Universidade Federal do Maranhão
Agência de Inovação, Empreendedorismo, Pesquisa, Pós-Graduação e Internacionalização
Programa de Pós-Graduação em Saúde do Adulto
Mestrado Acadêmico

KLOTHO NO CÂNCER : Uma revisão Sistematica

JUCILEIDE MOTA COSTA

São Luís 2023

JUCILEIDE MOTA COSTA

KLOTHO NO CÂNCER : Uma revisão Sistemática

Dissertação apresentada ao Programa de Pós-Graduação em Saúde do Adulto da Universidade Federal do Maranhão para obtenção do Grau de Mestre em Saúde do Adulto.
Área de Concentração: Processos biológicos em saúde.
Linha de Pesquisa: HPV e Câncer.
Orientador: Prof. Dr. Rui Miguel Gil da Costa Oliveira.
Coorientador: Profa. Dra. Fernanda Ferreira Lopes
Coordenador: Prof. Dr. Marcelo Souza de Andrade.

São Luís
2023

Ficha gerada por meio do SIGAA/Biblioteca com dados fornecidos pelo(a) autor(a). Diretoria Integrada de Bibliotecas/UFMA

MOTA COSTA, JUCILEIDE. Klotho no câncer: uma revisão sistemática / JUCILEIDE MOTA COSTA. - 2023.
35 f.

Coorientador(a): Fernanda Ferreira Lopes.
Orientador(a): Rui Miguel Gil da Costa Oliveira.
Dissertação (Mestrado) - Programa de Pós-graduação em Saúde do Adulto/ccbs, Universidade Federal do Maranhão, SÃO LUIS, 2023.

1. Cancer. 2. Diagnóstico Molecular. 3. Fator Prognóstico. 4. Proteínas Klotho. I. Ferreira Lopes, Fernanda. II. Gil da Costa Oliveira, Rui Miguel. III. Título.

JUCILEIDE MOTA COSTA

KLOTHO NO CÂNCER : Uma revisão Sistemática

Dissertação apresentada ao Programa de Pós-Graduação em Saúde do Adulto da Universidade Federal do Maranhão para obtenção do Grau de Mestre em Saúde do Adulto.

A Banca Examinadora da Defesa de Mestrado, apresentada em sessão pública, considerou o candidato aprovado em: ____/____/____.

_____

Prof. Dr. Rui Miguel Gil da Costa Oliveira (orientador)
Universidade Federal do Maranhão

_____

Profa. Dra. Fernanda Ferreira Lopes (co orientador)
Universidade Federal do Maranhão

_____

Prof. Dr. Marcelo Souza de Andrade
Universidade Federal do Maranhão

_____

Profa. Dra. Maria do Socorro de Sousa Cartágenes
Universidade Federal do Maranhão

_____

Profa. Dra Amanda Mara Teles
Universidade Estadual do Maranhão

_____

Profa. Dra. Haíssa Oliveira Brito (suplente)

AGRADECIMENTOS

Gostaria de começar expressando minha profunda gratidão a todas as pessoas que contribuíram para a conclusão desta tese de mestrado. É com imensa alegria que dedico um momento para expressar meus sinceros agradecimentos a todos que estiveram ao meu lado durante essa jornada desafiadora e enriquecedora. Primeiramente, gostaria de expressar minha gratidão ao meu orientador/professor Dr *Rui Miguel Gil da Costa Oliveira,* que me guiou ao longo deste processo com paciência, sabedoria e dedicação. Seu conhecimento especializado e suas valiosas sugestões foram fundamentais para o desenvolvimento desta pesquisa. Sou imensamente grato pela sua orientação e pelos valiosos conhecimentos que compartilhou comigo. A Professora *Haissa Brito* pela contribuição, nesse processo desde o início .Gostaria de agradecer também aos membros da banca examinadora pelo tempo e esforço dedicados à avaliação deste trabalho. Não posso deixar de expressar minha gratidão aos professores e funcionários do programa de Programa de Mestrado em Saúde do Adulto (PPGSAD)pela sua dedicação e apoio contínuos ao longo deste processo. Minha família e amigos em especial a meu amigo e irmão *Leandro Lima* pelo apoio e encorajamento constantes. Que esteve do meu lado, oferecendo seu apoio emocional e compreensão durante os momentos de pressão e desafios. A minha Querida professora Drª*Luciane Brito* Escrevo estas palavras com um coração repleto de emoções mistas, pois, ao mesmo tempo em que celebro minha jornada no mestrado, também sinto a sua falta hoje, desejo expressar meu mais profundo agradecimento àquela pessoa que foi tão significativa em minha vida e que infelizmente nos deixou antes de testemunhar este momento. Você foi uma presença constante em minha vida, a sua sabedoria foram pilares fundamentais na minha jornada acadêmica e pessoal. Enquanto trilhava o caminho do mestrado, você foi minha confidente, minha conselheira e minha maior defensora. Ela me ajudou a enfrentar os desafios acadêmicos, encorajando-me a persistir mesmo quando os obstáculos pareciam intransponíveis. Sua inteligência e perspicácia eram inigualáveis, e eu valorizava imensamente suas opiniões e conselhos, sei que seu espírito está comigo a cada passo do caminho. Agradeço a Professora *Dra.*

Fernanda Ferreira Lopes que não me deixou desistir, sem palavras para agradecer. Ao professor ao *prof. Dr. Marcelo Souza de Andrade*, pela paciência e cuidado. A meu filho *Matheus Costa dos Santos* por suportar a minha ausência te amo filho. Mais uma vez, meu sincero agradecimento a todos os envolvidos no programa de mestrado. Vocês fizeram a diferença em minha vida acadêmica e estou honrado por ter feito parte dessa comunidade acadêmica excepcional.

RESUMO

Introdução e objetivo: A proteina Klotho, codificada pelo gene KL, faz parte do sistema endócrino FGF-Klotho e desempenha um papel na fisiopatologia de distúrbios relacionados ao envelhecimento, incluindo o câncer. Klotho é considerada um supressor tumoral e tem potencial para o tratamento do câncer. Foi descoberto que ela reduz a proliferação de células cancerígenas e induz a apoptose em vários tipos de câncer. O mecanismo molecular subjacente ao efeito supressor de tumores de Klotho não é bem compreendido. Klotho forma um complexo com FGFRs e é necessário para a ligação de alta afinidade dos FGFs aos seus receptores. Ela é expressa em vários tecidos e órgãos, com níveis mais elevados no rim. Klotho interage com vários membros da família Wnt e pode ter um papel no envelhecimento. Alterações na sinalização Wnt estão implicadas no desenvolvimento e progressão do câncer. Nesta dissertação se discute o papel das proteínas Klotho em vários tipos de câncer e seu potencial uso como marcadores diagnósticos e prognósticos. **Metodologia:** Trata-se de uma revisão sistemática, que analisou artigos publicados entre 2012 e 2022, de acordo com os Itens Preferenciais de Relatórios para Revisões Sistemáticas e Meta-análises (PRISMA). A estratégia de busca foi realizada em três bases de dados: PubMed, Scielo e ScienceDirect, empregando as palavras-chave "cancer AND Klotho", acessadas em abril de 2023. A busca resultou em 836 artigos, sendo que 401 eram duplicados. Após aplicar os critérios de inclusão e exclusão, 26 artigos fizeram parte desta revisão sistemática.

Resultados: Dentre os 26 artigos selecionados, 21 tratavam de αKlotho, 5 de βKlotho e apenas 3 de γKlotho, sendo um artigo estudando α e βKlotho e outro estudando todas as três proteínas. Encontrou-se que a αKlotho está consistentemente associada a uma melhora no prognóstico e pode ser útil para estimar a sobrevida do paciente com câncer. αKlotho parece atuar como um supressor de tumor e sua regulação negativa foi associada a fenótipos tumorais agressivos e pior prognóstico. Nível elevado de γKlotho está associado à agressividade do câncer e ruim prognóstico, podendo ser úteis para prever a sobrevida do paciente e a resposta à terapia. βKlotho apresenta resultados mistos. Conclusão: Enquanto o αKlotho foi associado a um melhor prognóstico do paciente, o γKlotho foi associado ao aumento da agressividade

do câncer e o βKlotho apresentou resultados mistos. É fundamental identificar com precisão os subtipos de tumores e a expressão do Klotho para aplicabilidade ao máximo do seu potencial biológico. No entanto, a maioria dos estudos ainda não apresentou resultados tão detalhados e o uso clínico do Klotho exigirá mais estudos futuros.
Palavras-chave: Proteínas Klotho; Câncer; Fator Prognóstico; Diagnóstico Molecular.

