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CLARIANO PIRES DE OLIVEIRA NETO

AVALIAÇÃO DE BIOMARCADORES CELULARES E MOLECULARES NA FISIOPATOLOGIA DE CRANIOFARINGIOMAS

São Luís
2025

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde.

Orientador: Prof. Dr. Manuel dos Santos Faria
Co-orientadores: Prof. Dr. Gilvan Cortês Nascimento e Prof. Dr. Marcelo Magalhães Silva

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"A inteligência, quando não se curva à humildade,
corre o risco de se perder em sua própria vaidade."
— Josué Montello, Os Degraus do Paraíso

Para minha mãe,
que é farol, alento,
e fé onde havia dúvida.

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SUMÁRIO

LISTA DE SIGLAS E ABREVIATURAS

ACP	Craniofaringioma Adamantinomatoso
ACTH	Hormônio Adrenocorticotrófico
AVP	Arginina-Vasopressina
BMP	Bone Morphogenetic Protein (Proteína Morfogenética Óssea)
CK	Cytokeratin
CLDN1	Claudin-1
CXCL	Chemokine (C-X-C motif) ligand
CXCR	CXC chemokine receptor
DM2	Diabetes melito tipo 2
EGF	Epidermal Growth Factor (Fator de Crescimento Epidérmico)
EGFR	Epidermal Growth Factor Receptor (Receptor do Fator de Crescimento Epidérmico)
EMT	Epitelial-Mesenchymal Transition (Transição Epitelial-Mesenquimal)
ERK	Extracellular Signal-Regulated Kinase
FGF	Fibroblast Growth Factors (Fatores de Crescimento de Fibroblastos)
FFPE	Formalin-Fixed, Paraffin-Embedded
FSH	Hormônio Folículo-Estimulante
GH	Hormônio do Crescimento
H&E	Hematoxylin and eosin
HUUFMA	Hospital Universitário da Universidade Federal do Maranhão
ICC	Intraclass correlation coefficient
IFNα	Interferon-α
INF-peg-α-2b	Pegylated Interferon-α-2b
IGF-1	Fator de Crescimento Insulínico Tipo 1
IL	Interleucina
LH	Hormônio Luteinizante
MAPK	Mitogen-Activated Protein Kinase
MEK	Mitogen-Activated Protein Kinase Kinase
MMP	Metaloproteinase

PCP	Craniofaringioma Papilar
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein ligand 1
PI3K/AKT	Phosphoinositide 3-kinase/Protein kinase B
RAS	Rat Sarcoma
SHH	Sonic Hedgehog
T4L	Tiroxina Livre
TGF-β	Transforming Growth Factor-β (Fator de Crescimento Transformador β)
TNF	Fator de Necrose Tumoral
TSH	Hormônio Estimulador da Tireoide
VDAC2	Canal de Anion Dependente de Voltagem 2
VEGF	Fator de Crescimento Endotelial Vascular
WHO	World Health Organization (Organização Mundial da Saúde)
Wnt	Wingless-related integration site

LISTA DE QUADROS E TABELAS

LISTA DE FIGURAS

Resumo

Craniofaringiomas são tumores intracranianos raros e potencialmente debilitantes, originados na região selar e de comportamento clínico desafiador. Embora histologicamente benignos, podem apresentar crescimento invasivo, recorrência e causar disfunções visuais, hormonais e metabólicas significativas. Apesar dos avanços, a fisiopatologia desses tumores ainda não é completamente compreendida, e os tratamentos disponíveis frequentemente resultam em sequelas importantes. Hipotetiza-se que a expressão de proteínas como Ki-67, β-catenina e VDAC2 esteja associada a variações na agressividade tumoral e na resposta terapêutica. Este estudo teve como objetivo caracterizar os aspectos clínicos, histopatológicos e moleculares de pacientes portadores de craniofaringiomas. A metodologia consistiu na análise de amostras tumorais de pacientes submetidos à cirurgia no HUUFMA. Foram realizadas revisão clínica, avaliação histopatológica e análise imunohistoquímica para β-catenina, VDAC2 e Ki-67, com correlação estatística entre os achados clínicos, morfológicos e moleculares. Todos os tumores analisados foram do tipo adamantinomatoso, com predomínio em pacientes pediátricos. A imunomarcação para β-catenina revelou acúmulo nuclear e citoplasmático, confirmando a ativação da via Wnt/β-catenina. A expressão de VDAC2 foi observada em diferentes intensidades e apresentou correlação positiva estatisticamente significativa com o índice proliferativo Ki-67 e com a expressão de β-catenina, sugerindo seu papel como marcador de agressividade biológica. Conclui-se que a expressão de VDAC2 emerge como potencial biomarcador de agressividade em craniofaringiomas, representando um possível alvo terapêutico futuro. Esses achados contribuem para o avanço do conhecimento fisiopatológico e para o desenvolvimento de abordagens mais individualizadas no manejo desses tumores complexos.

Palavras-chave: Craniofaringioma, Neuroendocrinologia, Biomarcadores

Abstract

Craniopharyngiomas are rare and potentially debilitating intracranial tumors that originate in the sellar region and present a challenging clinical course. Although histologically benign, they can exhibit invasive growth, recurrence, and cause significant visual, hormonal, and metabolic dysfunctions. Despite recent advances, the pathophysiology of these tumors remains incompletely understood, and available treatments often result in substantial sequelae. It is hypothesized that the expression of proteins such as Ki-67, β-catenin, and VDAC2 is associated with variations in tumor aggressiveness and treatment response. This study aimed to characterize the clinical, histopathological, and molecular aspects of patients with craniopharyngiomas. The methodology involved the analysis of tumor samples from patients who underwent surgery at HUUFMA. Clinical review, histopathological examination, and immunohistochemical analysis for β-catenin, VDAC2, and Ki-67 were performed, with statistical correlation between clinical, morphological, and molecular findings. All analyzed tumors were of the adamantinomatous type, with a predominance in pediatric patients. Immunostaining for β-catenin revealed nuclear and cytoplasmic accumulation, confirming activation of the Wnt/β-catenin signaling pathway. VDAC2 expression was observed at varying levels and showed a statistically significant positive correlation with the proliferative index Ki-67 and β-catenin expression, suggesting its role as a marker of biological aggressiveness. In conclusion, VDAC2 expression emerges as a potential biomarker of aggressiveness in craniopharyngiomas, representing a possible future therapeutic target. These findings contribute to the advancement of pathophysiological understanding and support the development of more individualized approaches to the management of these complex tumors.

Keywords: Craniopharyngioma, Neuroendocrinology, Biomarkers

1 INTRODUÇÃO

Os craniofaringiomas constituem um grupo de tumores intracranianos raros, de origem epitelial, que se desenvolvem a partir de remanescentes embrionários da bolsa de Rathke (MÜLLER et al., 2019). Apesar de sua classificação como neoplasias benignas de grau I pela Organização Mundial da Saúde, apresentam comportamento clínico complexo e potencialmente agressivo, principalmente em razão de sua localização na região selar e suprasselar, próxima a estruturas neurológicas e endócrinas vitais. Representam entre 2 a 5% dos tumores intracranianos primários, com distribuição etária bimodal — acometendo principalmente crianças entre 5 e 15 anos e adultos entre a quinta e sexta décadas de vida — e sem predileção por sexo (PILONI et al., 2023).

A despeito de sua raridade, os craniofaringiomas impõem um enorme fardo clínico e psicossocial aos pacientes e seus familiares. Seu crescimento, ainda que lento, pode provocar efeitos compressivos sobre o quiasma óptico, o hipotálamo, a hipófise e o terceiro ventrículo, resultando em um amplo espectro de manifestações clínicas: desde distúrbios visuais e déficits hormonais múltiplos até alterações cognitivas, comportamentais e metabólicas graves (ERFURTH, 2023). Não raro, crianças afetadas apresentam retardo no crescimento, puberdade tardia, obesidade hipotalâmica e comprometimento do desenvolvimento neuropsicomotor (MARTINEZ-BARBERA; ANDONIADOU, 2020). Em adultos, o comprometimento da autonomia funcional e a alta taxa de recidiva impactam negativamente na qualidade de vida a longo prazo (PASCUAL; PRIETO, 2021).

Embora a ressecção cirúrgica continue sendo a principal modalidade terapêutica, ela raramente é curativa. O caráter infiltrativo dos craniofaringiomas, particularmente do tipo adamantinomatoso, e sua íntima relação com estruturas nobres do sistema nervoso central, tornam a cirurgia radical uma estratégia de alto risco, frequentemente associada a sequelas neurológicas, visuais e endócrinas permanentes (MÜLLER, 2011). A radioterapia, por sua vez, tem papel adjuvante importante na contenção do crescimento tumoral residual, mas também carrega risco de efeitos colaterais tardios, como disfunção hipofisária, comprometimento cognitivo e danos ao parênquima cerebral adjacente (ALBANO et al., 2020). Outras estratégias terapêuticas — como a aplicação intracística de agentes esclerosantes — são

indicadas apenas em contextos específicos, com eficácia limitada e resultados inconsistentes (KILDAY et al., 2017).

Nesse contexto, os craniofaringiomas permanecem como uma das neoplasias de tratamento mais desafiador na neuroendocrinologia, não apenas pelos limites impostos pelas terapias atuais, mas também pela insuficiência do conhecimento fisiopatológico que sustenta a abordagem clínica desses tumores. Embora avanços nas últimas décadas tenham permitido a identificação de diferenças morfológicas, epidemiológicas e genéticas entre os dois tipos histológicos principais, ainda existem lacunas significativas quanto aos mecanismos celulares e moleculares que regem seu comportamento biológico (MARTINEZ-BARBERA; ANDONIADOU, 2020).

Compreender a fisiopatologia dos craniofaringiomas não é um exercício acadêmico meramente descritivo, mas uma necessidade concreta para transformar o paradigma terapêutico atual. A variabilidade clínica observada entre os pacientes, mesmo dentro de um mesmo tipo histológico, indica que fatores intrínsecos ao tumor — como padrões de proliferação, diferenciação celular, interação com o microambiente e resistência à apoptose — influenciam diretamente o prognóstico e a resposta ao tratamento (ALBOQAMI et al., 2024). Identificar esses determinantes pode abrir caminho para intervenções mais eficazes, seletivas e menos invasivas.

Além disso, o entendimento aprofundado das vias de sinalização envolvidas na tumorigênese dos craniofaringiomas pode revelar pontos vulneráveis à intervenção farmacológica. No tipo papilar, por exemplo, a presença quase universal da mutação *BRAF V600E* e sua consequente ativação da via MAPK permitiram a introdução, em casos selecionados, de terapias-alvo com inibidores de BRAF e MEK, que demonstraram respostas clínicas expressivas em relatos de caso e estudos iniciais (BRASTIANOS et al., 2023). Já no tipo adamantinomatoso, caracterizado por mutações no gene *CTNNB1* e ativação da via Wnt/β-catenina, ainda não há terapias moleculares validadas, apesar dos crescentes esforços de pesquisa (HARA et al., 2019).

Outro aspecto relevante é a crescente evidência de que o microambiente tumoral, incluindo componentes inflamatórios, imunológicos e vasculares, desempenha papel ativo na manutenção e progressão dos craniofaringiomas (LIN et al., 2019). O reconhecimento de que esses tumores não são biologicamente inertes,

mas sim entidades dinâmicas e interativas com o tecido adjacente, impõe a necessidade de modelos experimentais mais complexos e análises integrativas, que extrapolem os limites da histologia convencional.

Nesse cenário, a investigação da fisiopatologia dos craniofaringiomas deve ser concebida como etapa essencial para viabilizar uma medicina de precisão — aquela que reconhece a heterogeneidade tumoral e propõe intervenções individualizadas com base no perfil biológico específico de cada paciente. Essa abordagem é particularmente promissora em tumores raros e recidivantes, nos quais a aplicação de terapias-padrão frequentemente fracassa ou impõe risco elevado de morbidade.

Portanto, ampliar o conhecimento sobre a fisiopatologia dos craniofaringiomas é uma estratégia fundamental não apenas para compreender os determinantes de sua agressividade e heterogeneidade, mas também para nortear a descoberta de biomarcadores prognósticos, preditores de resposta terapêutica e potenciais alvos farmacológicos. Trata-se de uma demanda urgente da prática clínica, que precisa ser respondida com ciência de qualidade, rigor metodológico e sensibilidade para os impactos reais na vida dos pacientes.

2 REFERENCIAL TEÓRICO

2.1 Definição e epidemiologia

Craniofaringiomas são tumores intracranianos raros originários de células remanescentes do epitélio do ducto craniofaríngeo, conhecido como bolsa de Rathke (FARIA; NASCIMENTO; VAZ, 2013). Representam 1,2 a 4,6% de todas as neoplasias primárias intracranianas, com distribuição bimodal (um pico na população pediátrica e outro pico em adultos na 6ª década de vida) e são responsáveis por 5 a 11% dos tumores intracranianos em crianças; acomete igualmente homens e mulheres (MÜLLER et al., 2019). Embora essas lesões sejam classificadas como benignas, sua capacidade invasiva e a proximidade com estruturas nobres como as vias ópticas, o 3º ventrículo, a hipófise e o hipotálamo, são associadas a déficits visuais, hormonais e neurológicos assim como perda da qualidade de vida conforme o crescimento tumoral, consistindo em uma doença agressiva e com terapêutica limitada (MARTINEZ-BARBERA; ANDONIADOU, 2020). A transformação maligna é extremamente incomum (FRANGOU et al., 2009; GUPTA et al., 1999).

2.2 Patologia e patogênese

Existem dois tipos de craniofaringioma – adamantinomatoso e papilar –, os quais diferem em sua apresentação clínica e radiológica, aspectos histopatológicos, gênese e genética (MÜLLER et al., 2019; PILONI et al., 2023). (Quadro 1).

O tipo adamantinomatoso é o mais comum, sendo responsável por até 90% dos casos, ocorrendo em todas as idades, em particular na população pediátrica, porém com distribuição bimodal apresentando um outro pico de incidência por volta da 6ª década de vida (MOMIN et al., 2021; MÜLLER et al., 2019). Apresenta histologia semelhante ao adamantinoma da mandíbula ou do tecido embrionário formador de dentina, com uma camada de células basais colunares com arranjo em paliçada que constituem o elemento principal no diagnóstico do adamantinoma (Figura 1). São tumores predominantemente císticos ou sólido-císticos, que podem ser multilobulados, apresentando um líquido de coloração escura de elevado teor proteico, calcificações, cristais de colesterol e queratina (KARAVITAKI et al., 2006).

O tipo papilar, quase exclusivo do adulto, é composto de epitélio escamoso papilífero, pode apresentar cistos, mas raramente calcifica, e tem como principal

diagnóstico diferencial o adenoma hipofisário, mais comum nessa faixa etária. Na histologia observa-se somente o epitélio escamoso, sem componente adamantinomatoso e apresenta maior tendência a ser sólido (Figura 2). O conteúdo cístico, quando presente, não contém cristais de colesterol, apresenta coloração amarelada e aspecto viscoso. A calcificação é um achado raro (JANNELLI et al., 2023). Diferentemente do tipo adamantinomatoso, apresenta discreta gliose peritumoral e sem evidência de ilhotas tumorais, o que justifica menor propensão a recidivas (WU et al., 2022).

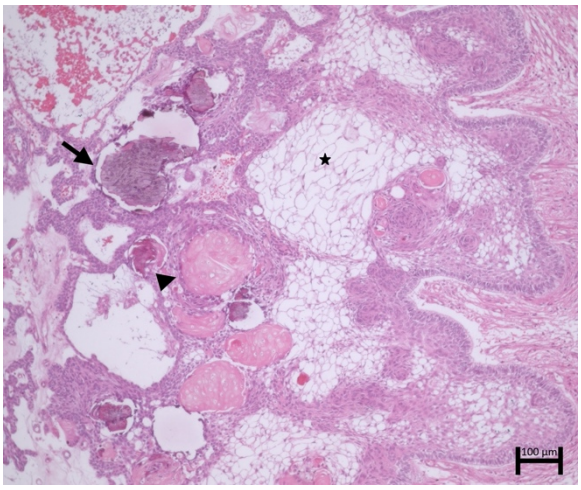

Figura 1. Tipo adamantinomatoso. Neoplasia composta por ilhas e cordões de células epiteliais com paliçada delimitando o estroma composto por células reticulo-estreladas (asterisco). Ninhos de células escamosas anucleadas (cabeça de seta) e focos de calcificação (seta).

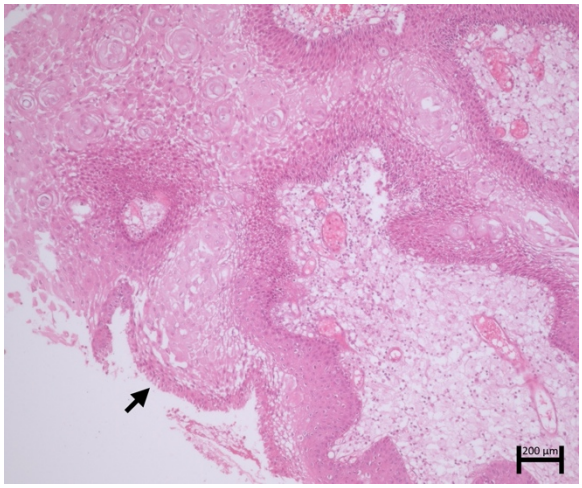

Figura 2. Tipo papilar. Eixos conjuntivos fibrovasculares formando papilas revestidas por epitélio escamoso bem diferenciado com paliçada (seta)

Embora de fisiopatologia não esclarecida, estudos recentes apontam para origem genética diferente entre os dois tipos histológicos. O comportamento agressivo, do ponto de vista clínico e biológico, aponta para uma doença molecular complexa. A rigor, os mecanismos biológicos envolvidos na base da natureza agressiva de uma lesão neoplásica são: proliferação celular, falha de via apoptótica e ativação de via antiapoptótica, anaplasia celular, invasividade local, neoangiogênese e resposta imune. Tais mecanismos parecem estar presentes no desenvolvimento de craniofaringiomas (CAMPANINI et al., 2023; GUADAGNO et al., 2017; LARKIN; KARAVITAKI, 2017; SHAH; JUNG, 2015).

