Metadichol-Induced KLF Expression in PBMC Cells. Links SIRTs, NRs, TLRs, and Circadian genes. A Systems-Wide Biology Approach

Palayakotai Raghavan *

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Article

Metadichol-Induced KLF Expression in PBMC Cells. Links SIRTs, NRs, TLRs, and Circadian genes. A Systems-Wide Biology Approach

P.R. Raghavan

Nanorx Inc., USA, PO Box 131, Chappaqua NY 10514; raghavan@nanorxinc.com

Abstract: Metadichol expresses all nuclear receptors, the TLR family (1-10), the Sirtuin family (1-7), all 48 nuclear receptors, and all Yamanaka factors: Oct 4, Sox2, KLF4, C-myc, and circadian genes: Per1, CRY1, BMAL1, CLOCK, and PPARGC1A. The transcription factor family Kruppel-like factors (KLFs) is essential for cell proliferation, differentiation, and development. Our tiny chemical, Metadichol, a long-chain lipid alcohol nanoemulsion, modulates KLF family 1-18 expression. Concentration dependent. At 1 ng/ml, 16 of 18 KLF transcription factors are downregulated, save KLF 4 and 18. Small chemical modulators of KLF expression and activity offer new therapeutic approaches for many disorders, including cancer. In immunotherapy, KLF10 inhibition may reduce T regulatory cell numbers or function to boost anti-tumor immunity. Small compounds substituting KLF4 in cellular reprogramming could increase iPSC production efficiency and safety in regenerative medicine. In addition to KLF family expression results, this research will examine the regulation mechanisms of KLFs, nuclear receptors, SIRTs, circadian genes, Toll-like receptors, Klotho, P53, and PPARGC1A in cancer. These protein families form a dynamic web of interconnected signaling networks that affect cancer biology, including the mechanisms of crosstalk between them, the roles of individual members in different cancer types, and the tumor microenvironment's effect on these interactions. Our research reveals that molecular biology, genetics, immunology, and clinical oncology must be integrated to understand cancer's intricate networks. This will allow novel therapeutic techniques to target gene family connections, improving cancer therapy results and tumor biology understanding.

Keywords: Metadichol KLF; Toll-like receptors; nuclear receptors; Sirtuins; Circadian genes; Klotho; TP53; telomerase; long-chain lipid alcohols

Introduction

We have 18 mammalian Kruppel-like factor KLFs [1]. The (KLF) family of zinc-finger transcription factors regulates several biological processes. KLFs regulate cell differentiation, proliferation, and development in response to environmental stress. The intricate role of KLFs in many cellular processes emphasizes their importance in homeostasis and their therapeutic potential.

KLF Structure and Classification

KLF proteins have a conserved C-terminal DNA-binding domain. The three C2H2 zinc fingers in this domain allow KLFs to bind to GC-rich DNA [2]. The N-terminal regulatory domains vary, contributing to family members' different roles [2]. The Sp/KLF family includes Sp1-4 and many Kruppel-like factors [3]. These factors bind to Sp1 sites—GC boxes, CACCC boxes, and basic transcription elementswith different affinities [4]. The DNA-binding domain's structural conservation and the N-terminal regulation domain's diversity allow for particular DNA binding and various functional activities.

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KLFs and Development Roles

KLFs regulate germ layer formation and body axis patterning during embryonic development. The Xenopus studies show KLF involvement in these processes [5,6]. KLF4 controls important developmental signals [5]. Zebrafish KLF genes regulate hematopoiesis, blood vessel function, fin and epidermal growth. Multiple KLFs' tissue-specific expression patterns in tree shrews highlight their different developmental roles [7]. MMP9 regulation by KLF5 is necessary for cartilage and bone formation [8]. Impaired cartilage breakdown delays bone regeneration in KLF5+/- mice [8]. KLFs' spatiotemporal expression during development shows their exact functions in tissue architecture and function.

Obesity, cardiovascular disease, cancer, and inflammatory disorders are linked to KLF expression dysregulation [1]. KLFs have important roles in renal fibrosis progression, tubulointerstitial inflammation regulation, and glomerular filtration barrier maintenance [9]. Inflammatory monocyte differentiation requires KLF4 [10]. KLF4 -/- chimeras have fewer resident and circulating inflammatory monocytes [10]. KLF10 controls colonic macrophages and DSS colitis risk [11]. KLF10-deficient animals produce less IL-10 and fewer colonic macrophages [11]. They also produce higher IL-12p70 after LPS [11]. KLFs are crucial to optimal physiological function due to their role in these different disease processes (Figure 1).

KLFs in Cancer

Cell growth and cancer progression are heavily regulated by KLFs [3]. They participate in many growth signal transduction pathways. Their overexpression can boost or hinder proliferation. KLFs affect oncogenes and tumor suppressors. They can even cause cancer. Multiple cancers have altered KLF expression. In gastric cancer, KLF8 is associated with poor prognosis [12]. It controls glycolysis via GLUT4 [12]. Higher KLF8 expression in gastric cancer is associated with larger tumors, advanced T and N stages, and shorter survival. Different forms of cancer are linked to KLF8 [12]. Its silencing slows cancer cell glycolysis. The context-dependent activities of KLFs in cancer highlight their intricacy in carcinogenesis.

Immune System and KLFs

KLFs are essential for immune cell growth and function. Inflammatory monocyte differentiation requires KLF4 [10]. KLF2 modulates chemokine receptor patterns to control T-cell trafficking and recirculation [13]. KLF10 controls intestinal macrophages and causes innate immune colitis [11]. Aortic lesions are more extensive in KLF2+/− ApoE−/− animals than in controls [14]. This shows KLF2 protects against atherosclerosis [14]. KLF2 expression is downregulated in monocytes from severe atherosclerosis patients. In endothelial and monocyte cells, KLF2 and KLF4 control shear-dependent genes [14]. The various actions of KLFs on immune cells show their role in innate and adaptive immunity.

Targeting KLFs Therapeutically

KLFs are attractive therapeutic targets due to their involvement in several illnesses. Modulating KLF expression or activity may treat cardiovascular disease, cancer, and inflammatory illnesses [1]. For instance, statins increase endothelium KLF expression, which protects against atherosclerosis [15]. Overexpression of KLF4 prevents atherothrombosis, pulmonary hypertension, and restenosis [1]. KLF13 deficiency in uterine endometrial cells impairs steroid hormone receptor signaling in a mouse model of endometriosis [16]. KLF9 null endometrium increases ectopic lesion formation in the same model [15]. Manipulating KLF activity offers promising treatment approaches to several disorders.

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KLFs and Cellular Processes

KLFs control proliferation, apoptosis, differentiation, and stem cell maintenance. [1] KLF4 and KLF5 regulate esophageal cancer cell growth, apoptosis, and invasion. KLF5 promotes invasion, while KLF4 suppresses proliferation and induces apoptosis [17]. KLF4 and KLF5 inhibit esophageal cancer [18]. Colorectal cancer tumor suppression by KLF6 [18]. In colon cancer cells, insulin promotes FASN expression and proliferation [19]. These essential cellular mechanisms demonstrate KLFs' role in tissue homeostasis and cell destiny regulation.

Genetic Regulation via KLFs

KLFs regulate gene expression by binding to DNA [19]. KLF1 is needed for proper globin synthesis [20,21]. KLF4 turns on HBG gene expression in primary erythroid cells [22]. KLF4 preferentially binds to the HBG promoter CACCC region and interacts with CREBBP [22]. In mice, KLF13 controls axonal growth, development, and regeneration [23]. Psychiatric symptoms and ADHD [23] were linked to a de novo heterozygous KLF13 gene variation. KLF5 transactivates MMP9 to degrade cartilage [8]. Adult corticospinal axon regeneration is promoted by KLF7 [23]. Overexpression of VP16-KLF7 stimulates adult mouse CST axon sprouting [24] and regeneration. Figure 1 summarizezs the role of the KLF family of genes in several cellular and physiological processes by regulating the expression of various target genes.

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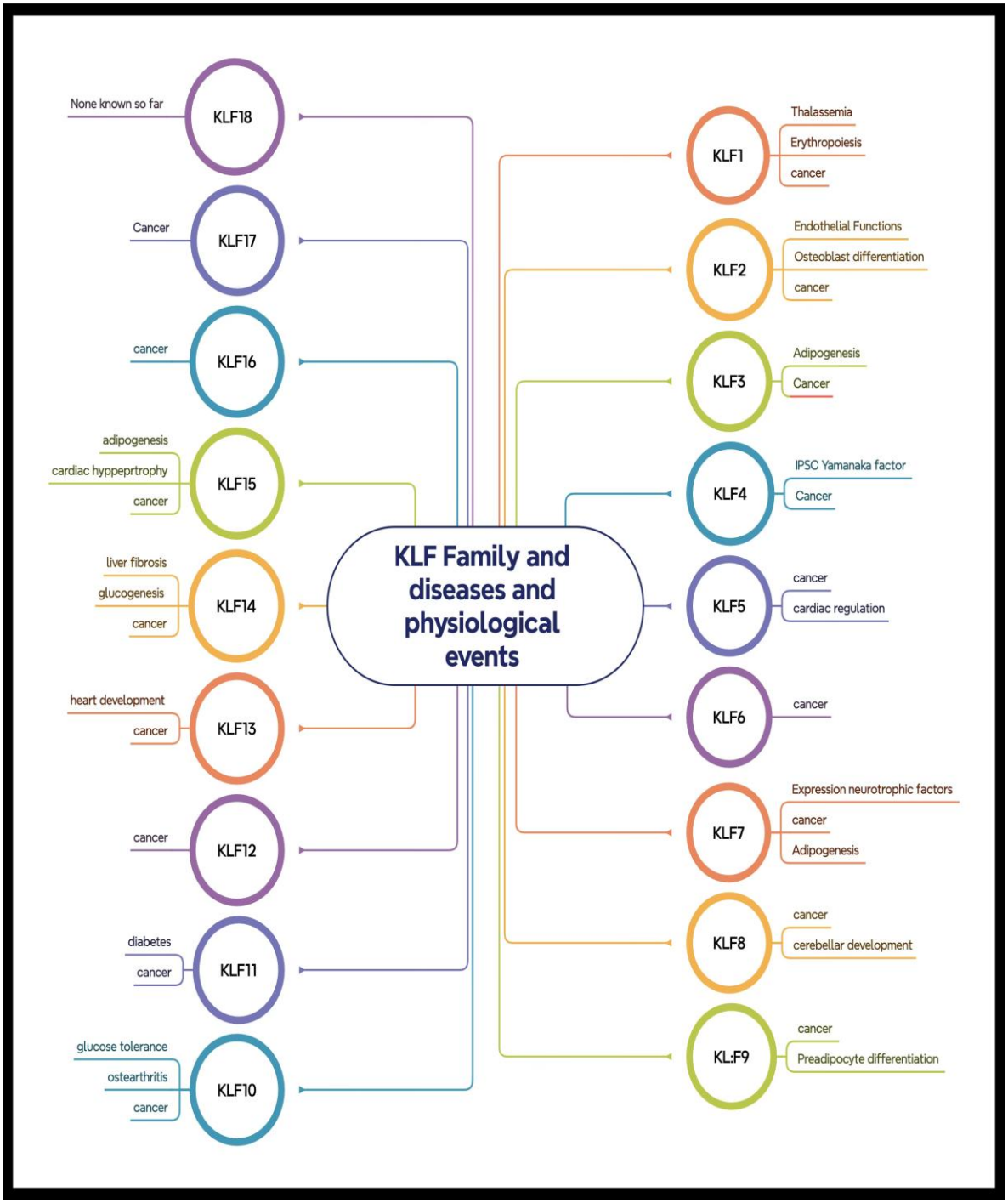

Figure 1. Diseases and KLFs.

Cross-Regulation and KLF Family Interactions

Cross-regulation between KLF family members complicates their regulatory networks. After arterial damage, KLF4 inhibits neointimal development [14]. It accomplishes this by working against NF-κB [25]. KLF7 indicates aggressive gastric cancer with bad prognosis [26]. KLF family members interact to add control to their regulatory roles.

Metadichol, a long-chain alcohol nanoemulsion [27]. Our prior study [28–32] indicated that Sirtuins, Klotho, Tp53, Telomerase (Tert), FoxO1, PPARGC1A, TLR family, nuclear receptors, Yamanaka factors (Oct 4, KLF2, c-MYC, Sox2), and circadian genes must be expressed and controlled to target cancer. We demonstrate for the first time that Metadichol, a nano formulation of long-chain

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alcohols, can express and regulate all KLF family members in PBMC cells using Q-RT-PCR at doses from 1 picogram to 100 nanograms.