ABSTRACT

Introduction and objective: The Klotho protein, encoded by the KL gene, is part of the FGF-Klotho endocrine system and plays a role in the pathophysiology of agerelated disorders, including cancer. Klotho is considered a tumor suppressor and has potential for treating cancer. It has been found to reduce the proliferation of cancer cells and induce apoptosis in several types of cancer. The molecular mechanism underlying the tumor suppressive effect of Klotho is not well understood. Klotho forms a complex with FGFRs and is required for the high affinity binding of FGFs to their receptors. It is expressed in various tissues and organs, with highest levels in the kidney. Klotho interacts with several members of the Wnt family and may play a role in aging. Alterations in Wnt signaling are implicated in cancer development and progression. This dissertation discusses the role of Klotho proteins in various types of cancer and their potential use as diagnostic and prognostic markers. **Methodology:** This is a systematic review, which analyzed articles published between 2012 and 2022, according to the Preferred Reporting Items for Systematic Reviews and Metaanalyses (PRISMA). The search strategy was carried out in three databases: PubMed, Scielo and ScienceDirect, using the keywords "cancer AND Klotho", accessed in April 2023. The search resulted in 836 articles, 401 of which were duplicates. the inclusion and exclusion criteria, 26 articles were part of this systematic review. **Results:** Among the 26 articles selected, 21 dealt with αKlotho, 5 with βKlotho and only 3 with γKlotho, one article studying α and βKlotho and another studying all three proteins. αKlotho was found to be consistently associated with improved prognosis and may be useful for estimating cancer patient survival. αKlotho appears to act as a tumor suppressor and its down-regulation has been associated with aggressive tumor phenotypes and worse prognosis. Elevated γKlotho levels are associated with cancer aggressiveness and poor prognosis, and may be useful in predicting patient survival and response to therapy. βKlotho shows mixed results. **Conclusion:** While αKlotho was associated with a better patient prognosis, γKlotho was associated with increased cancer aggressiveness, and βKlotho had mixed results. It is essential to accurately identify tumor subtypes and Klotho expression for maximum applicability of its biological potential. However, most studies have not yet provided such detailed results and the clinical use of Klotho will require further studies in the future.

Key words: Klotho proteins; Cancer; Prognostic Factor; Molecular Diagnostics.

SUMÁRIO

1. INTRODUÇÃO

Klotho é uma proteina que em humanos é codificada pelo gene KL e as três subfamílias de klotho são α-klotho, β-klotho e γ-klotho, que são componentes essenciais dos complexos endócrinos com Fatores de Crescimento de Fibroblastos (FGF) formando o sistema endócrino FGF-Klotho, que desempenha papel na fiosiopatologia de distúrbios relacionados ao envelhecimento, incluindo diabetes, doença renal cônica, arteriosclerose e câncer. (KURO-O, 2019).

O câncer é considerado uma neoplasia maligna, ocorrendo de forma agressiva com alta taxa de incidência e altos percentuais de mortalidade. De acordo com dados do Sistema de Informação de Mortalidade (SIM) do Ministério da Saúde, no ano de 2014, o câncer se tornou a segunda maior causa de mortalidade no país, com mais de 190 mil óbitos por ano. (HU et al., 2013; MEDICI et al., 2018). No Brasil, a maioria desses casos de câncer são apresentados em estágios avançados, e os métodos de prevenção ainda são escassos, e a quantidade de pessoas que necessitam do tratamento possui a estimativa de tendência elevada (ZENG et al., 2019). Segundo pesquisa, nos anos de 2010, 60,5% dos casos foram diagnosticados em estágios de 3 e 4, e nesses respectivos estágios, os custos para o tratamento ficaram de 60% e 80% maiores que nos estágios 1 e 2, sendo que as possibilidades de cura são bem menores (TALLON et al., 2020).

Ademais, vale ressaltar que o crescimento relativo ao câncer não tem ocorrido só no Brasil, mas em nível global. Conforme a Sociedade Norte-Americana de Câncer (American Cancer Society), entre os anos de 2010 e 2030, o nível mundial aumentará de 14,6 para 20,2 milhões, tendo como maioria os casos de câncer em países desenvolvidos como o Brasil.

Klotho é considerado como um supressor de tumor, sendo uma proteína que possui agentes potencias para o tratamento e intervenções terapêuticas para o câncer (CHEN et al., 2010;QU et al., 2013). A superexpressão da Klotho proporciona redução da proliferação de células cancerígenas produzindo a indução de apoptose no fígado, pulmão, rim e câncer de cólon (CHEN et al., 201, 0; SHU et al., 2013).

Klotho foi originalmente identificado como um gene antienvelhecimento e também tem sido relacionado com o prognóstico de vários tipos de cânceres, incluindo os de carcinoma hepatocelular, carcinoma gástrico e células escamosas do

esôfagono entanto, o mecanismo molecular subjacente do efeito supressor tumoral de Klotho não está bem esclarecido (ZHOU et al., 2021).

2. REFERENCIAL TEÓRICO

O nome Klotho foi escolhido com base na história de Cloto e suas irmãs Láquesis e Átropos, que na mitologia grega deu origem ao propósito e destino de todos os seres humanos em suas vidas. Nesse caso, Cloto é a deusa responsável pela proteção da vida (WELC et al., 2020).

O gene Klotho codifica uma glicoproteína transmembrana com um único canal do tipo I chamado Klotho, que possui um processo extracelular desenvolvido por duas repetições internas (KL1 e KL2) e possui cerca de 450 aminoácidos. (KURO-O et al., 2021). Diante do exposto, embora Klotho não permita a presença de resíduos de glutamato conservados durante sua reprodução, estes são essenciais para a atividade catalítica, e estudos têm relatado as atividades de sialidase e βglicuronidase dessa proteína (EWENDT et al., 2020).

Klotho forma um complexo obrigatório com os receptores do fator de crescimento de fibroblastos (FGFR), este último é responsável principalmente pela ação homeostática (IDE et al., 2016). As proteínas Klotho, αKlotho e βKlotho, são componentes essenciais dos complexos receptores do fator de crescimento de fibroblastos endócrinos (FGF), pois são necessários para a ligação de alta afinidade de FGF19, FGF21 e FGF23 aos seus receptores FGF (FGFRs). Klotho é necessário para que o FGF se ligue ao FGFR com alta afinidade, e é o complexo binário de Klotho e FGFR que funciona como o receptor fisiológico do FGF (KURO-O, 2019).

Além de serem reconhecidas na membrana plasmática, as proteínas Klotho foram encontradas no complexo de Golgi, e são expressas em múltiplos tecidos e órgãos sendo mais prevalentes no rim, onde são encontradas em níveis elevados, principalmente em túbulos contorcidos terminais distantes e túbulos proximais (ONISHI et al., 2020).

A proteína Klotho que foi identificada como o co-receptor FGF23 ficou conhecida como αKlotho, codificada pelo αKlotho gene, também conhecido como KL. (KUROSU et al, 2007). O FGF23 também atua na reabsorção renal de Ca2+ via

TRPV5, um receptor transitório para canais catiônicos, ativando e mediando a ação desse receptor. (WANG et al., 2021). O FGF23 se liga ao complexo αKlotho-FGFR e medeia vários processos fisiológicos para manter a homeostase do fosfato e do cálcio, principalmente regulando a função dos rins e das glândulas paratireoides, sendo que a interrupção do eixo endócrino FGF23-αKlotho tem um papel crucial na fisiopatologia dos distúrbios renais e ósseos. (KURO-O, 2019).

αKlotho é uma proteína transmembrana que consiste em 1012 aminoácidos e é expressa em células epiteliais renais tubulares distais e proximais, sendo que o gene αKlotho é abundantemente expresso em rins, glândulas paratireóides, plexo coróide, bem como no córtex cerebral, medula espinhal, cerebelo, hipotálamo, glândulas pituitárias, ovário, testículos, células epiteliais do seio, placenta, pâncreas, endotélio vascular do músculo liso e intestinal (TROCHEL et al., 2021).

