituitary tumours caused by promoter hypomethylation in comparison to the normal pituitary gland where this gene is not expressed. [8] Studies have also linked oestrogen treatment to the stimulation of *MAGE-A3* expression through epigenetic modifications, involving increased histone acetylation and decreased histone methylation.[18] *MAGE-A3* is usually downregulated in normal pituitary glands that express higher FGFR2 (particularly FGFR2-IIIb) levels, suggesting that FGFR is involved in the modulation of *MAGE-A3* expression.[25]

Cancer susceptibility candidate 2 (CASC2), a lncRNA capable of reducing HMGA2 expression and involved inhibition of cell growth and invasiveness, was shown to be decreased in different pituitary tumour subtypes, promoting proliferation. Expression of actin filament-associated protein 1 antisense RNA 1 (AFAP1-AS1) was increased in pituitary tumours when compared to normal pituitary tissue. *AFAP1-AS1* gene knockdown, leading to reduced expression, inhibited cell proliferation and increased apoptosis, acting as a suppressor.[26]

LncRNA RPSAP52 overexpression has been shown to promote oncogenesis. It was found to be the most upregulated lncRNA in pituitary tumours, increasing HMGA1 and HMGA2 expression, and therefore contributing to tumour growth.[26] IFNG-AS1 targets epithelial splicing regulatory protein 2 (ESRP2), a tumour growth inhibitor, acting as an oncogene in pituitary tumours. Overexpression of colon cancer associated transcript 2 (CCAT2) has also been linked to pituitary tumourigenesis leading to pituitary tumour-transforming gene 1 (PTTG1) overexpression and tumour growth. C5orf66-AS1 (C5orf66 antisense RNA 1), on the other hand, seems to have the opposite effect, and its expression was linked to inhibition of tumour progression and invasion, suggesting a tumour suppressor capacity.[27, 28]

2.1. Invasive *vs* non-invasive tumours

Around 35% of pituitary tumours are considered invasive tumours, defined by the invasion of structures adjacent to the sphenoid and cavernous sinus. As these tumours are harder to resect completely, recurrence rates are higher. In order to facilitate surgical planning, some classifications such as Hardy's or Knosp's are available. Although invasive tumours are frequently macroadenomas, a tumour can be invasive regardless of its size. The concept also

differs from carcinoma since tumours can be invasive in the absence of metastatic potential.[29]

DNMT1 and DNMT3A overexpression as well as general higher levels of methylation were associated with more aggressive behaviour (Table 1), particularly grade III and IV of the modified Hardy classification, with sellar invasion and poor prognosis.[3, 20] Different studies associated DNA hypomethylation with increased risk of malignancy, stating that hypomethylation of CpGs was more frequent than hypermethylation in invasive NFPAs.[1, 2]

Downregulation of polypeptide N-acetylgalactosaminyltransferase 9 (*GALNT9*) was observed in invasive tumours, [1] as was the downregulation of the gene *RIZ1* (retinoblastoma protein-interacting zinc-finger gene 1), an histone methyltransferase (HMT) and tumour suppressor.[2]

Pituitary tumour invasion has been associated with decreased expression of the TSG death associated protein kinase (*DAPK*) due to promoter CpG island methylation [14]. Cadherin-1 (*CDH1*) and cadherin-13 (*CDH13*) methylation was linked to aggressive tumours as well, mostly grade IV.[20]

The *RB1* gene is a tumour suppressor and was found to be silenced through DNA methylation in human pituitary tumours. Furthermore, neuron-glial antigen 2 (NG2)-driven Rb inactivation was associated with high penetrance tumours developing specifically from the Pit1-lineage.[14]

ESR1 (oestrogen receptor alpha) and RASSF1 hypomethylation was associated with more aggressive behaviour. *CASP8*, involved in apoptosis, was found to be silenced by hypermethylation in various pituitary tumours. This lack of expression was inconsistently correlated with more aggressive tumours and further studies are required.[16] However, different studies have linked *RASSF1* hypermethylation to more aggressive tumours (over 85% in grade IV tumours), correlating to a higher ki-67 index.[30]

As mentioned above, increased acetylation of H3K9 has been documented in pituitary tumours, activating chromatin and allowing for gene transcription and higher proliferation rates. This alteration has been linked to more invasive tumours with a higher ki-67 proliferation index, seemingly followed by some level of p53 mis-expression.[14] It has been demonstrated that acetylation is higher in tumours with increased proliferation index (MIB-1) and increased p53 levels.[31]

HMGA overexpression was associated with increased tumour size, invasiveness, aggressiveness (grade IV tumours) and the presence of metastases with higher Ki-67 index,

as a result the outcome is often poor.[26] Low levels of miRNA let-7 were associated with higher expression of the HMGA2.[14]

Another study linked downregulation of miR-183 with lactotroph-secreting and more aggressive pituitary tumours associated with expression of the gene *KIAA0101* and inhibition of p53-p21 mediated cell arrest leading to higher levels of proliferation.[3]

Decreased expression of miR-24, miR-93, miR-126 and miR-34a was documented in invasive pituitary tumours when compared to non-invasive ones, although no genes were described as being specifically targeted.[26]

Downregulation of miR-132, miR-15a and miR-16-1 was documented in association with invasive behaviour by targeting SRY-Box transcription factor 5 (*SOX5*), an oncogene associated with proliferation, invasion and migration.[19]

LncRNA H19 is paternally imprinted and maternally expressed, and its expression is regulated along with Insulin-like growth factor 2 (*IGF2*) gene. Some sources correlated overexpression of H19 with more invasive and aggressive secreting somatotroph tumours [26]. Other studies state this lncRNA is used as a tumour marker and that its expression was found to be decreased in pituitary tumour samples in general, decreasing further with tumour volume. Small nucleolar RNA host gene 1 (SNHG1) and X-inactive specific transcript (XIST) are two lncRNA found to be overexpressed in invasive pituitary tumours, suggesting the possibility of their use as markers for tumour progression and invasive potential.[28]

2.2. Micro *vs* macroadenoma

Microadenomas are tumours with a diameter inferior to 1 cm while macroadenomas are tumours of larger diameter. Understandably, larger tumours can cause symptoms despite their hormonal activity due to their growth and compression of adjacent structures, resulting in clinical manifestations such as visual disturbances (given their proximity to the optic chiasm) or headaches.[3]