Quadro 1 Comparação entre os craniofaringiomas adamantinomatosos e papilar.

Características	Adamantinomatoso	Papilar
Incidência	0,5 a 2,5 casos por milhão/ano	
Frequência	90%	10%
Faixa etária (anos)	5-15 e 45-60	40-55
Apresentação Clínica	Assintomático (incomum). Sintomas ao diagnóstico: hipertensão intracraniana, alteração visual, hipopituitarismo, obesidade	
Macroscopia	Tumor cístico com ou sem componente sólido	Predominantemente sólido
Microscopia	Multicístico, "retículo estrelado", "queratina úmida", calcificação ocasional, protrusões *finger-like* na borda cerebral delimitadas por células em paliçada, inflamação crônica no tecido cerebral peritumoral	Padrão de crescimento papilar com epitélio escamoso não queratinizado; sem queratina úmida; sem calcificação; epitélio neoplásico bem circunscrito, infiltração de tecido cerebral adjacente geralmente ausente
Localização	Intra/suprasselar	Suprasselar
Ressonância magnética	Cistos hiperintensos em T1	Cistos hipointensos em T1
Tomografia computadorizada	Presença de calcificações e predomínio de áreas císticas	Ausência de calcificações e predomínio de área sólida
Calcificações	Frequentes	Raras
Marcadores Moleculares	Mutação *CTNNB1*	Mutação *BRAF V600E*

Numerosas citocinas e mediadores inflamatórios são expressos em craniofaringiomas, tanto nos componentes sólidos quanto nos componentes císticos. Concentrações particularmente elevadas das citocinas IL-6, IL-8 e IL-10 são relatadas no fluido cístico de craniofaringiomas adamantinomatosos. Estas moléculas podem promover a fuga da vigilância imunológica em craniofaringiomas, favorecendo vias pró-tumorigênese (DONSON et al., 2017; MORI; TAKESHIMA; KURATSU, 2004; PETTORINI et al., 2010).

Estudos iniciais apontaram para associação entre mutações no gene *CTNNB1* e craniofaringioma adamantinomatoso, o que foi confirmado em estudos em ratos geneticamente modificados (MARTINEZ-BARBERA; BUSLEI, 2015; SEKINE et al., 2002). Esse gene codifica a β-catenina, que tem papel regulatório na via Wnt. Mutações no éxon 3 do *CTNNB1* são frequentes no craniofaringioma adamantinomatoso (prevalência de 56 a 92%) (ver Quadro 1) (HARA et al., 2019). Adicionalmente, foi observado acúmulo de β-catenina, nuclear e citoplasmática, em 90% a 94% desse tipo de craniofaringioma (CAMPANINI et al., 2010; JUCÁ et al., 2018; LARKIN; ANSORGE, 2013).

No tipo papilar são identificadas mutações no oncogene BRAFV600E em até 95% dos casos, resultando na ativação da via MAPK e culminando no processo de tumorigênese (BRASTIANOS; SANTAGATA, 2016; CIMINO et al., 2015; KIM; PAULUS; HEIM, 2015).

2.3 Diagnóstico

Paciente com craniofaringioma tipicamente apresentam, ao diagnóstico, características de aumento da pressão intracraniana e/ou anormalidades hormonais. Por apresentar crescimento lento, o diagnóstico desses tumores costuma ter atraso de meses a anos (HOFFMANN et al., 2015; MARTINEZ-GUTIERREZ et al., 2016; MÜLLER, 2020). O achado incidental dessa neoplasia em exames de imagem é incomum e ocorre em menos de 2% dos casos (BOEKHOFF et al., 2019). O diagnostico diferencial deve ser realizado com outros tumores da região hipotálamo-hipofisária, tais como adenomas hipofisários, gliomas de baixo grau, tumores de células germinativas e cistos da bolsa de Rathke (MÜLLER et al., 2012).

2.3.1 Manifestações Clínicas

A apresentação clínica é variável e está relacionada à idade do paciente, ao tamanho, à localização e à expansão do tumor e ao grau de comprometimento da função hipofisária (DEL CAN SÁNCHEZ et al., 2021; HOFMANN et al., 2010; LOSA et al., 2004; MÜLLER et al., 2019; PASCUAL; PRIETO, 2021). Na infância, sintomas secundários ao aumento da pressão intracraniana, notadamente cefaleia, mas também náuseas e vômitos, são muito comuns à apresentação clínica; a baixa estatura e o ganho ponderal podem estar presentes anos antes do diagnóstico ser estabelecido (HOFFMANN et al., 2015). Sintomas de aumento da pressão intracraniana, como cefaleia associada a náuseas e vômitos, também podem estar presentes (MÜLLER, 2020). Déficits visuais são comuns, podendo estar presentes em até 84% dos casos e com manifestação em qualquer faixa etária. O grau de deficiência visual depende da topografia anatômica do tumor no que diz respeito à compressão do quiasma óptico (WAN et al., 2018). (Quadro 2)

O excesso de peso decorrente de disfunção hipotalâmica também pode ser encontrado com frequência em pacientes com craniofaringioma, geralmente como

manifestação tardia, contudo pode estar presente em até 20% das crianças ao diagnóstico. Esse achado é atribuído a disfunção nos núcleos hipotalâmicos controladores da fome/saciedade e do gasto energético, bem como ao hipopituitarismo subjacente (IUGHETTI; BRUZZI, 2011). (Quadro 2)

2.3.2 Alterações Hormonais

Os déficits endócrinos são a primeira manifestação clínica na história da doença em 40% a 87% dos pacientes com diagnóstico de craniofaringioma, incluindo deficiência de arginina-vasopressina (AVP), que é observada em 17% a 27% dos pacientes antes do diagnóstico. As alterações hormonais são frequentemente causadas por distúrbios relacionados ao tumor e/ou ao tratamento no eixo hipotálamo-hipófise que afetam a secreção do hormônio do crescimento (GH) (75%), gonadotrofinas (40%), hormônio tireoestimulante (TSH) (25%) e hormônio adrenocorticotrófico (ACTH) (25%) (HOFFMANN et al., 2015; MÜLLER, 2020; MÜLLER et al., 2019).

Quadro 2 Manifestações Clínicas de Craniofaringiomas

Efeito de massa	Hipertensão intracraniana • Cefaleia • Náusea • Vômitos Déficit Visual
Alterações hormonais	Déficit somatotrófico • Baixa estatura Déficit Gonadotrófico • Atraso puberal Déficit Tireotrófico • Hipotireoidismo Déficit Corticotrófico • Insuficiência Adrenal
Alterações hipotalâmicas	Obesidade Hipotalâmica Deficiência de arginina-vasopressina (AVP) Desregulação da temperatura corporal Distúrbio do sono Disfunção cognitiva

2.3.3 Alterações Radiológicas

A avaliação radiológica é fundamental para a identificação, caracterização e planejamento terapêutico dos craniofaringiomas, dada sua ampla variedade de

apresentações morfológicas. Esses tumores podem ser exclusivamente sólidos (18% a 39%), císticos (46% a 64%) ou mistos (8% a 36%), com predominância de componente suprasselar (95% a 96%). A localização pode ser exclusivamente suprasselar (20% a 41%), intra e suprasselar (53% a 75%) ou puramente intrasselar (5% a 6%). Na ressonância magnética (RM) sem contraste, as porções sólidas e as paredes císticas podem apresentar sinais variados em T1, indo de hipointensos a hiperintensos. Tipicamente, os ACPs exibem cistos com conteúdo hiperintenso em T1, enquanto os PCPs, quando císticos, tendem a ter conteúdo hipointenso (Figuras 3 e 4). Nas imagens ponderadas em T2, os tumores apresentam sinal heterogêneo, devido à distribuição irregular das calcificações e à variabilidade individual. As calcificações estão presentes em 45% a 57% dos casos, sendo menos frequentes em adultos. Embora possam auxiliar no diagnóstico diferencial — especialmente com adenomas hipofisários — sua identificação por RM pode ser limitada. Sequências ponderadas em T2* são mais sensíveis, mas podem ser prejudicadas pela interferência dos seios paranasais. Por esse motivo, a tomografia computadorizada (TC) permanece como o método mais sensível e específico para detecção de calcificações em craniofaringiomas, sendo considerada o padrão ouro. Em alguns casos, essas calcificações podem até ser visualizadas em radiografias simples de crânio. (LIM et al., 2022; MÜLLER et al., 2019; MÜLLER; MARTINEZ-BARBERA, 2019).

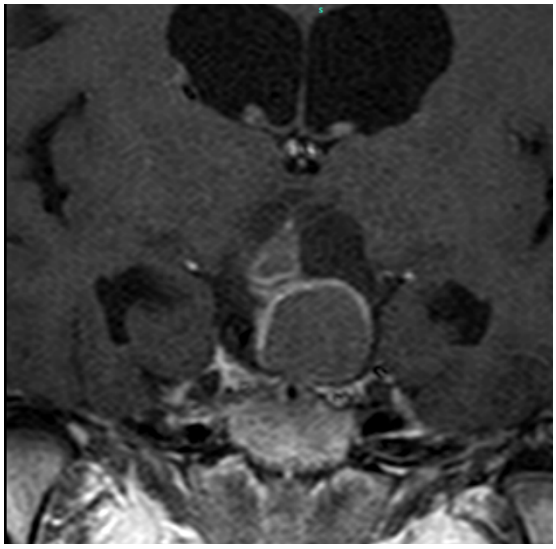

Figura 3. Imagem de Ressonância Magnética. Corte coronal mostrando craniofaringioma adamantinomatoso cístico na fase T1 pós contraste. O conteúdo do cisto é hiperintenso

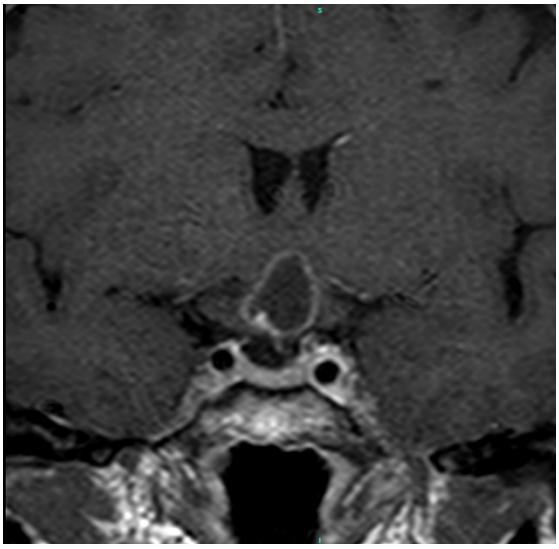

Figura 4. Imagem de Ressonância Magnética. Corte coronal mostrando craniofaringioma papilar cístico na fase T1 pós contraste. O conteúdo do cisto é hipointenso.

2.4 Tratamento

À exceção de tumores pequenos e estáveis, o tratamento convencional para craniofaringiomas geralmente envolve uma abordagem multimodal que pode consistir em uma ou mais intervenções cirúrgicas, combinadas ou não com radioterapia e tratamentos intracísticos. Dentre os objetivos do tratamento, são listados: confirmação diagnóstica, resolução do quadro neurológico e oftalmológico, recuperação ou preservação da função hipofisária, prevenção de recidivas e preservação da qualidade de vida (MÜLLER, 2020). (Quadro 3)

Quadro 3 Opções Terapêuticas em Craniofaringiomas

Cirurgia	Vantagens • Possibilidade de ressecção tumoral completa • Redução do efeito de massa • Avaliação histológica Desvantagens • Risco operatório • Risco de hipopituitarismo e disfunção hipotalâmica • Recorrência
Radioterapia	Vantagens • Tratamento conservador (tumores inoperáveis) • Evita riscos relacionados à cirurgia • Redução do tamanho tumoral Desvantagens • Complicações a longo prazo no tecido adjacente • Tempo prolongado para resposta ao tratamento • Necessidade de múltiplas sessões • Recorrência
Terapia Intracística	Vantagens • Abordagem direta em tumores císticos • Aplicação de substâncias esclerosantes da parede cística Desvantagens • Efetividade variável • Neurotoxicidade • Recorrência
Terapias-alvo (Inibidores de Tirosinoquinase)	Vantagens • Tratamento personalizado • Menor toxicidade • Redução tumoral (tipo papilar) Desvantagens • Resistência tumoral • Custo elevado • Necessidade de testes moleculares • Restrito ao tipo papilar

2.4.1 Cirurgia

A cirurgia atualmente é o tratamento de escolha para craniofaringiomas, sobretudo quando há possibilidade de ressecção tumoral completa, o que diminui drasticamente o risco de recidiva. No entanto, cirurgias mais amplas são relacionadas a maior morbidade hipofisária e hipotalâmica, além de comprometimento da qualidade de vida. Dessa forma, tem se considerado terapia cirúrgica parcial associada a outras estratégias de tratamento, como a radioterapia, com o intuito de preservar as estruturas adjacentes ao tumor, diminuindo o impacto da cirurgia na promoção de morbidades, na qualidade de vida e no prognóstico ao custo do aumento do risco de recidiva (HONG; OMAY, 2022).

O risco de complicações induzidas pelo grau de ressecção cirúrgica pode ser avaliado pela ressonância magnética pré-operatória, que permite a visualização do grau de adesão ou compressão das estruturas hipotalâmicas. Qualquer cirurgia para recorrência do tumor está associada a uma menor eficácia cirúrgica do que a cirurgia inicial e a um risco aumentado de complicações, incluindo mortalidade, o que leva cada vez mais a uma preferência pela radioterapia adjuvante como terapia complementar. Para craniofaringiomas puramente císticos, uma abertura cística no terceiro ventrículo seguida por uma estratégia expectante também pode representar uma opção segura, principalmente naqueles pacientes com predomínio de hipertensão intracraniana. Em alguns casos, cirurgia de emergência para descompressão quiasmática ou redução da pressão intracraniana podem ser necessárias (YAXIAN et al., 2021).

A via de acesso para ressecção cirúrgica será escolhida na dependência de características do tumor, como tamanho, localização (selar ou suprasselar), expansão (linha média ou fora da linha média) e presença de calcificações, entre outras, e de características da sela túrcica e do seio esfenoidal. Em casos de tumores predominantemente císticos, com sela túrcica aumentada, poucas calcificações e tumor sem grande extensão fora da linha média, a via transesfenoidal com abordagem microscópica e/ou endoscópica é a principal opção. Para casos com sela túrcica pequena, com hipófise normal presente e funcionante inferior ao tumor, ou em casos com grande extensão fora da linha média, a via transcraniana é geralmente a melhor opção. Acesso endoscópico estendido com abertura ampla do plano esfenoidal ou

clivo tem sido indicado mesmo em tumores com expansão fora da linha média, mas os autores têm tentado identificar critérios que poderiam auxiliar no prognóstico do resultado cirúrgico, especialmente para evitar dano hipotalâmico, além do desenvolvimento de técnicas de correção da fístula liquórica intraoperatória provocada por esse acesso (CUNY et al., 2022; MÜLLER, 2020).

2.4.2 Radioterapia

A radioterapia é importante arma terapêutica nos craniofaringiomas, seja por técnica estereotáxica fracionada, seja em dose única ou intracística. Quando combinada com a cirurgia, a radioterapia pode ser empregada imediatamente após uma cirurgia parcial, ou após a cirurgia total, durante o acompanhamento do paciente, quando surge um novo remanescente tumoral, ou se um remanescente tumoral previamente conhecido demonstra progressão. A radioterapia externa é geralmente indicada após ressecção parcial ou no momento de recidiva/recrescimento, mas pode ser ainda tratamento primário após confirmação do diagnóstico por biopsia estereotáxica em casos em que a localização da lesão leve a aumento da morbidade cirúrgica e o tumor não apresente efeito de massa que indique urgência na redução do volume tumoral. A radioterapia externa pode ser indicada também em lesões císticas que tenham tido seu conteúdo aspirado por punção estereotáxica ou pela colocação de cateteres intracísticos. Existe ainda a opção de radioterapia intracística com a utilização de material radioativo coloidal aplicado por meio de cateteres intracísticos. Em crianças menores, geralmente com idade inferior a 7 anos, a radioterapia é evitada pelos riscos de alteração cognitiva (ALBANO et al., 2020).

A radioterapia não tem efeito imediato no volume tumoral, com diminuição do tumor geralmente tendo início em 6 a 12 meses após o procedimento e continuando ao longo do tempo (SHI et al., 2012).

2.4.3 Terapia intracística

Colocação de cateter intracístico que permita esvaziamento por punção de câmara localizada no subcutâneo do couro cabeludo também é alternativa em cistos recidivados até que a radioterapia alcance o objetivo de evitar recidiva. O cateter pode

permitir também aplicação de substâncias que poderiam levar à esclerose da parede e evitar recrescimento do cisto (BIANCHI; BENATO; MASSIMI, 2022).

Os tratamentos intracísticos são uma opção alternativa à ressecção cirúrgica em pacientes bem selecionados com craniofaringiomas predominantemente císticos. Particularmente úteis nos pacientes mais jovens, as terapias intracísticas podem auxiliar no adiamento da radioterapia e devem ser realizadas apenas por equipes experientes. O tratamento intracístico com interferon-α (IFNα) proporciona um menor risco de efeitos adversos, mas é limitado à porção cística, sem efeito no componente sólido do tumor. O uso de bleomicina intracística é associado a complicações graves ligadas a extravasamento pela parede do cisto, resultando em neurotoxicidade irreversível ou mesmo morte, com revisões recentes não apoiando essa terapia em crianças com base nos benefícios e efeitos nocivos. Até o momento, os estudos sobre terapias intracísticas são pequenos e com pouco poder estatístico, com dados limitados para apoiar a sua utilização (SCHMUTZER-SONDERGELD et al., 2024).

Em casos que se apresentem com hidrocefalia, a derivação ventriculoperitoneal pode ser indicada como procedimento de urgência, até mesmo antes de qualquer tratamento dirigido ao craniofaringioma (PILONI et al., 2023).

2.5 Complicações

Morbidade aumentada é igualmente constatada com relação a hipopituitarismo, lesão hipotalâmica, déficit visual e cognitivo, declínio na qualidade de vida e do desenvolvimento de fatores de risco cardiovascular, consequentes à obesidade hipotalâmica. Outras manifestações de disfunção hipotalâmica que também estão ligadas sobretudo à redução da qualidade de vida são distúrbios da sede e da termorregulação, sonolência e apneia do sono (WIJNEN et al., 2018).