As citocinas pró-inflamatórias, como IL-1, IL-6 e fator de necrose tumoral, podem induzir a expressão de FGF23 por meio da ativação de NF-κB (fator nuclear kappa B), que desempenha funções como fator de transcrição na regulação da resposta imunitária, via de sinalização da inflamação (LAWRENCE, 2009). Quaisquer fatores que aumentam a expressão de FGF23, incluindo aumento da ingestão de fosfato, podem indiretamente diminuir a expressão de αKlotho in vivo, portanto o FGF23 é considerado um potente regulador negativo da expressão de αKlotho (KURO-O, 2019). Estudos evidenciam o possível envolvimento de αKlotho em muitas vias, como a reabsorção de fosfato no rim, o metabolismo da vitamina D e a regulação dos níveis intra e extracelulares de Ca^{2+}. (PESHES-YELOZ et al., 2019).

Três Klothos formam um complexo com os receptores do fator de crescimento de fibroblastos (FGFRs), proporcionando assim a afinidade de ligação seletiva dos FGFRs aos FGFs endócrinos, sendo que outros membros da família Klotho são βKlotho e γKlotho (KIM et al., 2015).

βKlotho é composto por um domínio semelhante a β-glicosidase (domínio KL1 e 2) é uma proteína transmembrana de passagem única, amplamente expressa em vários tecidos, como tecido adiposo, pâncreas e intestino, para regular a glicose e o metabolismo lipídico (Hou et al., 2022). A proteína homóloga βKlotho é codificada pelo βKlotho gene; também conhecido como KLB. βKlotho forma complexos binários com FGFR1c (expresso por adipócitos) e FGFR4 (expresso por hepatócitos), que

funcionam como receptores fisiológicos para FGF21 e FGF19, respectivamente (KUROSU et al, 2007).

γKlotho, uma proteína transmembrana de passagem única tipo 1 mais curta, é feita de um domínio extracelular semelhante à glicosidase (domínio KL1) e um domínio intracelular curto, sendo que é altamente expressa no rim e na pele (ITO et al, 2002). γKlotho é expressa em tecido adiposo e o olho e pode funcionar como um co-receptor adicional para FGF19, formando complexos com FGFR1b, FGFR1c, FGFR2c e FGFR4, no entanto, a função biológica de γKlotho permanece indefinida (KIM et al., 2015).

Klotho também faz interação com vários membros da família Wnt, sendo que animais com deficiência de klotho mostraram aumento da sinalização de Wnt, tanto in vitro como in vivo, e a exposição contínua a Wnt desencadeou a senescência celular acelerada. Assim, klotho parece ser um antagonista Wnt secretado e as proteínas Wnt têm um papel no envelhecimento dos mamíferos (Liu et al, 2007).

Considera-se que alteração na sinalização Wnt é um fator chave na iniciação e/ou manutenção e desenvolvimento de muitos cânceres, afetando o comportamento das células-tronco cancerígenas, que são consideradas como responsáveis pelo estabelecimento do tumor e também pela recidiva da doença, portanto o desenvolvimento de novos compostos terapêuticos direcionados à via de sinalização Wnt prometem ser uma nova esperança para terapia oncológica (Duchartre et al., 2016).

Ao estudar a relação de klotho com a via Wnt em carcinoma hepatocelular humano, TANG et al. (2016) observaram que os níveis de expressão de klotho foram significativamente menores no câncer do que em tecidos não cancerosos adjacentes, sendo que os pacientes com tumores que expressavam klotho tiveram períodos de sobrevida mais longos do que aqueles com tumores klotho-negativos. (ABOLGHASEMI et al., 2019).

Klotho é um gene antienvelhecimento que recentemente demonstrou estar envolvido no desenvolvimento de tumores hepáticos humanos. Sabe-se que a sinalização Wnt é antagonizada pelas proteínas klotho, sendo que a sinalização Wnt desempenha um papel importante na tumorigênese do carcinoma hepatocelular (CHC) humano (PESHES-YELOZ et al., 2019). Klotho exibe múltiplas funções, que

incluem a melhora do estresse oxidativo e a inibição de vias de sinalização do fator de crescimento da insulina, Wnt/β-catenina (TROCHEL et al., 2021).

Klotho dispõe de múltiplas atividades pleiotrópicas, contendo a inibição das basilares ferramentas de sinalização, redução do estresse oxidativo e supressão da inflamação, sendo que essas atividades estão fortemente relacionadas ao câncer (LIGUMSKY et al., 2022.) Portanto, o papel de Klotho como um gene supressor de tumor tem sido estudado desde a década de 1960, sendo importante na progressão do câncer, na mitose e na previsão do prognóstico do paciente, associado a muitos tumores hematológicos e sólidos. (GUNES et al., 2021).

3. OBJETIVOS

Objetivo Geral

Realizar uma revisão sistemática sobre o papel das proteínas Klotho em vários tipos de câncer e seu potencial uso como marcadores diagnósticos e prognósticos**.**

Objetivos Específicos

- Demonstrar o papel das proteínas αKlotho, βKlotho e γKlotho em vários tipos de câncer e seu potencial uso como marcador diagnóstico.
- Identificar o papel das proteínas αKlotho, βKlotho e γKlotho em vários tipos de câncer e seu potencial uso como marcador prognóstico.

4. ARTIGO

Artigo científico a ser submetido para a Revista ***Pharmaceuticals*** (ISSN: 14248247) Impact Factor: 4.6 (2022); 5-Year Impact Factor: 4.9 (2022) – Qualis A1.

Review

Klotho in cancer: a systematic review.

Jucileide Mota[1], Alice Marques Moreira Lima[2], Marcelo Souza de Andrade[1], Haissa O. Brito[1], Fernanda F. Lopes[1] Paula A. Oliveira[3,4], and Rui M. Gil da Costa [1,3,5,6,7,8]*

[1] Post-Graduate Programme in Adult Health (PPGSAD), Federal University of Maranhão, São Luís 65085580, Brazil; haissa.brito@ufma.br; rui.costa@ufma.br; marcelo.andrade@ufma.br; fernanda.ferreira@ufma.br [2] State University of the Tocantins Region of Maranhão (UEMASUL), Imperatriz 6591-480, Brazil; alice_mmlima@outlook.com

[3] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

[4] Inov4Agro—Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

[5] Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[6] Associate Laboratory in Chemical Engineering, Faculty of Engineering (ALiCE), University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[7] Molecular Oncology and Viral Pathology Group, Portuguese Oncology Institute of Porto (IPO Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

[8] Health Research Network, Research Center of Portuguese Oncology Institute of Porto (CI-IPOP/RISE@CIIPOP), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

* Correspondence: rui.costa@ufma.br

Abstract: Klotho proteins, αKlotho, βKlotho and γKlotho exert tumor suppressive activities via the fibroblast growth factor receptors and multiple cell signaling pathways. There is a growing interest in Klotho proteins as potential diagnostic and prognostic biomarkers for multiple diseases. However, recent advances regarding their roles and potential applications in cancer remain disperse and require systematic analysis. The present review analyzed research articles published between 2012 and 2022 in the Cochrane and Scopus scientific databases, to study the role of Klotho in cancer and their potential as tools for diagnosing specific cancer types, predicting tumor aggressiveness and prognosis. Twenty-six articles were selected, dealing with acute myeloid leukemia and with bladder, breast, colorectal, esophageal, gastric, hepatocellular, ovarian, pancreatic, prostatic, pulmonary, renal and thyroid cancers. αKlotho was consistently associated with improved prognosis and may be useful to estimate patient survival. A single study reported the use of soluble αKlotho levels in blood serum as a tool to aid the diagnosis of esophageal cancer. γKlotho was associated with increased aggressiveness of bladder, breast and prostate cancer and βKlotho showed mixed results. The further clinical development of Klotho-based assays will require careful identification of specific tumor subtypes where Klotho proteins may be most valuable as diagnostic or prognostic tools.

Keywords: liquid biopsy; cancer; klotho; prognosis; diagnosis

1. Introduction

The Klotho proteins, alpha(α)Klotho [1-2] and beta(β)Klotho [3] are encoded by the *KLA* and *KLB* genes located in chromosomes 4 and 13, respectively. αKlotho was originally identified in mice and elicited great interest due to its anti-ageing properties [1]. It is expressed in a variety of tissues and is located in the cell membrane as a type I single-pass 135 kDa protein containing an N-terminal sequence, two extracellular domains (designated KL1 and KL2) with glycosidase activity, a transmembrane helix and an intracellular domain consisting of only 10 aminoacids [2]. The αKlotho protein is also present in blood as a secreted protein generated by alternative mRNA splicing containing the KL1 domain only [1] and as a soluble protein that may contain KL1 alone or both the KL1 and Kl2 extracellular domains [4]. Cleavage of the αKlotho extracellular domains is mediated by a disintegrin and metalloproteinase domain-containing (ADAM) proteins ADAM10 and ADAM17 [4]. The βKlotho protein shares structural similarities with αKlotho and is also located in the cell's plasma membrane [3, 5], and soluble βKlotho has also been reported [6]. Another membrane-bound glycosidase-like protein, designated Klotho-lactase phlorizin hydrolase was first identified in mice and is encoded by the *LCTL* gene on chromosome 15 in humans [7]. The functions of this protein, also referred to as γKlotho, are less clear than those of αKlotho and βKlotho.