Although DNMT1, DNMT3A and DNMT3B were found to be overexpressed in all pituitary tumours, DNMT1 overexpression was particularly associated with macroadenomas (Table 2).[20]

Promoter hypermethylation of the MutS Homolog 6, part of the mismatch repair system (*MSH6*) and involved in cell adhesion (*CADM1*) genes was linked to pituitary macroadenomas of different subtypes [16], but not correlated with the presence or absence of hormone production. The *MSH6* gene methylation and expression is regulated by the mRNAs miR-21

and miR-155. Overexpression of these mRNAs has been associated with poor prognostic in other tumours and could be used as a prognostic marker in pituitary tumours.[3]

Increased diameter of somatotroph and lactotroph tumours has been associated with lower expression of miR-15a and miR-16-1, both miRNAs target cell-cycle regulation genes.[2, 14]

2.3. Recurrent *vs* non-recurrent tumours

A comparison between recurrent and non-recurrent pituitary tumours identified 68 genes that were differentially expressed in these two groups, enrichment of genes carrying reactome pathway chemokine receptors bind chemokines (R-HSA-380108) appeared to be relevant to tumour recurrence, specifically chemokine related genes interleukin 8 (*IL8*=, C-X-C chemokine receptor 1 (*CXCR1*) and 2 (*CXCR2*).[4]

2.4. Treatment resistant *vs* non-resistant

Concerning the effect of treatment in the epigenomics of pituitary tumours, one study recently compared the methylation and expression status of GH-secreting tumours treated with octreotide/lanreotide to those of untreated samples. The results showed that the expression of proliferation marker Ki-67 was lower in the samples of the patients who received treatment. The expression of gene Mucin1 (*MUC1*) was higher in the same group, and this increase in expression could be related to the success of treatment. B-cell surface antigen CD40 (*CD40*), involved in the modulation of B-cell activation and differentiation, was also upregulated in the treated samples.[4]

Lactotroph tumours treated with bromocriptine showed upregulation of miR-206, miR-516b and miR-550 and downregulation of miR-671-5p. Another study also identified miR-93, miR-17 and miR-126 as being upregulated in lactotroph tumours resistant to bromocriptine, and that the silencing of these miRNAs could increase bromocriptine treatment effectiveness. Particularly, the silencing of miR-93 resulted in upregulation of p21 and increased bromocriptine sensitivity in rat prolactinomas cell line MMQ.[32] Similar data was found in lactotroph tumours treated with bromocriptine where the same miRNAs were deregulated.[19]

Patients that did not respond to somatostatin analogue (SSA) treatment were found to have lower rates of *SSTR2* expression, a gene targeted by miR-185, suggesting that miRNAs can also be used to predict treatment response.[33] Another study also showed that miRNA-1299 expression was decreased in lactotrophs resistant to pharmacological treatment, associated with forkhead box protein O1 (*FOXO1*) gene silencing.[28]

Downregulation of miR-524-5p was identified in patients with somatotroph tumours that responded to somatostatin analogues (defined as a decrease in GH secretion of over 50%) treatment in comparison to non-responders. However, patients treated with lanreotide were found to have miR-524-5p upregulation when compared to the ones with no pre-surgical treatment. SSA response is determined by the number of somatostatin receptors expressed in the tumour cells as well as their subtype. Various theories were presented to explain this difference in expression but further research is necessary to better understand the influence of miR-524-5p expression in tumour response.[34]

2.5. Functional *vs* non-functioning tumours

NFPAs represent up to 35% of pituitary tumours and can have clinical manifestations derived from lack of hormone production, mass growth or they can be incidental findings.[35]

A possible explanation for the lack of hormone production and secretion in pituitary tumours could be the DNA hypermethylation, and therefore silencing, of genes encoded in the promoter region.[16]

Non-functioning tumours showed global higher rates of methylation when compared to functioning ones.[1] Genes such as voltage-gated potassium channel subunit beta-2 (*KCNAB2*), calcium-activated potassium channel subunit beta-4 (*KCNMB4*) and calcium voltage-gated channel subunit alpha 1C (*CACNA1C*) were shown to be hypermethylated (Table 3). Silent gonadotroph tumours had the higher methylation rates while functioning corticotroph-secreting tumours were the least methylated.[36]

The *ESR1* gene was found to be hypermethylated in functioning corticotroph tumours when compared to silent ones, this modification did not show implications in tumour size or aggressiveness.[15]

Hypermethylation of genes echinoderm microtubule associated protein like 2 (*EML2*), homeobox B1 (*HOXB1*) and Rho related GTP-binding protein (*RHOD*) was associated with reduced expression of these genes in NFPAs, somatotroph and lactotroph-secreting tumours.[1] Serine/threonine kinase 26 (STK26), a protein that stimulates factors involved in the tumourigenesis, was shown to be upregulated in NFPAs.[37]

miR-135a, miR-140-5p, miR-582-3p, miR-582-5p and miR-938 were all found to be overexpressed in NFPAs, and they appear to be involved in the downregulation of the TGF-β signalling pathway.[2, 8] Upregulation of miR-20a, miR-106b and miR-17-5p was described in non-functioning carcinomas, decreasing phosphatase and tensin homolog (PTEN) TSG and

tissue inhibitor of metalloproteinases 2 (TIMP2), involved in the inhibition of MMPs that promote cell migration and metastasis expression.[3]

Hox transcript antisense intergenic RNA (HOTAIR) is the most studied lncRNA and it was found to be overexpressed in various tumours involved in chromatin remodelling, sometimes associated to larger size, invasion and metastatic potential, particularly in NFPAs.[26, 27]

MEG3 is a maternally imprinted gene that encodes lncRNAs, it functions as a tumour suppressor and prompts p53 dependent transcription. *MEG3* was found to be downregulated in pituitary tumours, particularly NFPAs, promoting their development.[8] Silencing of the *MEG3* gene, through promoter hypermethylation, was documented in NFPAs [26]. Some studies have associated both MEG3 and HOTAIR with invasion in NFPAs.[27]

2.6. Specific subtypes

2.6.1. **Somatotroph tumours**

Somatotrophs represent around 15-20% of pituitary tumours. Tumours associated with GH secretion frequently present with increased height in childhood and adolescence (gigantism), while in adults clinical manifestations are of acromegaly, resulting in bone and cartilage overgrowth, hypertension, resistance to insulin, cardiovascular and respiratory complications, and a higher risk of developing other tumours.[38, 39]