Experimental

All work was outsourced to Skanda Labs, Bangalore, India. Saha Biologics, Hyderabad, India, supplied all primers.

Isolation of Human WBCs by Cell Line and Condition

Blood sample preparation: Fresh human blood was taken in EDTA tubes, diluted 1:1 with PBS, and mixed by inverting.

Isolation of Mononuclear Cells- In a 15 ml centrifuge tube, 5 ml of Histopaque-1077 was added and 5 ml of prepared blood was progressively put on top without disturbing it. Tubes were then centrifuged at 400 X g for 30 min at room temperature with brake off. Upper layer was removed using Pasteur pipette without disrupting interphase layer after centrifugation. The interphase layer was carefully placed in a clean centrifuge tube. After washing with 1X PBS, cells will be centrifuged at 250 X g for 10 min. (2X). After centrifugation, supernatant was discarded and pellet was recovered in 10% FBS-RPMI medium. Hemo-cytometers counted and verified cell viability.

Cell maintenance and seeding: 1 X 106 cells/ml medium were sown into 6 well plates and incubated for 24 h at 37oC with 5% CO2. After 24 hours of seeding, the medium was carefully removed, and cells were treated with MTT-selected concentrations and kept at 37℃ in a CO2 incubator for 24 hours.

Table 1. Treatment concentrations.

Cell line	Sample name	Treatment details
Human PBMC	Metadichol	Control
		1 pg/ml
		100 pg/ml
		1 ng/ml
		100ng/ml

Sample Preparation and RNA Isolation

We detached and washed treated cells with sterile 1X PBS and centrifuged. Decant the supernatant and add 0.1 ml of TRIzol, mixing gently by inversion for 1 min. Samples rested at room temperature for 10 minutes. Per 0.1 ml of TRIzol, 0.75 ml chloroform was added. The contents vortexed for 15 seconds. The tube rested at room temperature for 5 minutes. The mixture was centrifuged at 12,000 rpm for 15 minutes at 4˚C. The upper aqueous phase was placed in a sterile micro-centrifuge tube, added 0.25 ml of isopropanol, and incubated at -20˚C for 20 minutes. The contents were centrifuged at 12,000 rpm for 10 minutes at 4˚C. The RNA pellet was rinsed with 0.25 ml of 70% ethanol after discarding supernatant. The RNA mixture was centrifuged at 12,000 rpm at 4˚C. Supernatant was carefully eliminated and pellet air-dried. Re-suspend the RNA pellet in 20 µl of DEPC-treated water. Total RNA yield was measured by Spectra drop (Spectramax i3x, Molecular Devices, USA).

cDNA Production

According to the manufacturer's instructions, 500 ng of RNA was used to synthesize cDNA using Prime script RT reagent kit (TAKARA) and oligo dT primer. A 20 µl reaction volume was used for cDNA synthesis at 50°C for 30 min, followed by RT inactivation at 85°C for 5 min utilizing Veritii biosystems. Real-time PCR was performed on cDNA.

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RT-qPCR Primers and Analysis

The PCR mixture (final volume 20 µl) included 1.4 µl cDNA, 10 µL SyBr green Master solution, and 1 µM complementary forward and reverse primers (table 3) for target genes. Enzyme activation at 95°C for 2 minutes was followed by a 2-step reaction with initial denaturation and an annealing cum extension step at 95°C for 5 seconds, annealing for 30 seconds at the appropriate temperature amplified for 39 cycles, secondary denaturation at 95°C for 5 seconds, and 1 cycle with melt curve capture step from 65°C to 95°C for 5 seconds each. Results were examined, and fold expression or regulation was estimated using CFX Maestro software. The comparative CT approach was utilized to compare target gene expression to β-actin and untreated control cells.

The delta CT for each therapy was computed using a formula.

(CT Method)

The comparative CT approach compared target gene expression to β-actin and untreated control cells.

Delta CT was determined for each treatment using the formula.

Delta Ct = target gene – reference gene.

Ct was subtracted from control to get delta delta CT to compare treated samples to untreated controls. Delta delta Ct = treatment group – control group.

Each treatment's target gene expression fold change was estimated using the formula. Fold change = 2^(−delta delta Ct)

Results

Q-RT-PCR (Table 2) showed that at 1 ng/ml, all transcription factors except KLF4, KLF15, KLF 17, and 18 were downregulated or repressed. These findings imply metadichol may be important in various malignancies. According to the literature, many KLF family members are oncogenic. [1] These techniques could treat cancer by suppressing oncogenic gene expression. KLF4, a Yamanaka factor, is increased in fibroblasts and primary cancer cells and is an anticancer agent and cell reprogramming. Figures 5 and 6 show various KLF factor functions in cancer.

Table 2. RNA yields.

	Test concentrations				
RNA yield (ng/µl)	0	1 pg/ ml	100 pg/ ml	1 ng/ ml	100 ng/ ml
Human PBMC's	623.120	343.123	792.123	673.111	611.123

Table 3. Primer details.

Primer	Sequence	Amplicon size	Annealing temperature
GAPDH	GTCTCCTCTGACTTCAACAGCG	186	60
	ACCACCCTGTTGCTGTAGCCAA		
KLF1	CAGGTGTGATAGCCGAGACC	111	65
	TCTTGGTGTAGCTCTTGCCG		
KLF2	CCAAGAGTTCGCATCTGAAGGC	131	65
	CCGTGTGCTTTCGGTAGTGGC		
KLF3	CTCATGGTCTCCTTATCGGAGG	131	65
	TGTCCTCTGTGGTTCGATCCCA		
KLF4	CCTTCCTGCCCGATCAGATG	132	62
	TGAGCATCATCCCGTGTGTC		

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KLF5	GGAGAAACGACGCATCCACTAC	140	65
	GAACCTCCAGTCGCAGCCTTC		
KLF6	GACAGCTCCGAGGAACTTTCT	156	65
	CACGCAACCCCACAGTTGA		
KLF7	CTCACGAGGCACTACAGGAAAC	135	67
	TGGCAACTCTGGCCTTTCGGTT		
KLF8	CCTGAAAGCTCACCGCAGAATC	113	61
	TGCTTGCGGAAATGGCGAGTGA		
KLF9	GGGAAACACGCCTCCGAAAA	110	65
	CGTTCACCTGTATGCACTCTGTA		
KLF10	AGGAGTCACATCTGTAGCCACC	139	67
	GAACGGGCAAACCTCCTTTCAC		
KLF11	ATGGATGCAGCCACACCTGAAC	115	65
	GGAGAAACAGGTGTCCTTGTCG		
KLF12	CCTTTCCATAGCCAGAGCAGTAC	130	65
	CTGGCGTCTTGTGCTCTCAATAC		
KLF13	CAGAGGAAGCACAAGTGCCACT	137	65
	CGCGAACTTCTTGTTGCAGTCC		
KLF14	CATCCAGATATGATCGAGTACCG	163	65
	CCTTGAGGGTAAGACTGACAGC		
KLF15	GTGAGAAGCCCTTCGCCTGCA	114	67
	ACAGGACACTGGTACGGCTTCA		
KLF16	GACTGCGCCAAAGCCTACTACA	171	65
	CCTGCCAGTCACAAGCAAAAGG		
KLF17	GCTGCCCAGGATAACGAGAAC	128	67
	ATCTCTGCGCTGTGAGGAAAG		
KLF18	TCCATGGGCCAGAAAGTGAC	197	67
	GGGTGTTCAGCTGGCTACTT		

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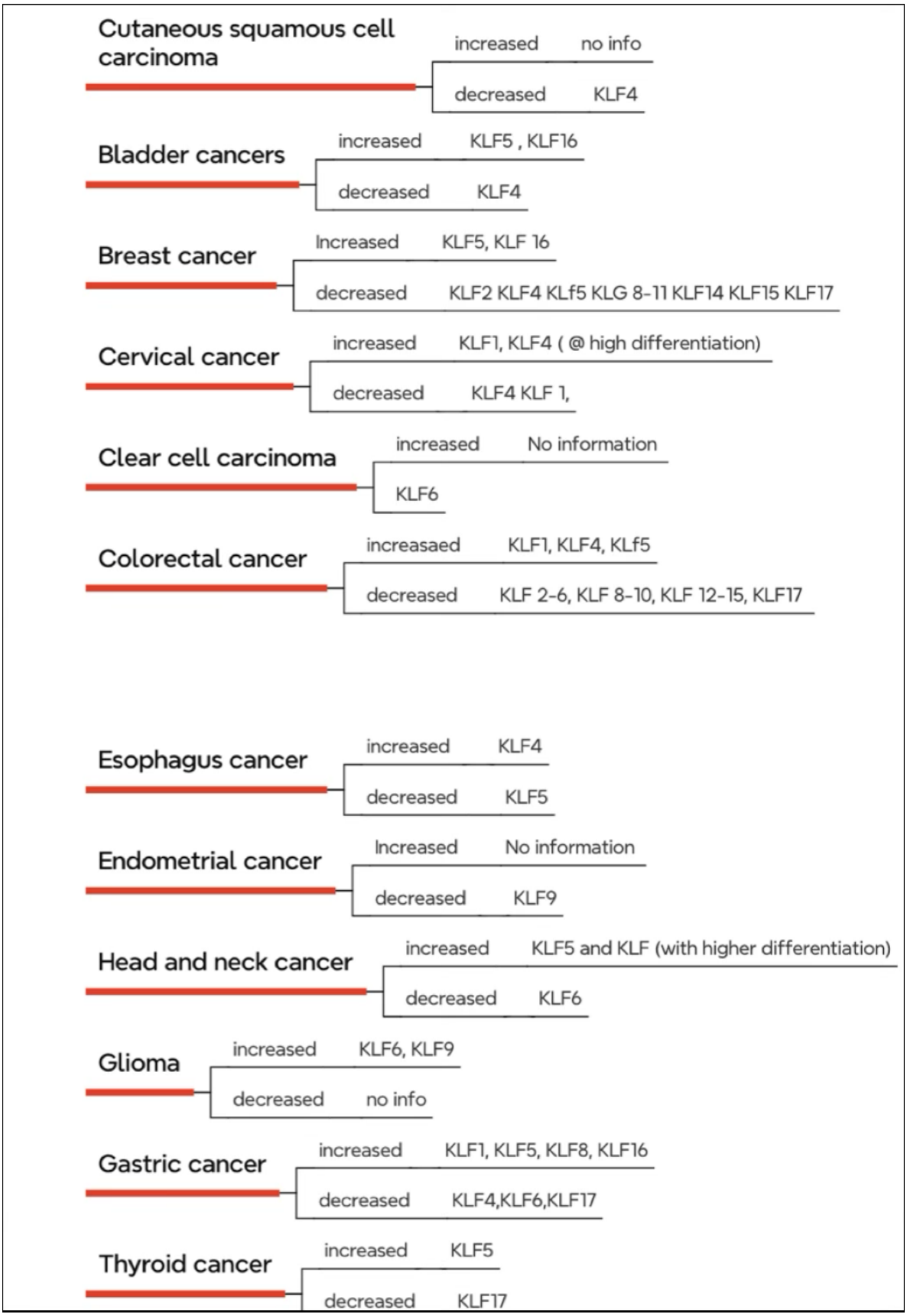

Figure 5. KLF and expression levels in malignancies.