αKlotho binds to FGR receptors acting as a co-receptor for FGF23 and playing a key role on the renal regulation of phosphate levels [8-9]. βKlotho acts as a co-receptor for fibroblast growth factor 19 and 21 (FGF19 and FGF21), by forming binary complexes with FGFR4 and FGFR1c, respectively [10-12]. Binding of βKlotho with FGFR1c in adipose tissue or FGFR4 in the liver and with endocrine ligands FGF21 and FGF19 triggers multiple intracellular responses, as previously reviewed [13]. Canonically, the binding of FGF21 to the βKlotho-FGFR1c complex activates ERK1/2 downstream signaling and regulates the synthesis of biliary acids in hepatocytes, while FGF19 binds to βKlothoFGFR4 complexes to downregulate Cyp17a1, also regulating hepatic bile production [11-15].

Loss of αKlotho has been consistently linked with chronic kidney disease and phosphate metabolism dysfunction [16-17]. αKlotho downregulation was also associated with pleiotropic effects associated with aging [1, 13] and is proposed to act as a tumor suppressor, as recently reviewed [18]. Interestingly, βKlotho has been associated with both tumorigenic and tumor-suppressive effects in different types of cancer, suggesting a more complex scenario with multiple contextspecific activities [19-21]. γKlotho expression has also been studied in multiple types of cancer [22-23]. Accumulating data suggests that the tissue expression of Klotho proteins and, especially, the detection and quantitation of their soluble forms in body fluids like blood serum may be useful for establishing the diagnosis and prognosis of some types of cancer [6, 24-25]. The present review aims to systematically analyze scientific data regarding the role of Klotho proteins in cancer and to retrieve information regarding their potential use as diagnostic and prognostic biomarkers.

2. Materials and Methods

The systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [61] according to the following parameters: population: cancer patients, *in vivo* and *in vitro* cancer models. Intervention: expression of Klotho genes. The search strategy contemplated three standard databases on biomedicine: PubMed, Scielo, and ScienceDirect, accessed in April 2023. The keywords "cancer AND Klotho" were applied. The following inclusion criteria were established concerning: type of study (case series and case–control studies in humans; experimental *in vitro* and *in vivo* studies) and outcomes (effects of Klotho gene products in cancer). Exclusion criteria were lack of clear definition of cancer type or controls, lack of Klotho gene product quantification, case reports, review articles, commentaries, hypothesis and meta-analyses, languages other than English. The abstracts and, when necessary, the materials and methods, were analyzed to apply inclusion and exclusion criteria.

3. Results

Most publications were excluded due to duplication between databases, or by applying exclusion criteria. Many articles dealt with other pathologies where Klotho proteins are thought to play significant roles, most prominently renal diseases. Overall, after applying inclusion and exclusion criteria, 26 articles were selected for further analysis (Figure 1). Fourteen of the articles were published in the first half of the 10-years period included in this systematic review (2012-2017) (Table 1). Most studies used *in vitro* and/or clinical observational approaches, with only 7 articles using *in vivo* studies with animal models. Clinical observational studies often described the expression of Klotho genes at the RNA and/or protein levels and provided correlations between these marker's expression levels and relevant clinical parameters. Remarkably, none of the clinical studies adopted an interventional approach. *In vitro* studies provided insights into the regulation of Klotho protein's expression and their effects in cancer cell. Among the 26 selected articles, 21 dealt with αKlotho, 5 with βKlotho and only 3 with γKlotho, with one article studying α and βKlotho and another studying all the three proteins.

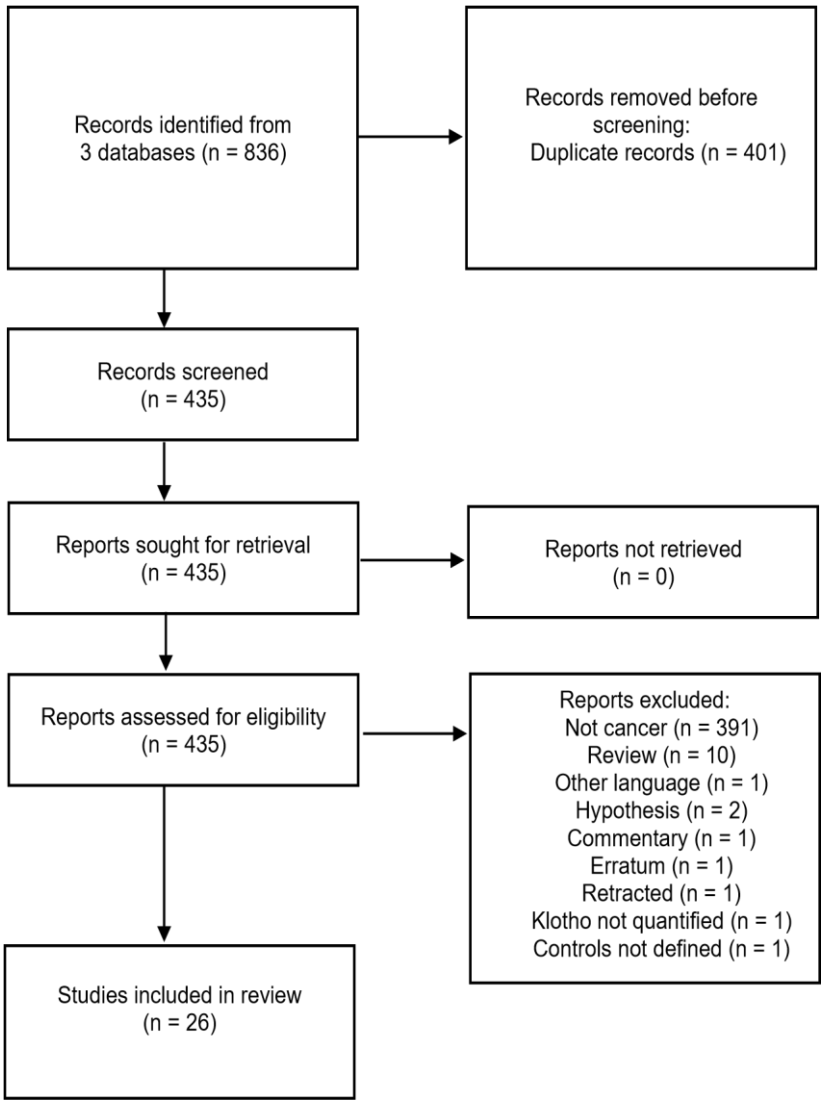

Figure 1. Systematic review of Klotho in cancer and resulting publications for analysis.

Table 1. Characteristics of the 26 articles included in the systematic review.

Reference	Year	Type of Cancer	*In vitro*	*In vivo*	Clinical (observational)
[26]	2012	Hepatocellular carcinoma	x		x
[27]	2013	Prostate cancer	x		x
[28]	2013	Lung cancer	x	x	
[29]	2013	Hepatocellular carcinoma	x		x
[30]	2013	Renal cell carcinoma	x		x
[31]	2015	Renal cell carcinoma			x
[32]	2015	Ovarian cancer	x		x
[33]	2015	Acute myeloid leukemia	x		

[34]	2015	Hepatocellular carcinoma	x		
[22]	2015	Breast cancer	x		x
[35]	2016	Thyroid cancer	x		
[36]	2016	Esophageal cancer			x
[37]	2017	Pulmonary squamous cell carcinoma	x		x
[38]	2017	Ovarian cancer	x	x	x
[39]	2018	Pancreatic adenocarcinoma			x
[23]	2018	Bladder cancer	x	x	x
[40]	2019	Large cell neuroendocrine lung cancer			x
[41]	2019	Colorectal cancer	x	x	x
[42]	2020	Prostate cancer		x	x
[43]	2020	Colorectal cancer	x		
[44]	2020	Gastric adenocarcinoma	x		
[45]	2021	Colorectal cancer	x		
[46]	2021	Pancreatic cancer		x	x
[47]	2021	Gastric cancer	x		
[48]	2022	Hepatocellular carcinoma	x	x	
[49]	2022	Colorectal cancer	x		

The main findings of the 21 articles addressing αKlotho in cancer are summarized in Table 2. Four studies were focused on colorectal cancer, another three on lung cancer, two on hepatocellular carcinoma, two on ovarian cancer, two on renal cell carcinoma, and two on gastric cancer. Prostate cancer, acute myeloid leukemia, thyroid cancer, esophageal cancer, breast cancer and pancreatic cancer were each studied by a single article. αKlotho was generally found to act as a tumor suppressor and its downregulation was consistently associated with aggressive tumor phenotypes and worse prognosis. Importantly, the quantitation of αKlotho levels on tumor tissues using immunohistochemistry (IHC) was of prognostic significance in multiple types of cancer. Soluble αKlotho can be quantified in blood serum using ELISA and αKlotho levels were also suggested to have diagnostic and prognostic value for esophageal and renal cancer, respectively. *KLA* promoter methylation and mRNA expression levels by quantitative real-time PCR (qRT-PCR) were also reported to have prognostic value in prostate cancer, hepatocellular carcinoma and pancreatic cancer.