Most (~95%) somatotrophs are sporadic and the pathogenesis is largely unknown. Gain-of-function mutations in *GNAS* gene occur in ~40-50% of cases, resulting in constitutive synthesis of cyclic adenosine monophosphate (cAMP), but various studies also linked epigenetic changes to their tumourigenesis (Table 4).[40-43]

Somatotroph tumours are highly sensitive to cAMP activity, affecting proliferation and GH production, suggesting that cAMP deregulation could be involved in the pathogenesis of these tumours. The activation of Gαi via SSTR2 and SSTR5 inhibits cAMP production and reduces the secretion of GH.[44]

A genetic mutation on *GNAS* gene results in gain of function of the Gαs protein, activating cAMP synthesis and protein kinase A pathway, leading to tumour growth in somatotrophs. Epigenetic studies showed strong differences in the methylation patterns of tumours according to *GNAS* mutation status. *GNAS*-mutated tumours showed substantially higher rates of hypomethylation (close to 98%) when compared to wild type ones. DNMT1 was found to be upregulated, maintaining methylation patterns, suggesting it is also a factor promoting tumour proliferation.[39]

The imprinting of the *GNAS* gene is tissue specific, in the normal pituitary it is almost exclusively maternally expressed due to the process of imprinting that relies on epigenetic mechanisms such as DNA methylation and histone modifications. Studies have shown that the *GNAS* mutation occurs mostly on the maternal allele. The lack of maternal imprinting allows for the transcription of the mutated gene and subsequent protein expression, showing that somatic mutations and epigenetic modifications can be associated with the process of tumourigenesis. Additionally, an imprinting relaxation has been documented in somatotroph tumours, regardless of the *GNAS* mutation, enabling the expression of at least some amount of the normal protein.[14, 45]

Ras association domain family member 3 (*RASSF3*) is a TSG and its promoter hypermethylation has been documented in somatotroph tumours, leading to decreased expression and promoting tumour growth by inhibiting apoptosis through the p53 pathway.[46]

miR-34b, miR-326, miR-432, miR-548c-3p, miR-570 and miR-603 downregulation was identified and resulted in downregulation of the genes *HMGA1*, *HMGA2* and E2F transcription factor 1 (*E2F1*), which leads to increased expression and cellular proliferation.[10, 19, 26] In addition, let-7 was associated with rat sarcoma (*RAS*) oncogene targeting and downregulation of the *HMGA2*.[3] Somatotrophs also presented with drastic upregulation of miR-320 when compared to other tumour subtypes although the mechanisms leading to tumour development were not clear.[47] BMI1 polycomb ring finger oncogene 1 downregulation was found in somatotrophs through miR-128 targeting, causing *PTEN* silencing.[8]

PTTG1 is an oncogene involved in tumour proliferation. Downregulation of miR-126 and miR-381 were shown to target this gene, promoting tumourigenesis. Overexpression of miR-300, miR-329, miR-381 and miR-655 on the other hand, also target *PTTG1* gene but they seem to have a tumour suppressor effect, inhibiting tumour proliferation.[19, 26]

A positive correlation between HMGA and pituitary transcription factor 1 (*PIT1*) expression was reported in somatotrophs and lactotrophs. *PIT1* encodes a transcriptional factor specific to the pituitary that is crucial in *GH* and *PRL* gene activation and it was found that *PIT1* was overexpressed in these tumours when compared to normal pituitary glands.[26]

Both miR-23b and miR-130b showed lower expression in somatotrophs, gonadotroph and NFPAs [14]. MiR-23b seems to target *HMGA2* gene expression while miR-130b targets cyclin-A2 (*CCNA2*).[26]

Somatotroph tumours seem to present with higher rates of hypomethylation and higher expression rates in comparison to corticotroph and NFPAs. Promoter hypomethylation of

somatostatin receptor 5 (*SSTR5*) and growth hormones 1 (*GH1*) and 2 (*GH2*) resulted in their overexpression.[4]

2.6.2. **Corticotroph tumours**

Corticotroph tumours represent 15% of pituitary adenomas, these ACTH-secreting tumours result in excess cortisol secretion and Cushing's disease, characterized by centripetal fat distribution, moon face, hirsutism, facial plethora along with cardiovascular, respiratory, metabolic or psychiatric manifestations.[48]

Corticotrophs presented with proopiomelanocortin (*POMC*) promoter hypomethylation resulting in its overexpression (Table 5).[4] CDK5 and ABL1 enzyme substract 1 (CABLES1) protein, encoded by the TSG of the same name negatively correlates with tumour progression, and it has been shown to be activated by glucocorticoids.[12] CABLES1 expression was found to be decreased in 55% of corticotrophs and is associated with low levels of p27.[37]

Overexpression of miR-26a in corticotroph tumours seems to target protein kinase C delta (PRKCD), a serine/threonine kinase implicated in proliferation, apoptosis and cell cycle regulation.[8]

In corticotroph tumours low levels of miR-145 were associated to reduced expression of oncogenic MYC proto-oncogene (*MYC*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), fos proto-oncogene (*FOS*) and yes proto-oncogene (*YES*).[19] Low expression of various miRNAs was also documented, including miR-16, miR-21, miR-141, miR-143, miR-150 and let-7a, most of them acting through downregulation of the *AIP* gene; additionally let-7a also targeted *HMGA2*, decreasing its expression.[3, 14] Corticotroph, somatotroph and lactotroph tumours all showed decreased levels of miR-15a, miR-16 and let-7 associated with deficient secretion of p43, a potentially an anticancer cytokine.[3]

Histone modifications, particularly increased H3K27 methylation) in the bone morphogenetic protein 4 (*BMP-4*) have been described in association with non-functioning, somatotroph and corticotroph tumours, resulting in gene downregulation and cell proliferation.[2, 14]

2.6.3. **Lactotroph tumours**

Besides the previous epigenetic modifications associated with lactotrophs, BMP-4 expression was found to be increased, stimulating cell proliferation.[17] This alteration was found to be more frequent in women, possibly associated to higher oestrogen levels. BMP-4 inhibition with antioestrogens further supports this theory.[49]