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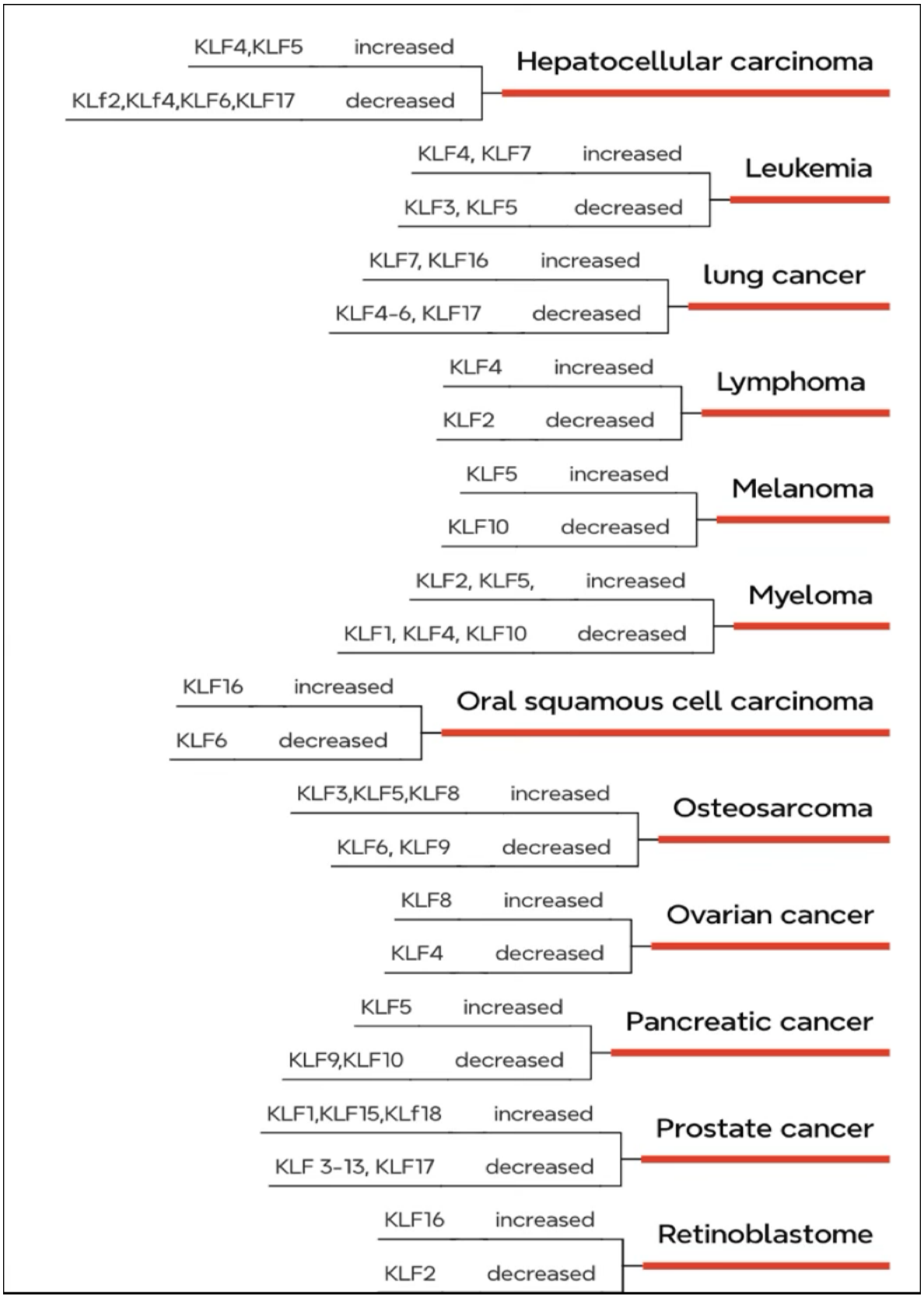

Figure 6. Klf expression levels in various malignancies.

The experimental results (Table 4) and literature-based results (Figures 5 and 6) are well correlated. In esophageal cancer, KLF4 expression [33] decreases and KLF5 expression increases [34]. .Metadichol enhanced KLF4 expression at one nanogram per ml and decreased KLF5 expression, as seen in Table 4. In bladder cancer, Metadichol raises KLF4 expression and downregulates KLF5 and

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KLF6 expression at 100 ng/ml, as indicated in Table 4 and Figure 5 and 6. .The third example is ovarian cancer, where KLF8 expression is elevated (Table 1) and decreased [35] This is important to combat ovarian cancer expression. We can compare our experimental results to those in the literature.[36–40]

Table 4. Metadichol-induced expression of various KLF family members.

	Control	1 pg/ml	100 pg/ml	1 ng/ml	100 ng/ml
KLF1	1	1.42	0.94	0.22	0.7
KLF2	1	1.03	0.67	0.27	0.77
KLF3	1	1.63	1.35	0.38	1.35
KLF4	1	1.1	0.24	1.56	0.8
KLF5	1	0.98	0.7	0.3	0.96
KLF6	1	0.57	0.54	0.46	0.47
KLF7	1	0.87	0.5	0.12	1.02
KLF8	1	4.99	0.85	0.34	1.02
KLF9	1	3.02	1.3	0.41	1.08
KLF10	1	1.4	1.38	0.26	1.15
KLF11	1	1.45	1.5	0.28	0.91
KLF12	1	0.59	0.76	0.22	0.88
KLF13	1	1.84	0.92	0.27	1.12
KLF14	1	1.45	1.05	0.32	1.51
KLF15	1	2.9	1.49	0.89	2.14
KLF16	1	2.31	1.47	0.36	0.93
KLF17	1	0.39	0.41	0.66	2.47
KLF18	1	1.23	0.87	1.75	1.91

By activating the PI3K/AKT pathway, KLF1 promotes cervical cancer dissemination and invasion [41–42].

The tumor suppressor KLF2 is found in breast, colorectal, gastric, and lung malignancies. PI3K/AKT and HIF-1α/Notch-1 pathways are regulated to limit proliferation, invasion, and metastasis [43].

Based on the kind of cancer, KLF3 has two functions. It suppresses and promotes colorectal, lung, cervical, pancreatic, and prostate cancers [44].

Context-dependent dual role for KLF4. In some cases, it increases esophageal squamous cell carcinoma but suppresses colorectal, stomach, and lung malignancies [45].

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KLF5 works as a tumor suppressor and promoter depending on cancer type and stage. It affects breast, pancreatic, colorectal, gastric, and endometrial cancers [46].

In prostate cancer, glioblastoma, hepatocellular carcinoma, and lung cancer, KLF6 suppresses tumor growth. A KLF6-SV1 splice variation promotes metastasis [47].

KLF7 is an oncogene in colon, pancreatic, and high-grade serous ovarian cancers [48].

In breast, colorectal, gastric, and liver malignancies, KLF8 promotes proliferation, invasion, and metastasis [49].

KLF9 suppresses breast, pancreatic, hepatocellular, and colorectal cancer growth and metastasis [50].

KLF10 suppresses pancreatic, leukemia, and cervical cancers. In certain cancers, it inhibits growth and induces apoptosis. [51]

In lung, pancreatic, breast, and ovarian cancers, KLF11 plays two roles. In certain situations, it promotes or suppresses cancers [52].

KLF12 increases proliferation and suppresses apoptosis in breast cancer, acting as an oncogene [53].

KLF13 suppresses prostate cancer but may promote other cancers [54].

In breast, colon, and cervical malignancies, KLF14 suppresses tumor growth via the PI3K/AKT pathway [55].

KLF15 suppresses breast, colorectal, and gastric cancer proliferation and metastasis [56].

Oncogene KLF16 promotes proliferation and invasion in breast, bladder, gastric, and pancreatic adenocarcinoma [57].

KLF17 regulates EMT and metastasis to suppress breast, lung, gastric, and esophageal cancers [58].

KLF18's role in cancer is unknown.

The Table 4 and Figures 5 and 6 above imply that targeting all Krüppel-like factor (KLF) family members in cancer therapy may have several benefits:

Antitumor Synergy

Targeting several KLFs can have synergistic antitumor effects due to their different cancer progression functions [59–60]. Different KLFs influence cancer growth, apoptosis, metastasis, and stemness.

Past Compensatory Mechanisms

Targeting one KLF may result in family compensation because KLFs commonly overlap. Inhibiting numerous KLFs simultaneously can overcome compensatory processes and improve treatment efficacy.

Multiple Signaling Pathway Modulation

KLFs regulate key cancer formation pathways like NF-κB, PI3K/AKT, and WNT [61]. Targeting multiple KLFs disrupts oncogenic pathways better than targeting one.

Addressing Tumor Heterogeneity

Cancer subtypes and even cells within the same tumor may depend on various KLFs for survival and progression. A multi-KLF targeting method may address heterogeneity and provide a more holistic therapeutic plan.

Making therapy more effective

Pancreatic cancer treatment resistance, especially radioresistance, is linked to KLFs like KLF10 [62]. Targeting these KLFs with other family members may improve therapy efficacy and overcome resistance.

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Potential for Personalized Medicine

KLF expression and roles vary by cancer type and patient [3,12].. Based on a patient's tumor's KLF expression profile, targeting various KLFs provides for a more personalized strategy.

Targeting numerous KLF family genes in cancer treatment may be promising, however KLFs also regulate cellular activities and homeostasis [8].

Genetic and epigenetic changes throughout cancer genesis and progression cause uncontrolled cell proliferation and metastasis. This complex process involves several molecular regulators with unique but interrelated roles. In cancer, we must study the synergistic interactions between Kruppel-like factors (KLFs), sirtuins, Toll-like receptors (TLRs), nuclear receptors, circadian clocks, Klotho, p53, and TERT. They each contribute to cancer etiology, but more crucially, their complicated interconnections determine their synergistic effects. Understanding these complex interactions is essential for creating new cancer treatments.

We previously demonstrated that somatic cell lines express all sirtuins toll receptors nuclear receptors circadian transcription factors , Klotho TP53 , and telomerase [28–32,63–65]. Figures 7–10 show how sirtuins, TLRs, and nuclear receptors affect cancer.

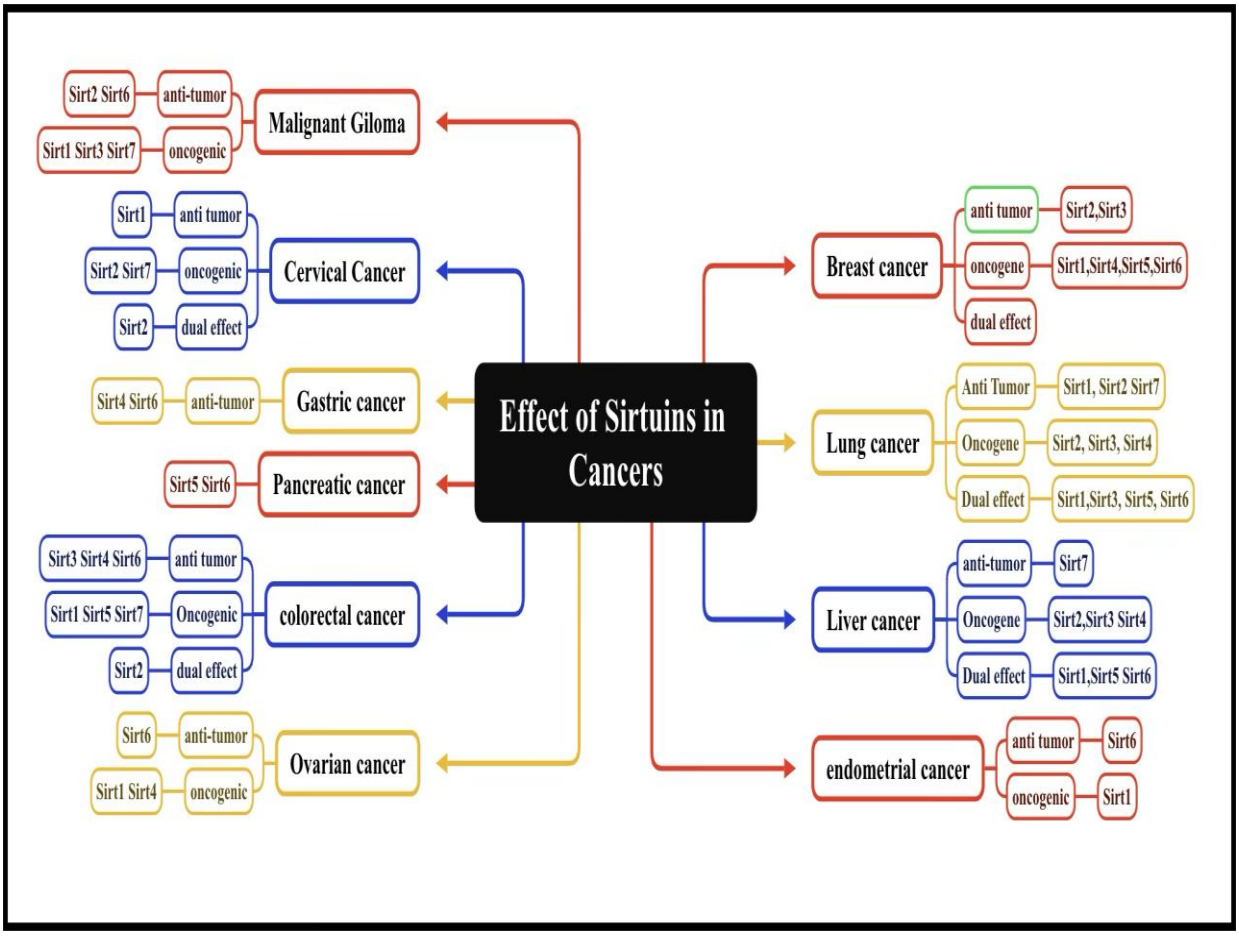

Figure 7. Sirtuin expressions in cancer.