Table 2. Studies dealing with αKlotho.

Cancer type	Reference	Type of sample	Main findings	Potential applications
Prostate cancer	[27]	Frozen and FFPE cancer tissues. PC3, DU145, VCaP, LNCaP cancer cell lines, PNT1a normal prostate cells.	*KLA* gene expression detected in all cell lines by qRT-PCR and FGF19 stimulates PCa cells in vitro. αKlotho detected by IHC in 50% primary and 90% metastatic PCa samples.	Screening of patients who may benefit from anti-FGFR therapies and may using IHC on tumor tissues.
Lung cancer	[28]	A549 and H460 tumor cells and xenografts.	αKlotho downregulation promotes cisplatin resistance *in vitro* and *in vivo*.	
	[37]	FFPE cancer tissues (centrally located early lung cancer and SCC), only in 13% SCC lines. A549 and SQ5 tumor cell lines.	αKlotho expressed in 100% centrally located early lung cancer samples but N-cadherin expression *in vitro*.	
	[40]	FFPE cancer tissues and prognosis (survival).	Tissue expression (large cell neuroendocrine lung cancer) may predict	αKlotho expressed in ¾ patients associated with survival.
Hepatocellular carcinoma	[29]	Frozen and FFPE tumor and adjacent tissues. HRPG2, BEL-7402, SMMC-7721, HL7702, HUH-7, MHCC-97-H cancer cell lines and L-02 hepatocytes.	αKlotho is downregulated at mRNA and protein levels in HCC versus adjacent tissue; promoter methylation and reduced protein expression correlate with reduced survival.	αKlotho promoter methylation and protein expression may predict prognosis (survival)
	[34]	HepG2 and SMMC-7721 cancer cell lines, L-02 hepatocytes.	Recombinant αKlotho downregulates Wnt/β-catenin signaling suppressing proliferation and inducing apoptosis.	
Renal cell carcinoma	[30]	786-O, OS-RC-2, ACHN, Caki-1 and Renca cancer cell lines. Tumor tissue.	αKlotho tissue expression (IHC) inversely correlated with tumor size, TNM stage and nuclear grade. *In vitro* blocked EMT via PI3K/Akt/GSK3 β/Snail.	Potential IHC marker of tumor aggressiveness.
	[31]	Frozen tumor and adjacent tissue (clear cell RCC). Preoperative blood serum.	αKlotho is downregulated in tumor tissue at RNA (qRT-PCR) and protein (IHC) levels. Reduced serum levels (ELISA) associated with higher tumor volume, Fuhrman grade, clinical stage, reduced cancer-specific survival and progression-free survival.	Serum αKlotho levels using ELISA may predict prognosis including survival.

Ovarian cancer	[32]	Tumor (high-grade papillary-serous adenocarcinoma) and adjacent tissues (IHC) and in 16/19 cell lines (qRT-PCR).	αKlotho was reduced in tumor versus adjacent ovarian tissues. 19 cancer cell lines.	
	[38]	FFPE and frozen tumor and adjacent tissues. 7 cancer cell lines.	αKlotho was reduced in tumor versus adjacent tissues (IHC). Reduction correlates with low survival. Tumor xenografts expressing αKlotho had lower size. *KLA*$^{-/-}$ mice showed higher IL-6 levels in response to xenografts.	Tissue expression using IHC may predict survival.
Acute myeloid leukemia	[33]	KG-1 cells	Exposure to miR-126-5p decreased αKlotho levels, induced Akt phosphorylation and cytarabine resistance.	αKlotho may predict cytarabine resistance
Breast cancer	[22]	Frozen tumor and adjacent tissues. MDAMB-231 and H357T cancer cell lines.	αKlotho was downregulated in cancer versus adjacent tissue. Undetectable in both cell lines.	
Follicular thyroid carcinoma	[35]	FTC133 and FTC238 cancer cell lines	αKlotho reduced cell proliferation and induced apoptosis *in vitro*.	
Esophageal cancer	[36]	FFPE cancer and adjacent tissues. Blood serum from patients/controls.	αKlotho was downregulated in cancer versus adjacent tissue (IHC). Correlates with improved survival, inversely correlated with staging, grade, lymph node metastasis, β-catenin. Serum levels higher in patients versus controls.	Tissues levels (IHC) may predict prognosis including survival. Serum 327 pg/mL cut-off (ELISA) is diagnostic with sensitivity 81% and specificity 81%.
Colorectal cancer	[41]	FFPE tumor tissue. RKO and LoVo cancer cell lines, Wi-38 and HUVEC cells.	Lower αKlotho (IHC) associates with lower patient survival. αKlotho prevents pro-tumorigenic effects of senescent cells *in vitro* and *in vivo* via NFκB/CCL2 blockade.	Tissues levels (IHC) may predict survival.
	[43]	Six cancer cell lines and normal cells.	FL-1 regulates αKlotho expression in cancer cells.	cancer
	[45]	CaCo-2 cells	αKlotho induces apoptosis via TRAIL death receptor.	
	[49]	HT29 cancer cell line, CCD841 cells.	αKlotho induces apoptosis specifically in cancer cells	cancer
Gastric cancer	[44]	6 cancer cell lines and normal cells	SOX17 regulates αKlotho expression in cancer cells *in vitro*.	cancer
	[47]	HGC-27, AGS, MKN-45, HE-293-T cancer cell lines, GES-1 cells	Circular RNA ITCH upregulates αKlotho by sponging out miR-199-5p, inhibiting cell proliferation, migration, invasion and EMT	MGC-803,

Pancreatic cancer	[46]	TCGA pancreatic ductal adenocarcinoma datasets, 3 mouse models	Promoter methylation and mRNA downregulation associated with reduced survival. αKlotho knockdown synergized with Kras mutation to promote carcinogenesis. Soluble αKlotho inhibited xenograft growth and promoted survival of KPC mice.	Methylation and expression levels may predict survival.

The 5 articles focused on βKlotho are addressed in Table 3, which summarizes their main findings. Two articles dealt with hepatocellular carcinoma, while prostate cancer, breast cancer and pancreatic adenocarcinoma were studied in one article each. In hepatocellular carcinoma, βKlotho was proposed to mediate tumor aggressiveness via FGFR signaling and may be useful in selecting patients who may benefit from anti-FGFR therapies. A similar scenario was suggested by the single study focused on prostate cancer. Conversely, in breast and pancreatic cancers, βKlotho was proposed to act as tumor suppressor.

Table 3. Studies dealing with βKlotho.

Cancer type	Reference	Type of sample	Main findings	Potential applications
Hepatocellular carcinoma	[26]	Tumor and adjacent tissue in Trizol	*KLB* gene expression is upregulated in cancer tissues. A >2-fold increase correlates with development of multiple lesions.	Screening of patients who could benefit from anti-FGFR therapies. Prediction of lesion multiplicity.
	[48]	Cell lines and xenograft mouse model	βKlotho mediates FGF9 pro-survival functions via FGFR3 and FGFR4. Inhibiting βKlotho was more effective than inhibiting FGFR4.	Screening of patients who could benefit from anti-FGFR therapies.
Prostate cancer	[27]	Frozen primary tumor tissue, FFPE metastases. PC3, DU145, VCaP, LnCaP cancer cell lines, PNT1a cells.	KLB gene expression observed with qRT-PCR in DU145 and VCaP only and FGF19 showed stimulatory effects. βKlotho was detected in a majority of primary and metastatic lesions using IHC.	βKlotho IHC may be useful for screening patients that could benefit from antiFGFR therapy.
Breast cancer	[22]	Frozen tumor and adjacent tissue. MDAMB-231 and HS578T cancer cell lines.	βKlotho was downregulated in cancer versus normal tissues and was undetectable in both cell lines, suggesting tumor suppressor role.	
Pancreatic adenocarcinoma	[39]	Gene expression data from Gene Expression Omnibus database.	High *KLB* mRNA expression is associated with increased overall survival.	*KLB* gene expression may be useful to predict patient survival.