Downregulation of the lncRNA clarin 1 antisense RNA 1 (CLRN1-AS1) was documented, this lncRNA is generally linked to cell growth suppression.[28]

2.6.4. **Gonadotroph tumours**

Downregulation of miR-410 was found to be present in gonadotroph tumours, promoting tumourigenesis. It targets cyclin-B1 (*CCNB1*) gene that encodes cyclin B, a cell regulator, and restoration of the expression of miR-410 inhibited cell proliferation in pituitary tumour cells. Other cell cycle regulators such as cyclins A and D and CDKs are also targeted by miR-410, suggesting that the mechanism that leads to tumour growth depends on cyclin level amplification.[26]

2.7. Carcinomas

O-6-methylguanine-DNA methyltransferase (*MGMT*) hypermethylation was linked to pituitary carcinomas but it did not correlate completely with gene expression.[2] Overexpression of miR-122 was identified in corticotroph carcinomas and upregulation of miR-20a, miR-106b and miR-17-5p was associated with metastases in pituitary carcinomas, interfering with PTEN and TIMP2 expression. Upregulation of miR-122 and miR-493 was found in corticotroph carcinomas when compared to adenomas or normal pituitary glands.[3]

When compared to non-metastatic corticotroph tumours, miR-122 and miR-493 upregulation was also identified in metastatic corticotroph tumours.[14] These miRNAs could be used as biomarkers to predict recurrence in aggressive corticotroph secreting tumours.[50]

3. Clinical application

3.1. Diagnostic value

Histological samples are the standard method of diagnosing and studying tumour genotype. However, only a part of the tumour is studied and therefore crucial information may be neglected, since tumours are not homogeneous tissues and they can evolve over time. Additionally, the procedure to obtain the tissue samples implies some risks. In recent years, liquid biopsies have emerged as an alternative option, since they may be a more accessible and reliable method to study pituitary tumours. These liquid samples can be obtained from body fluids such as blood, urine or saliva, enabling repeated testing, that are valuable for diagnostic purposes but also for treatment efficiency/follow-up and prognosis. On the downside, this test requires a high sensitivity rate since pituitary tumours are usually small and the levels of released biomarkers may be low.[28]

Different molecules have been studied as potential biomarkers: circulating tumour DNA (ctDNA) from dead tumour cells, cell-free RNA (cfRNA), lncRNAs, miRNAs, circulating tumour cells (CTCs) and genetic information contained in exosomes.[28]

The half-life of ctDNAs is approximately 150 minutes, which implies that the detected ctDNAs are always an accurate representation of the current tumour characteristics. It has also been reported that malignant tumours release higher amounts of ctDNA when compared to benign ones. Diagnosis of a tumour cannot be inferred based only on the presence of the ctDNA, since healthy individuals also release it, but consecutive measuring of ctDNA levels could be an option to evaluate tumour growth or recurrence.[51]

Extracellular miRNAs are released from normal and tumour cells and may be identified in blood and other fluids. Questions were raised concerning the mechanisms and stability of these circulating markers, rather than passive leakage of these miRNAs to the bloodstream, the data points to specific transport mechanisms responsible for their secretion, mainly through exosomes or bound to proteins. This information is relevant as the stability of cell-free miRNAs means they have the potential of being used as biomarkers to determine diagnosis, prognosis and treatment follow-up. Extracellular miRNAs can also be involved in the tumourigenesis process and may be a target for cancer therapy.[52] Despite this evidence, further studies are required to determine the exact release mechanisms and the function of cell-free miRNAs and their accuracy as biomarkers. Some aspects to take into consideration are that these markers are not suitable for population screening since miRNA alterations vary according to the characteristics of each specific tumour. Furthermore, some of the miRNAs detected were considered to be “passenger alterations” since they did not seem to have implications in tumourigenesis.[28]

LncRNAs are expressed within the cells but some of them, called circulating lncRNAs, can be found in biological fluids such as blood associated with proteins or exosomes. These lncRNAs could potentially be used as markers in disease diagnosis since they are resistant to nucleases, and could even be used for prognostic and disease monitoring in pituitary tumours.[27]

3.2. Treatment opportunities

Somatotrophs have higher rates of overall hypomethylation and this could mean that the treatment for acromegaly using epigenetic targets may be more attainable.[4] Similar therapies could be considered in both secreting and silent-corticotroph tumours since their genetic patterns showed similarities.

Therapies targeting epigenetic alterations have been divided into two groups: broad reprogrammers and targeted compounds. Broad reprogrammers reverse genome-wide specific epigenetic alterations having large-scale effects on the epigenome, while targeted compounds are directed at specific epigenetic altering enzymes.[2]

DNMT and HDAC inhibitors (DNMTi and HDACi) are broad reprogrammers. Two of the first DNMTi approved by the United States Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome and acute myeloid leukemia are azacytidine and decitabine, which induce gene re-expression of hypermethylated genes.[28, 53] HDACi drugs have also been approved for use in cutaneous T-cell lymphoma and multiple myeloma treatment. These drugs affect the whole genome which puts into question the possible side effects. SGI-110, whose active metabolite is decitabine, was shown to have few side effects, mainly gastrointestinal. Hydralazine, a non-nucleoside analogue DNMTi, was also suggested as a possible epigenetic drug.[3]

On the contrary, targeted compounds are selective and target specific epigenetic altering enzymes. In this group of drugs are included HMT inhibitors (HMTi) and histone demethylase inhibitors (HDMi), still being studied for their use in haematological malignancies and also solid tumours.