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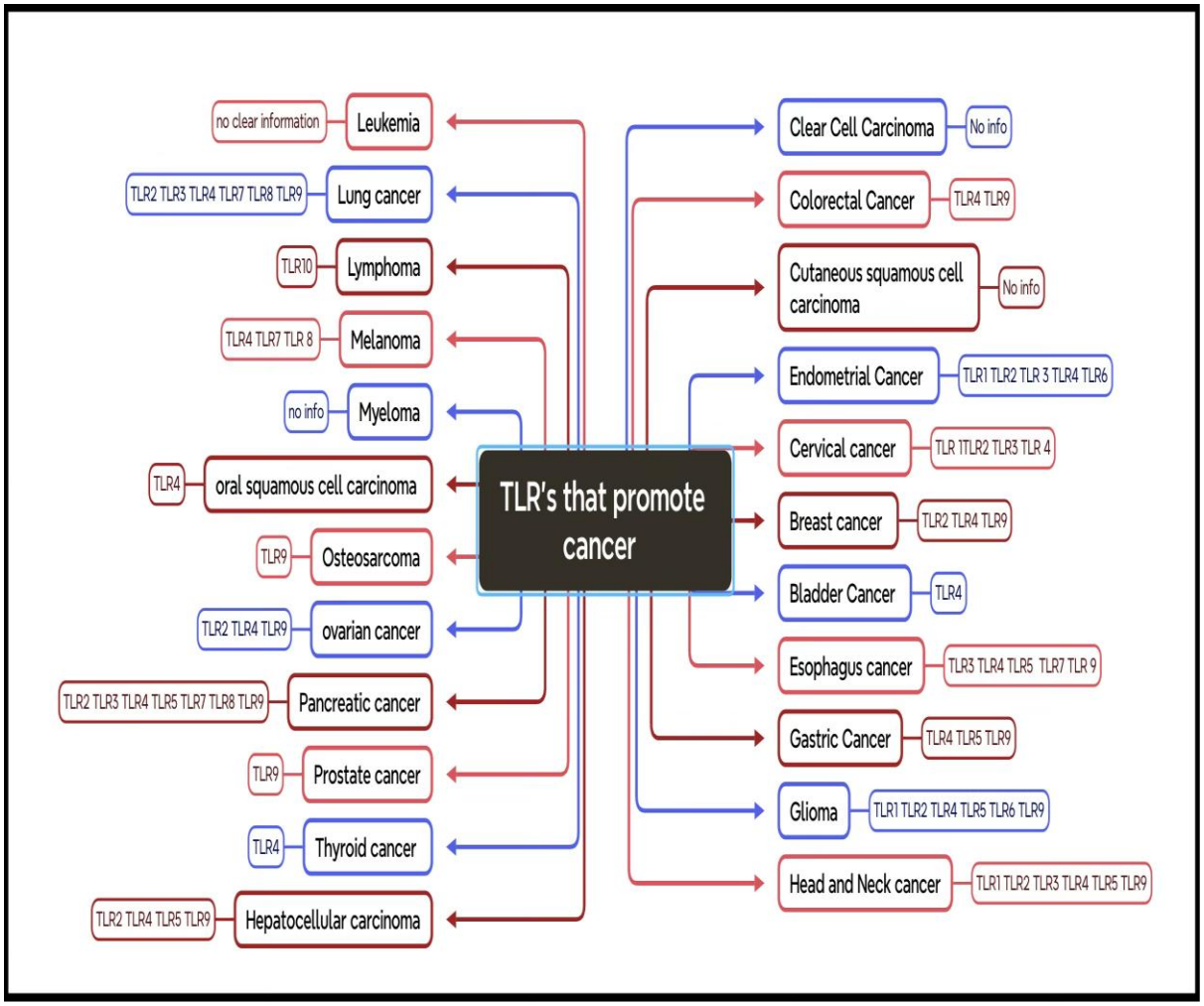

Figure 8. TLRs oncogenic role.

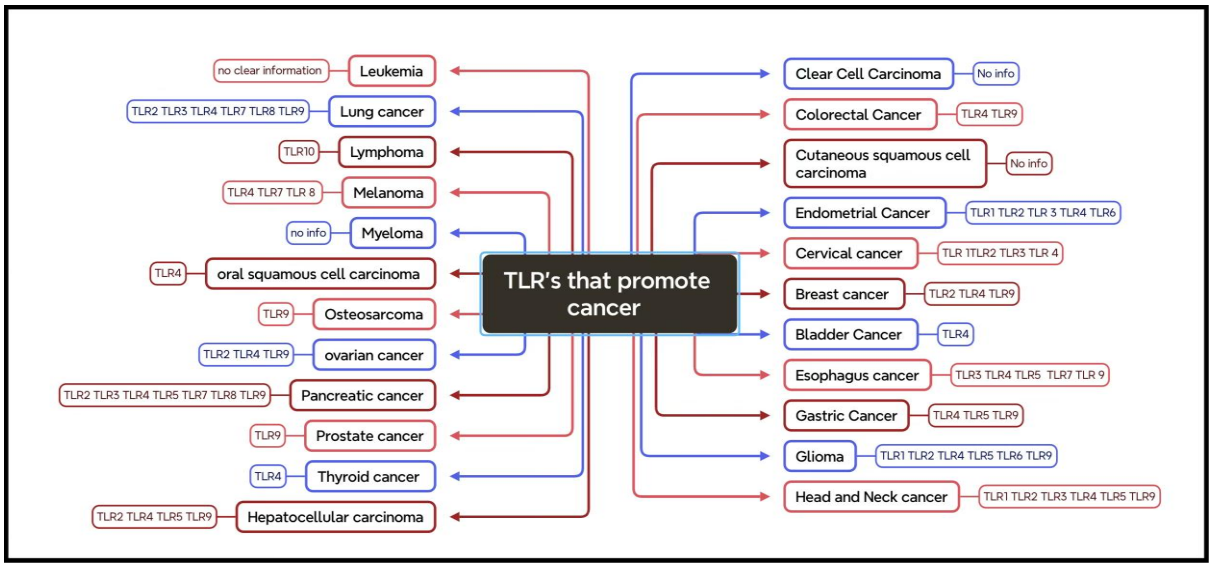

Figure 9. TLRs anti-tumor effects.

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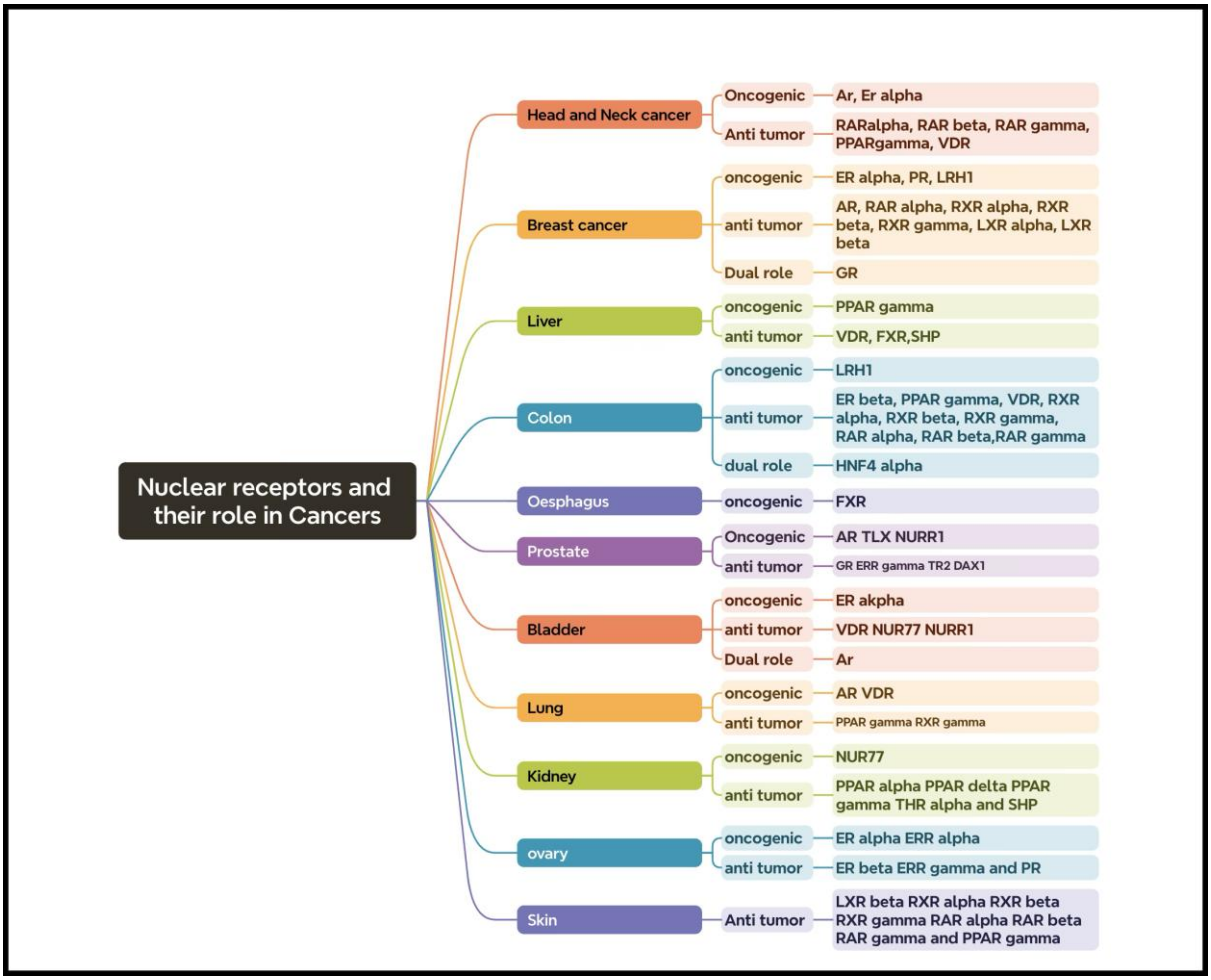

Figure 10. Nuclear Receptors in Cancer.

KLF and Regulatory Pathway Interactions

Complex diseases characterized by uncontrolled cell growth and spread involve intricate interactions between signaling pathways and regulatory molecules and four key protein families in cancer: Kruppel-like factors (KLFs), nuclear receptors (NRs), sirtuins, and Toll-like receptors.

The Klf and Sirtuin families regularly influence cancer-related pathways. Their effects on metabolic processes, especially glycolysis, overlap [66–68]. As previously discussed, cancer cells often modify glycolysis, a key metabolic process, to facilitate their uncontrolled growth and survival. Both families affect EMT [69–70], a crucial cancer metastatic mechanism. Additionally, the Klf and Sirtuin families regulate cell cycle and apoptosis [65–66], affecting tumor growth and cell survival.

Klf and sirtuin proteins may interact synergistically or antagonistically due to common regulatory mechanisms. These interactions may rely on the Klf and Sirtuin members and cellular environment. For instance, a tumor suppressor Klf protein could work with sirtuin to limit tumor growth. Conversely, an oncogenic Klf protein and sirtuin may stimulate tumor growth. Klf, a tumor suppressor, could oppose an oncogenic sirtuin, creating a complicated interaction that affects cancer formation. These interactions are complex and require further study to understand their effects on cancer.

Klf and Sirtuin activities rely on cell type, tissue microenvironment, and other signaling molecules [71–73]. This explains the seemingly contradicting literature, where the same protein can be a tumor suppressor and an oncogene. For instance, the same Klf protein can prevent tumor growth in one cancer cell but increase it in another.

NRs (figure 10) interact with many signaling pathways dysregulated in cancer. [74]. This interaction greatly complicates cancer genesis and progression. For instance, ERs interact with the

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PI3K/AKT pathway, which controls cell growth and survival. This connection boosts cell proliferation and survival, promoting tumor growth. ARs affect cell cycle progression and apoptosis via many signaling pathways. PRs regulate uterine and mammary gland cell proliferation and differentiation. [74]. NRs' functional interaction with various signaling pathways is context-dependent, contributing to cancer formation and progression heterogeneity.

Established KLF-NR Interactions in Cancer

Several investigations have shown direct physical interactions between specific KLFs and NRs, though the field is still evolving. In endometrial epithelial cells, KLF9 interacts with PR isoforms A and B. [74]. KLF9 directly modulates PR transcription and affects PR target genes implicated in cancer development and progression. [75] KLFs may directly influence NR activity, affecting cancer-related NR target gene expression. However, direct interactions between other KLFs and NRs are understudied, providing a promising study topic.