2.5.1 Obesidade e Craniofaringioma

Obesidade é uma complicação frequente e grave do craniofaringioma em si e de seu tratamento. O ganho de peso é comum, especialmente após a cirurgia, e ocorre em 26% a 61% dos pacientes cirurgicamente tratados. Mecanismos diversos podem estar envolvidos, mas entre os mais importantes está a destruição ou grave comprometimento pelo tumor ou pela cirurgia mais agressiva dos núcleos

hipotalâmicos que controlam a ingestão alimentar e o gasto energético. Redução da saciedade e hiperfagia são achados frequentes. Como consequência à obesidade, é comum o surgimento de diabetes melito tipo 2 (DM2), dislipidemia, hipertensão arterial e síndrome metabólica (IUGHETTI; BRUZZI, 2011). Em comparação à população geral, foi relatada na Suécia uma incidência aumentada em 7 vezes para infarto cerebral e em 5,6 vezes para DM2 (WIJNEN et al., 2018).

O tratamento da obesidade hipotalâmica se baseia nas mudanças do estilo de vida, que isoladamente não costumam ser efetivas, associadas ao uso de medicações para controle do peso. Pacientes com craniofaringioma e obesidade parecem apresentar uma boa resposta aos análogos do peptídeo semelhante ao glucagon 1 (GLP-1) (exenatida e liraglutida), mesmo em doses relativamente baixas. Cirurgia bariátrica está indicada para os casos de obesidade grave refratária ao tratamento clínico (MÜLLER, 2020).

2.5.2 Deficiências Hormonais

As deficiências endócrinas do eixo hipotálamo-hipófise resultam na necessidade de substituição hormonal ao longo da vida. Deficiências hormonais hipofisárias estão presentes em 54–100% dos pacientes. A deficiência pós-operatória de ACTH ocorre em 55–88% dos pacientes, GH em 88–100% dos pacientes, TSH em 39–95% dos pacientes, gonadotrofinas em 80–95% dos pacientes e AVP em 25–86% dos pacientes. Em caso de hipopituitarismo não tratado ou reposição hormonal insuficiente, são comuns efeitos adversos graves, como baixa estatura (para deficiência de GH) ou situações de emergência com risco de vida, como crise adrenal (para deficiência de ACTH). A terapia de reposição com GH recombinante é segura em relação aos riscos de progressão e recorrência tumoral, com melhora da qualidade de vida, peso e altura (BOGUSZEWSKI et al., 2022; CUNY et al., 2022).

2.5.3 Distúrbios Hidroeletrolíticos

No pós-operatório, as alterações hidroeletrolíticas são as de manejo mais difícil e importante. A presença de deficiência de AVP antes da cirurgia deve ser tratada com reposição adequada de desmopressina, que deve ser mantida no pré e pós-operatório imediato. No intra e pós-operatório imediato, a necessidade de

administração de desmopressina injetável (por via subcutânea ou intravenosa) em pequenas doses (0,5 ou 1 μg) deverá ser monitorada a intervalos curtos (2 a 4 horas) a partir da avaliação do sódio sérico e do volume urinário. Em pacientes com consciência preservada, a presença de sede pode auxiliar a indicação da administração da desmopressina e de líquidos que possam ser liberados por via oral o mais precocemente possível (MUSOLINO; CESCATO, 2021).

Alterações visual e neurológica são complicações dependentes da compressão do tumor ou do tratamento, como cirurgia e radioterapia, sendo também significativas para o comprometimento da qualidade de vida desses pacientes (CHEN; AI; SUN, 2023).

2.5.4 Mortalidade e Craniofaringioma

A mortalidade geral em craniofaringiomas é relatada como três a cinco vezes maior do que aquela observada na população geral. A sobrevida global descrita em coortes pediátricas varia de 83% a 96% em 5 anos, de 65% a 100% em 10 anos, e é, em média, de 62% em 20 anos. Em coortes mistas de pacientes pediátricos e adultos, a sobrevida global está na faixa de 54% a 96% em 5 anos, 40% a 93% em 10 anos e 66% a 85% em 20 anos. Se a idade no diagnóstico de craniofaringioma é um fator prognóstico para a sobrevivência ainda é uma questão controversa. Vários estudos demonstraram que os pacientes mais jovens apresentam melhor sobrevida do que pacientes adolescentes e adultos. Além disso, foi relatado um melhor prognóstico para os craniofaringiomas papilares do que para os craniofaringiomas adamantinomatosos, porém com resultados ainda inconsistentes (MÜLLER et al., 2019; WIJNEN et al., 2018).

A mortalidade a longo prazo está associada a fatores de risco relacionados ao tumor e/ou ao tratamento, como doença progressiva com múltiplas recorrências, doença cerebrovascular, deficiências neuroendócrinas crônicas, síndrome hipotalâmica, DM2 e convulsões. A mortalidade global padronizada variou de 2,88 vezes a 9,28 vezes em estudos de coorte publicados; pacientes com craniofaringiomas apresentam taxa de mortalidade cardiovascular associada à síndrome metabólica aumentada de 3 a 19 vezes quando comparados à população geral. Uma taxa de risco cardiovascular ainda maior é observada em pacientes do

sexo feminino com craniofaringioma, provavelmente causada por deficiência de estrogênio devido ao hipogonadismo secundário (PEREIRA et al., 2005; WIJNEN et al., 2018).

2.6 Terapias-Alvo

Embora representem apenas 10% de todos os craniofaringiomas, o tipo papilar apresenta a mutação BRAFV600E na quase totalidade das vezes. Por esse motivo, inibidores da tirosinoquinase (sobretudo dabrafenibe, isolado ou em combinação com trametinibe) têm despontado como fármacos potencialmente úteis nos casos mais graves, com múltiplas recorrências. Existem vários relatos de casos na literatura que mostraram uma redução tumoral de até 90% em pacientes com craniofaringioma papilar tratados com essas drogas, levantando a questão do uso de inibidores de MAPK como terapia neoadjuvante antes de considerar redução cirúrgica e/ou radioterapia (AGOSTI et al., 2024; FASANO et al., 2021; RAO et al., 2019). (Ver Figura 5)

Ao considerar os craniofaringiomas adamantinomatosos atualmente não existem terapias médicas disponíveis que tenham mostrado resultados semelhantes aos observados com inibidores BRAF/MEK em craniofaringiomas papilar. No entanto, a recente demonstração de que a via MAPK/ERK é ativada nas protrusões finger-like do craniofaringioma adamantinomatoso, seguida por uma redução da proliferação e um aumento da apoptose em culturas de células desses tumores tratadas com trametinibe, indica que novas vias médicas podem surgir em o futuro próximo (APPS et al., 2018; CUNY et al., 2022; IGLESIAS, 2022). Além disso, persistem lacunas nos mecanismos patogenéticos dos craniofaringiomas e estudos vem sendo realizados, com o intuito de proporcionar uma maior compreensão das vias fisiopatológicas implicadas na oncogênese desses tumores, de modo a oferecer opções terapêuticas mais eficazes podendo, desta forma, contribuir com melhora significativa do resultado terapêutico, possibilitando maior racionalidade na abordagem dessa patologia de difícil manejo médico e de escassas opções terapêuticas.

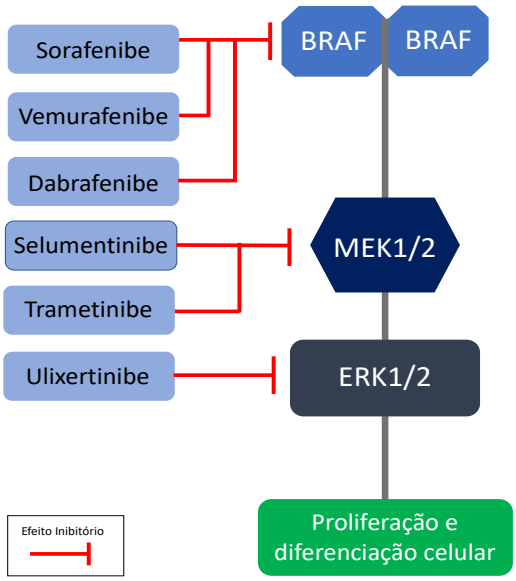

Figura 5. Componentes da via MAPK e inibidores de intermediários da via. O uso isolado ou combinado dessas substâncias tem sido estudado no tratamento craniofaringiomas papilares.

3 OBJETIVOS

3.1 Geral

Caracterizar os aspectos clínicos, histopatológicos e moleculares dos pacientes portadores de craniofaringiomas.

3.2 Específicos

- Identificar aspectos clínicos dos pacientes com craniofaringioma;
- Analisar a expressão de β-catenina e VDAC em craniofaringiomas e sua relação com tipos histológicos e marcador de agressividade Ki-67;
- Correlacionar achados de imunohistoquímica com características clínicas e histopatológicas de craniofaringiomas;
- Inferir vias fisiopatológicas envolvidas na gênese de craniofaringiomas e potenciais preditores de agressividade e alvos terapêuticos.

4 RESULTADOS

4.1 Artigo de Revisão submetido na Revista Frontiers in Endocrinology (Qualis A3 / Fator de Impacto 3,9)

Recent Advances in Craniopharyngioma Pathophysiology and Emerging Therapeutic Approaches

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Abstract:

Craniopharyngiomas are rare intracranial tumors originating from the Rathke's pouch, affecting the sellar and parasellar regions. Despite their benign nature, they cause significant morbidity and mortality due to their proximity to vital structures such as the optic pathways and the hypothalamic-pituitary axis, resulting in endocrine, visual, neurological impairment, and hypothalamic syndrome. Classified into adamantinomatous (ACP) and papillary (PCP), these tumors differ in epidemiology, histology, and pathophysiology. ACP, the most common type, presents a bimodal peak incidence between 5 and 15 years of age and 45 and 60 years of age, while PCP is more restricted to adults. Traditional treatments such as surgery and radiotherapy face significant challenges, including high recurrence rates. Intracystic chemotherapy is used in monocystic ACP but with limited efficacy and adverse effects related to toxicity. Recent advances in molecular biology have introduced targeted therapies, such as BRAF and MEK inhibitors, which show potential benefits in craniopharyngioma

patients, particularly in the PCP. For ACP, however, therapeutic outcomes remain limited despite advances in molecular understanding, including mutations in the *CTNNB1* gene and growth factors. Increasing investigation into the inflammatory microenvironment and immune response of these tumors presents new therapeutic possibilities and promising alternatives for tumor control, such as the use of anti-IL-6R, anti-VEGF agents and immune checkpoints inhibitors. This review aims to synthesize advancements in the pathophysiology of craniopharyngiomas and explore emerging therapeutic implications, focusing on precision medicine approaches for the management of this challenging disease.

Keywords: Craniopharyngioma; Adamantinomatous craniopharyngioma; Papillary craniopharyngioma; Target therapies; Precision medicine.

1 Introduction

Craniopharyngiomas are rare intracranial epithelial tumors that develop from remnants of Rathke's pouch, predominantly located in the sellar and parasellar regions (1,2). Two main histological types are recognized: adamantinomatous craniopharyngioma (ACP) and papillary craniopharyngioma (PCP) (1). These tumors account for 1–3% of all primary intracranial tumors in adults and 5–10% of intracranial tumors in children, with an annual incidence ranging from 0.13 to 2 per 100,000 individuals and no gender predilection (2).

ACP is more common in children and young adults, displaying a bimodal age distribution (5–15 years and 45–60 years) and is frequently associated with somatic mutations in the *CTNNB1* gene encoding β-catenin. In contrast, PCP predominantly occurs in adults, especially in the fifth and sixth decades of life, and is associated with BRAF V600E mutations (1,3).

Although histologically classified as WHO low grade I benign tumors, craniopharyngiomas exhibit complex clinical behavior and significant morbidity and mortality (4). Treatment typically involves a multidisciplinary approach combining surgery and radiotherapy to control the tumor while preserving hypothalamic and pituitary functions. Total resection is preferred when feasible, but subtotal resection followed by radiotherapy is often used due to proximity to critical neurovascular

structures, especially in cases of hypothalamic invasion (3,5). While total resection can provide local control, it is associated with a high risk of morbidity, including hypothalamic and pituitary dysfunction (6). Subtotal resection with radiotherapy offers comparable control rates with fewer complications (7,8). Various radiotherapy modalities are effective but may cause side effects like visual deterioration and endocrinopathies, depending on tumor characteristics and patient factors (9,10). The limited efficacy and considerable side effects of traditional therapies underscore the need for innovative strategies to improve therapeutic outcomes and mitigate adverse effects (11).

Recent advances in molecular biology have provided new insights into the genetic basis of craniopharyngiomas. Notably, the discovery of the BRAF V600E mutation in PCP has enabled the development of targeted therapeutic interventions. The use of BRAF inhibitors, such as vemurafenib and dabrafenib, either alone or in combination with MEK inhibitors, has shown efficacy in specific cases. This represents a paradigm shift in treatment, as these therapies offer more precise and less invasive alternatives compared to traditional approaches (12,13).

Targeted therapies offer multiple advantages in the management of craniopharyngiomas. Unlike conventional methods, which often harm surrounding healthy tissues, these therapies specifically inhibit molecular pathways driving tumor growth. Consequently, they promote significant reductions in tumor size and improved clinical outcomes while minimizing complications and preserving essential neurological and endocrine functions. This highlights their potential for achieving better long-term results (12). Understanding the genetic alterations underlying craniopharyngiomas enables a personalized approach, tailoring treatment to the molecular profile of each patient. Furthermore, positive responses in cases refractory to conventional therapies reinforce the potential of these interventions as rescue options, offering new hope to patients with limited therapeutic alternatives (12,14,15).

This review aims to provide a comprehensive analysis of the current understanding of craniopharyngioma pathophysiology, with a particular emphasis on targeted therapies. By compiling and synthesizing available evidence, it seeks to highlight targeted therapies evaluated in craniopharyngioma patients with a

pathophysiological basis, identify knowledge gaps, address emerging challenges, and delineate areas requiring further research to guide future directions.

2 Papillary Craniopharyngioma (PCP)

PCPs account for approximately 10% of craniopharyngiomas and almost exclusively occur in adults, predominantly in the fifth and sixth decades of life, with a mean age of 44.7 years (16,17). These tumors typically present as large masses in the suprasellar region, often located above a preserved infundibulum or in the infundibulo-tuberal region of the third ventricle`s floor. Clinically, patients may present with visual deficits, hormonal alterations, memory impairment, and symptoms related to intracranial hypertension (17).

Macroscopically, PCPs consist of solid or mixed round masses, containing yellowish viscous cysts and rare calcifications. Histologically, these tumors are composed of well-defined neoplastic epithelium with cauliflower-shaped papillary projections, without infiltration into adjacent brain tissue. The histological component of PCPs includes pseudopapillae of mature squamous epithelium and an anastomosing fibrovascular stroma with fine capillaries and scattered immune cells, such as macrophages and neutrophils (16,18). (Figure 1)

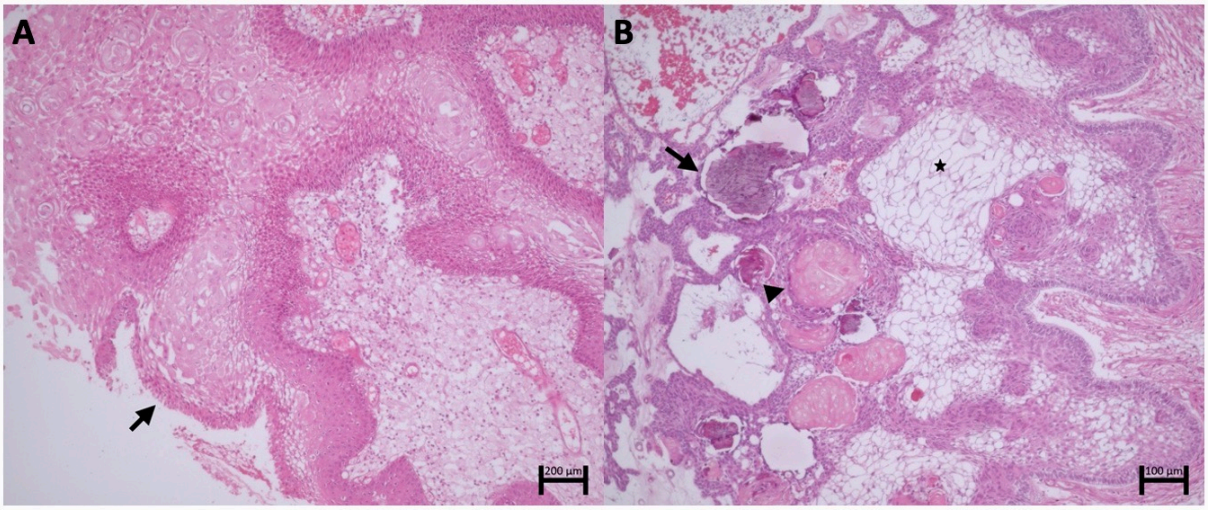

Figure 1. **A.** Papillary Craniopharyngioma. Fibrovascular connective axes forming papillae lined by well-differentiated squamous epithelium with palisading (arrow). **B.** Adamantinomatous Craniopharyngioma. Islands and cords of epithelial cells with palisading delineating the stroma composed of stellate reticulum cells (asterisk). Clusters of anucleated squamous cells (arrowhead) and foci of calcification (arrow).

Unlike ACPs, PCPs do not present palisading reticular cells, wet keratin, or collagenous whorls, which are rare and small when present (15). Distinguishing PCPs from other suprasellar masses, such as Rathke's pouch cysts, can be challenging (1).