γKlotho was studied in three articles, summarized in Table 4. Breast, prostate and bladder cancers were studied in one article each. All three articles found that higher γKlotho expression is associated with cancer aggressiveness and poor prognosis, suggesting that γKlotho levels assessed at the mRNA or at the protein level may be useful to predict patient survival and response to therapy.

Table 4. Studies dealing with γKlotho.

Cancer type	Reference	Type of sample	Main findings	Potential applications
Breast cancer	[22]	Frozen tumor and adjacent tissue. MDA-MB-231 and HS578T cancer cell lines.	*LCTL* gene expression is upregulated in cancer versus normal tissues, especially in triple-negative lesions, using qRT-PCR, correlating with increased cell proliferation, histological grade, TNM stage and reduced progression-free survival.	*LCTL* gene expression using qRT-PCR may be useful to predict patient survival.
Prostate	[42]	FFPE tumor tissue from castration-resistant prostate cancer and cell lines.	Higher γKlotho expression observed by IHC in tumor tissue correlates with reduced overall survival and poor response to docetaxel in patients and in a mouse xenograft model.	γKlotho IHC may predict overall survival and response to docetaxel in castration-resistant prostate cancer.
Bladder cancer	[23]	FFPE pre-treatment tumor tissue. UMUC3, MGH-U3 and J82 cells.	Higher γKlotho expression observed by IHC in muscle invasive versus non-muscle-invasive lesions. In non-muscle-invasive lesions γKlotho levels correlated with poor progression-free survival.	γKlotho IHC may predict overall survival in patients with non-muscle invasive bladder cancer.

3. Discussion

The three Klotho proteins have complex roles in different types of cancer. The role of γKlotho is less well defined than that of its related Klotho proteins, partially because of its unusual molecular structure and also because it was discovered more recently. The present review

systematic organized data from scientific articles published between 2012 and 2022, regarding the roles of Klotho proteins in cancer and their potential use as diagnostic and prognostic tools.

The role of all three proteins was studied in prostate cancer. This is a highly prevalent disease in middle aged to older men, which usually develops as an androgen dependent adenocarcinoma, but may progress to an androgen-independent castration-resistant phenotype and to a small-cell neoplasia, often displaying neuroendocrine markers, which are associated with poor patient prognosis [50]. αKlotho and βKlotho expression was detected in prostate cancer cell lines representing prostate adenocarcinoma and small cell carcinoma, as well as in tumor tissues from primary tumors and metastasis, where they seem to mediate FGFR signaling [27]. It was further suggested that IHC tests for detecting αKlotho and βKlotho in tumor tissue may be of use to predict response to anti-FGFR therapies [27]. γKlotho expression in castrationresistant prostate cancer was associated with reduced survival and resistance to docetaxel [42], which is used as chemotherapy for such advanced cases [51]. Taken together, these results suggest that the immunoexpression patterns of Klotho proteins on prostate cancer tissues may be a valuable tool for tailoring treatment regimens for specific patients.

Lung cancer is also a common and aggressive malignancy, which includes multiple subtypes with distinct biological behavior [52]. Loss of αKlotho expression was consistently associated with increased tumor aggressiveness in three studies using *in vitro* and *in vivo* models [28] and clinical observational studies of neuroendocrine tumors [40], early centrally-located cancers and squamous cell carcinomas [37]. The observation that αKlotho may predict survival in patients with large cell neuroendocrine lung cancer is of particular interest, as it suggests that this marker has prognostic value in this specific lung cancer subtype [40]. Additionally, limited *in vivo* and *in vitro* data suggests that αKlotho downregulation may predict resistance to cisplatin-based chemotherapy [28], but additional studies are required to confirm this hypothesis. Hepatocellular carcinoma is the most common type of liver cancer [53]. While αKlotho was reported to act as a tumor suppressor [29, 34], βKlotho showed oncogenic activity via enhanced FGFR signaling [26, 48]. Importantly, αKlotho gene promoter methylation and protein expression may be if use as prognostic markers to estimate patient survival [29], while βKlotho may be a useful marker to predict response to anti-FGFR therapies [26].

In renal cell carcinoma, αKlotho downregulation was also reported to act as a tumor suppressor, and its loss was associated with tumor aggressiveness [30, 31]. Of particular interest is the use of ELISA tests to detect soluble αKlotho in blood serum samples, as reduced levels of this protein were significantly associated with the of patients with the clear cell subtype of RCC [31]. These findings suggest that such tests may be used in liquid biopsies to help establishing the prognosis of specific RCC patient subgroups.

Ovarian cancer is a frequent malignancy in women [54], and αKlotho was reported to act as a tumor suppressor in this type of cancer using experimental and clinical approaches [32, 39]. Importantly, one study suggested that reduced αKlotho immunoexpression in cancer tissues may be useful as a prognostic marker to predict poor patient survival [39]. The same study reported that αKlotho associated with higher interleukin-6 (IL-6) circulating levels. IL-6 is a pro-inflammatory cytokine that mediates

some paraneoplastic syndromes like cancer cachexia [55], so it is interesting to speculate that αKlotho expression levels may also be used to predict the development of such syndromes.

In acute myeloid leukemia, loss of αKlotho was reported to associate with cytarabine resistance *in vitro*, suggesting its possible use as a tool to design tailored therapies for leukemia patients [33]. Additional studies are needed to test this hypothesis, as cytarabine remains an important drug for treating this type of leukemia [56].

Breast cancer is highly prevalent in women and is often lifethreatening [57]. In one study, αKlotho and βKlotho were downregulated in tumor tissue versus adjacent tissue, suggesting they act as tumor suppressors [22]. Conversely, higher γKlotho (*LCTL*) gene expression levels using qRT-PCR were found in cancer versus adjacent tissue, specifically in the aggressive triple-negative cancer subtype [22, 58], suggesting it is associated with tumor aggressiveness. Interestingly, it was suggested that qRT-PCR for *LCTL* may be useful as a prognostic marker to estimate patient survival in patients with triple-negative breast cancer [22].

In papillary thyroid cancer, a single study [35] reported that αKlotho was able to reduce cell proliferation and induce apoptosis in vitro. The potential use of this protein for diagnostic and prognostic purposes in thyroid cancer remains to be determined.

In esophageal cancer, an interesting study [36] reported that the levels of soluble αKlotho in blood serum as detected by ELISA were higher in patients versus healthy controls. A cut-off value was estimated that allowed researchers to distinguish between patients and controls with approximately 81% sensitivity and specificity. Interestingly, in tissue samples, αKlotho was expressed at higher levels in adjacent versus tumor samples, and αKlotho down-regulation correlated with increased tumor aggressiveness and reduced patient survival. These data highlight the potential of αKlotho as a marker in liquid biopsies for the diagnosis of esophageal cancer, while tissue levels may have prognostic significance.

Colorectal cancer is highly prevalent in multiple world regions and large bowel carcinogenesis is associated with chronic inflammation [59]. In this type of cancer, 4 studies consistently reported that αKlotho acts as a tumor suppressor [41, 43, 45, 49]. *In vitro* tests revealed new regulatory pathways that control αKlotho expression via FL-1 [43] and support the pro-apoptotic role of αKlotho via TRAIL [45]. Interestingly, one study described how αKlotho downregulation promotes a senescenceassociated secretory phenotype in mesenchymal cells that may contribute to tumorigenesis via the nuclear factor kappa-light-chainenhancer of activated B cells (NFκB) signaling pathway [41]. This is a pivotal mediator of inflammation and tissue repair, but also of carcinogenesis in specific settings. Chronic inflammation is a key player in colon cancer and the secretion of NFκB-controlled C-C motif chemokine ligand 2 (CCL2) by senescent stromal cells was proposed to promote carcinogenesis of the colon. αKlotho abrogated CCL2 signaling and was associated with improved patient survival, suggesting it may be of use as a prognostic marker.

Two *in vitro* studies addressed the role of αKlotho in gastric cancer, further associating αKlotho downregulation with aggressive cancer phenotypes [44, 47]. SOX17 and an epigenetic pathway involving circular RNA ITCH and miR-199-5p were shown to regulate αKlotho expression in gastric cancer cells. While these findings support the role of αKlotho as

a tumor suppressor, further developments are needed to explore its potential role as a diagnostic or prognostic marker in gastric cancer.