KLFs and NRs can influence each other through shared signaling pathways and regulatory networks without direct physical contact. [76–81]. KLFs can indirectly affect NR activity by regulating NR or co-regulator expression. Conversely, NRs can regulate KLF expression or activity. This complicated interaction provides regulatory loops that fine-tune gene expression and shape cellular response to inputs. This intricate interaction affects cancer growth and progression, making it an essential research field.

KLF-TLR Interactions in Cancer

There is some evidence that KLFs directly regulate TLR expression, however this is not well documented. Studies have suggested that KLFs regulate TLR gene transcription [82] to determine which KLF isoforms are involved, which TLR genes they target, and the molecular processes of this control. Identification of KLF-binding sites in TLR gene promoters would prove this regulation.

KLFs and TLRs may indirectly affect downstream targets like inflammatory cytokines. Both KLFs and TLRs regulate NF-κB, a transcription factor crucial for inflammation and cancer. NF-κB activation causes pro-inflammatory cytokines to promote tumor development and angiogenesis [83–86]. The convergence of KLF and TLR signaling on NF-κB is a critical interaction site that affects cancer formation. Further research into the processes of this convergence is needed to completely comprehend KLF-TLR interactions in cancer.

KLF-TLR interactions are especially important during macrophage polarization. TLRs, essential components of the innate immune system, initiate immunological responses and shape the TME. TLR stimulation drives macrophage polarization toward the pro-inflammatory M1 phenotype, while KLF4, a major regulator of macrophage polarization, can balance M1 and M2 phenotypes. [87] TLR-mediated activation and KLF4-mediated macrophage polarization affect TME and cancer progression

PTEN-deficient prostate cancer cells express TLR3, TLR4, and TLR9 differently [88]. PTEN, a tumor suppressor gene, controls cell growth and survival. PTEN-deficient cells express TLRs differently, suggesting they may be involved in prostate cancer growth. The relationship between TLRs and KLFs in prostate cancer needs additional study. This interaction may reveal prostate cancer growth and progression processes. KLF modulation of TLR expression and signaling in prostate cancer cells needs further study to completely grasp this connection. The effect of this interaction on treatment response needs additional study.

According to breast cancer study, TLR3 signaling increases IL-6 and STAT3 phosphorylation [88]. Both IL-6 and STAT3, a transcription factor important in cell proliferation and survival, increase cancer progression. This shows that TLR3 signaling promotes breast cancer growth and inflammation. In cancer, KLFs and TLRs affect TME inflammation and immunological responses [89–96].

Cancer, KLFs, and Circadian Clock

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KLFs, core circadian genes (CRY1, PER1, CLOCK, BMAL1), and PPARG1CA (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) play intricate functions in cancer pathways. The circadian clock, a crucial timekeeping mechanism, is increasingly implicated in cancer development and progression [97–99]. PPARG1CA, a key regulator of energy metabolism and lipid homeostasis, influences cellular processes like inflammation, cell proliferation, and apoptosis [100]. A key transcriptional coactivator, PPARG1CA regulates energy metabolism and lipid homeostasis [101]. This function is closely linked to PPARγ, a nuclear receptor involved in several metabolic processes. By increasing the transcriptional activity of PPARγ, PPARG1CA improves its effects on gene expression. This coactivator regulates glucose, lipid, mitochondrial, and adipogenesis genes. Through its effects on inflammation, cell proliferation, and apoptosis, PPARG1CA dysregulation may contribute to many malignancies. PPARG1CA's involvement in cancer varies by type and tumor microenvironment. PPARG1CA can prevent or accelerate tumor development. Its effects on inflammation, a cancer driver, are important. PPARG1CA affects the tumor microenvironment via modulating inflammatory cytokines and signaling pathways. Its regulation of cell proliferation and apoptosis also affects cancer cell growth and survival. More research is needed to understand PPARG1CA's various effects in different cancer types and situations.

Circadian clocks and KLFs interact through complicated regulatory networks. KLF10's liver rhythmic expression, depending on BMAL1, a circadian clock component, is a good illustration of this connection [102–103]. This regulatory link shows the circadian clock regulates KLF activity and cancer pathways. KLF10's rhythmic expression may help maintain liver metabolic homeostasis[104] by coordinating hepatic metabolic activities. Circadian clock dysfunction can disrupt this rhythmic expression, causing metabolic abnormalities and metabolic-associated malignancies.

KLF10 is not the only circadian gene-KLF relationship. Other KLF isoforms may be regulated by the circadian clock through complex transcriptional and post-transcriptional processes. KLFs may also affect circadian gene expression, producing feedback loops that regulate cellular functions.[105]

The complicated relationship between circadian genes and KLF10 in hepatic metabolism [104] shows this context dependence. KLF10's rhythmic expression depends on BMAL1, but other factors may potentially affect it, resulting in different consequences. These synergistic and antagonistic interactions will determine cancer pathway effects.

Understanding how circadian genes and KLFs affect cancer growth and progression opens new treatment possibilities. These parameters may be modulated to reduce tumor development, improve therapy efficacy, or improve patient outcomes [102–106].

KLF-TP53, TERT, FOXO1, Klotho Interactions in Cancer

KLFs like KLF4 and KLF5 directly interact with TP53 to boost its tumor suppressor function. These interactions can synergistically promote apoptosis and decrease cell growth [105–107] The mechanisms of these interactions are still being studied, but transcriptional control and protein-protein interactions are presumably involved. To develop new cancer therapeutics targeting TP53-KLF pathways, these interactions must be understood.

The link between KLFs and TERT is unclear. However, indirect interactions are possible because KLFs regulate cell growth and senescence, and TERT maintains cancer cell telomeres. KLFs may indirectly affect cancer cell telomerase activity and length by modulating TERT expression or activity.

Interactions between KLF and FOXO1 in cancer pathways are complex.

Many studies show a complex relationship between KLFs and FOXO1. [108]. KLFs like KLF4 may complement FOXO1 to prevent tumor growth by inducing apoptosis and reducing cell proliferation. Other KLFs may interact with FOXO1 antagonistically, encouraging tumor development.

These genes' critical involvement in controlling cancer pathways make them intriguing cancer therapy targets. Strategies to restore tumor suppressor KLFs or inhibit oncogenic KLFs are being

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studied. Gene therapy or small molecule inhibitors may be used. Modulating TP53, TERT, or FOXO1 activity may also be helpful.

Klotho-KLF Interactions in Cancer: An Indirect Influence Network

Since Klotho and KLF proteins are involved in several cancer-relevant pathways, indirect interactions are likely. Direct physical interactions are not yet known. Klotho and other KLFs modulate signaling cascades, including IGF-1, Wnt/β-catenin, and TGF-β pathways (109, 20, 110.111). Klotho and KLFs may interact functionally and crosstalk in cancer due to their overlapping regulatory impact. [112–113] These interactions may vary by KLF family member, malignancy kind, and cellular microenvironment.

Synergistic and Antagonistic Effects: Context-Dependent Results

Based on the situation, Klotho and KLFs can synergistically or antagonistically affect cancer pathways. Klotho's inhibitory action on Wnt/β-catenin signaling may boost the synergistic anti-cancer effects of KLFs targeting this pathway. [114–116] This intricate interaction emphasizes the importance of KLF and cancer type for predicting Klotho and KLF effects on cancer progression. [117–119]

Klotho and KLF family member expression levels may be cancer biomarkers. [120]. Low Klotho expression or activity often indicates a worse prognosis and more aggressive tumors. Specific KLF expression patterns [120–122] can predict tumor behavior and patient survival.Klotho and KLFs are promising therapeutic targets due to their cancer pathway functions. Strategies to restore Klotho expression or activity may help malignancies with downregulated Klotho. (109,110)

Inputting all the genes expressed by Metadichol into the STRING database network analysis program [123] shows literature-curated interactions between and relationships of the gene families.

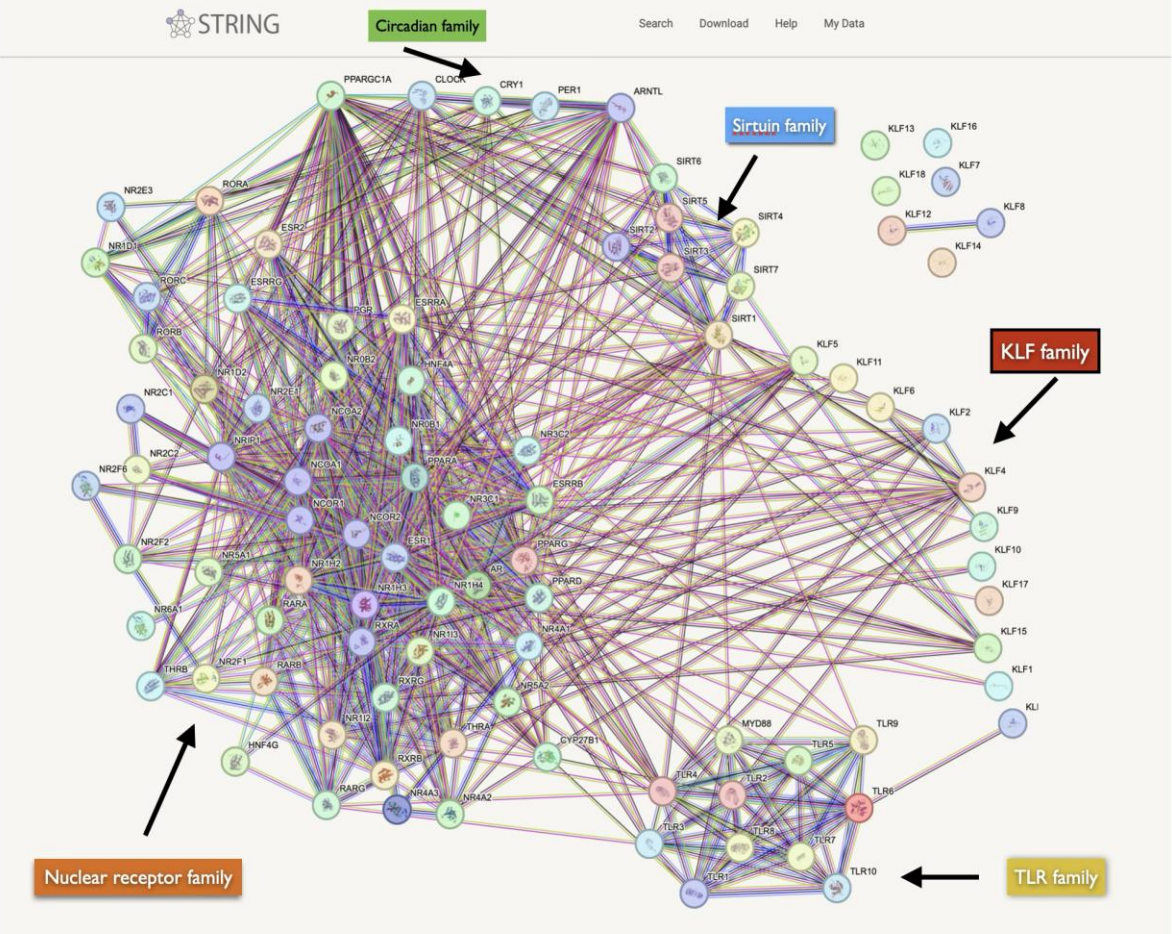

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Figure 11. KLF, NR, TLR, Sirtuins, and circadian family gene network connections.

Figure 12. Metadichol gene expression and its impact on cancer processes .

Conclusions

Metadichol has the capacity to affect various molecular families implicated in cancer formation and progression (figure 12) and offers a unique potential for cancer research and treatment. This

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multidimensional approach may be able to target numerous cancer hallmarks, outperforming single-pathway therapies.

Metadichol's capacity to regulate several actors in the complex and dynamic interaction of these molecular families in cancer presents interesting new cancer treatment possibilities. Metadichol can now be used to treat many cancers due to its nontoxicity [124–126].