In recent years, the genetic characterization of PCPs has advanced significantly. In 2014, the BRAF V600E mutation was identified through exome sequencing in three PCP samples, later confirmed in 36 of 39 additional samples (19,20). The BRAF V600E mutation constitutively activates the MAPK/ERK pathway, an oncogenic signaling pathway that promotes the proliferation of SOX2+ embryonic cells, transforming them into tumor-initiating cells and stimulating processes such as angiogenesis and apoptosis inhibition (21,22). (Figure 2)

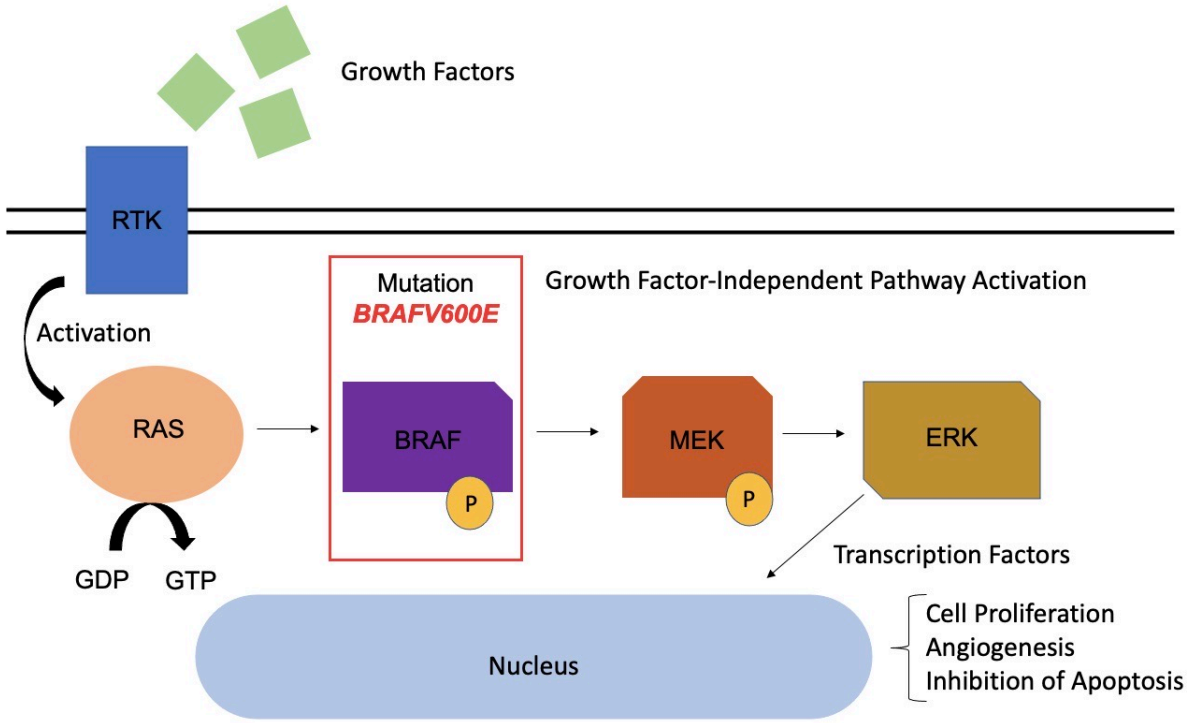

Figure 2. MAPK pathway with presence of BRAF mutation in the pathophysiology of papillary craniopharyngioma. RTK: Receptor tyrosine kinase; RAS: Rat Sarcoma; GDP: Guanosine Diphosphate; GTP: Guanosine Triphosphate; BRAF: B-Rapidly Accelerated Fibrosarcoma; MEK: Mitogen-Activated Protein Kinase Kinase; ERK: Extracellular Signal-Regulated Kinase.

BRAF V600E mutation is identified in 81% to 100% of PCPs, serving as a genetic marker for this type (20,22). Immunoreactivity for the VE1 antibody using immunohistochemistry confirms the presence of this mutation, while β-catenin remains

localized to the cell membranes (19). This mutation is also observed in melanomas, and BRAF and MEK inhibitors such as vemurafenib, dabrafenib, and trametinib have revolutionized the treatment of these neoplasms, with promising results also for PCPs (23). (Figure 3)

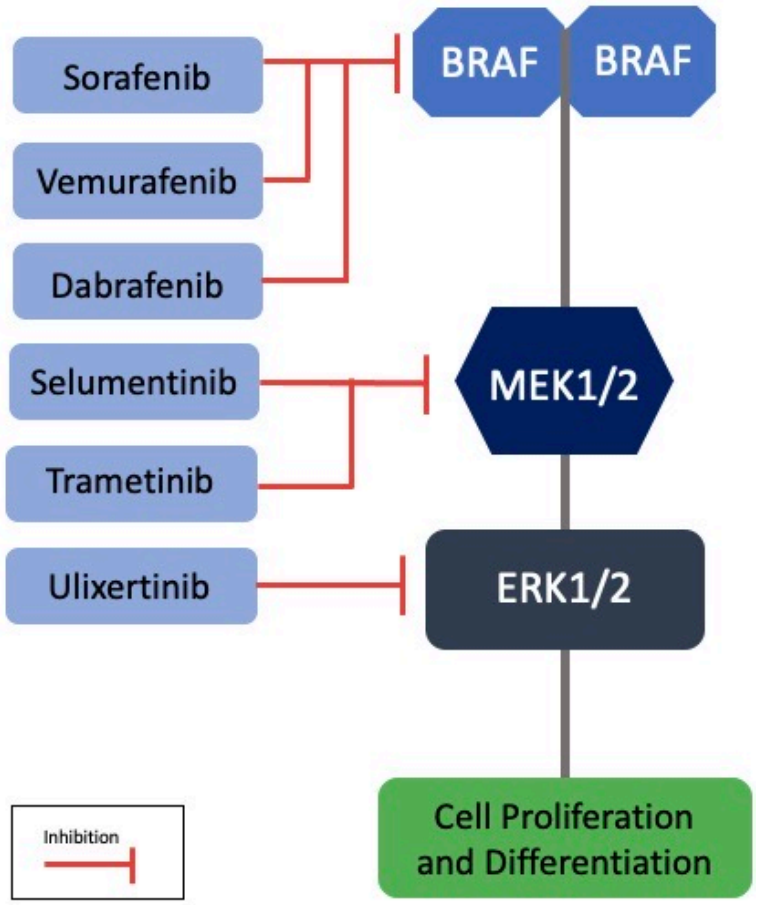

Figure 3. Targeted therapies in the MAPK pathway with potential application in papillary craniopharyngiomas. BRAF: B-Rapidly Accelerated Fibrosarcoma; MEK: Mitogen-Activated Protein Kinase; ERK: Extracellular Signal-Regulated Kinase.

Recent studies report significant responses to treatment with BRAF and MEK inhibitors in cases of PCPs with the BRAF V600E mutation. In one of the first reported cases, there was an 85% reduction in the solid portions and 81% reduction in the cystic portions of the tumor following treatment with MEK/BRAF inhibitors (24). In a series with six patients, treatment with dabrafenib, trametinib, or vemurafenib, either alone or in combination, resulted in a reduction of 80% to 91% of the tumor masses, allowing for subsequent surgical or radiotherapeutic interventions (23).

The efficacy of these therapies with BRAF and MEK inhibitors has been extensively studied. In a phase II study, 94% of patients treated with the combination of vemurafenib and cobimetinib achieved a durable partial response, with a median tumor volume reduction of 91%. Disease progression-free survival was 87% at 12 months and 58% at 24 months, indicating significant tumor control (25). Dabrafenib, in combination with trametinib, has shown promise as a treatment option for papillary craniopharyngiomas with the BRAFV600E mutation. Studies and case reports suggest that this combination may lead to significant reductions in tumor volume and improvement in clinical symptoms, such as visual deficits and neurological dysfunctions (24,26,27). In a cohort study, the combination of dabrafenib and trametinib resulted in a partial response or better in 94% of patients, with an average tumor volume reduction of up to 91.8% in some treatment groups (26). A systematic review highlighted that treatment response may range from 24% to 100% volumetric reduction, with near-complete response observed in many cases (28). (Table 1)

Table 1: Targeted Therapies for Papillary Craniopharyngioma (PCP)

Agent	n	Duration	Mechanism	Efficacy	Adverse effects
Vemurafenib + Cobimetinib Brastianos et al., 2023 (BRASTIANOS et al., 2023)	16	8 cycles (28 days each)	BRAF and MEK Inhibition	Partial response in 94%, average tumor volume reduction of 91%, progression-free survival of 87% at 12 months and 58% at 24 months.	Rash, elevated creatine kinase, hyperglycemia
Dabrafenib + Trametinib Alcubierre et al., 2024 (DE ALCUBIERRE et al., 2024)	16	7.6 ± 5.3 months	BRAF and MEK Inhibition	Tumor volume reduction of 73.3% to 91.8%. Improvement in neurological symptoms.	Low-grade fever, fatigue, cough, peripheral edema, skin rash, anemia, elevated liver enzymes, verrucous keratoses, hyperglycemia

These findings strengthen the potential of targeted therapies in the management of PCPs, especially in patients refractory to traditional treatments. The accurate identification of the genetic mutations involved not only facilitates differential diagnosis but also opens new perspectives for personalized and more effective therapeutic approaches (19).

3 Adamantinomatous Craniopharyngioma (ACP)

ACPs represent 90% of craniopharyngiomas and can affect individuals of any age, showing a bimodal distribution with peaks between 5–15 and 45–60 years (29). The mean age at diagnosis in children under 15 years is 8.8 years (1,16). Studies have shown no significant differences between pediatric and adult ACPs (22,30).

These tumors are commonly cystic, with calcifications and cholesterol-rich contents resembling “motor oil” (31). Their irregular margins, with palisading basal layers infiltrating into surrounding tissues in finger-like projections, are surrounded by intense gliosis, complicating surgical identification of planes (32). The heterogeneous tumor epithelium is juxtaposed with stellate reticulum and wet keratin nodules, composed of ghost and squamous cells, often associated with calcifications, cholesterol crystals, and hemosiderin deposits from chronic hemorrhage (1). (Figure 1)

In their pathophysiology, the Sonic Hedgehog (SHH) pathway is highly active, particularly in cell clusters and palisaded basal layers marked by Ki-67 positivity (33,34). The SHH pathway, linked to pituitary embryogenesis, appears to sustain tumor stem cells and promote tumor growth, infiltration, and angiogenesis via autocrine and paracrine mechanisms (35–37).

The pathogenesis of ACPs involves somatic mutations in the *CTNNB1* gene, which encodes β-catenin (38). Normally, the Wnt pathway regulates crucial processes like growth and metabolism, with β-catenin localized to cell membranes (39). β-catenin also participates in adhesion complexes with E-cadherin, maintaining cellular architecture (40). Activating mutations in *CTNNB1* are reported with prevalence rates ranging from 16% to 100%, depending on sequencing techniques (1,15,41).

Mutations in *CTNNB1* affect exon 3, encoding β-catenin's degradation complex, leading to aberrant nucleocytoplasmic accumulation in 96% of ACPs (36). This

accumulation hyperactivates the Wnt pathway, critical for cell proliferation and pituitary embryogenesis, as evidenced by targets like AXIN2, LEF1, and BMP4 (34,42). In ACPs, β-catenin accumulates in small whorled clusters or near infiltrative edges, acting as signaling centers for cell proliferation and differentiation (42,43). (Figure 4).

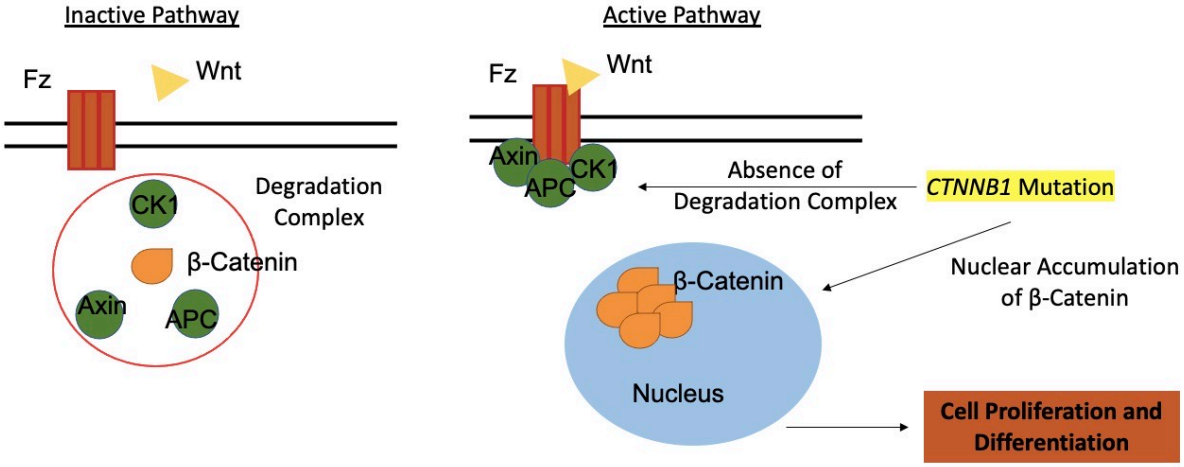

Figure 4. The Wnt Pathway and Its Role in Tumorigenesis of Adamantinomatous Craniopharyngiomas. Wnt: Wingless-Integration site; Fz: Frizzled receptor; CK1: Casein Kinase 1; APC: Antigen-presenting Cell; AXIN: Axin protein.

CTNNB1 mutations are specific to ACPs and absent in other sellar region tumors, such as PCPs (15,43,44). However, simultaneous mutations in *CTNNB1* and BRAF have been identified in tumors with mixed adamantinomatous and papillary features (45). Wnt pathway hyperactivation correlates with aggressive disease and poorer overall survival rates (46).

Further studies identified fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), and transforming growth factor-β (TGF-β) as mediators of tumor growth in ACPs (36,42). Activation of the epidermal growth factor receptor (EGFR) stabilizes β-catenin in tumor cells coexpressing fascin, an actin-binding protein linked to adhesion, migration, invasion, and cytoskeletal reorganization. Fascin expression correlates with invasive growth and poor prognosis in ACP patients (47).

Moreover, reports suggest MAPK/ERK pathway activation in ACPs, with FGF, EGF and ERK1/2 expression colocalized with Ki-67 in palisaded epithelium. Ex vivo experiments on ACPs treated with the MEK inhibitor trametinib demonstrated

increased apoptosis and reduced cell proliferation, highlighting the relevance of this pathway (48).

Markers like cytokeratins (CK), particularly CK8 and CK18, are elevated in β-catenin-positive cells, indicating dedifferentiation and tumor progression. Claudin-1 (CLDN1), a tight junction component, is downregulated in β-catenin-positive clusters and finger-like projections, suggesting a role in invasiveness. Claudins also influence cyst formation through fluid accumulation from endothelial leakage, impairing cellular adhesion (49). In reactive tissues, cholesterol crystals trigger interleukin-1β (IL-1β) secretion, activating immune cells and causing inflammation (50).

Treating ACPs remains challenging, with no clear consensus on the optimal therapeutic approach. Since β-catenin is critical for normal processes, nonspecific inhibitors could harm healthy tissues (51). Therapeutic approaches vary widely, reflecting the complexity of managing ACPs. Interferon-α (INF-α) shows mixed outcomes, ranging from complete response to disease progression (52,53). Pegylated Interferon-α-2b (INF-peg-α-2b) appears more effective, with studies showing complete responses or significant cystic reduction (54,55). Other inflammatory mediators like tocilizumab (anti-IL-6R) and bevacizumab (anti-VEGF) are promising, with reports of significant tumor volume reduction after recurrences (56–58). Understanding the role of the MEK/ERK pathway in ACPs may also enable therapeutic targeting. MEK inhibitor binimetinib reduced tumor volume by over 95% after 21 months of treatment (59). (Table 2)

These findings underscore the need for individualized treatments for ACPs due to disease heterogeneity and variable responses. Further studies are essential to determine the best therapeutic combinations and sequences (61).

Table 2: Targeted Therapies for Adamantinomatous Craniopharyngiomas (ACP)

Agent	n	Duration	Mechanism	Efficacy	Adverse effects
Interferon-α Kilday et al., 2017 (KILDAY et al., 2017)	56	5,1 years	Immunomodulation	Varied response (ranging from complete response to disease progression)	Fever, chills, myalgias, hypotension, lethargy, nausea, vomiting, elevated transaminases, thrombocytopenia, seizures, hyperpigmentation, weight loss
Pegylated Interferon-α-2b Goldman et al., 2020 (GOLDMAN et al., 2020)	19	108 weeks	Immunomodulation	Partial response in 28%. Median disease-free survival of 19.5 months.	Nausea, fever, constitutional symptoms, elevated transaminases, thrombocytopenia, fatigue, neutropenia
Tocilizumab Grob et al., 2019 (GROB et al., 2019)	2	6 months	Anti-IL-6R	Reduction of cystic volume and disease stability	Neutropenia
Bevacizumab De Rosa et al., 2023 (DE ROSA et al., 2023)	1	6 weeks	Anti-VEGF	Partial response (66% tumor volume reduction after 3 months)	Not reported
Binimetinib Patel et al., 2021 (PATEL et al., 2021)	1	8 months	MEK Inhibition	Disease stability	Skin rash, nail dystrophy, hyponatremia, venous stasis, fatigue, daytime sleepiness, weight gain.

4 Inflammatory Mediators and Immune Response

Inflammation plays a significant role in the pathophysiology of craniopharyngiomas, influencing both the biological behavior of the tumor and the clinical prognosis of patients (11,48). Several inflammatory mediators, including interleukins (IL-6, IL-8, IL-10), tumor necrosis factor (TNF), and chemokines (CXCL12,

CXCR4), are implicated in tumor progression and the pathological inflammatory microenvironment of these tumors (62). The presence of a pronounced inflammatory response correlates with a higher incidence of hypopituitarism and a lower likelihood of recurrence-free survival (62). Moreover, β-catenin, frequently mutated in ACPs, not only participates in Wnt signaling but also interacts with inflammatory mediators, amplifying cytokine and chemokine production in the tumor microenvironment. This process establishes a vicious cycle wherein inflammation perpetuates cellular proliferation and fosters tumor recurrence (63).

4.1 Interleukins

Interleukins, such as IL-6 and IL-8, are often found in elevated levels within tumor tissues and are associated with the promotion of a pro-tumorigenic microenvironment (64). IL-6 is known for its ability to enhance tumor invasion and angiogenesis while mediating communication between tumor cells and immune cells, such as tumor-associated macrophages, which can increase tumor aggressiveness. This occurs via epithelial-mesenchymal transition (EMT), a process that boosts the migratory capacity of tumor cells, facilitating local invasion (65). Furthermore, elevated levels of IL-6 and its receptor (IL-6R) suggest that this signaling pathway could be a potential therapeutic target, as demonstrated in studies exploring the use of tocilizumab, a monoclonal antibody that blocks IL-6R, to reduce cystic volume in ACP patients (57).