A single study addressed the role of αKlotho in pancreatic adenocarcinoma, and concluded that *KLA* gene expression levels and promoter methylation may have prognostic value, as increased *KLA* promoter methylation and decreased mRNA expression levels were associated with lower patient survival [46]. This was further supported by tests in three complementary mouse models, where αKlotho decreased cancer growth and improved survival. Another study using expression data from the GEO database also suggested that *KLB* upregulation is associated with improved survival in pancreatic cancer patients [39]. Taken together, these data provide evidence to support the further development of Klotho as prognostic markers in pancreatic adenocarcinoma.

Urothelial carcinoma of the urinary bladder is a common malignancy that includes highly aggressive forms which invade the bladder's muscular layer, and non-muscle invasive forms associated with local recurrence [60]. One study reported that γKlotho expression was observed in both muscle-invasive and non-muscle invasive bladder cancer using IHC, and that expression levels were associated with poor overall survival among patients with non-muscle-invasive cancer [23].

4. Conclusions

Overall, the data sets published between 2012 and 2022 provide evidence supporting the development of klotho genes and their mRNA and protein products as potential prognostic markers. in multiple types of cancer, especially in the prediction of patient survival. While αKlotho was consistently associated with improved patient prognosis, γKlotho was associated with increased cancer aggressiveness and βKlotho showed mixed results. It is critical to accurately identify specific tumor subtypes where Klotho is of interest (muscle-invasive versus non-muscle invasive urothelial carcinoma) to take most advantage of its potential. The use of klotho levels as diagnostic markers was less frequently observed in the literature, although one study provided detailed data regarding soluble αKlotho levels in blood serum and the diagnosis of esophageal cancer. However, most studies still did not present such detailed results and the clinical use of Klotho will require additional development.

Author Contributions: Conceptualization, R.G.C. and H.O.B.; methodology, M.S.A., A.M.M.L.; validation, F.F.L., investigation, J.M. and L.L.S.; resources, P.A.O., R.G.C..; data curation, M.S.A. and F.F.L.; writing—original draft preparation, R.G.C.; writing—review and editing, H.O.B.; M.S.A. and F.F.L.; supervision, F.F.L. and R.G.C..; funding acquisition, M.S.A., P.A.O. and H.O.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAPES - finance code 001 and grant 13/2020 - PDPG Amazônia Legal 0810/2020/88881.510244/2020-01, by grant IECT-FAPEMA-05796/18 and FAPEMA IECT 30/2018 - IECT Saúde, grant PPSUS-02160/20 financed by FAPEMA, CNPq and the Brazilian Ministry of Health, by the Research Center of the Portuguese Oncology Institute of Porto (project no. PI86-CI-IPOP-66-2017); by European Investment Funds by FEDER/COMPETE/POCI - Operational Competitiveness and Internationalization Program, and national funds by FCT - Portuguese Foundation for Science and Technology under projects UID/AGR/04033/2020,

UIDB/CVT/00772/2020. This work was also supported by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), 2SMART (NORTE-01-0145-FEDER000054), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Rui Gil da Costa received a FAPEMA postdoctoral grant (BPD-01343/23).

Data Availability Statement: the data produced in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

5. CONCLUSÕES

Klotho e suas proteínas parecem desempenhar papel como potenciais marcadores de prognóstico em vários tipos de câncer. Enquanto, o αKlotho foi consistentemente associado a um melhor prognóstico do paciente, o γKlotho foi associado ao aumento da agressividade do câncer e o βKlotho apresentou resultados mistos. É fundamental identificar com precisão os subtipos de tumores e a expressão do Klotho para aplicabilidade ao máximo do seu potencial biológico. No entanto, a maioria dos estudos ainda não apresentou resultados tão detalhados e o uso clínico do Klotho exigirá mais estudos futuros.

REFERÊNCIAS

Kuro-o, M. **The Klotho proteins in health and disease**. Nat **Rev Nephrol** 15, 27–44 (2019).

Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova AV, Mohammadi M, Rosenblatt KP, Kliewer SA, Kuro-O M. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem. 2007 Sep 14;282(37):26687-26695.

Lawrence T. **The nuclear factor NF-kappaB pathway in inflammation**. Cold Spring Harb Perspect Biol. 2009 Dec;1(6):a001651.

Liu H, Fergusson MM, Castilho RM, Liu J, Cao L, Chen J, Malide D, Rovira II, Schimel D, Kuo CJ, Gutkind JS, Hwang PM, Finkel T. **Augmented Wnt signaling in a mammalian model of accelerated aging**. Science. 2007 Aug 10;317(5839):803-6.

Duchartre Y, Kim YM, Kahn M. **The Wnt signaling pathway in cancer**. **Crit Rev Oncol Hematol.** 2016 Mar;99:141-9.

Tang X, Wang Y, Fan Z, Ji G, Wang M, Lin J, Huang S, Meltzer SJ. **Klotho: a tumor suppressor and modulator of the Wnt/β-catenin pathway in human hepatocellular carcinoma.** Lab Invest. 2016 Feb;96(2):197-205.

Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. **Biological Role of Anti-aging Protein Klotho**. J Lifestyle Med. 2015 Mar;5(1):1-6.

Ito S, Fujimori T, Hayashizaki Y, Nabeshima Y. **Identification of a novel mouse membrane-bound family 1 glycosidase-like protein, which carries an atypical active site structure.** Biochim Biophys Acta. 2002 Jul 19;1576(3):341-5.

Hou Z, Ding Q, Li Y, Zhao Z, Yan F, Li Y, Wang X, Xu J, Chen W, Wu G, Ruan X, Zhao L**. Intestinal epithelial β Klotho is a critical protective factor in alcoholinduced intestinal barrier dysfunction and liver inju**ry. EBioMedicine. 2022 Aug;82:104181.

Kuro-o, M. et al. **Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature** 390, 45–51 (1997).

Matsumura, Y.; Aizawa, H.; Shiraki-Iida, T.; Nagai, R.; Kuro-O, M.; Nabeshima, Y.-I. **Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein**. Biochem. Biophys. Res. Commun. 1998, 242, 626–630.

Ito, S.; Kinoshita, S.; Shiraishi, N.; Nakagawa, S.; Sekine, S.; Fujimori, T.; Nabeshima, Y.-I. **Molecular cloning and expression analyses of mouse βklotho, which encodes a novel Klotho family protein.** Mech. Dev. 2000, 98, 115–119.

Chen, C.-D.; Podvin, S.; Gillespie, E.; Leeman, S.E.; Abraham, C.R. **Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17.** Proc. Natl. Acad. Sci. USA 2007, 104, 19796–19801.

Kuro-o M. **The Klotho proteins in health and disease**. **Nature Reviews Nephrology 15, 27-44. 2019**
Bednarska S., Fryczak J., Siejka A. Serum β-Klotho concentrations are increased in women with polycystic ovary syndrome. Cytokine. 134, 155188, 2020.

Ito S., Fujimori T., Hayashizaki Y., Nabeshima Y. **Identification of a novel mouse membrane-bound family 1 glycosidase-like protein, which carries an atypical active site structure.** Biochim Biophys Acta 1676, 341-345, 2022.

Kurosu, H. et al. **Regulation of fibroblast growth factor-23 signaling by klotho**. J. Biol. Chem. 281, 6120–6123 (2006).
Urakawa, I. et al. Klotho converts canonical **FGF receptor into a specific receptor for FGF23**. Nature 444, 770–774 (2006).

Lee, S. et al. **Structures of β-klotho reveal a 'zip code'- like mechanism for endocrine FGF signalling.** Nature 553, 501–505 (2018)
Lin BC, Wang M, Blackmore C, Desnoyers LR. Liver-specific activities of FGF19 require klotho beta. J Biol Chem 2007;282:27277-84.

Ogawa, Y. et al. **βKlotho is required for metabolic activity of fibroblast growth factor** 21. Proc. Natl Acad. Sci. USA 104, 7432–7437 (2007).

Kuro-o M. **The Klotho proteins in health and disease.** Nature Reviews Nephrology 15, 27-44. 2019.
Kurosu, H. et al. **Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J. Biol. Chem.** 282, 26687–26695 (2007).

Kharitonenkov, A.; Dunbar, J.D.; Bina, H.A.; Bright, S.; Moyers, J.S.; Zhang, C.; Ding, L.; Micanovic, R.; Mehrbod, S.F.; Knierman, M.D.; et al. FGF-21/FGF-21 **receptor interaction and activation is determined by βKlotho**. J. Cell. Physiol. 2008, 215, 1–7.