Another new possible category of epigenetic treatment are drugs that target and inhibit epigenetic readers such as the bromodomain and extraterminal domain (BET) family of proteins, known to be involved in the transcription process, upregulating oncogene expression in different cancers. However, they have not been researched in pituitary tumours specifically [54]. Neither of these drugs has successfully completed clinical studies in the treatment of pituitary tumours.[2]

SAHA, an HDACi, showed to reduce proliferation and increase apoptosis in early studies in GH- and PRL-secreting GH3 rat pituitary adenoma cell lines. SAHA also showed benefits in murine-derived corticotroph tumours and human-derived corticotroph tumour (hCtT) cells by decreasing cell viability and ACTH secretion due to POMC downregulation. Another HDACi, trichostatin A (TSA), was associated with decreased ACTH production by downregulation of PTTG1 and interference in histone modifications.[2, 14] These results suggest that HDACi may potentially produce favourable outcomes in the treatment of Cushing's disease. TSA along with zebularine, a DNA demethylating agent, was also shown to interfere with the histones tail modifications, enhancing H3K9c and decreasing H3K27me3 and resulting in re-expression of HMGA targeting miRNAs.[14]

A study performed on colon cancer cell line LS 180 compared the effects on gene re-expression of zebularine and TSA used isolated and in combination.[55] It showed that both

drugs were successful in up-regulating $p21^{Cip1/Waf1/Sdi1}$, $p27^{Kip1}$, $p57^{Kip2}$ and downregulating DNMTs and HDACs expression, leading to inhibition of cell proliferation, and monotherapy with TSA had a more pronounced effect than zebularine. Additionally, combined treatment with both drugs was most effective in promoting gene re-expression, inhibiting cell growth and inducing apoptosis. This study was not carried out on pituitary tumour cells but, since similar epigenetic mechanisms are involved, the results are encouraging and further research could verify the impact of this treatment in pituitary tumours.

In pituitary tumours with decreased EFEMP1 expression, zebularine and TSA were showed to reverse epigenetic silencing and induce re-expression. These drugs could potentially be therapeutic options for the treatment of pituitary tumours, such as somatotrophs or corticotrophs, showing high BMP-4 methylation levels as they resulted in BMP-4 re-expression. Furthermore, retinoic acid has also been considered a potential treatment option by inhibiting cell proliferation and decreasing GH and ACTH secretion, especially when combined with the epidrugs to induce BMP-4 re-expression.[17]

Methylation of *RASSF3* was linked to somatotroph tumourigenesis. Treatment with decitabine in somatotroph cell lines restored *RASSF3* re-expression. Although TSA treatment alone did not induce re-expression, combined therapy with TSA followed by decitabine had an synergic effect, increasing protein expression even further than decitabine alone.[46]

Since serine/threonine kinase 26 (STK26) was found to be upregulated in NFPAs, STK26 inhibition could be a therapeutic option for these tumours with some in vitro studies revealing favourable results using Hesperadin, an aurora kinase inhibitor.[56]

As mentioned before, MEG3 is a tumour suppressor whose expression is often decreased in pituitary NFPAs. Although no studies were carried out in this area so far, and preparations developed with lncRNAs MEG3 could be a possible therapy for NFPAs.[27]

HMGAs have an essential role in pituitary tumourigenesis and are largely associated with epigenetic regulation, and most pituitary tumours show HMGA overexpression through non-coding RNAs. Therefore, the treatment of these tumours could rely on drugs directed to epigenetic modifications (re-establishing the expression of miRNAs targeting HMGAs) or interfering with HMGA function. Trabectedin, a drug that reportedly inhibits HMGA function, by interfering with its transcriptional activity, could be used as a therapy in these cases.[26, 57]

Not only have DNMTi and HDACi treatments, individually and in combination, had positive results, but they have also been shown to increase somatostatin receptors (particularly SSTR2) mRNA expression, improving the response to SSA treatment, acting as adjuvant therapy.[58]

Regarding ACTH-secreting pituitary carcinomas, an upregulation of the miR-122 and miR-493 has been documented. miR-122 has also been linked with hepatitis C virus (HCV) replication and hepatocellular carcinoma tumourigenesis. Miravirsen, an miR-122 inhibitor is being studied for the treatment of the HCV infection and could be an option for the treatment of ACTH-secreting carcinomas, but further studies are required to evaluate its efficacy and side effects.[3]

MGMT is an enzyme that repairs DNA damage from environmental factors. In glioblastomas, clinical response to temozolomide has been connected to *MGMT* promoter methylation, studies are still required to evaluate this correlation in pituitary carcinomas.[3]

3.3. Prognostic value

Since different tumour characteristics and subtypes are associated with different epigenetic changes, these could be used not only to assist in the diagnostic and guide therapy but also as prognostic markers to predict how the tumour might behave.

As mentioned earlier, decreased MSH6, due to overexpression of miR-21 and miR-155, has been used as a poor prognostic marker in unresectable colorectal cancer and chemotherapy resistance in Lynch Syndrome.[3] Since miRNA dysregulation has been documented in association with various characteristics of pituitary tumours, this method could similarly be applied in these cases. For instance, decreased expression of miR-15a and miR-16-1 was linked to increased tumour diameter and invasive behaviour, the detection of this downregulation when studying a new tumour suggests the prognostic could be worse than if the expression of these miRNAs wasn't altered.[19] MiR-122 and miR-493 upregulation was linked to corticotroph carcinomas, so these miRNAs are also being looked into as possible biomarkers to predict recurrence in aggressive corticotroph secreting tumours.[50]

Since the correlation between increased H3K9 acetylation status and tumour invasiveness has been established, this data could be used as a biomarker in predicting pituitary tumour behaviour.[31]

The epigenetic alterations mentioned previously may also be used to predict the behaviour of the tumour, but the ability of access to the epigenetic code in liquid biopsies represents an even greater advantage because of the easy and minimally invasive access to samples. Also, since epigenetics are changeable over time, it allows for a much more reliable source of the tumour characteristics, for example, in response to therapy.

4. Future perspectives

A substantial amount of pituitary tumours have no clinical relevance, and the detection of molecular alterations in asymptomatic patients, who may remain so for the entire life, could result in overtreatment, possibly causing more harm than the tumour itself. Nevertheless, epigenetic biomarkers have been shown to be useful in complementing current strategies for diagnosis, prognosis, and treatment, as well as assist with therapeutic decision-making and prediction of treatment responses.[28]

Molecules such as lncRNAs are being studied as possible tumour biomarkers. Certain epigenetic changes have been linked to different tumour subtypes or biological behaviour, allowing for prognostic assessment, and new drugs are being tested for their use in reversing specific epigenetic changes in pituitary tumours. Although a lot more research is still required, some of these studies present promising results.

In recent years, as technology has evolved, studies on epigenetic deregulation in pituitary tumours have also developed rapidly, focusing on uncovering patterns that can be used to diagnose, treat and predict tumour behaviour. The encouraging results achieved so far will promote further studies in this area, uncovering the full potential of epigenomics in pituitary tumour development.

CONCLUSION

The lack of a clear association between genetic abnormalities and tumourigenesis in pituitary tumours led to increased interest in the research of epigenetic mechanisms in an attempt to better understand its pathogenesis.