IL-8 also plays a significant role in the tumor microenvironment of craniopharyngiomas, contributing to local invasion and adhesion to surrounding tissues (66). Studies indicate that IL-8, alongside other pro-inflammatory cytokines such as IL-6, is elevated in the plasma of patients and brain tumor tissues (62,67). IL-8 is further recognized for its pro-tumorigenic functions across various cancers, including promoting angiogenesis, increasing cellular proliferation and survival, and facilitating cell migration (66). Although specific literature on craniopharyngiomas is limited, IL-8's role in other tumors suggests that it may contribute to tumor progression through similar mechanisms by modulating the tumor microenvironment (62,68).

Vascular Endothelial Growth Factor (VEGF), a key mediator of angiogenesis, is an essential component in craniopharyngioma pathophysiology, driving the formation

of new blood vessels that supply the tumor with nutrients and oxygen (69). It is present in epithelial cells of both ACP and PCP, and microvascular density related to VEGF expression correlates with tumor recurrence, suggesting the prognostic value of angiogenesis extent. VEGF is also linked to cyst formation in craniopharyngiomas, with its expression appearing to correlate with tumor size (70). Regulated by β-catenin and other molecular pathways, VEGF interacts with matrix metalloproteinases (MMPs), such as MMP-9, to remodel the extracellular matrix and facilitate tumor invasion. This remodeling may be critical for craniopharyngioma progression. MMP-1, for instance, can enhance VEGF-2R expression on endothelial cells, promoting cellular proliferation and angiogenesis via intracellular signaling pathways (71). Evidence also suggests that MMPs may induce the production of the anti-apoptotic protein Bcl-2, which regulates tumor growth and recurrence through autocrine and paracrine mechanisms (72). Dysregulation of apoptosis-related genes, including members of the Bcl-2 family, seems to play a significant role in the pathogenesis of pituitary adenomas (73). Although the specific role of MMPs in craniopharyngiomas remains underexplored, their involvement in angiogenesis and VEGF modulation suggests they contribute to tumor pathogenesis.

The interplay between IL-6 and VEGF establishes a pathological axis that amplifies inflammation and vascularization in the tumor microenvironment. IL-6 can upregulate VEGF expression, creating a feedback loop that intensifies these processes (74). This interaction also contributes to resistance to conventional therapies, highlighting the need for integrated approaches to disrupt this cycle.

While studies on anti-IL-6 and anti-VEGF therapies in craniopharyngiomas remain limited, preliminary evidence suggests therapeutic potential. A study reported the combined use of tocilizumab (anti-IL-6R) and bevacizumab (anti-VEGF) in two patients with recurrent cystic ACPs, resulting in significant responses, including reduced cystic tumor burden and tumor stability, suggesting a viable therapeutic alternative (57).

Inflammatory mediators also play a significant role in PCPs, influencing both tumor invasiveness and the immune microenvironment. The use of preoperative inflammatory markers, such as neutrophil counts and the neutrophil-to-lymphocyte ratio, can aid in the differential diagnosis of PCPs from other sellar region tumors, with

higher levels observed in PCPs. These tumors exhibit high immune infiltration but with low activity, attributed to extensive neutrophil infiltration that creates an inactive immune microenvironment (75). Furthermore, elevated IL-6 expression is positively correlated with PCP tumor invasion into the hypothalamus, suggesting that these inflammatory mediators could serve as potential therapeutic targets to prevent tumor invasion (76).

4.2 Chemokines

Chemokines from the CXC and CC families play significant roles in craniopharyngioma pathophysiology, particularly in ACP. The CXCL12/CXCR4 axis is specifically implicated in tumor progression. Overexpression of CXCL12 and CXCR4 in ACP promotes tumor cell proliferation, migration, and invasion, primarily via the activation of the PI3K/AKT signaling pathway (77,78). This pathway is critical in regulating cell growth and survival, indicating that the CXCL12/CXCR4 axis contributes to tumor aggressiveness. These chemokines are also recognized for their capacity to influence the tumor microenvironment by modulating angiogenesis and immune responses, potentially affecting tumor progression (79). Although the impact of other CC and CXC chemokines on craniopharyngiomas is less studied, their roles in other cancers suggest similar contributions to tumor growth and invasion (80).

4.3 Immune Checkpoint

Immune checkpoints are essential molecules and pathways in the immune system that regulate immune responses, preventing them from becoming destructive to the body's healthy cells. These include inhibitory receptors and ligands that play a crucial role in modulating T-cell activation and function. Under normal conditions, these checkpoints help maintain immune tolerance and prevent collateral damage during antimicrobial immune responses (81). In the context of cancer, tumor cells can exploit these checkpoints to evade immune destruction. This occurs when proteins on the surface of T-cells, known as immune checkpoint proteins, recognize and bind to partner proteins on cancer cells, sending inhibitory signals that suppress the immune response (82). Therapies that block these checkpoints, known as immune checkpoint

inhibitors, have shown significant efficacy in restoring T-cell capability to attack tumor cells, leading to robust tumor regressions (83).

Immune checkpoint inhibitors have demonstrated significant potential in the treatment of various cancer types. Blocking programmed cell death protein 1 (PD-1) and its ligand (PD-L1) with agents such as nivolumab and pembrolizumab has improved survival in cases of melanoma and non-small cell lung adenocarcinoma (84,85). The availability of these agents, combined with their relatively favorable side effect profiles, has prompted numerous studies investigating their efficacy across various tumor types.

Recent studies have demonstrated PD-L1 expression in the epithelial cells lining cysts and intrinsic PD-1 expression in cell clusters in ACPs with β-catenin overexpression. These clusters play a central role in tumor growth in ACPs through various mechanisms, making PD-1 targeting a promising therapy (86,87). Two more studies also demonstrated elevated PD-L1 expression in ACPs, revealing this as a potential therapeutic target in craniopharyngiomas (88,89).

5 Ongoing Clinical Trials

In recent years, the multi-omic characterization of craniopharyngiomas has provided significant insights into their pathogenesis, driving clinical trials aimed at developing more personalized and lower-risk therapeutic approaches (Table 3). Ongoing studies with binimetinib (NCT05286788), tocilizumab (NCT05233397), and nivolumab (NCT05465174) for ACP as well as vemurafenib and cobimetinib (NCT03224767) for the PCP, offer promising therapeutic alternatives and may help define optimal patient profiles (90) (Figure 5). However, some challenges still remain regarding the optimal timing for introducing these treatments—whether as neoadjuvant therapy to reduce tumor volume before surgery or as adjuvant therapy to minimize recurrence and improve disease control—as well as the appropriate duration of treatment. Furthermore, uncertainties persist regarding their use as monotherapy, in combination with other agents, or in conjunction with surgery and radiotherapy, underscoring the need for further studies to refine the management of these rare tumors.

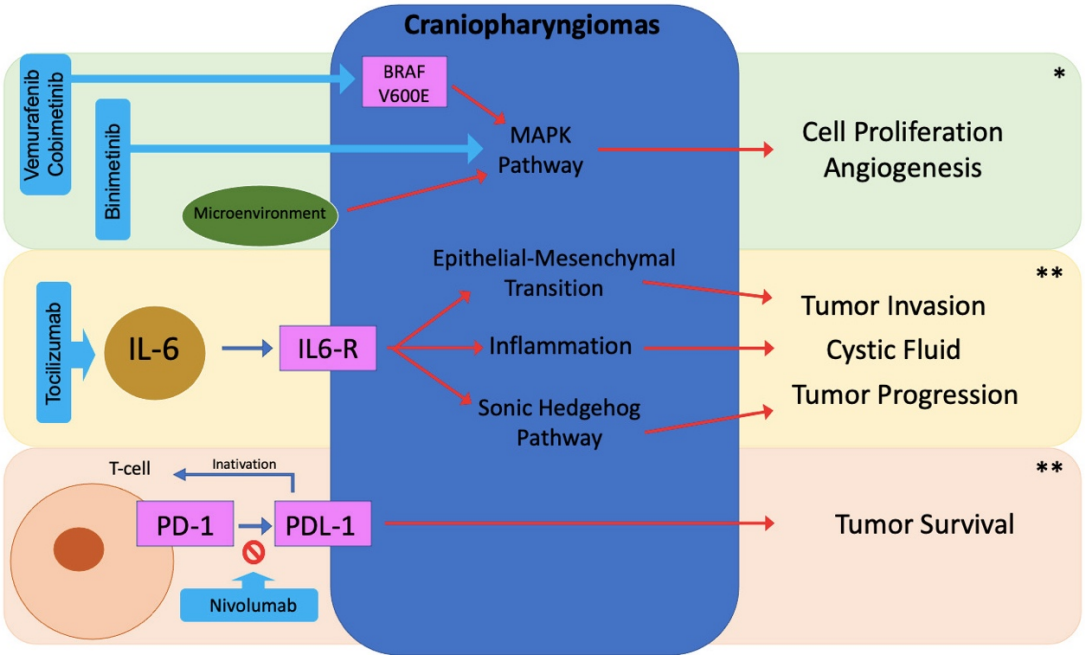

Figure 5. Potential Targeted Therapies in Craniopharyngiomas. *: ACP/PCP; **: ACP; BRAF: B-Rapidly Accelerated Fibrosarcoma; IL-6: Interleukin 6; PD-1: Programmed Cell Death Protein 1; PD-L1: Programmed Cell Death Protein Ligand 1.

Table 3. Targeted Therapies for Inflammatory Mediators and for Immune Response

Inflammatory and Immune Target	Mechanism	Therapeutic Potential
IL-6 (GROB et al., 2019)	Tumor invasion, angiogenesis, immune cell/tumor communication, and epithelial-to-mesenchymal transition (EMT).	Anti-IL-6R monoclonal antibodies (e.g., tocilizumab) reduce tumor invasion and cystic volume.
IL-8 (PENG et al., 2021)	Angiogenesis, cell proliferation, migration, and cell adhesion.	Blocking IL-8-related pathways to reduce local invasion and angiogenesis.
VEGF (DE ROSA et al., 2023)	Angiogenesis and cyst formation; interacts with MMPs for extracellular matrix remodeling.	VEGF inhibitors (e.g., bevacizumab) to reduce angiogenesis and tumor growth.
CXCL12/CXCR4 (GONG et al., 2014; YIN et al., 2019)	Tumor progression, migration, invasion, and angiogenesis through activation of CXCR4 signaling pathways.	Limit tumor progression and invasion.
PD-1/PD-L1 (BANDAY; ABDALLA, 2023)	Inhibits the immune response, allowing tumor evasion.	Immune checkpoint inhibitors (e.g., nivolumab, pembrolizumab) to reactivate the immune system against the tumor.

6 Conclusion

Recent advances in understanding the pathophysiology of craniopharyngiomas provide a promising foundation for developing more effective therapeutic strategies. Given the limitations of conventional approaches, targeted therapies, such as BRAF and MEK inhibitors for the PCP, have shown encouraging results. Recently, the growing understanding of the inflammatory behavior and immune response of these tumors has highlighted the therapeutic potential of anti-IL-6R and anti-VEGF agents and immune checkpoints inhibitors, signaling a promising future for the application of precision medicine in this field in both, APC and PCP.

Significant gaps still remain, particularly in managing more aggressive or resistant tumors. Robust clinical trials and interinstitutional collaborations are crucial to validate these therapies on a larger scale and standardize treatment protocols. Additionally, identifying predictive biomarkers and elucidating molecular interactions within the tumor microenvironment may offer new therapeutic opportunities, improve prognostic outcomes, and reduce the morbidity and mortality associated with craniopharyngiomas.

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4.2 Artigo Original submetido na Revista New England Journal of Medicine (Qualis A1 / Fator de Impacto 96,2)

Overexpression of VDAC2 in Adamantinomatous Craniopharyngiomas: Potential Role as a Biomarker of Tumor Aggressiveness

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Abstract

Background: Adamantinomatous craniopharyngiomas (ACPs) are rare intracranial tumors that exhibit clinically aggressive behavior despite their benign classification. The variability in tumor aggressiveness highlights the need for additional biomarkers. VDAC2, a protein involved in apoptosis regulation, has been implicated in the pathogenesis of various neoplasms and suggested as a key player in tumorigenesis; however, its expression and clinical significance in ACPs remain unknown. Objective: To evaluate the immunohistochemical expression of VDAC2 in ACP samples,

correlating it with β-catenin expression and the Ki-67 proliferation index, in order to identify potential biomarkers of tumor aggressiveness. Methods: This is a retrospective observational study including 12 ACP samples from patients who underwent surgical treatment. VDAC2 and β-catenin expression were quantified by immunohistochemistry using the H-score. The Ki-67 proliferative index was determined by calculating the percentage of positively stained tumor cell nuclei. Correlations between variables were analyzed using Spearman's rank correlation test. Results: VDAC2 was expressed in 83.3% of the cases. A significant positive correlation was observed between VDAC2 expression and Ki-67 (r = 0.62, p = 0.032), as well as between VDAC2 and β-catenin expression (r = 0.78, p = 0.003). Tumors with high VDAC2 expression showed significantly higher levels of Ki-67 (p = 0.0455) and β-catenin (p = 0.0411). Conclusion: VDAC2 overexpression in ACPs is associated with increased cellular proliferation and activation of the Wnt/β-catenin pathway, suggesting its potential as a biomarker of tumor aggressiveness. The identification of VDAC2 may represent an advance in prognostic stratification and support the development of more targeted therapeutic approaches.

Key-words: Adamantinomatous craniopharyngioma; VDAC2; Apoptosis; β-catenin; Ki-67.

Introduction

Craniopharyngiomas are rare epithelial neoplasms, histologically benign, that originate from embryonic remnants of Rathke's pouch. Despite being classified as World Health Organization (WHO) grade I tumors, they often exhibit clinically aggressive behavior, with high recurrence rates and significant morbidity (1–3). Their location in the sellar and suprasellar region, often involving critical neurovascular structures, contributes to the complexity of clinical and surgical management (4).

The global incidence of craniopharyngiomas is estimated at 0.13–2 cases per million people per year, with a bimodal distribution primarily affecting children between 5 and 15 years of age and adults between 50 and 70 years (3,5). These tumors account for 2–5% of all primary intracranial tumors and are responsible for up to 10% of pediatric brain tumors (6,7). The clinical relevance of craniopharyngiomas extends

beyond their rarity, as patients often experience long-term endocrine, visual, and neurological sequelae after treatment, which significantly impact their quality of life (8).

Two main histological types of craniopharyngiomas are recognized: adamantinomatous and papillary. The adamantinomatous type (ACP) is more common in children, whereas the papillary type (PCP) typically occurs in adults. ACPs are characterized by a distinct histological pattern, including stratified squamous epithelium, peripheral palisading cells, and “wet” keratin nodules. Notably, ACPs tend to exhibit more aggressive behavior compared to the PCP, with a higher propensity for local invasion and recurrence (9).

In recent decades, advances in the molecular characterization of ACPs have revealed that activation of the Wnt/β-catenin signaling pathway is a central event in the pathogenesis of these tumors (10,11). Studies have shown that over 90% of ACPs harbor somatic mutations in exon 3 of the *CTNNB1* gene, which encodes β-catenin. These mutations result in the stabilization and nuclear accumulation of the protein, promoting the transcription of genes associated with cell proliferation and inhibition of apoptosis (12,13). The dysregulation of this pathway not only contributes to tumor initiation but is also implicated in disease maintenance and progression (11).

Despite advances in the understanding of ACP pathophysiology, the clinical management of these tumors remains challenging, with recurrence rates reaching up to 50% in some series (14,15). The clinical variability observed among cases with *CTNNB1* mutations suggests the presence of additional molecular factors modulating the biological behavior of these tumors (13). In this context, the investigation of additional biomarkers that may reflect tumor aggressiveness, assist in prognostic stratification, or represent potential therapeutic targets is of great relevance.

Among the emerging candidates is Voltage-Dependent Anion Channel 2 (VDAC2), an isoform of the VDAC family located on the outer mitochondrial membrane. Traditionally associated with the transport of ions and metabolites between the cytosol and the mitochondrial intermembrane space, VDAC2 has garnered increasing attention for its role in the regulation of apoptosis, particularly through its interaction with Bcl-2 family proteins such as Bax and Bak (16–18). Studies in other tumor types suggest that alterations in VDAC2 expression may contribute to apoptotic evasion, one of the hallmarks of tumorigenesis (19,20).

The interface between mitochondrial metabolism and pro-proliferative signaling pathways, such as the Wnt/β-catenin pathway, represents a promising area for understanding mechanisms of tumor aggressiveness. The hypothesis that VDAC2 may be differentially expressed in ACPs and correlate with histological markers of aggressiveness, such as the proliferative index Ki-67, has not yet been explored. Furthermore, its potential association with aberrant β-catenin accumulation could offer insights into functional interactions relevant to tumor biology. In this context, the present study aimed to evaluate the immunohistochemical expression of VDAC2 in ACP samples, correlating it with β-catenin expression and the Ki-67 index.

Methods

Study Design and Samples

This is an observational, retrospective study conducted with 12 samples of ACPs from patients who underwent surgical treatment at the University Hospital of the Federal University of Maranhão (HUUFMA) between January 2016 and December 2024. Cases with a confirmed histopathological diagnosis of ACP and availability of formalin- fixed, paraffin-embedded (FFPE) tumor tissue blocks properly stored at the Pathology Service of HUUFMA were included. Inclusion criteria were histopathological diagnosis of ACP confirmed by an experienced pathologist, availability of FFPE blocks with adequate morphological integrity, and complete medical records containing clinical and demographic data. Exclusion criteria included samples unsuitable for immunohistochemical analysis due to autolysis or fixation artifacts, incomplete medical records, or uncertain histopathological diagnosis. Clinical and demographic data (age at diagnosis, sex, pituitary function assessment, tumor size, and adjuvant treatments) and histopathological findings were obtained through the review of medical records.