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References

1. Yuce K, Ozkan AI. The kruppel-like factor (KLF) family, diseases, and physiological events, . Gene. 2024 Feb 15;895:148027. doi: 10.1016/j.gene.2023.148027.
2. Swamynathan SK. Krüppel-like factors: three fingers in control. Hum Genomics. 2010 Apr;4(4):263-70. doi: 10.1186/1479-7364-4-4-263.
3. Black AR, Black JD, Azizkhan-Clifford J. Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol. 2001 Aug;188(2):143-60. doi: 10.1002/jcp.1111.
4. Gao Y, Cao Q, Lu L, Zhang X, Zhang Z, Dong X, Jia W, Cao Y. Kruppel-like factor family genes are expressed during Xenopus embryogenesis and involved in germ layer formation and body axis patterning. Dev Dyn. 2015 Oct;244(10):1328-46. doi: 10.1002/dvdy.24310. Epub 2015 Aug 10. PMID: 26198170.
5. Oates AC, Pratt SJ, Vail B, Yan Yl, Ho RK, Johnson SL, Postlethwait JH, Zon LI. The zebrafish KLF gene family. Blood. 2001 Sep 15;98(6):1792-801. doi: 10.1182/blood.v98.6.1792. PMID: 11535513.
6. Oates AC, Pratt SJ, Vail B, Yan Yl, Ho RK, Johnson SL, Postlethwait JH, Zon LI. The zebrafish KLF gene family. Blood. 2001 Sep 15;98(6):1792-801. doi: 10.1182/blood.v98.6.1792. PMID: 11535513.
7. Shao M., Ge G., Liu W., Xiao J., Xia H., Fan Y., Zhao F., He B.Chen C. Characterization and phylogenetic analysis of Krüppel-like transcription factor (KLF) gene family in tree shrews (*Tupaia belangeri chinensis*). Oncotarget. 2017; 8: 16325-16339.
8. Shinoda Y, Ogata N, Higashikawa A, Manabe I, Shindo T, Yamada T, Kugimiya F, Ikeda T, Kawamura N, Kawasaki Y, Tsushima K, Takeda N, Nagai R, Hoshi K, Nakamura K, Chung UI, Kawaguchi H. Kruppel-like factor 5 causes cartilage degradation through transactivation of matrix metalloproteinase 9. J Biol Chem. 2008 Sep 5;283(36):24682-9. doi: 10.1074/jbc.M709857200.
9. Mallipattu SK, Estrada CC, He JC. The critical role of Krüppel-like factors in kidney disease. Am J Physiol Renal Physiol. 2017 Feb 1;312(2):F259-F265. doi: 10.1152/ajprenal.00550.2016.
10. Alder JK, Georgantas RW 3rd, Hildreth RL, Kaplan IM, Morisot S, Yu X, McDevitt M, Civin CI. Kruppel-like factor 4 is essential for inflammatory monocyte differentiation in vivo. J Immunol. 2008 Apr 15;180(8):5645-52. doi: 10.4049/jimmunol.180.8.5645.
11. Papadakis Konstantinos, Krempski James, P-201 Krüppel-Like Factor KLF10 Regulates Intestinal Macrophages and Innate Immune Colitis, *Inflammatory Bowel Diseases*, Volume 20, Issue suppl_1, December 2014, Page S106, https://doi.org/10.1097/01.MIB.0000456963.59724.80
12. Mao A, Zhou X, Liu Y, Ding J, Miao A, Pan G. KLF8 is associated with poor prognosis and regulates glycolysis by targeting GLUT4 in gastric cancer. J Cell Mol Med. 2019 Aug;23(8):5087-5097. doi: 10.1111/jcmm.14378.
13. Sebzda E, Zou Z, Lee JS, Wang T, Kahn ML. Transcription factor KLF2 regulates the migration of naive T cells by restricting chemokine receptor expression patterns. Nat Immunol. 2008 Mar;9(3):292-300. doi: 10.1038/ni1565
14. Tugal D, Jain MK, Simon DI. Endothelial KLF4: crippling vascular injury? J Am Heart Assoc. 2014 Feb 26;3(1):e000769. doi: 10.1161/JAHA.113.000769.
15. Liu, C., Shen, M., Tan, W.L.W. *et al.* Statins improve endothelial function via suppression of epigenetic-driven End MT. *Nat Cardiovasc Res* **2**, 467–485 (2023). https://doi.org/10.1038/s44161-023-00267-1

16. Melissa E. Heard, Michael C. Velarde, Linda C. Giudice, Frank A. Simmen, Rosalia C.M. Simmen, Krüppel-Like Factor 13 Deficiency in Uterine Endometrial Cells Contributes to Defective Steroid Hormone Receptor Signaling but Not Lesion Establishment in a Mouse Model of Endometriosis, Biology of Reproduction, Volume 92, Issue 6, 1 June 2015, 140, 1–9, https://doi.org/10.1095/biolreprod.115.130260
17. Athon W. Homeister and Cam Patterson. Zinc Fingers in the Pizza Pie, Circulation Research, 2008, Volume 103, Number 7. /doi.org/10.1161/CIRCRESAHA.108.18576
18. Yang Y, Goldstein BG, Chao HH, Katz JP. KLF4 and KLF5 regulate proliferation, apoptosis and invasion in esophageal cancer cells. Cancer Biol Ther. 2005 Nov;4(11):1216-21. doi: 10.4161/cbt.4.11.2090.
19. Brown, A. R., Simmen, R., and Simmen, F.. 2011. "Abstract A44: Suppression of insulin-induced fatty acid synthase gene expression and colon cancer cell proliferation by members of the Krppel-like family of transcription factors". Cancer Prev Res 2011;4(10 Suppl):A44.
20. Bieker JJ. Krüppel-like factors: three fingers in many pies. J Biol Chem. 2001 Sep 14;276(37):34355-8. doi: 10.1074/jbc.R100043200..
21. Pang CJ, Lemsaddek W, Alhashem YN, Bondzi C, Redmond LC, Ah-Son N, Dumur CI, Archer KJ, Haar JL, Lloyd JA, Trudel M. Kruppel-like factor 1 (KLF1), KLF2, and Myc control a regulatory network essential for embryonic erythropoiesis. Mol Cell Biol. 2012 Jul;32(13):2628-44. doi: 10.1128/MCB.00104-12.
22. Kalra IS, Alam MM, Choudhary PK, Pace BS. Krüppel-like Factor 4 activates HBG gene expression in primary erythroid cells. Br J Haematol. 2011 Jul;154(2):248-59. doi: 10.1111/j.1365-2141.2011.08710.x.
23. Vinci, M.; Greco, D.; Treccarichi, S.; Chiavetta, V.; Figura, M.G.; Musumeci, A.; Greco, V.; Federico, C.; Calì, F.; Saccone, S. Bioinformatic Evaluation of *KLF13* Genetic Variant: Implications for Neurodevelopmental and Psychiatric Symptoms. Genes **2**024, 15, 1056. https://doi.org/10.3390/genes15081056.
24. Blackmore MG, Wang Z, Lerch JK, Motti D, Zhang YP, Shields CB, Lee JK, Goldberg JL, Lemmon VP, Bixby JL. Krüppel-like Factor 7 engineered for transcriptional activation promotes axon regeneration in the adult corticospinal tract. Proc Natl Acad Sci U S A. 2012 May 8;109(19):7517-22. doi: 10.1073/pnas.1120684109.
25. Shaverdashvili K, Padlo J, Weinblatt D, Jia Y, Jiang W, Rao D, Laczkó D, Whelan KA, Lynch JP, Muir AB, Katz JP. KLF4 activates NFκB signaling and esophageal epithelial inflammation via the Rho-related GTP-binding protein RHOF. PLoS One. 2019 Apr 18;14(4):e0215746. doi: 10.1371/journal.pone.0215746.
26. Jiang Z, Yu T, Fan Z, Yang H, Lin X. Krüppel-Like Factor 7 is a Marker of Aggressive Gastric Cancer and Poor Prognosis. Cell Physiol Biochem. 2017;43(3):1090-1099. doi: 10.1159/000481748..
27. P.R. Raghavan US patents 8,722,093 (2014) and 9,006,292 (2015).
28. P.R. Raghavan . Metadichol®-induced expression of Sirtuins 1-7 in somatic and cancer cells, Medical Research Archives, (online), 2024 12(6). https://doi.org/10.18103/mra.v12i6.5371.
29. P.R. Raghavan . Metadichol-induced expression of Toll receptor family members in peripheral blood mononuclear cells. Medical research archives, 2024. (online) 12(8). https://doi.org/10.18103/mra.v12i9.5610.
30. P.R. Raghavan. Metadichol® A Nano Lipid Emulsion that Expresses All 49 Nuclear Receptors in Stem and Somatic Cells. Archives of Clinical and Biomedical Research. 7 2023: 524-536. Doi.10.26502/acbr.50170368.
31. P.R. Raghavan, Metadichol, a Natural Ligand for the Expression of Yamanaka Reprogramming Factors in Human Cardiac, Fibroblast, and Cancer Cell Lines. Medical Research Archives, (online) 2024, 12(6). https://doi.org/10.18103/mra.v12i6.
32. P.R. Raghavan PR., 2024. Metadichol®-induced expression of Circadian clock transcription factors in human fibroblasts. Medical Research Archives, online) 2024, 12(6). https://doi.org/10.18103/mra.v12i6.5371.
33. Tetreault MP, Wang ML, Yang Y, Travis J, Yu QC, Klein-Szanto AJ, Katz JP. Klf4 overexpression activates epithelial cytokines and inflammation-mediated esophageal squamous cell cancer in mice. Gastroenterology. 2010 Dec;139(6):2124-2134.e9. doi: 10.1053/j.gastro.2010.08.048. PMID: 20816834; PMCID: PMC3457785.
34. Tarapore RS, Yang Y, Katz JP. Restoring KLF5 in esophageal squamous cell cancer cells activates the JNK pathway leading to apoptosis and reduced cell survival. Neoplasia. 2013 May;15(5):472-80. doi: 10.1593/neo.122126.

35. Lahiri SK, Zhao J. Krüppel-like factor 8 emerges as an important regulator of cancer. Am J Transl Res. 2012;4(3):357-63.
36. Li ZY, Zhu YX, Chen JR, Chang X, Xie ZZ. The role of KLF transcription factor in the regulation of cancer progression. Biomed Pharmacother. 2023 Jun;162:114661. doi: 10.1016/j.biopha.2023.114661.
37. McConnell B.B, Yang VW. Mammalian Krüppel-like factors in health and diseases. Physiol Rev. 2010 Oct;90(4):1337-81. doi: 10.1152/physrev.00058.2009.
38. Yang, Y. & Katz, J. P. in The Biology of Krüppel-like Factors (eds. Nagai, R. F., Friedman, S. L. & Kasuga, M.) 2009, 67–82 (Springer).
39. Tetreault, MP., Yang, Y. & Katz, J. Krüppel-like factors in cancer. *Nat Rev Cancer*, 2013 **13**, 701–713 . https://doi.org/10.1038/nrc3582.
40. Zhang Y, Yao C, Ju Z, Jiao D, Hu D, Qi L, Liu S, Wu X, and Zhao C. Krüppel-like actors in tumors: Key regulators and therapeutic avenues. Front. Oncol. 2023, 13:1080720. doi: 10.3389/fonc.2023.1080720.
41. Rascio F, Spadaccino F, Rocchetti MT, Castellano G, Stallone G, Netti GS, Ranieri E. The Pathogenic Role of PI3K/AKT Pathway in Cancer Onset and Drug Resistance: An Updated Review. Cancers (Basel). 2021 Aug 5;13(16):3949. doi: 10.3390/cancers13163949. PMID: 34439105; PMCID: PMC8394096.
42. Zhu B, Liu Q, Han Q, Zeng B, Chen J, Xiao Q. Downregulation of Krüppel-like factor 1 inhibits the metastasis and invasion of cervical cancer cells. Mol Med Rep. 2018 Oct;18(4):3932-3940. doi: 10.3892/mmr.2018.9401.
43. Xu R, Chen Y, Wei S, Chen J. Comprehensive Pan-Cancer Analysis of the Prognostic Role of KLF Transcription Factor 2 (KLF2) in Human Tumors. Onco Targets Ther. 2024 Nov 2;17:887-904. doi: 10.2147/OTT.S476179.
44. Zhu J, Teng H, Zhu X, Yuan J, Zhang Q, Zou Y. Pancancer analysis of Krüppel-like factor 3 and its carcinogenesis in pancreatic cancer. Front Immunol. 2023 Aug 3;14:1167018. doi: 10.3389/fimmu.2023.1167018.
45. He, Z., He, J. & Xie, K. KLF4 transcription factor in tumorigenesis. Cell Death Discov. 2023, 9, 118. https://doi.org/10.1038/s41420-023-01416-y.
46. Luo Y, Chen C. The roles and regulation of the KLF5 transcription factor in cancers. Cancer Sci. 2021 Jun;112(6):2097-2117. doi: 10.1111/cas.14910.
47. DiFeo A, Martignetti JA, Narla G. The role of KLF6 and its splice variants in cancer therapy. Drug Resist Updat. 2009 Feb-Apr;12(1-2):1-7. doi: 10.1016/j.drup.2008.11.001.
48. Li Z, Liu Q. The Oncogenic Role of KLF7 in Colon Adenocarcinoma and Therapeutic Perspectives. Int J Genomics. 2023 Dec 12;2023:5520926. doi: 10.1155/2023/5520926.
49. Lahiri SK, Zhao J. Krüppel-like factor 8 emerges as an important regulator of cancer. Am J Transl Res. 2012;4(3):357-63.
50. Ying M, Sang Y, Li Y, Guerrero-Cazares H, Quinones-Hinojosa A, Vescovi AL, Eberhart CG, Xia S, Laterra J. Krüppel-like family of transcription factor 9, a differentiation-associated transcription factor, suppresses Notch1 signaling and inhibits glioblastoma-initiating stem cells. Stem Cells. 2011 Jan;29(1):20-31. doi: 10.1002/stem.561.
51. Memon A, Lee WK. KLF10 as a Tumor Suppressor Gene and Its TGF-β Signaling. Cancers (Basel). 2018 May 25;10(6):161. doi: 10.3390/cancers10060161.
52. Xi Z, Zhang R, Zhang F, Ma S, Feng T. KLF11 Expression Predicts Poor Prognosis in Glioma Patients. Int J Gen Med. 2021 Jun 28;14:2923-2929. doi: 10.2147/IJGM.S307784.
53. Li, Y., Li, S., Shi, X. et al. KLF12 promotes the proliferation of breast cancer cells by reducing the transcription of p21 in a p53-dependent and p53-independent manner. Cell Death Dis, 2023, 14, 313. https://doi.org/10.1038/s41419-023-05824-x.
54. Chen CC, Xie XM, Zhao XK, Zuo S, Li HY. Krüppel-like Factor 13 Promotes HCC Progression by Transcriptional Regulation of HMGCS1-mediated Cholesterol Synthesis. J Clin Transl Hepatol. 2022 Dec 28;10(6):1125-1137. doi: 10.14218/JCTH.2021.00370..
55. Wang X, Qu X, Liu X, Wang K, Yang Y, Zhang Y, Wang Z, Fan G, Li Y, Zeng Y, Chen H, Zhu T. KLF14 inhibits tumor progression via FOSL1 in glioma. Biochem Biophys Rep. 2024 Nov 27;41:101885. doi: 10.1016/j.bbrep.2024.101885.