Epigenetic alterations such as DNA methylation, histone modifications and miRNAs were all studied in association with pituitary tumourigenesis in an attempt to identify patterns that could be linked to specific tumour characteristics and potentially be used as diagnostic and prognostic biomarkers, similarly to other tumours. Various studies have demonstrated solid correlations linking epigenetic modifications to increased tumour size, invasiveness, response to treatment or hormonal secretion. DNMT1 and DNMT3A overexpression are associated with larger size and invasive behaviour, as was the upregulation of HMGA. Decreased expression of SSTR2 correlates with resistance to SSA treatment. NFPAs overall show higher rates of methylation that could be the explanation for the lack of hormone production. *GNAS* gene is associated to somatotroph tumourigenesis, both through genetic and epigenetic mechanisms through hypomethylation, indicating that both mechanisms can concur in promoting tumour development.

At the same time, improving understanding of these mechanisms and their reversibility enabled the development of new drugs like azacytidine, decitabine, SAHA, TSA and zebularine, that are being studied as individual or combined therapy alternatives for pituitary tumours, especially those that do not respond to SSA treatment or that can not be surgically resected. The results have been very promising.

Adding epigenetic screening to pituitary tumour workups, once the correlations between tumour epigenetics and their correspondence to histological or clinical manifestations are verified, can change decision-making. Although further research is required, the field of epigenomics has the potential to be a turning point in comprehending and approaching pituitary tumours.

TABLES

Table 1. Epigenetic dysregulation in invasive tumours

DNA Methylation	Gene	Gene expression level	Gene Function	
	DNMT1	Increased	Maintenance of DNA methylation	
	DNMT3A	Increased	*De novo* DNA methylation	
	ESR1	Increased	Transcription regulation	
	RASSF1	Undetermined*	TSG, cell cycle arrest	
	GALNT9	Decreased		
	RIZ1	Decreased	HMT, TSG	
	DAPK	Decreased	Apoptosis, TSG	
	CDH1	Decreased	Cell adhesion	
	CDH13	Decreased	Cell adhesion	
	RB1	Decreased	TSG	
	CASP8	Decreased	Apoptosis	
Histone Modifications	Gene or histone-modifying enzyme	Altered histone mark	Gene or histone-modifying enzyme function	
	Unspecified	H3K9 acetylation	Transcription activation	
miRNAs	miRNA	Target gene	miRNA expression level	Gene function
	Let-7	*HMGA2*	Decreased	Transcription regulation
	miR-183	*KIAA0101*	Decreased	Cell proliferation and cell cycle progression
	miR-24	Unspecified	Decreased	
	miR-93			
	miR-126			
	miR-34a			
	miR-132	*SOX5*	Decreased	Oncogene, cell proliferation
	miR-15a			
	miR-16-1			
lncRNAs	lncRNA	Level of expression		
	H19	Increased		
	SNHG1	Increased		
	XIST	Increased		

*Some studies stated hypermethylation of *RASSF1* gene while hypomethylation was documented in others, further studies are required to settle the influence of *RASSF1* in tumour development.

Table 2. Epigenetic dysregulations in macroadenomas

DNA Methylation	Gene	Gene expression level	Gene Function	
	DNMT1	Increased	Maintenance of DNA methylation	
	MSH6	Decreased	Mismatch repair system	
	CADM1	Decreased	Cell adhesion	
miRNAs	miRNA	Target gene	miRNA expression level	Gene function
	miR-21	*MSH6*	Increased	Mismatch repair system
	miR-155			
	miR-15a	Unspecified	Decreased	Cell-cycle regulators
	miR-16-1			

Table 3. Epigenetic dysregulations in non-functioning pituitary tumours

DNA Methylation	Gene	Gene expression level	Gene Function	
	ESR1	Increased	Transcription regulation	
	KCNAB2	Decreased	Regulation of voltage-gated potassium channel	
	KCNMB4	Decreased	Regulation of voltage and calcium-sensitive potassium channel	
	CACNA1C	Decreased	Regulation of voltage-dependent calcium channel	
	HOXB1	Decreased	Transcription regulation	
	RHOD	Decreased	Regulation of membrane transport	
	MEG3	Decreased	TSG, p53 dependent transcription	
miRNAs	miRNA	Target gene/pathway	miRNA expression level	Gene function
	miR-135a	*TGF-β pathway*	Increased	
	miR-140-5p			
	miR-582-3p			
	miR-938			
	miR-20a	*PTEN, TIMP2*	Increased	TSG, inhibition of MMPs
	miR-106b			
	miR-17-5p			
lncRNAs	lncRNA	Level of expression		
	HOTAIR	Increased		

Table 4. Epigenetic dysregulations in somatotroph tumours

DNA Methylation	Gene	Gene expression level	Gene Function	
	GNAS	Increased	cAMP synthesis	
	SSTR5	Increased	Somatostatin receptor	
	GH1	Increased	Growth hormone production	
	GH2	Increased		
	POMC	Increased	ACTH precursor	
	RASSF3	Decreased	TSG, apoptosis	
miRNAs	miRNA	Target gene	miRNA expression level	Gene function
	Let-7	*RAS*	Increased	oncogene
	miR-320	Unspecified	Increased	
	miR-128	*PTEN*	Increased	Regulation of PI3K signalling
	miR-34b	*HMGA1* *HMGA2* *E2F1*	Decreased	Cell-cycle and transcription regulators
	miR-326			
	miR-432			
	miR-548c-3p			
	miR-570			
	miR-603			
	miR-126	*PTTG1*	Decreased	oncogene
	miR-23b	*HMGA2*	Decreased	transcription regulator
	miR-130b	*CCNA2*	Decreased	Cell-cycle regulator (G1/S and G2/M phases)

Table 5. Epigenetic dysregulations in corticotroph tumours

DNA Methylation	Gene	Gene expression level		Gene Function
	CABLES1	Decreased		TSG
Histone Modifications	Gene or histone-modifying enzyme	Altered histone mark		Gene or histone-modifying enzyme function
	BMP-4	Increased H3K27 methylation		Cell proliferation
miRNAs	miRNA	Target gene	miRNA expression level	Gene function
	miR-26a	*PRKCD*	Increased	Apoptosis, cell-cycle regulation
	miR-145	*MYC, KRAS, FOS, YES*	Decreased	oncogenes
	miR-16	*AIP*	Decreased	Receptor-mediated signalling
	miR-21			
	miR-141			
	miR-143			
	miR-150			
	let-7a			
	let-7a	*HMGA2*	Decreased	Transcription regulation
	miR-15a	Unspecified	Decreased	
	miR-16			
	Let-7			

AGRADECIMENTOS

Aos meu pais. O amor e o apoio incondicionais.