Histopathological Analysis

Hematoxylin and eosin (H&E)-stained slides were reviewed by two experienced pathologists to confirm the diagnosis of ACP and to select representative areas for immunohistochemical analysis. Histological sections of 4 μm were stained with H&E following a standard protocol, including deparaffinization in xylene, rehydration through graded ethanol series, staining with Harris hematoxylin for 5 minutes, differentiation in acid alcohol, eosin staining for 2 minutes, and mounting with a permanent mounting

medium. Classic morphological criteria of ACP were considered, including palisading basal cells, loosely cohesive squamous epithelium, presence of “wet” keratin nodules, and absence of features characteristic of the PCP.

Immunohistochemistry (VDAC2 and β-catenin)

Histological sections of 4 μm were subjected to immunohistochemical staining for the detection of VDAC2 and β-catenin proteins. After deparaffinization in xylene and rehydration through graded ethanol series, antigen retrieval was performed by heating in citrate buffer (pH 6.0) in a water bath for 30 minutes. The slides were then incubated with specific primary antibodies against VDAC2 (Abcam, ab126120, 1:50 dilution) and β- catenin (Dako, IR70261-2, 1:50 dilution) for 1 hour at room temperature. Following PBS washes, the slides were incubated with a polymer-based peroxidase detection system conjugated to a secondary antibody (EnVision+, Dako) for 30 minutes. Signal development was carried out using diaminobenzidine (DAB) for 5 minutes, followed by counterstaining with Mayer’s hematoxylin. Positive and negative controls were used to validate the specificity of the immunostaining.

VDAC2 and β-catenin expression was quantified using the H-score, a semi-quantitative method that considers both the staining intensity (0 = absent, 1 = weak, 2 = moderate, 3 = strong) and the percentage of positively stained cells in each category, generating a final score ranging from 0 to 300 (Figure 1). Evaluation was performed by two independent observers, blinded to the patients’ clinical data, using a light microscope at 400× magnification and 300 dpi resolution. Interobserver agreement was assessed using the intraclass correlation coefficient (ICC), with values > 0.75 considered indicative of good concordance.

Ki-67 Proliferative Index

The nuclear expression of Ki-67 was assessed by immunohistochemistry, following a protocol similar to the one described above, using the MIB-1 monoclonal antibody clone (Dako). The proliferative index was determined by calculating the percentage of positively stained tumor nuclei in representative areas of the sample (hot spots), defined as regions with the highest density of labeled cells. Counting was performed on at least 1,000 tumor cells per case using a light microscope at 400× magnification.

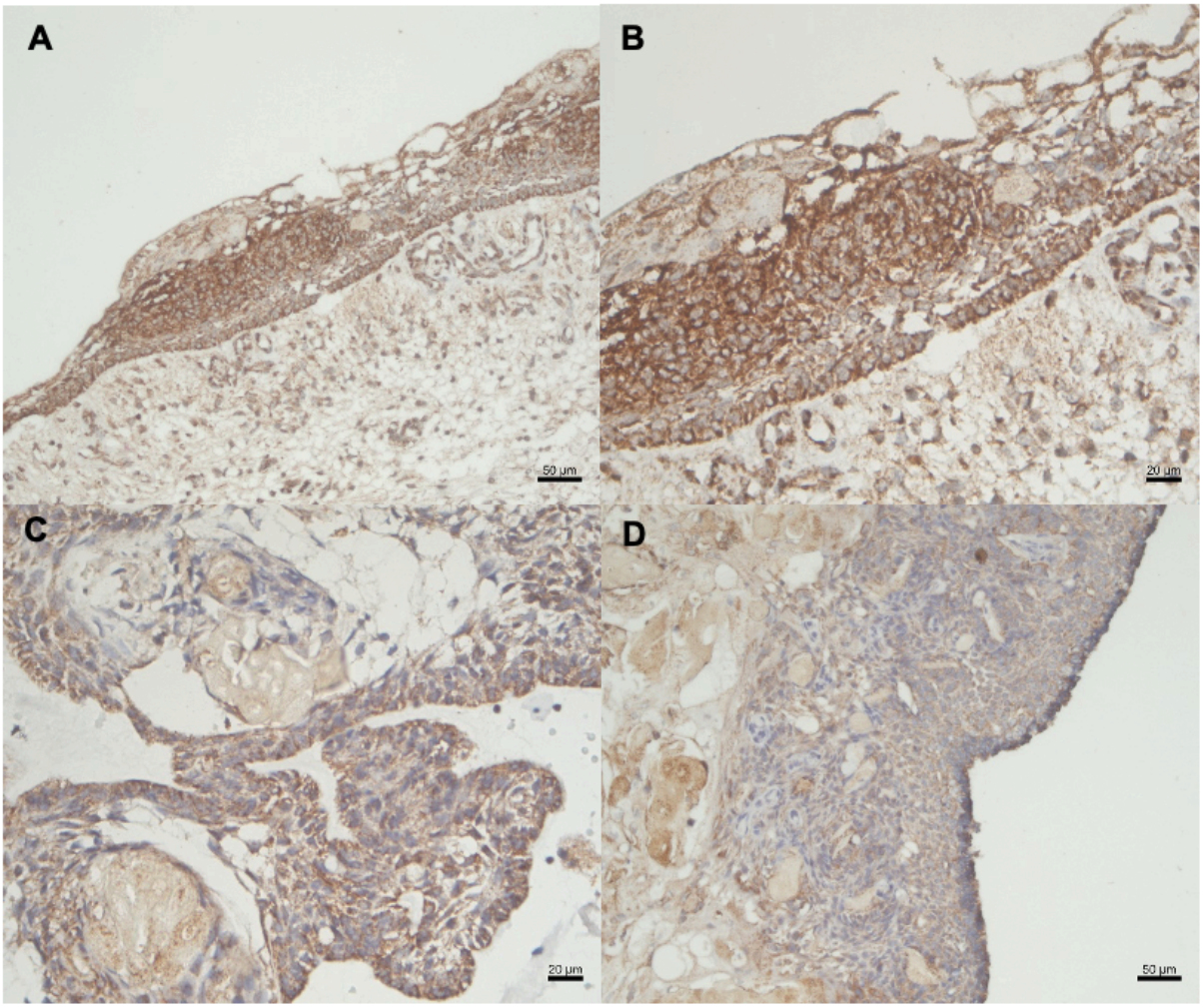

Figure 1. Immunohistochemistry for VDAC2. A and B: Neoplastic cells with squamoid appearance, showing cytoplasmic positivity throughout the full thickness of the epithelial lining, with moderate to strong intensity (++/+++) using anti-VDAC2 antibody. C: Neoplastic cells with squamoid appearance, showing cytoplasmic positivity throughout the full thickness of the epithelial lining (++/+++), and faint positivity (+) in ghost cells. D: Diffuse cytoplasmic positivity in neoplastic cells with weak intensity (+) using anti-VDAC2 antibody, with stronger staining observed in ghost cells.

Statistical Analysis

Data were analyzed using STATA software, version 16.0 (Stata Corp., College Station, United States). Continuous variables were expressed as median and interquartile range due to the non-parametric distribution of the data, as assessed by the Shapiro–Wilk test. Categorical variables were presented as frequencies and

percentages. The correlation between VDAC2 and β-catenin H-scores and the Ki-67 index was assessed using Spearman's rank correlation test. The Mann–Whitney U test was used to compare medians between groups for continuous variables. Fisher's exact test was applied to compare proportions between groups for categorical variables. A p- value < 0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the Research Ethics Committee of the Federal University of Maranhão (CAAE: 63982722.2.0000.5086). All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the national regulations for research involving human subjects.

Results

Sample Characteristics

The clinical and demographic characteristics of the sample are summarized in Table 1. The median age at diagnosis was 9.5 years (range: 2–54), with 7 female and 5 male patients. Four patients (33.3%) presented with preoperative hypopituitarism. The mean preoperative maximum tumor diameter was 3.8 ± 0.8 cm. Calcifications were present in 10 tumors (83.3%), and all tumors exhibited a cystic component (100%). The mean tumor reduction six months after surgery was 43.2% ± 22.7%. Notably, all patients developed postoperative hypopituitarism and experienced tumor recurrence. Seven patients (58.3%) received adjuvant therapy.

Table 1. Clinical and demographic characteristics of the sample (n = 12)

Characteristic	**Value**
Age at diagnosis in years (median)(range)	9,5 (2-54)
Sex (Women/Men)	7/5
Preoperative Hypopituitarism	4 (33,3%)
Preoperative maximum tumor diameter (cm)	3,8 ± 0,8
Calcification	10 (83,3%)
Cystic component	12 (100%)
Tumor reduction (6 months after surgery)	43,2% ± 22,7%
Post-operative Hypopituitarism	12 (100%)
Adjuvant therapy	7 (58,3%)

VDAC Expression and Correlations

VDAC positivity was observed in 10 out of 12 cases (83.3%), with a median H-score of 70 (range: 0–250). Spearman's correlation analysis revealed a significant positive correlation between VDAC expression and Ki-67 (r = 0.62, *p* = 0.032), as well as between VDAC and β-catenin (r = 0.78, *p* = 0.003), as shown in Table 2. No significant correlation was found between Ki-67 and β-catenin (r = 0.56, p = 0.058).

Table 2. Correlations between VDAC2, Ki-67, and β-catenin

Variables	**Correlation Coeficient**	***p*-value**
VDAC2 vs Ki-67	0,62	0,032*
VDAC2 vs β-catenin	0,78	0,003*
Ki-67 vs β-catenin	0,56	0,058

*Statistically significant (*p* < 0,05)

Comparison between VDAC2 groups

The sample was divided into two groups based on the VDAC2 H-score: VDAC2 < 70 (n = 6) and VDAC2 ≥ 70 (n = 6). Comparison between the groups revealed that tumors with higher VDAC expression (H-score ≥ 70) showed significantly higher levels of Ki-67 (median 1.5% vs. <1%, *p* = 0.0455) and β-catenin (median H-score 80 vs. 25, *p* = 0.0411) compared to tumors with low VDAC2 expression (Table 3). No significant differences were observed between the VDAC2 groups regarding age at diagnosis (*p* = 0.8728), sex (*p* = 1.000), or tumor size (*p* = 0.3785). However, a trend toward lower tumor reduction was noted in the high VDAC2 expression group (33.0% vs. 53.4%, *p* = 0.0649).

Table 3. Comparison of variables between VDAC expression groups

Variables	**VDAC2 < 70 (N = 6)**	**VDAC2 ≥ 70 (N = 6)**	***p*-value**
Age at diagnosis (years)	10 (3-54)	9 (2-19)	0,8728
Sex (Women/Men)	4/2	3/3	1,000
Preoperative maximum tumor diameter (cm)	3,6 ± 0,9	4,0 ± 0,7	0,3785
Tumor reduction (6 months after surgery) (%)	53,4 ± 20,8	33,0 ± 20,6	0,0649
Ki-67 (%)	<1 (<1-1)	1,5 (<1-5)	0,0455*
β-catenin (H-score)	25 (5-50)	80 (10-110)	0,0411*

*Statistically significant (*p* < 0,05)

Discussion

The present study investigated the expression of the VDAC2 protein in ACPs and its relationship with the proliferative index Ki-67 and β-catenin expression, aiming to identify potential biomarkers of tumor aggressiveness. Our results demonstrated that VDAC2 is frequently expressed in ACPs and shows a significant positive correlation with both Ki-67 and β-catenin, suggesting a relevant role in the biology of these tumors.

VDAC2 expression was observed in 83.3% of the analyzed cases, with a median H-score of 70. This finding indicates that VDAC2 is a protein frequently expressed in ACPs, which may reflect its importance in mitochondrial function and the energy metabolism of these tumor cells. VDAC2 is a mitochondrial outer membrane protein that regulates the transport of ions and metabolites across the membrane, playing a crucial role in cellular respiration, ATP production, and mitochondrial homeostasis (16,21,22). In various types of cancer, VDAC2 overexpression has been associated with tumor progression, resistance to apoptosis, and worse prognosis (19,23–26).

Ki-67 is widely used as a marker of cell proliferation in cancers, as its expression is strongly associated with tumor growth and cell cycle progression. High levels of Ki-67 generally indicate greater tumor aggressiveness and a poorer prognosis (27). In craniopharyngiomas, tumors with higher Ki-67 expression may have an increased risk of recurrence after treatment, although the exact relationship still needs to be better defined (28). The significant positive correlation between VDAC2 and Ki-67 ($r = 0.62$, $p = 0.032$) suggests that VDAC2 expression is associated with cellular proliferation in ACPs. We believe that, in ACPs, VDAC2 overexpression may provide tumor cells with a metabolic advantage by increasing energy production and facilitating cell proliferation. Additionally, VDAC2 may be involved in the regulation of apoptosis, protecting tumor cells from programmed cell death and promoting tumor growth (29).

ACPs are frequently characterized by mutations in the *CTNNB1* gene, which encodes β-catenin, leading to aberrant activation of the Wnt/β-catenin signaling pathway. (30). This pathway is crucial for the development and progression of these tumors, with nuclear accumulation of β-catenin being considered an important diagnostic marker for ACPs (31). High β-catenin expression, especially in younger

patients (under 15 years old), is associated with a more aggressive tumor behavior, including larger and more invasive tumors, and a significantly higher risk of recurrence (32). The significant positive correlation between VDAC2 and β-catenin (r = 0.78, *p* = 0.003) suggests that VDAC2 expression is associated with activation of the Wnt/β-catenin pathway in ACPs. One hypothesis for this relationship is that VDAC2, by modulating mitochondrial membrane permeability, directly interacts with members of the Bcl-2 family—particularly Bak, Bax, and Bcl-2—which are key regulators of mitochondrial apoptosis. Bak and Bax are pro-apoptotic proteins that promote the release of cytochrome c through oligomerization and permeabilization of the mitochondrial membrane. (17,33). On the other hand, Bcl-2 is anti-apoptotic and inhibits mitochondrial membrane permeabilization. VDAC2 may inhibit Bak oligomerization, contributing to a cellular phenotype that is more resistant to apoptosis. (34). Thus, VDAC2 overexpression could promote tumor cell survival even in the presence of pro-apoptotic stimuli. As a result, cells harboring mutations or aberrant activation of the Wnt/β-catenin pathway may evade programmed cell death, accumulate, and contribute to tumor growth (Figure 2).

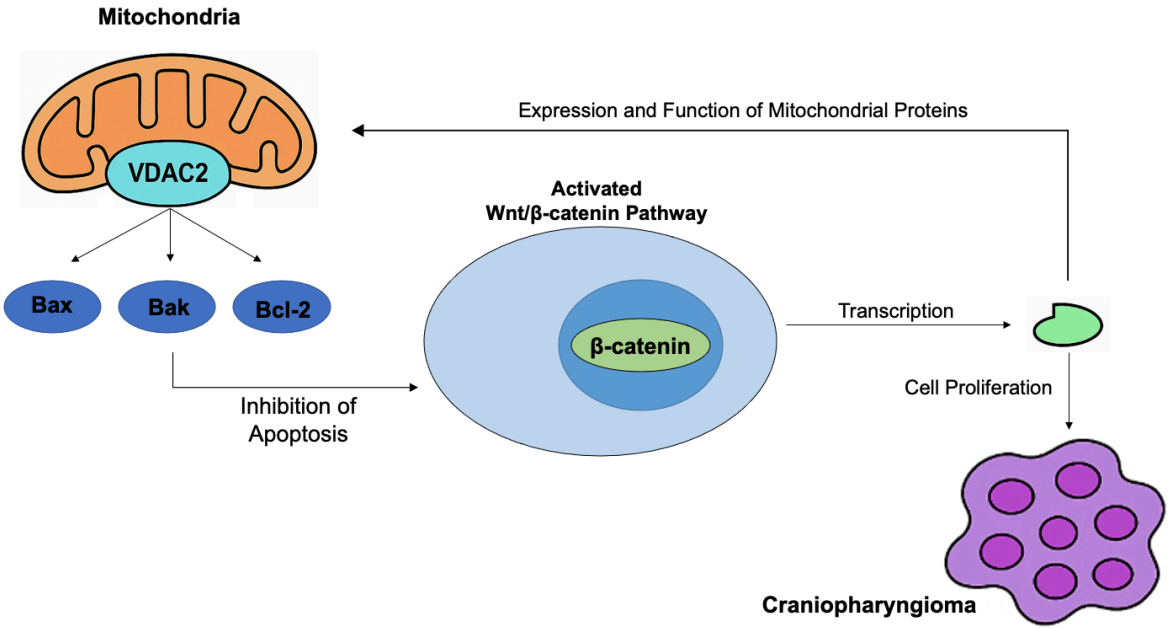

Figure 2. Feedback loop between β-catenin and mitochondrial proteins in the inhibition of apoptosis and tumorigenesis of craniopharyngioma.

On the other hand, growing evidence suggests that the Wnt/β-catenin pathway may also indirectly regulate the expression and function of mitochondrial proteins, such as VDAC2. This interaction could establish a feedback loop that promotes cell proliferation and resistance to apoptosis, thereby contributing to a more aggressive and therapy-resistant tumor phenotype. β-catenin plays critical roles in both cell adhesion and the transcriptional regulation of nuclear genes involved in proliferation, differentiation, and metabolism. Recent studies suggest that activation of the Wnt/β-catenin pathway can modulate mitochondrial activity and cellular metabolism in a context-dependent manner (35,36). In melanoma cells, for instance, Wnt/β-catenin signaling has been associated with the regulation of mitochondrial function, with implications for oncogenic potential (37). In breast cancer models, β-catenin suppression led to a reduction in mitochondrial mass, indicating its role in mitochondrial biogenesis. (38). Moreover, β-catenin can translocate to the mitochondria in intestinal epithelial and colon cancer cells, where it associates with survival proteins such as Bcl-2, suggesting a direct role in the regulation of mitochondrial apoptosis. (39). These findings reinforce the hypothesis of a functional interconnection between the Wnt/β-catenin pathway and mitochondrial physiology, with important implications for tumor cell survival and aggressiveness.

The association with Ki-67 supports the hypothesis that the overexpression of VDAC2 and β-catenin is linked to a higher proliferative potential in ACPs. Since the proliferative index is one of the main predictors of tumor recurrence, these findings suggest that VDAC2 and β-catenin may be explored as potential biomarkers of aggressiveness and prognosis in ACPs.