 doi:10.20944/preprints202502.0271.v1

56. Kanyomse, Q., Le, X., Tang, J. et al. KLF15 suppresses tumor growth and metastasis in Triple-Negative Breast Cancer by downregulating CCL2 and CCL7. Sci Rep, 2022, 12, 19026. https://doi.org/10.1038/s41598-022-23750-4.
57. Ma P, Sun CQ, Wang YF, Pan YT, Chen QN, Liu WT, Liu J, Zhao CH, Shu YQ, Li W. KLF16 promotes proliferation in gastric cancer cells by regulating p21 and CDK4. Am J Transl Res. 2017 Jun 15;9(6):3027-3036. PMID: 28670390; PMCID: PMC5489902.
58. Jiang X, Shen TY, Lu H, Shi C, Liu Z, Qin H, Wang F. Clinical significance and biological role of KLF17 as a tumor suppressor in colorectal cancer. Oncol Rep. 2019 Nov;42(5):2117-2129. doi: 10.3892/or.2019.7324.
59. Zhang Y, Yao C, Ju Z, Jiao D, Hu D, Qi L, Liu S, Wu X, Zhao C. Krüppel-like factors in tumors: Key regulators and therapeutic avenues. Front Oncol. 2023 Jan 25;13:1080720. doi: 10.3389/fonc.2023.1080720..
60. Simmen FA, Alhallak I, Simmen RCM. Krüppel-like Factor-9 and Krüppel-like Factor-13: Highly Related, Multi-Functional, Transcriptional Repressors and Activators of Oncogenesis. Cancers (Basel). 2023 Nov 30;15(23):5667. doi: 10.3390/cancers15235667..
61. Fleming-de-Moraes CD, Rocha MR, Tessmann JW, de Araujo WM, Morgado-Diaz JA. Crosstalk between PI3K/Akt and Wnt/β-catenin pathways promote colorectal cancer progression regardless of mutational status. Cancer Biol Ther. 2022 Dec 31;23(1):1-13. doi: 10.1080/15384047.2022.2108690..
62. Tsai YC, Hsin MC, Liu RJ, Li TW, Ch'ang HJ. Krüppel-like Factor 10 as a Prognostic and Predictive Biomarker of Radiotherapy in Pancreatic Adenocarcinoma. Cancers (Basel). 2023 Oct 30;15(21):5212. doi: 10.3390/cancers15215212. PMID: 37958386; PMCID: PMC10648792.
63. P.R. Raghavan. Metadichol, A Modulator that Controls Expression of Toll-Like Receptors in Cancer Cell Lines British Journal of Cancer Research. 2024, 7(3): 720-732. doi: 10.31488/bjcr.198
64. P.R. Raghavan. Metadichol: An Agonist that Expresses the Anti-Aging Gene Klotho in Various Cell Lines. Fortune Journal of Health Sciences. 2023, 6: 357-362. DOI:10.26502/jbsb.5107066.
65. P.R. Raghavan.The Quest for Immortality: Introducing Metadichol®, a Novel Telomerase Activator. Stem Cell Res Ther 2019, 9: 446. doi: 10.4172/2157-7633.1000446.
66. Mao A, Zhou X, Liu Y, Ding J, Miao A, Pan G. KLF8 is associated with poor prognosis and regulates glycolysis by targeting GLUT4 in gastric cancer. J Cell Mol Med. 2019 Aug;23(8):5087-5097. doi: 10.1111/jcmm.14378.
67. Tsai, YiChih, Chen, Suliang, Peng, Shu-Ling, Tsai, Ya-Li, Chang, Zuong-Ming, Chang, Vincent, and Chang, Hui-Ju. "Upregulating sirtuin 6 ameliorates glycolysis, EMT and distant metastasis of pancreatic adenocarcinoma with krppel-like factor 10 deficiency". Springer Nature. https://doi.org/10.1038/s12276-021-00687-8
68. Ansari A, Rahman MS, Saha SK, Saikot FK, Deep A, Kim KH. Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. Aging Cell. 2017 Feb;16(1):4-16. doi: 10.1111/acel.12538.
69. Limame R, Op de Beeck K, Lardon F, De Wever O, Pauwels P. Krüppel-like factors in cancer progression: three fingers on the steering wheel. Oncotarget. 2014 Jan 15;5(1):29-48. doi: 10.18632/oncotarget.1456..
70. Bureau C, Hanoun N, Torrisani J, Vinel JP, Buscail L, Cordelier P. Expression and Function of Kruppel Like-Factors (KLF) in Carcinogenesis. Curr Genomics. 2009 Aug;10(5):353-60. doi: 10.2174/138920209788921010
71. Zhao E, Hou J, Ke X, Abbas MN, Kausar S, Zhang L, Cui H. The Roles of Sirtuin Family Proteins in Cancer Progression. Cancers (Basel). 2019 Dec 5;11(12):1949. doi: 10.3390/cancers11121949..
72. Black AR, Black JD, Azizkhan-Clifford J. Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol. 2001 Aug;188(2):143-60. doi: 10.1002/jcp.1111.
73. Stünkel W, Campbell RM. Sirtuin 1 (SIRT1): the misunderstood HDAC. J Biomol Screen. 2011 Dec;16(10):1153-69. doi: 10.1177/1087057111422103..
74. Zhang XL, Zhang D, Michel FJ, Blum JL, Simmen FA, Simmen RC. Selective interactions of Kruppel-like factor 9/basic transcription element-binding protein with progesterone receptor isoforms A and B determine transcriptional activity of progesterone-responsive genes in endometrial epithelial cells. J Biol Chem. 2003 Jun 13;278(24):21474-82. doi: 10.1074/jbc.M212098200. Epub 2003 Apr 2. PMID:

75. Knoedler JR, Denver RJ. Krüppel-like factors are effectors of nuclear receptor signaling. Gen Comp Endocrinol. 2014 Jul 1;203:49-59. doi: 10.1016/j.ygcen.2014.03.003.
76. Luo Y, Chen C. The roles and regulation of the KLF5 transcription factor in cancers. Cancer Sci. 2021 Jun;112(6):2097-2117. doi: 10.1111/cas.14910. Epub 2021 May 3. PMID: 33811715; PMCID: PMC8177779.
77. Fan L, Sweet DR, Fan EK, Prosdocimo DA, Madera A, Jiang Z, Padmanabhan R, Haldar SM, Vinayachandran V, Jain MK. Transcription factors KLF15 and PPARδ cooperatively orchestrate genome-wide regulation of lipid metabolism in skeletal muscle. J Biol Chem. 2022 Jun;298(6):101926. doi: 10.1016/j.jbc.2022.101926.
78. Shrestha S, Sewell JA, Santoso CS, Forchielli E, Carrasco Pro S, Martinez M, Fuxman Bass JI. Discovering human transcription factor physical interactions with genetic variants, novel DNA motifs, and repetitive elements using enhanced yeast one-hybrid assays. Genome Res. 2019 Sep;29(9):1533-1544. doi: 10.1101/gr.248823.119. PMID: 31481462; PMCID: PMC6724672.
79. Giarrizzo M, LaComb JF, Bialkowska AB. The Role of Krüppel-like Factors in Pancreatic Physiology and Pathophysiology. Int J Mol Sci. 2023 May 11;24(10):8589. doi: 10.3390/ijms24108589..
80. Yea S, Narla G, Zhao X, Garg R, Tal-Kremer S, Hod E, Villanueva A, Loke J, Tarocchi M, Akita K, Shirasawa S, Sasazuki T, Martignetti JA, Llovet JM, Friedman SL. Ras promotes growth by alternative splicing-mediated inactivation of the KLF6 tumor suppressor in hepatocellular carcinoma. Gastroenterology. 2008 May;134(5):1521-31. doi: 10.1053/j.gastro.2008.02.015.
81. Sun H, Gao Y, Lu K, Zhao G, Li X, Li Z, Chang H. Overexpression of Klotho suppresses liver cancer progression and induces cell apoptosis by negatively regulating wnt/β-catenin signaling pathway. World J Surg Oncol. 2015 Oct 24;13:307. doi: 10.1186/s12957-015-0717-0.
82. Ozkan AD, Eskiler GG, Kazan N, Turna O. Histone deacetylase inhibitor sodium butyrate regulates the activation of toll-like receptor 4/interferon regulatory factor-3 signaling pathways in prostate cancer cells. J Cancer Res Ther. 2023 Oct 1;19(7):1812-1817. doi: 10.4103/jcrt.jcrt_2032_21.
83. Luo, Xinjing, Chen, Jie, Ruan, Jianwei, Chen, Yongfeng, Mo, Xuanrong, Xie, Jiangwen, and Lv, Guoju. 2015. "Krüppel-Like Factor 4 Is a Regulator of Proinflammatory Signaling in Fibroblast-Like Synoviocytes through Increased IL-6 Expression". Mediators of Inflammation. https://doi.org/10.(82)55/2016/1062586
84. Tsai, Kuo-Wang. 2017. "Identify metastasis-associated lnc RNA in triple-negative breast cancer". Journal of Cancer Science & Therapy. https://doi.org/10.4172/1948-5956-c5-136
85. Ma L, Feng L, Ding X, Li Y. Effect of TLR4 on the growth of SiHa human cervical cancer cells via the MyD88-TRAF6-TAK1 and NF-κB-cyclin D1-STAT3 signaling pathways. Oncol Lett. 2018 Mar;15(3):3965-3970. doi: 10.3892/ol.2018.7801.
86. Fakhri S, Moradi SZ, Yarmohammadi A, Narimani F, Wallace CE, Bishayee A. Modulation of TLR/NF-κB/NLRP Signaling by Bioactive Phytocompounds: A Promising Strategy to Augment Cancer Chemotherapy and Immunotherapy. Front Oncol. 2022 Mar 1;12:834072. doi: 10.3389/fonc.2022.834072..
87. De Paoli F, Staels B, Chinetti-Gbaguidi G. Macrophage phenotypes and their modulation in atherosclerosis. Circ J. 2014;78(8):1775-81. doi: 10.1253/circj.cj-14-0621.
88. Troutman TD, Bazan JF, Pasare C. Toll-like receptors, signaling adapters and regulation of the pro-inflammatory response by PI3K. Cell Cycle. 2012 Oct 1;11(19):3559-67. doi: 10.4161/cc.21572. Epub 2012 Aug 16. PMID: 22895011; PMCID: PMC3478307.
89. Szebeni GJ, Vizler C, Kitajka K, Puskas LG. Inflammation and Cancer: Extra- and Intracellular Determinants of Tumor-Associated Macrophages as Tumor Promoters. Mediators Inflamm. 2017;2017:9294018. doi: 10.1155/2017/9294018.
90. Zou J, Shankar N. Roles of TLR/MyD88/MAPK/NF-κB Signaling Pathways in the Regulation of Phagocytosis and Proinflammatory Cytokine Expression in Response to E. faecalis Infection. PLoS One. 2015 Aug 28;10(8):e0136947. doi: 10.1371/journal.pone.0136947..
91. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, Paruchuri K, Mahabeleshwar GH, Dalmas E, Venteclef N, Flask CA, Kim J, Doreian BW, Lu KQ, Kaestner KH, Hamik A, Clément K, Jain MK. Krüppel-like factor 4 regulates macrophage polarization. J Clin Invest. 2011 Jul;121(7):2736-49. doi: 10.1172/JCI45444..