Brownstein, C. A. et al. **A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism**. Proc. Natl Acad. Sci. USA 105, 3455–3460 (2008).

Stenvinkel, P. et al. Novel treatment strategies for chronic kidney disease: insights from the animal kingdom. Nat. Rev. Nephrol. 14, 265–284 (2018)

Ligumsky H., Merenbakh-Lamin K., Keren-Khadmy N., Wolf I., Rubinek T. **The role of α-klotho in human cancer: molecular and clinical aspects. Oncogene 41**, 4487-4489. 2022.
Ye X, Guo Y, Zhang Q, et al. **βKlotho suppresses tumor growth in hepatocellular carcinoma by regulating Akt/ GSK-3β/cyclin D1 signaling pathway**. PLoS One 2013;8:e55615.

Liu Z, Qi S, Zhao X, et al. **Metformin inhibits 17β-estradiol-induced epithelial-tomesenchymal transition via βKlotho-related ERK1/2 signaling and AMPKα signaling in endometrial adenocarcinoma cells.** Oncotarget 2016;7:21315-31. Cui G. et al. Up-regulation of FGF15/19 signaling promotes hepatocellular carcinoma in the background of fatty liver. J Exp Clin Cancer Res 2018, 37: 136.

Trost N., et al. **γKlotho is a novel marker and cell survival factor in a subset of triple negative breast cancers.** Oncotarget 7, 2611-2628, 2015.

Hori S., et al. **Gamma-klotho exhibits multiple roles in tumor growth of human bladder cancer.** Oncotarget 9, 19508-19524.

Hori S. et al. Clinical **significance of α- and β-Klotho in urothelial carcinoma of the bladder.** Oncology Reports 2016, 36:2117-2125.

Zhou J., Ben S., Xu T., Xu L., Yao X. **Serum β-Klotho is a potential biomarker in the prediction of clinical outcomes among patients with NSCLC**. Journal of Thoracic Diseases 2021, 13: 3137-3150.

Poh W., et al. **Klotho-beta over expression as a novel target for suppressing proliferation and fibroblast growth factor receptor-4** signalling in hepatocellular carcinoma. Molecular Cancer 11, 14. 2012.

Feng S., Dakhova O., Creighton C.J., Ittman M. **Endocrine fibroblast growth factor FGF19 promotes prostate cancer progression**. Cancer Research 73, 2551-2562, 2013.

Wang Y., et al. **Klotho sensitizes human lung cancer cell lines to cisplatin via PI3k/Akt pathway.** PLoS One 8, e57391, 2013.

Xie B., et al. **Epigenetic silencing of Klotho expression correlates with poor prognosis of human hepatocellular carcinoma**. Human Pathology 44, 795-801, 2013.

Zhu Y., et al. **Klotho suppresses tumor progression via inhibiting PI3K/Akt/GSK3β/Snail signalling in renal cell carcinoma**. Cancer Science 104, 663-671.
Gigante M., et al. **Soluble sérum αKlotho is a potential predictive marker of disease progression ij clear cell renal cell carcinoma**. Medicine 94, e1917, 2015.

Lojkin I., et al. **Reduced expression and growth inhibitory activity of the aging suppressor klotho in the epithelial ovarian cancer**. Cancer Lett 362, 149-157, 2015.
Shibayama Y., et al. **Upregulation of microRNA-125-59 is associated with drug resistance to cytarabine and poor prognosis in AML patients**. Oncology Reports 33, 2176-2182.

Sun H., et al. **Over expression of Klotho suppresses liver cancer progression and induces cell apoptosis by negatively regulating wnt/β-catenin signaling pathway.** World Journal of Surgical Oncology 13, 307, 2015.

Dai D., et al. **Klotho inhibits human follicular thyroid cancer cell growth and promotes apoptosis through regulation of the expression of stanniocalcin-1. Oncology** Reports 35, 552-558, 2016.

Tang X., et al. **Expression of klotho and β-catenin in eso phageal squamous cell carcinoma, and their clinicopathological and prognostic significance**. Diseases of the Esophagus 29, 207-214, 2016.

Ibi T., et al. **Klotho expression is correlated to molecules associated with epithelial-mesenchymal transition in lung squamous cell carcinoma**. Oncology Letters 14, 5526-5532, 2017.

Yan Y., et al. **Reduced Klotho expression contributes to poor survival rates in human patients with ovarian cancer, and overexpression of Klotho inhibits the progression of ovarian cancer partly via the inhibition of systemic inflammation in nude mice**. Molecular Medicine Reports 15, 1777-1785.

Haq F., et al. FGFR1 **expression defines clinically distinct subtypes in pancreatic cancer. Journal of Trranslational Medicine** 16, 374, 2018.
Brominska B., et al. **Klotho expression and nodal involvement as predictive factors for large cell lung carcinoma.** Archives of Medical Sciences 15, 10101016.

Liu Y., et al. (2019) **Klotho-mediated targeting of CCL2 suppresses the induction of colorectal cancer progression by stroma cell senescent microenvironments.** Molecular Oncology 13, 2460-2475, 2019.

Onishi K., et al. **γ-Klotho is correlated with resistance to docetaxel in castrationresistant prostate cancer**. Oncology Letters 19, 2306-2316, 2020.

Xie B., et al. FL-1 **mediates tumor suppressor function via Klotho signalling in regulating CRC**. Cell Biology International 44, 1514-1522, 2020.

Yang L., et al. **Delivery of BR2-SOX17 fusion protein can inhibit cell survival, proliferation and invasion in gastric cancer cells through regulating Klotho gene expression**. Cell Biology International 44, 2011-2020, 2020.

Gunes S., et al., **Enhancement of Apo2L/TRAIL signalling pathway receptors by the activation of Klotho gene with** CRISPR/Cas9 in Caco-2 colon cancer cells. Medical Oncology 38, 146, 2021.

Rubinstein T.A., **A transgenic model reveals the role of Klotho in pancreatic cancer development and paves the way for new Klotho-based therapy**. Cancers 13, 6297, 2021.

Wang Y., et al. **Circular RNA ITCH suppresses metastasis of gastric cancer via regulating miR-199a-5p/Klotho axis**. Cell Cycle 20, 522-536, 2021.

Tao Z., Cui Y., Xu X., **Han T. FGFR redundancy limits the efficacy of FGFR4selective inhibitors in hepatocellular carcinoma**. Proceedings of the National Academy of Sciences of the USA 119, e2208844119, 2022.

Sariboyaci A.E., Uysal O., Soykan M.N., **Gunes S. The potential therapeutic effect of klotho on cell viability in human colorectal adenocarcinoma HT-29 cells.** Medical Oncology 39, 191, 2022.

Labrcque et al. **Characterizing the phenotypic diversity of treatment-refractory metastatic castration-resistant prostate cancer**. Journal of Clinical Investigation 130, 4492-4505, 2019.

Tannock I.F., et al. **Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer**. New England Journal of Medicine 351, 1502-1512.

Rodriguez-Canales J, Parra-Cuentas E., Wistuba I.I. **Diagnosis and molecular classification of lung cancer**. Cancer Treatment Research170, 25-46.

Forner A., Reig M., Bruix J. **Hepatocellular carcinoma. Lancet 391, 1301-1314. Lheureux S., Braunstein M., Oza A.M. Epithelial ovarian cancer: evolution of management in the era of precision medicine**. CA A Cancer Journal for Clinicians 69, 280-304, 2019.

Santos et al. **Towards drug repurposing in cancer cachexia: potential targets and candidates.** Pharmaceuticals 14, 1084, 2021.

Liu H. **Emerging agents and regimens for AML**. Journal of Hematology and Oncology 14, 49.

Harbeck N., Gnant M. Breast cancer. Lancet 389, 1134-1150. Bianchini G., de Angelis C., Licata L., Gianni L. **Treatment landscape of triple-negative breast cancer – expanded options, evolving needs.** Nature Reviews Clinical Oncology 19, 91-113, 2022.

Patel et al., The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention and early detection. Lancet. Gastroenterology and Hepatology 7, 262-274, 2022.

Compérat E., et al. **Current best practice for bladder cancer: a narrative review of diagnostics and treatments**. Lancet 400, 1712-1721, 2022.

Page M.J., et al. **The PRISMA 2020 statement: an updated guideline for reporting systematic reviews.** British Medical Journal 372, n71, 2021.