À minha irmã. A amizade e a cumplicidade.

Aos meus avós. A ternura e a sabedoria.

Aos amigos. O carinho e a companhia.

Ao Dr. Luís Cardoso e Doutora Leonor Gomes. O incentivo e a disponibilidade.

REFERENCES

1. Hauser, B.M., et al., *The Epigenomics of Pituitary Adenoma.* Front Endocrinol (Lausanne), 2019. **10**: p. 290.
2. Shariq, O.A. and K.E. Lines, *Epigenetic dysregulation in pituitary tumors.* International Journal of Endocrine Oncology, 2019. **6**(3): p. IJE19.
3. Shen, A.J.J., et al., *Insights into pituitary tumorigenesis: from Sanger sequencing to next-generation sequencing and beyond.* Expert Rev Endocrinol Metab, 2019. **14**(6): p. 399-418.
4. Salomon, M.P., et al., *The Epigenomic Landscape of Pituitary Adenomas Reveals Specific Alterations and Differentiates Among Acromegaly, Cushing's Disease and Endocrine-Inactive Subtypes.* Clin Cancer Res, 2018. **24**(17): p. 4126-4136.
5. Lloyd, R.V., et al., *WHO classification of tumours of endocrine organs*. 4th edition ed, ed. R.V. Lloyd, et al. 2017, Lyon: IARC Press.
6. Pepe, S., M. Korbonits, and D. Iacovazzo, *Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects.* J Endocrinol, 2019. **240**(2): p. R21-R45.
7. Ezzat, S. and S.L. Asa, *Mechanisms of disease: The pathogenesis of pituitary tumors.* Nat Clin Pract Endocrinol Metab, 2006. **2**(4): p. 220-30.
8. Fukuoka, H. and Y. Takahashi, *The role of genetic and epigenetic changes in pituitary tumorigenesis.* Neurol Med Chir (Tokyo), 2014. **54**(12): p. 943-57.
9. Aflorei, E.D. and M. Korbonits, *Epidemiology and etiopathogenesis of pituitary adenomas.* J Neurooncol, 2014. **117**(3): p. 379-94.
10. Tatsi, C. and C.A. Stratakis, *The Genetics of Pituitary Adenomas.* J Clin Med, 2019. **9**(1).
11. Mindermann, T. and C.B. Wilson, *Age-related and gender-related occurrence of pituitary adenomas.* Clin Endocrinol (Oxf), 1994. **41**(3): p. 359-64.
12. Lim, C.T. and M. Korbonits, *Update on the Clinicopathology of Pituitary Adenomas.* Endocr Pract, 2018. **24**(5): p. 473-488.
13. Li, E., *Chromatin modification and epigenetic reprogramming in mammalian development.* Nat Rev Genet, 2002. **3**(9): p. 662-73.
14. Ezzat, S., S. Cheng, and S.L. Asa, *Epigenetics of pituitary tumors: Pathogenetic and therapeutic implications.* Mol Cell Endocrinol, 2018. **469**: p. 70-76.
15. Duong, C.V., et al., *Quantitative, genome-wide analysis of the DNA methylome in sporadic pituitary adenomas.* Endocr Relat Cancer, 2012. **19**(6): p. 805-16.
16. Garcia-Martinez, A., et al., *DNA Methylation of Tumor Suppressor Genes in Pituitary Neuroendocrine Tumors.* J Clin Endocrinol Metab, 2019. **104**(4): p. 1272-1282.
17. Farrell, W.E., *Epigenetics of pituitary tumours: an update.* Curr Opin Endocrinol Diabetes Obes, 2014. **21**(4): p. 299-305.
18. Dudley, K.J., et al., *Pituitary tumours: all silent on the epigenetics front.* J Mol Endocrinol, 2009. **42**(6): p. 461-8.
19. Wierinckx, A., et al., *MicroRNAs in pituitary tumors.* Mol Cell Endocrinol, 2017. **456**: p. 51-61.
20. Ma, H.S., et al., *Overexpression of DNA (Cytosine-5)-Methyltransferase 1 (DNMT1) And DNA (Cytosine-5)-Methyltransferase 3A (DNMT3A) Is Associated with Aggressive Behavior and Hypermethylation of Tumor Suppressor Genes in Human Pituitary Adenomas.* Med Sci Monit, 2018. **24**: p. 4841-4850.
21. Zhu, X., et al., *Deoxyribonucleic acid methyltransferase 3B promotes epigenetic silencing through histone 3 chromatin modifications in pituitary cells.* J Clin Endocrinol Metab, 2008. **93**(9): p. 3610-7.
22. Yoshino, A., et al., *Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas.* J Neurooncol, 2007. **83**(2): p. 153-62.
23. Wang, W., et al., *Histone deacetylase 11 suppresses p53 expression in pituitary tumor cells.* Cell Biol Int, 2017. **41**(12): p. 1290-1295.