92. Saheed Oluwasina Oseni, Rolando Branly, Mirjana Pavlovic, James Kumi-Diaka; Abstract 5730: Co-targeting toll-like receptor and PI3K survival signaling pathways in stem-like castration resistant prostate cancer cells. *Cancer Res* 1 July 2018; 78 (13_Supplement): 5730. https://doi.org/10.1158/1538-7445.AM2018-5730
93. Schwartz AL, Dickerson E, Dagia N, Malgor R, McCall KD. TLR signaling inhibitor, phenylmethimazole, in combination with tamoxifen inhibits human breast cancer cell viability and migration. Oncotarget. 2016 Jul 1;8(69):113295-113302. doi: 10.18632/oncotarget.10358. PMID: 29371911; PMCID: PMC5768328.
94. Wang H, Flannery SM, Dickhöfer S, Huhn S, George J, Kubarenko AV, Lascorz J, Bevier M, Willemsen J, Pichulik T, Schafmayer C, Binder M, Manoury B, Paludan SR, Alarcon-Riquelme M, Bowie AG, Försti A, Weber ANR. A coding IRAK2 protein variant compromises Toll-like receptor (TLR) signaling and is associated with colorectal cancer survival. J Biol Chem. 2014 Aug 15;289(33):23123-23131. doi: 10.1074/jbc.M113.492934..
95. Chu Y, Liu Z, Liu J, Yu L, Zhang D, Pei F. Characterization of lncRNA-Perturbed TLR-Signaling Network Identifies Novel lncRNA Prognostic Biomarkers in Colorectal Cancer. Front Cell Dev Biol. 2020 Jun 18;8:503. doi: 10.3389/fcell.2020.00503. PMID: 32626715; PMCID: PMC7314994.
96. Sedighzadeh SS, Khoshbin AP, Razi S, Keshavarz-Fathi M, Rezaei N. A narrative review of tumor-associated macrophages in lung cancer: regulation of macrophage polarization and therapeutic implications. Transl Lung Cancer Res. 2021 Apr;10(4):1889-1916. doi: 10.21037/tlcr-20-1241. PMID: 34012800; PMCID: PMC8107755.
97. Yong Zhu, Richard G. Stevens, Aaron E. Hoffman, Liesel M. FitzGerald, Erika M. Kwon, Elaine A. Ostrander, Scott Davis, Tongzhang Zheng, Janet L. Stanford; Testing the Circadian Gene Hypothesis in Prostate Cancer: A Population-Based Case-Control Study. *Cancer Res* 15 December 2009; 69 (24): 9315–9322. https://doi.org/10.1158/0008-5472.CAN-09-0648
98. Dierickx P, Van Laake LW, Geijsen N. Circadian clocks: from stem cells to tissue homeostasis and regeneration. EMBO Rep. 2018 Jan;19(1):18-28. doi: 10.15252/embr.201745130.
99. Wu Y, Tao B, Zhang T, Fan Y, Mao R. Pan-Cancer Analysis Reveals Disrupted Circadian Clock Associates With T Cell Exhaustion. Front Immunol. 2019 Oct 24;10:2451. doi: 10.3389/fimmu.2019.02451. PMID: 31708917; PMCID: PMC6821711.
100. Subramaniam M, Hawse JR, Rajamannan NM, Ingle JN, Spelsberg TC. Functional role of KLF10 in multiple disease processes. Biofactors. 2010 Jan-Feb;36(1):8-18. doi: 10.1002/biof.67.
101. Léveillé M, Besse-Patin A, Jouvet N, Gunes A, Sczelecki S, Jeromson S, Khan NP, Baldwin C, Dumouchel A, Correia JC, Jannig PR, Boulais J, Ruas JL, Estall JL. PGC-1α isoforms coordinate to balance hepatic metabolism and apoptosis in inflammatory environments. Mol Metab. 2020 Apr;34:72-84. doi: 10.1016/j.molmet.2020.01.004.
102. Angelousi A, Kassi E, Ansari-Nasiri N, Randeva H, Kaltsas G, Chrousos G. Clock genes and cancer development in particular in endocrine tissues. Endocr Relat Cancer. 2019 Jun;26(6):R305-R317. doi: 10.1530/ERC-19-0094..
103. Sulli G, Lam MTY, Panda S. Interplay between Circadian Clock and Cancer: New Frontiers for Cancer Treatment. Trends Cancer. 2019 Aug;5(8):475-494. doi: 10.1016/j.trecan.2019.07.002. Epub 2019 Aug 3. PMID: 31421905; PMCID: PMC7120250.
104. Guillaumond, F., Gréchez-Cassiau, A., Subramaniam, M., Brangolo, S., Peteri-Brünback, B., Staels, B., … Teboul, M.. Krüppel-Like Factor KLF10 Is a Link between the Circadian Clock and Metabolism in Liver. *Molecular and Cellular Biology*, 2010, *30*(12), 3059–3070. https://doi.org/10.1128/MCB.01141-09
105. Chen P, Hsu WH, Chang A, Tan Z, Lan Z, Zhou A, Spring DJ, Lang FF, Wang YA, DePinho RA. Circadian Regulator CLOCK Recruits Immune-Suppressive Microglia into the GBM Tumor Microenvironment. Cancer Discov. 2020 Mar;10(3):371-381. doi: 10.1158/2159-8290.CD-19-0400..
106. Kiss Z, Ghosh PM. WOMEN IN CANCER THEMATIC REVIEW: Circadian rhythmicity and the influence of 'clock' genes on prostate cancer. Endocr Relat Cancer. 2016 Nov;23(11):T123-T134. doi: 10.1530/ERC-16-0366. Epub 2016 Sep 22. PMID: 27660402; PMCID: PMC5148656.

107. Zhou S, Tang X, Tang F. Krüppel-like factor 17, a novel tumor suppressor: its low expression is involved in cancer metastasis. Tumour Biol. 2016 Feb;37(2):1505-13. doi: 10.1007/s13277-015-4588-3. Epub 2015 Dec 12. PMID: 26662959; PMCID: PMC4842221.
108. Yusuf I, Kharas MG, Chen J, Peralta RQ, Maruniak A, Sareen P, Yang VW, Kaestner KH, Fruman DA. KLF4 is a FOXO target gene that suppresses B cell proliferation. Int Immunol. 2008 May;20(5):671-81. doi: 10.1093/intimm/dxn024.
109. Ligumsky H, Rubinek T, Merenbakh-Lamin K, Yeheskel A, Sertchook R, Shahmoon S, Aviel-Ronen S, Wolf I. Tumor Suppressor Activity of Klotho in Breast Cancer Is Revealed by Structure-Function Analysis. Mol Cancer Res. 2015 Oct;13(10):1398-407. doi: 10.1158/1541-7786.MCR-15-0141.
110. Ewendt F, Feger M, Föller M. Role of Fibroblast Growth Factor 23 (FGF23) and αKlotho in Cancer. Front Cell Dev Biol. 2021 Jan 14;8:601006. doi: 10.3389/fcell.2020.601006.
111. Melemadathil, Karthika, Udupa, Karthik, Belle, V., Udupa, C. K. K., Pai, Ananth, and Mailankody, S.. 2024. "Role of serum -Klotho level in patients with breast carcinoma.". 2924, Journal of Clinical Oncology. DOI: 10.1200/jco.2024.42.16_suppl.e15031
112. Raj, S.; Ahuja, M. The Versatility of Klotho Protein: Insights into Its Multifaceted Functions in Health and Disease. *World Journal of Current Med and Pharm Research***2024**, *6*, 12-17.
113. Farrugia MK, Vanderbilt DB, Salkeni MA, Ruppert JM. Kruppel-like Pluripotency Factors as Modulators of Cancer Cell Therapeutic Responses. Cancer Res. 2016 Apr 1;76(7):1677-82. doi: 10.1158/0008-5472.CAN-15-1806..
114. Abramovitz L, Rubinek T, Ligumsky H, Bose S, Barshack I, Avivi C, Kaufman B, Wolf I. KL1 internal repeat mediates klotho tumor suppressor activities and inhibits bFGF and IGF-I signaling in pancreatic cancer. Clin Cancer Res. 2011 Jul 1;17(13):4254-66. doi: 10.1158/1078-0432.CCR-10-2749..
115. Wu Q, Jiang L, Wu J, Dong H, Zhao Y. Klotho Inhibits Proliferation in a RET Fusion Model of Papillary Thyroid Cancer by Regulating the Wnt/β-Catenin Pathway. Cancer Manag Res. 2021 Jun 17;13:4791-4802. doi: 10.2147/CMAR.S295086. PMID: 34168498; PMCID: PMC8216664.
116. Black AR, Black JD, Azizkhan-Clifford J. Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol. 2001 Aug;188(2):143-60. doi: 10.1002/jcp.1111.
117. Simmen RC, Pabona JM, Velarde MC, Simmons C, Rahal O, Simmen FA. The emerging role of Krüppel-like factors in endocrine-responsive cancers of female reproductive tissues. J Endocrinol. 2010 Mar;204(3):223-31. doi: 10.1677/JOE-09-0329.
118. Bureau C, Hanoun N, Torrisani J, Vinel JP, Buscail L, Cordelier P. Expression and Function of Kruppel Like-Factors (KLF) in Carcinogenesis. Curr Genomics. 2009 Aug;10(5):353-60. doi: 10.2174/138920209788921010. PMID: 20119532; PMCID: PMC2729999.
119. Mota J, Lima AMM, Gomes JIS, Souza de Andrade M, Brito HO, Silva MMAL, Faustino-Rocha AI, Oliveira PA, Lopes FF, Gil da Costa RM. Klotho in Cancer: Potential Diagnostic and Prognostic Applications. Diagnostics (Basel). 2023 Oct 31;13(21):3357. doi: 10.3390/diagnostics13213357.
120. Mao A, Zhou X, Liu Y, Ding J, Miao A, Pan G. KLF8 is associated with poor prognosis and regulates glycolysis by targeting GLUT4 in gastric cancer. J Cell Mol Med. 2019 Aug;23(8):5087-5097. doi: 10.1111/jcmm.14378. Epub 2019 May 24. PMID: 31124603; PMCID: PMC6653475.
121. Jiang Z, Yu T, Fan Z, Yang H, Lin X. Krüppel-Like Factor 7 is a Marker of Aggressive Gastric Cancer and Poor Prognosis. Cell Physiol Biochem. 2017;43(3):1090-1099. doi: 10.1159/000481748.
122. Wang Y, Chen L, Huang G, He D, He J, Xu W, Zou C, Zong F, Li Y, Chen B, Wu S, Zhao W, Wu J. Klotho sensitizes human lung cancer cell line to cisplatin via PI3k/Akt pathway. PLoS One. 2013;8(2):e57391. doi: 10.1371/journal.pone.0057391
123. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023 Jan 6;51(D1):D638-D646. doi: 10.1093/nar/gkac1000. PMID: 36370105; PMCID: PMC9825434
124. Aleman C, Mas R, Hernandez C, Rodeiro I, Cerejido E, Noa M, Capote A, Menendez R, Amor A, Fraga V. A 12-month study of policosanol oral toxicity in Sprague Dawley rats. Toxicol Lett , 1994, 70:77–87.

 doi:10.20944/preprints202502.0271.v1

125. Alemán CL, Ferreiro RM, Puig MN, Guerra IR, Ortega CH, Capote A. Carcinogenicity of policosanol in sprague dawley rats: a 24 month study. Teratog Carcinog Mutagen, 1994, 14:239–249.
126. Alemán CL, Puig MN, Elias EC, Ortega CH, Guerra IR, Ferreiro RM, Briñis F. Carcinogenicity of policosanol in mice: an 18-month study. Food Chem Toxicol, 1995 33:573–578.