It is important to note that all patients in our study developed postoperative hypopituitarism and experienced tumor recurrence. Hypopituitarism is a common complication of ACP surgery due to the tumor's proximity to the pituitary gland and the risk of injury during surgical resection (40). Tumor recurrence is also a major challenge in the management of ACPs, with recurrence rates ranging from 20% to 50% within 10 years (14). We believe that tumor recurrence in our study may be related to several factors, including incomplete tumor resection, the biological aggressiveness of the tumor, and the incomplete response to adjuvant therapy.

We observed a trend toward a smaller tumor reduction in the group with high VDAC2 expression (33.0% vs. 53.4%, p = 0.0649), although this difference did not reach statistical significance. This finding suggests that VDAC2 overexpression may be associated with a poorer response to surgical treatment in ACPs. We hypothesize that tumor cells with high VDAC2 expression may be more resistant to treatment-induced apoptosis, which could lead to a lower degree of tumor reduction.

Our study has some limitations that should be considered. First, the sample size is relatively small (n = 12), which may limit the statistical power of our analyses. Future studies with larger cohorts are needed to confirm our findings and investigate the relationship between VDAC2 and ACPs with greater accuracy. Second, our study is retrospective, which may introduce biases in data collection and patient follow-up. Prospective studies are needed to evaluate the prognostic value of VDAC2 more robustly in ACPs. Third, our study did not investigate the molecular mechanisms underlying the relationship between VDAC2 and ACPs. Functional in vitro and in vivo studies are necessary to elucidate the mechanisms by which VDAC2 influences cell proliferation, activation of the Wnt/β-catenin pathway, and treatment response in ACPs.

Despite these limitations, the VDAC2 overexpression observed in ACPs in this study supports the hypothesis that alterations in mitochondrial metabolism and apoptotic mechanisms may be involved in the pathophysiology of these tumors. Considering that VDAC2 acts as a key regulator of mitochondrial permeability and the release of pro-apoptotic factors, its overexpression may contribute to tumor cell survival and greater biological aggressiveness. These findings not only enhance our understanding of the molecular mechanisms of craniopharyngioma but also suggest that VDAC2 may represent a biomarker of interest and, in the future, a potential therapeutic target. Moreover, modulation of the Wnt/β-catenin pathway emerges as a promising therapeutic strategy in ACPs, and the combination of VDAC2 inhibitors with modulators of this pathway may enhance therapeutic outcomes. Further investigations using experimental models and functional studies will be essential to validate these findings and explore their potential clinical applications.

Conclusion

In this study, we found VDAC2 overexpression in ACP samples. This finding expands our understanding of the molecular mechanisms associated with the pathophysiology of this rare neoplasm, suggesting that VDAC2 may play a relevant role in the tumor biology of ACPs. Considering the role of VDAC2 in the regulation of mitochondrial metabolism, metabolite transport, and apoptosis control, its overexpression may be related to the maintenance of cell viability, evasion of programmed cell death, and metabolic adaptation of tumor cells.

The presence of high VDAC2 expression in the analyzed samples raises the hypothesis that this mitochondrial channel may contribute to the local aggressiveness of the tumor—a hallmark of craniopharyngiomas—particularly regarding their tendency to recur and infiltrate adjacent structures, given the correlations found with Ki-67 and β-catenin. Moreover, the identification of biomarkers such as VDAC2 may represent a relevant advance in prognostic stratification and in the development of more targeted therapeutic approaches.

The results obtained provide a foundation for future investigations. Additional studies exploring the signaling pathway associated with VDAC2, its interaction with other elements of the tumor microenvironment, and its impact on therapeutic response may help clarify the true potential of this protein as a therapeutic target in craniopharyngiomas.

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Anexo – Parecer do Comitê de Ética em Pesquisa

HOSPITAL UNIVERSITÁRIO DA UNIVERSIDADE FEDERAL DO MARANHÃO / HU - UFMA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação de biomarcadores moleculares e celulares na fisiopatologia de craniofaringiomas

Pesquisador: Manuel dos Santos Faria

Área Temática:

Versão: 2

CAAE: 63982722.2.0000.5086

Instituição Proponente: Hospital Universitário da Universidade Federal do Maranhão/HU/UFMA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.748.361

Apresentação do Projeto:

As informações elencadas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas do arquivo Informações Básicas da Pesquisa (PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1931590. Datado de 08/11/2022).

INTRODUÇÃO

Craniofaringiomas são tumores cerebrais raros originários de células remanescentes do epitélio do ducto craniofaríngeo, conhecido como bolsa de Rathke (Faria et al., 2013). Representam 2 a 5% de todas as neoplasias primárias intracranianas, com distribuição bimodal (um pico na população pediátrica e outro pico em adultos na 6ª década de vida) e são responsáveis por 5 a 10% dos tumores intracranianos; não apresentam predileção por sexo, acometendo igualmente homens e mulheres (Muller et al., 2019).Embora essas lesões sejam classificadas como benignas, sua capacidade invasiva e a proximidade com estruturas nobres como as vias ópticas, o 3º ventrículo, a hipófise e o hipotálamo, são associadas a déficits visuais, hormonais (somatotrófico, gonadotrófico, corticotrófico, tireotrófico e diabetes insipidus) e neurológicos assim como perda da qualidade de vida conforme o tumor cresce, consistindo em uma doença agressiva e com terapêutica limitada (Martinez-Barbera & Andoniadou, 2020).Existem duas variantes histológicas para os craniofaringiomas: o adamantinomatoso (CFA) e o papilar (CFP), que diferem em sua gênese e distribuição etária. Os CFAs acometem todas as faixas etárias e são o subtipo mais

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comum, enquanto os CFPs são restritos a adultos. CFAs podem estar relacionados ao surgimento de mutações somáticas no gene CTNNB1 (que codifica -catenina) que promovem acúmulo de -catenina, levando à ativação da via WNT. Com base em características moleculares e histológicas, essa variante tumoral parece ter origem embrionária (Karavitaki & Larkin, 2017). Por outro lado, estudos sugerem que mutações somáticas do gene BRAF estariam associadas à patogênese dos CFPs, resultando na ativação contínua da via de sinalização da proteína quinase ativada por mitogênio (MAPK) (Brastianos & Santagata, 2016). Ao exame de imagem, esses tumores também apresentam diferenças marcantes sendo que os CFAs são predominantemente císticos com calcificações enquanto os CFPs apresentam-se comumente como estruturas sólidas e não calcificadas (Muller et al., 2019).Quanto a terapia, o tratamento de escolha para craniofaringiomas é a completa ressecção tumoral. No entanto, nem sempre esta intervenção é possível devido à proximidade e envolvimento de vias ópticas, vasos cerebrais, hipófise e hipotálamo. Danos a estas estruturas podem acarretar elevada morbidade e perda de qualidade de vida aos pacientes (Muller et al., 2011). Quando há impossibilidade de ressecção tumoral completa, a radioterapia adjuvante, a despeito de sua eficácia limitada e efeitos adversos, deve ser considerada no seguimento terapêutico (Ajithkumar et al., 2018). A administração intracística de substâncias esclerosantes, como bleomicina e INF- tem sido sugerida para atenuar a formação de fluidos e diminuir o tamanho do cisto. Contudo, o uso da bleomicina é limitado por sua toxicidade e o IFN- apresenta pouca eficácia, restrita a tumores monocísticos, sem efeito sobre o componente sólido do tumor (Kilday et al., 2017).Várias descobertas têm impactado no conhecimento acerca da tumorigênese na perspectiva de gerar opções terapêuticas personalizadas. O Ki-67 é uma proteína de ligação ao DNA nuclear que é expressa em todos os vertebrados amplamente utilizado para classificar tumores por seu papel marcador de proliferação celular e agressividade tumoral, geralmente associado a mau prognóstico e presente em neoplasias de pulmão, bexiga e mamas, com potencial interesse terapêutico (Menon et al., 2019). O MGMT (O-6-metilguanina DNA metiltransferase), uma enzima de reparo de DNA que desempenha importante papel na quimiorresistência a agentes alquilantes, é utilizado como um marcador prognóstico independente nos glioblastomas, com poucos estudos em craniofaringiomas, embora tenha se mostrado um alvo terapêutico promissor (Zuhur et al., 2011; Hussain et al., 2013; Yu et al., 2020). O VDAC2 (voltage-dependent anion channel 2) é um poro localizado na membrana externa da mitocôndria que permite o fluxo de metabólitos entre o citosol e a mitocôndria, portanto, um determinante na escolha celular sobre vias apoptóticas, o que é relevante para as células cancerosas e por esta razão se tornou um alvo terapêutico potencial no combate ao câncer (Mazure, 2017). A TERT

(transcriptase reversa da telomerase), uma unidade catalítica funcional da telomerase que alonga os telômeros, normalmente é transcrita apenas no início do desenvolvimento embrionário em células com alto potencial proliferativo; porém, na maioria dos cânceres, sofre reativação e, por alongar os telômeros, contribui para formação e progressão dos tumores, sendo um alvo de possíveis implicações terapêuticas com drogas antitelomerásicas (Dratwa et al., 2020). Por fim, as proteínas RAS desempenham papel crucial no controle da proliferação celular por meio da transdução de sinais e ativação das vias MAPK e PI3K/AKT. Mutações no gene RAS, especialmente envolvendo códons 12/13 e 61, são predominantemente observadas em tumores de tireoide. Desse modo, acredita-se que as informações sobre tais alterações possam ser importantes no prognóstico dos tumores (Xing, 2013). O progresso da biologia molecular na descoberta de novas vias fisiopatológicas tem possibilitado a identificação de moléculas como alvos terapêuticos, permitindo o surgimento de tratamentos medicamentosos direcionados (medicina de precisão) no intuito de minimizar efeitos adversos associados à cirurgia e radioterapia, principalmente em tumores recorrentes em que há poucas ou nenhuma opção terapêutica.

Hipótese:
Alterações na expressão de Ki-67, MGMT, VDAC2 e a presença de mutação TERT e da família RAS estão envolvidas na fisiopatologia de craniofaringiomas.

Metodologia Proposta: Será realizado um estudo analítico transversal retrospectivo e prospectivo. O estudo será realizado no Hospital Universitário da Universidade Federal do Maranhão (HUUFMA). O atendimento clínico aos pacientes e pesquisa de prontuários será realizado no Serviço de Endocrinologia do HUUFMA. As cirurgias para remoção dos tumores hipofisários e avaliação histopatológica das amostras serão realizados em colaboração com Centro Cirúrgico e Serviço de Anatomia Patológica do HUUFMA. Os ensaios de Biologia Molecular serão realizados no Centro de Pesquisa Clínica (CEPEC) do HUUFMA e Laboratório de Estudos Genômicos e de Histocompatibilidade (LEGH).A população será composta de crianças e adultos. Será utilizada uma amostra de conveniência estimada em 50 participantes Serão incluídos no estudo pacientes de cinco a 80 anos, de ambos os sexos, que apresentem diagnóstico de craniofaringioma. Os voluntários dispostos a participar desta pesquisa ou seus responsáveis legais devem assinar o Termo de Consentimento Livre e Esclarecido (TCLE). A coleta de dados consistirá na consulta ao prontuário ou entrevista com paciente para obtenção de dados do protocolo de pesquisa. Será realizada ainda avaliação histológica, imunohistoquímica e de biologia molecular para

determinação dos marcadores que se propõe estudar com essa pesquisa.Os pacientes serão submetidos a um inquérito clínico-demográfico através de questionário padronizado no qual serão coletados dados relativos a sexo, idade (anos), naturalidade, cor (autorreferida), tempo de evolução, uso de medicações, doenças associadas e antecedentes familiares. Serão avaliadas variáveis antropométricas (peso, altura, índice de massa corpórea e circunferência abdominal). Será realizada coleta de sangue para dosagens hormonais dos eixos hipofisários pré e pós operatórios. Serão dosados ACHT, TSH, GH, FSH, LH, prolactina, cortisol, T4L, IGF-1, testosterona e estradiol.Serão realizados exames de imagem (Ressonância Nuclear Magnética e Tomografia Computadorizada) dos pacientes incluídos na pesquisa para avaliação de características tumorais e evolução tumoral pós-operatória.Os ácidos nucleicos do tecido neoplásico serão isolados e quantificados em espectrofotômetro. Para a síntese do cDNA ,1 g de RNA será submetido a reação de transcrição reversa (RT-PCR). A amplificação por PCR será realizada usando primers que abrangem os locais de fosforilação da glicogênio sintase quinase-3b (GSK-3b) do CTNNB1/ -catenina, mutação em BRAF, no promotor TERT e genes da família RAS. As reações de amplificação ocorrerão em termociclador com desnaturação inicial a 95ºC por 5 minutos, 35 ciclos de 30 segundos a 95ºC (desnaturação), 45 segundos a 55ºC (anelamento) e 45 segundos a 72ºC (extensão). A extensão final será realizada a 72°C durante 2 minutos. Os produtos de PCR serão visualizados em gel de agarose 2%, purificados e posteriormente sequenciados pelo método de terminação de cadeia dideoxi. O sequenciamento das amostras será realizado por empresa especializada. Os eletroferogramas obtidos pelo sequenciamento serão editados utilizando os programas BioEdit 7.0.9.0 e ChromasPro 2.33 e as sequências consensos dos contigs serão alinhadas utilizando a ferramenta de bioinformática Clustal X. A expressão tecidual de Ki-67, b-catenina, MGMT e VDAC2 será avaliada por imuno-histoquímica. Para as reações de Imunoperoxidase serão utilizados anticorpos primários anti-humano para Ki-67, b-catenina, MGMT e VDAC2.

Critério de Inclusão:
Serão incluídos no estudo pacientes de cinco a 80 anos, de ambos os sexos, que apresentem diagnóstico de craniofaringioma.

Critério de Exclusão:
Não serão incluídos pacientes com análise histopatológica incompatível com craniofaringioma.

Metodologia de Análise de Dados:
As variáveis quantitativas serão expressas por média ± desvio padrão. A comparação dos dados entre dois grupos será realizada por meio do teste Mann-Whitney U e o teste de Kruskal-Wallis será utilizado para a comparação entre mais de dois grupos. A normalidade da distribuição será verificada pelo teste de Shapiro-Wilk. As variáveis categóricas serão analisadas através do teste de qui-quadrado ou Fisher. Valores de p0,05 serão considerados estatisticamente significativos. Os dados estatísticos serão analisados e visualizados utilizando o programa GraphPad Prism v. 7.

Desfecho Primário:
Presença de expressão de Ki-67, MGMT, VDAC2 e a presença de mutação TERT e da família RAS em amostras tumorais de craniofaringiomas

Tamanho da Amostra no Brasil: 50

Objetivo da Pesquisa:
Objetivo Primário:
Caracterizar os aspectos clínicos, histopatológicos e moleculares dos pacientes portadores de craniofaringiomas.

Objetivo Secundário:
- Identificar aspectos clínicos dos diferentes subtipos de craniofaringioma;
- Analisar a expressão de -catenina, MGMT e VDAC em craniofaringiomas e sua relação com subtipos histológicos e marcador de agressividade Ki-67;
- Determinar a presença de mutação CTNNB1, BRAF, RAS e TERT em craniofaringiomas e sua relação com subtipos histológicos e marcador de agressividade Ki-67;
- Correlacionar achados de biologia molecular com características clínicas e histopatológicas de craniofaringiomas;
- Inferir vias fisiopatológicas envolvidas na gênese de craniofaringiomas e potenciais preditores de agressividade e alvos terapêuticos.

Avaliação dos Riscos e Benefícios:
De acordo com o pesquisador:

Riscos: Os riscos envolvidos na pesquisa compreendem potenciais alterações físicas, psicológicas e sociais a que o participante está exposto ao participar da pesquisa. Portanto, durante esta a

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pesquisa, o mesmo pode apresentar: cansaço ou aborrecimento ao responder os questionários da pesquisa; constrangimento durante a realização de exame físico e antropométrico; desconforto durante coleta de exames de sangue, com risco de acidente de punção (dolorimento, hematoma, etc). Tais riscos serão minimizados com a aplicação de questionários curtos e objetivos, presença de um acompanhante durante a realização de exame físico e coleta de sangue por profissional experiente. Há ainda o risco de quebra de sigilo, ainda que involuntária e não intencional. Tal risco será minimizado pela codificação pelo número do prontuário e a restrição do número de pesquisadores envolvidos na pesquisa.

Benefícios: Os benefícios dessa pesquisa são principalmente voltados à sociedade, com poucos benefícios diretos, a curto prazo, ao participante. A este pode ser citada a facilidade na realização de exames laboratoriais e de imagem implicado no escopo da pesquisa, contribuindo no processo diagnóstico e terapêutico do mesmo.

Comentários e Considerações sobre a Pesquisa:

Estudo analítico transversal retrospectivo e prospectivo que será realizado no Hospital Universitário da Universidade Federal do Maranhão (HUUFMA) na cidade de São Luís, no Maranhão. O atendimento clínico aos pacientes e pesquisa de prontuários será realizado no Serviço de Endocrinologia do HUUFMA. A população será composta de crianças e adultos. Será utilizada uma amostra de conveniência estimada em 50 participantes. Serão incluídos no estudo pacientes de cinco a 80 anos, de ambos os sexos, que apresentem diagnóstico de craniofaringioma. Não serão incluídos pacientes com análise histopatológica incompatível com craniofaringioma. A coleta de dados consistirá na consulta ao prontuário ou entrevista com paciente para obtenção de dados do protocolo de pesquisa. Será realizada ainda avaliação histológica, imunohistoquímica e de biologia molecular para determinação dos marcadores que se propõe estudar com essa pesquisa.

Considerações sobre os Termos de apresentação obrigatória:

O protocolo apresenta documentos referente aos "Termos de Apresentação Obrigatória": Folha de rosto, Orçamento financeiro detalhado, Cronograma com etapas detalhada, Termo de Consentimento Livre e Esclarecido (TCLE), Autorização do Gestor responsável do local para a realização da coleta de dados e Projeto de Pesquisa Original na íntegra em Word. Atende à Norma Operacional no 001/2013 (item 3/ 3.3).

Recomendações:

Após o término da pesquisa o CEP-HUUFMA solicita que se possível os resultados do estudo sejam

devolvidos aos participantes da pesquisa ou a instituição que autorizou a coleta de dados de forma anonimizada.