24. Trivellin, G., et al., *MicroRNA miR-107 is overexpressed in pituitary adenomas and inhibits the expression of aryl hydrocarbon receptor-interacting protein in vitro.* Am J Physiol Endocrinol Metab, 2012. **303**(6): p. E708-19.
25. Zhu, X., S.L. Asa, and S. Ezzat, *Fibroblast growth factor 2 and estrogen control the balance of histone 3 modifications targeting MAGE-A3 in pituitary neoplasia.* Clin Cancer Res, 2008. **14**(7): p. 1984-96.
26. D'Angelo, D., et al., *Emerging Role of USP8, HMGA, and Non-Coding RNAs in Pituitary Tumorigenesis.* Cancers (Basel), 2019. **11**(9).
27. Beylerli, O., et al., *The role of lncRNAs in the biology of pituitary adenomas.* World Neurosurg, 2019.
28. Gossing, W., M. Frohme, and L. Radke, *Biomarkers for Liquid Biopsies of Pituitary Neuroendocrine Tumors.* Biomedicines, 2020. **8**(6).
29. Yang, Q. and X. Li, *Molecular Network Basis of Invasive Pituitary Adenoma: A Review.* Front Endocrinol (Lausanne), 2019. **10**: p. 7.
30. Qian, Z.R., et al., *Inactivation of RASSF1A tumor suppressor gene by aberrant promoter hypermethylation in human pituitary adenomas.* Lab Invest, 2005. **85**(4): p. 464-73.
31. Ebrahimi, A., et al., *Histone acetylation patterns of typical and atypical pituitary adenomas indicate epigenetic shift of these tumours.* J Neuroendocrinol, 2011. **23**(6): p. 525-30.
32. Wu, Z.B., et al., *MicroRNA expression profile of bromocriptine-resistant prolactinomas.* Mol Cell Endocrinol, 2014. **395**(1-2): p. 10-8.
33. Fan, X., et al., *Expression of somatostatin receptor subtype 2 in growth hormone-secreting pituitary adenoma and the regulation of miR-185.* Journal of Endocrinological Investigation, 2015. **38**(10): p. 1117-1128.
34. Mao, Z.G., et al., *Differential expression of microRNAs in GH-secreting pituitary adenomas.* Diagn Pathol, 2010. **5**: p. 79.
35. Chang, M., et al., *Genetic and Epigenetic Causes of Pituitary Adenomas.* Front Endocrinol (Lausanne), 2020. **11**: p. 596554.
36. Ling, C., et al., *A pilot genome-scale profiling of DNA methylation in sporadic pituitary macroadenomas: association with tumor invasion and histopathological subtype.* PLoS One, 2014. **9**(4): p. e96178.
37. Caimari, F. and M. Korbonits, *Novel Genetic Causes of Pituitary Adenomas.* Clin Cancer Res, 2016. **22**(20): p. 5030-5042.
38. Melmed, S., *Acromegaly pathogenesis and treatment.* J Clin Invest, 2009. **119**(11): p. 3189-202.
39. Valimaki, N., et al., *Genetic and Epigenetic Characterization of Growth Hormone-Secreting Pituitary Tumors.* Mol Cancer Res, 2019. **17**(12): p. 2432-2443.
40. Srirangam Nadhamuni, V. and M. Korbonits, *Novel Insights into Pituitary Tumorigenesis: Genetic and Epigenetic Mechanisms.* Endocr Rev, 2020. **41**(6).
41. Neou, M., et al., *Pangenomic Classification of Pituitary Neuroendocrine Tumors.* Cancer Cell, 2020. **37**(1): p. 123-134 e5.
42. Bi, W.L., et al., *Landscape of Genomic Alterations in Pituitary Adenomas.* Clin Cancer Res, 2017. **23**(7): p. 1841-1851.
43. Song, Z.J., et al., *The genome-wide mutational landscape of pituitary adenomas.* Cell Res, 2016. **26**(11): p. 1255-1259.
44. Ben-Shlomo, A., et al., *DNA damage and growth hormone hypersecretion in pituitary somatotroph adenomas.* The Journal of Clinical Investigation, 2020. **130**(11): p. 5738-5755.
45. Hayward, B.E., et al., *Imprinting of the G(s)alpha gene GNAS1 in the pathogenesis of acromegaly.* J Clin Invest, 2001. **107**(6): p. R31-6.
46. Peng, H., et al., *Silencing of RASSF3 by DNA Hypermethylation Is Associated with Tumorigenesis in Somatotroph Adenomas.* PLOS ONE, 2013. **8**(3): p. e59024.

47. D'Angelo, D., et al., *Altered microRNA expression profile in human pituitary GH adenomas: down-regulation of miRNA targeting HMGA1, HMGA2, and E2F1.* J Clin Endocrinol Metab, 2012. **97**(7): p. E1128-38.
48. Buliman, A., et al., *Cushing's disease: a multidisciplinary overview of the clinical features, diagnosis, and treatment.* J Med Life, 2016. **9**(1): p. 12-18.
49. Paez-Pereda, M., et al., *Involvement of bone morphogenetic protein 4 (BMP-4) in pituitary prolactinoma pathogenesis through a Smad/estrogen receptor crosstalk.* Proc Natl Acad Sci U S A, 2003. **100**(3): p. 1034-9.
50. Stilling, G., et al., *MicroRNA expression in ACTH-producing pituitary tumors: up-regulation of microRNA-122 and -493 in pituitary carcinomas.* Endocrine, 2010. **38**(1): p. 67-75.
51. Frattini, M., et al., *Quantitative and qualitative characterization of plasma DNA identifies primary and recurrent colorectal cancer.* Cancer Lett, 2008. **263**(2): p. 170-81.
52. Ortiz-Quintero, B., *Cell-free microRNAs in blood and other body fluids, as cancer biomarkers.* Cell Prolif, 2016. **49**(3): p. 281-303.
53. Berdasco, M. and M. Esteller, *Clinical epigenetics: seizing opportunities for translation.* Nat Rev Genet, 2019. **20**(2): p. 109-127.
54. Filippakopoulos, P., et al., *Selective inhibition of BET bromodomains.* Nature, 2010. **468**(7327): p. 1067-73.
55. Sanaei, M. and F. Kavoosi, *Investigation of the Effect of Zebularine in Comparison to and in Combination with Trichostatin A on p21Cip1/Waf1/ Sdi1, p27Kip1, p57Kip2, DNA Methyltransferases and Histone Deacetylases in Colon Cancer LS 180 Cell Line.* Asian Pac J Cancer Prev, 2020. **21**(6): p. 1819-1828.
56. Xiong, W., et al., *Structure-Based Screen Identification of a Mammalian Ste20-like Kinase 4 (MST4) Inhibitor with Therapeutic Potential for Pituitary Tumors.* Mol Cancer Ther, 2016. **15**(3): p. 412-20.
57. D'Angelo, D., F. Esposito, and A. Fusco, *Epigenetic Mechanisms Leading to Overexpression of HMGA Proteins in Human Pituitary Adenomas.* Frontiers in Medicine, 2015. **2**(39).
58. Veenstra, M.J., et al., *Targeting the somatostatin receptor in pituitary and neuroendocrine tumors.* Expert Opin Ther Targets, 2013. **17**(11): p. 1329-43.