Conclusões ou Pendências e Lista de Inadequações:

O PROTOCOLO não apresenta óbices éticos, portanto atende aos requisitos fundamentais da Resolução CNS/MS nº 466/12 e suas complementares. sendo considerado APROVADO.

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa–CEP-HUUFMA, de acordo com as atribuições definidas na Resolução CNS nº.466/2012 e Norma Operacional nº. 001 de 2013 do CNS, manifesta-se pela APROVAÇÃO do projeto de pesquisa proposto.

Eventuais modificações ao protocolo devem ser inseridas à plataforma por meio de emendas de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Relatórios parcial e final devem ser apresentados ao CEP, inicialmente após a coleta de dados e ao término do estudo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1931590.pdf	08/11/2022 22:45:12		Aceito
Outros	Carta_resposta.pdf	08/11/2022 22:44:54	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado.docx	08/11/2022 22:31:01	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
Cronograma	Cronograma.pdf	08/11/2022 22:29:26	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	08/11/2022 22:28:09	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
Declaração de concordância	Declaracao_de_concordancia.pdf	23/06/2022 10:33:15	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termos_de_Assentimento.pdf	23/06/2022 10:31:58	CLARIANO PIRES DE OLIVEIRA NETO	Aceito
Orçamento	Orcamento.pdf	23/06/2022 10:22:55	CLARIANO PIRES DE OLIVEIRA NETO	Aceito

Continuação do Parecer: 5.748.361

Folha de Rosto	Folha_de_rosto.pdf	23/06/2022 10:22:10	CLARIANO PIRES DE OLIVEIRA NETO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO LUIS, 09 de Novembro de 2022

Assinado por:
Camiliane Azevedo Ferreira
(Coordenador(a))

Endereço: Rua Barão de Itapary nº 227
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Anexo – Normas para Submissão Frontiers in Endocrinology

Title

The title should be concise, omitting terms that are implicit and, where possible, be a statement of the main result or conclusion presented in the manuscript. Abbreviations should be avoided within the title.

Witty and creative titles are welcome, but only if relevant and within measure. Consider if a title meant to be thought-provoking might be misinterpreted as offensive or alarming. In extreme cases, the editorial office may veto a title and propose an alternative.

Authors should avoid:

- titles that are a mere question without giving the answer
- unambitious titles, for example starting with 'Towards,' 'A description of,' 'A characterization of' or 'Preliminary study on'
- vague titles, for example starting with 'Role of', 'Link between', or 'Effect of' that do not specify the role, link, or effect
- including terms that are out of place, for example the taxonomic affiliation apart from species name.

Abstract

As a primary goal, the abstract should make the general significance and conceptual advance of the work clearly accessible to a broad readership. The abstract should be no longer than a single paragraph and should be structured, for example, according to the IMRAD format. For the specific structure of the abstract, authors should follow the requirements of the article type or journal to which they're submitting. Minimize the use of abbreviations and do not cite references, figures or tables.

For clinical trial articles, please include the unique identifier and the URL of the publicly accessible website on which the trial is registered.

Manuscript length

We encourage you to closely follow the article word count lengths given in the 'Article types' page of the journals. The manuscript length includes only the main body of the text, footnotes, and all citations within it, and excludes the abstract, section titles,

figure and table captions, funding statement, acknowledgments, and references in the bibliography. Please indicate the number of words and the number of figures and tables included in your manuscript on the first page.

Sections

The manuscript is organized by headings and subheadings. The section headings should be those appropriate for your field and the research itself. You may insert up to 5 heading levels into your manuscript (i.e.,: 3.2.2.1.2 Heading Title).

For Original Research articles, it is recommended to organize your manuscript in the following sections or their equivalents for your field.

Introduction

Succinct, with no subheadings.

Materials and methods

This section may be divided by subheadings and should contain sufficient detail so that when read in conjunction with cited references, all procedures can be repeated. For experiments reporting results on animal or human subject research, an ethics approval statement should be included in this section (for further information, see the 'Bioethics' section of our policies and publication ethics.)

Results

This section may be divided by subheadings. Footnotes should not be used and must be transferred to the main text.

Discussion

This section may be divided by subheadings. Discussions should cover the key findings of the study: discuss any prior research related to the subject to place the novelty of the discovery in the appropriate context, discuss the potential shortcomings and limitations on their interpretations, discuss their integration into the current understanding of the problem and how this advances the current views, speculate on

the future direction of the research, and freely postulate theories that could be tested in the future.

For further information, please check the descriptions defined in the journal's 'Article types' page, in the 'For authors' menu on every journal page.

Language editing

Frontiers requires manuscripts submitted to meet international English language standards to be considered for publication.

For authors who would like their manuscript to receive language editing or proofreading to improve the clarity of the manuscript and help highlight their research, we recommend the language-editing services provided by the following external partners.

Note that sending your manuscript for language editing does not imply or guarantee that it will be accepted for publication by a Frontiers journal. Editorial decisions on the scientific content of a manuscript are independent of whether it has received language editing or proofreading by these partner services or other services.

Editage

We recommend the language-editing service provided by our external partner Editage. These services may be particularly useful for researchers for whom English is not the primary language. They can help to improve the grammar, syntax, and flow of your manuscript prior to submission. Frontiers' authors will receive a 10% discount by visiting the following link:
editage.com/frontiers

Language style

The default language style at Frontiers is American English. If you prefer your article to be formatted in British English, please specify this on the first page of your manuscript. For any questions regarding style, we recommend authors to consult the Chicago Manual of Style

Inclusive language guidelines

Frontiers is an inclusive publisher and we ask that all submissions are in line with our inclusive language policy. When preparing your manuscript for submission, take a mindful approach towards personal biases and a concerted effort to limit their influence. Authors should remove any suggestion or implication of superiority or inferiority of one person over another based on age, gender, race, ethnicity, culture, sexual orientation, disability, religion, or socio-economic class. We ask authors to use inclusive language practices and awareness of diversity, equity, and inclusion into their research and keep it at the forefront during the composition of their findings.

External guidance that may be useful is available at C4DISC's Guidelines on Inclusive Language and Images in Scholarly Communication.

Furthermore, when drafting your work, please take into account the following considerations

In general, seek to avoid

- language that could be deemed insulting, profane, or derogatory.
- descriptors that identify personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability, or health conditions, where they are not critically relevant to the discussion.
- any form of language that suggests a particular culture or group as the default or standard.

And where feasible:

- proactively ask individuals or groups how they would prefer to be referenced.
- adhere to the SAGER guidelines for reference to sex and gender in research.

Remember, the language we use can influence perceptions, evoke emotions, and shape perspectives. Let's work together to nurture an inclusive, respectful, and empowering discourse in science.

Guidelines for artificial intelligence and related technologies

These guidelines cover acceptable uses of generative AI technologies such as Large Language Models (ChatGPT, Jasper) and text-to-image generators (DALL-E 2,

Midjourney, Stable Diffusion) in the writing or editing of manuscripts submitted to Frontiers.

Use of AI to create written or visual content

Authors should not list a generative AI technology as a co-author or author of any submitted manuscript. Generative AI technologies cannot be held accountable for all aspects of a manuscript and consequently do not meet the criteria required for authorship.

If the author of a submitted manuscript has used written or visual content produced by or edited using a generative AI technology, this use must follow all Frontiers guidelines and policies. Specifically, the author is responsible for checking the factual accuracy of any content created by the generative AI technology. This includes, but is not limited to, any quotes, citations or references. Figures produced by or edited using a generative AI technology must be checked to ensure they accurately reflect the data presented in the manuscript. Authors must also check that any written or visual content produced by or edited using a generative AI technology is free from plagiarism.

If the author of a submitted manuscript has used written or visual content produced by or edited using a generative AI technology, such use must be acknowledged in the acknowledgements section of the manuscript and the methods section if applicable. This explanation must list the name, version, model, and source of the generative AI technology.

We encourage authors to upload all input prompts provided to a generative AI technology and outputs received from a generative AI technology in the supplementary files for the manuscript.

The entire document should be single-spaced and must contain page and line numbers in order to facilitate the review process. The manuscript should be written using either Word or LaTeX. See above for templates.

Abbreviations and nomenclatures

The use of abbreviations should be kept to a minimum. Non-standard abbreviations should be avoided unless they appear at least four times, and must be

defined upon first use in the main text. Consider also giving a list of non-standard abbreviations at the end, immediately before the acknowledgments.

Equations should be inserted in editable format from the equation editor.

Italicize gene symbols and use the approved gene nomenclature where it is available. For human genes, please refer to the HUGO Gene Nomenclature Committee (HGNC). New symbols for human genes should be submitted to the HGNC here. Common alternative gene aliases may also be reported, but should not be used alone in place of the HGNC symbol. Nomenclature committees for other species are listed here. Protein products are not italicized.

We encourage the use of Standard International Units in all manuscripts.

Chemical compounds and biomolecules should be referred to using systematic nomenclature, preferably using the recommendations by the International Union of Pure and Applied Chemistry (IUPAC).

Astronomical objects should be referred to using the nomenclature given by the International Astronomical Union (IAU) provided
Here.

Life Science Identifiers (LSIDs) for ZOOBANK registered names or nomenclatural acts should be listed in the manuscript before the keywords. An LSID is represented as a uniform resource name (URN) with the following format: urn:lsid:<Authority>:<Namespace>:<ObjectID>[:<Version>]

For more information on LSIDs please see the 'Code' section of our policies and publication ethics.

Enhancing search engine optimization (SEO)

There are a few simple ways to maximize your article's discoverability and search results.

- Include a few of your article's keywords in the title of the article
- Do not use long article titles
- Pick 5-8 keywords using a mix of generic and more specific terms on the article subject(s)
- Use the maximum amount of keywords in the first two sentences of the abstract

- Use some of the keywords in level 1 headings

Preparing and formatting references

Submissions to Frontiers must be grounded in relevant and up to date peer-reviewed, academic research, and this should be reflected in the accompanying reference lists.

Authors are welcome to use online referencing tools in preparation of their manuscript. Some useful resources include RefMe, Zotero, and Mendeley.

- The citation of non-academic and non-peer-reviewed sources (e.g. blog posts, website content), as well as anonymous sources or commercial websites should be avoided or kept to a minimum
- Authors should avoid citing content that is not directly relevant to the scope of the article and the journal
- Reference lists should reflect the current status of knowledge in the field, avoid bias, and not include a high proportion of citations to the same authors or sources, school of thought, etc.
- The length of the reference list should be appropriate depending on the article type, covering the relevant literature through sufficient referencing
- Authors should ensure that references are accurate, that all links are accessible, and that the citations/references adhere to the reference styles outlined below

Frontiers' journals use one of two reference styles, either Harvard (author-date) or Vancouver (numbered). These formats should be adhered to for the in-text citations and the reference lists. Please check our help center to find the correct style for the journal to which you're submitting.

- All citations of published works in the text, figures, or tables must be in the reference list and vice-versa.
- The names of the first six authors followed by et al. and the DOI (when available) should be provided.
- Given names of authors should be abbreviated to initials (e.g. Smith, J., Lewis, C.S., etc.).
- The reference list should only include articles that are published or accepted.

- Unpublished data, submitted manuscripts, or personal communications should be cited within the text only, for article types that allow such inclusions. Where additional details are available, these will be included as footnotes.
- For accepted but unpublished works use 'in press' instead of page numbers.
- Data sets that have been deposited to an online repository should be included in the reference list. Include the version and unique identifier when available.
- Personal communications should be documented by a letter of permission.
- Website URLs should be included as footnotes.
- Any inclusion of verbatim text must be contained in quotation marks and should clearly reference the original source.
- Preprints can be cited provided that a DOI or archive URL is available, and the citation clearly mentions that the contribution is a preprint. If a peer-reviewed journal publication for the same preprint exists, the official journal publication is the preferred source. See the preprints section for each reference style below for more information.

Templates

If working with Word please use our Word templates. If you wish to submit your article as LaTeX, we recommend our LaTeX templates.

For LaTeX files, please ensure all relevant manuscript files are uploaded: .tex file, PDF, and .bib file (if the bibliography is not already included in the .tex file).

During the interactive review, authors are encouraged to upload versions using track changes. Editors and reviewers can only download the PDF file of the submitted manuscript.

Preparing figures, tables, and images

Figures, tables, and images: rights and permissions

All figures, tables, and images will be published under a Creative Commons CC-BY license, and permission must be obtained for use of copyrighted material from other sources (including re-published/adapted/modified/partial figures and images

from the internet). It is the responsibility of the authors to acquire the licenses, follow any citation instructions requested by third-party rights holders, and cover any supplementary charges.

For additional information, please see the 'Image manipulation' section of our policies and publication ethics.

Figures and images: style guidelines

We require figures to be submitted individually, in the same order as they are referred to in the manuscript; the figures will then be automatically embedded at the end of the submitted manuscript. Ensure that each figure is mentioned in the text and in numerical order.

For figures with more than one panel, panels should be clearly indicated using labels (A), (B), (C), (D), etc. However, do not embed the part labels over any part of the image. These labels will be replaced during typesetting according to Frontiers' journal style. For graphs, there must be a self-explanatory label (including units) along each axis.

For LaTeX files, figures should be included in the provided PDF. In case of acceptance, our production office might require high-resolution files of the figures included in the manuscript in EPS, JPEG or TIF/TIFF format.

To upload more than one figure at a time, save the figures (labeled in order of appearance in the manuscript) in a zip file and upload them as 'Supplementary material presentation.'

Please note that figures not in accordance with the guidelines will cause substantial delay during the production process.

Captions

Captions should be preceded by the appropriate label, for example 'Figure 1.' Figure captions should be placed at the end of the manuscript. Figure panels are referred to by bold capital letters in brackets: (A), (B), (C), (D), etc.

Image size and resolution requirements

Figures should be prepared with the PDF layout in mind. Individual figures should not be longer than one page and with a width that corresponds to one column (85 mm) or two columns (180 mm).

All images must have a resolution of 300 dpi at final size. Check the resolution of your figure by enlarging it to 150%. If the image appears blurry, jagged, or has a stair-stepped effect, the resolution is too low.

The text should be legible and of high quality. The smallest visible text should be no less than eight points in height when viewed at actual size.

Solid lines should not be broken up. Any lines in the graphic should be no smaller than two points wide.

Please note that saving a figure directly as an image file (JPEG, TIF) can greatly affect the resolution of your image. To avoid this, one option is to export the file as PDF, then convert into TIFF or EPS using a graphics software.

Format and color image mode

The following formats are accepted: TIF/TIFF (.tif/.tiff), JPEG (.jpg), and EPS (.eps) (upon acceptance). Images must be submitted in the color mode RGB.

Images of chemical structures

Chemical structures should be prepared using ChemDraw or a similar program. If working with ChemDraw please use our ChemDraw template. If working with another program please follow the guidelines below.

- Drawing settings: chain angle, 120° bond spacing, 18% width; fixed length, 14.4 pt; bold width, 2.0 pt; line width, 0.6 pt; margin width, 1.6 pt; hash spacing, 2.5 pt. Scale 100% Atom Label settings: font, Arial; size, 8 pt
- Assign all chemical compounds a bold, Arabic numeral in the order in which the compounds are presented in the manuscript text.

Table requirements and style guidelines

Tables should be inserted at the end of the manuscript in an editable format. If you use a word processor, build your table in Word. If you use a LaTeX processor, build your table in LaTeX. An empty line should be left before and after the table.

Table captions must be placed immediately before the table. Captions should be preceded by the appropriate label, for example 'Table 1.' Please use only a single paragraph for the caption.

Ensure that each table is mentioned in the text and in numerical order.

Large tables covering several pages cannot be included in the final PDF for formatting reasons. These tables will be published as supplementary material.

Tables which are not according to the above guidelines will cause substantial delay during the production process.

During production, tables will be formatted according to Frontiers' house style.

Anexo – Normas para Submissão New England Journal of Medicine

NEJM uses highly rigorous editorial, peer, and statistical review processes to evaluate manuscripts for scientific accuracy, novelty, and importance.

Step 1: Acquaint yourself with NEJM Editorial Policies, Article Types, options for Presubmission Inquiry and Rapid Review, Statistical Reporting Guidelines (if applicable), and Key Journal Style Elements.

Step 2: Prepare materials for submission including cover letter (optional), main text, tables, figures, supplementary appendix, clinical trial protocol and statistical analysis plan (if applicable).

Step 3: Submit your manuscript to the NEJM online submission system by clicking on the red button at the top left hand side of this page.

Cover Letter

Though cover letters are not required, the NEJM Online Submission system contains a text field through which important information that is not in the metadata, such as a meeting presentation date or a major conflict of interest not in the manuscript, should be communicated with initial manuscript submissions.

Manuscript Text File

Compile all text, references, figure legends, and tables into a single double-spaced digital file (preferably an MS Word document). NEJM will also accept text (.txt), or Rich Text Format (.rtf) files.

Title Page

Create a title page that includes:

- Manuscript title
- Each author's name, highest degree, and affiliation/institution
- Contact information for one corresponding author

Abstract

Provide an abstract of not more than 250 words with four labeled paragraphs containing the following:

- Background: Problem being addressed in the study
- Methods: How the study was performed
- Results: Salient results
- Conclusions: What the authors conclude from study results
- Trial registration number

Identifying Data

At appropriate places in the manuscript, please provide the following items:

- If applicable, a statement that the research protocol was approved by relevant institutional review boards or ethics committees and that all human participants gave written informed consent
- Identities of those who analyzed the data
- For clinical trials, registration number and registry name (see: N Engl J Med 2004;351:1250-1)
- For studies containing microarrays, accession numbers and repository name

References

References must be double-spaced and numbered consecutively as they are cited. References first cited in a table or figure legend should be numbered so they will be in sequence with references cited in the text at the point where the table or figure is first mentioned. List all citation authors when there are six or fewer; when there are seven or more, list the first three, followed by et al.

Numbered references to personal communications, unpublished data, or manuscripts either “in preparation” or “submitted for publication” are unacceptable. If essential, such materials can be incorporated at appropriate places in the text.

Tables

All tables should be included at the end of the manuscript text file. Double-space tables (including footnotes) and provide a title for each table. For Original Articles, there is normally a limit of five figures and tables (total) per manuscript. Extensive tables or supplementary materials will be published as supplemental materials with the digital version of the article.