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REVIEW ARTICLE

The KiSS-1/GPR54 system: Essential roles in physiological homeostasis and cancer biology

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Abstract *KiSS-1*, first identified as an anti-metastasis gene in melanoma, encodes C-terminally amidated peptide products, including kisspeptin-145, kisspeptin-54, kisspeptin-14, kisspeptin-13 and kisspeptin-10. These products are endogenous ligands coupled to G protein-coupled receptor 54 (GPR54)/hOT7T175/AXOR12. To date, the regulatory activities of the KiSS-1/GPR54 system, such as puberty initiation, antitumor metastasis, fertility in adulthood, hypothalamic-pituitary-gonadal axis (HPG axis) feedback, and trophoblast invasion, have been investigated intensively. Accumulating evidence has demonstrated that *KiSS-1* played a key role in reproduction and served as a promising biomarker relative to the diagnosis, identification of therapeutic targets and prognosis in various carcinomas, while few studies have systematically summarized its subjective factors and concluded the functions of KiSS-1/GPR54 signaling in physiology homeostasis and cancer biology. In this review, we retrospectively summarized the regulators of the KiSS-1/GPR54 system in different animal models and reviewed its functions according to physiological homeostasis regulations and above all, cancer biology, which provided us with a profound understanding of applying the KiSS-1/GPR54 system into medical applications.

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Introduction

Discovery of KiSS-1 and GPR54

KiSS-1 was first identified as a metastasis suppressing gene in a melanoma patient in Hershey, Pennsylvania, through modified subtractive hybridization by Lee JH *et al* in 1996.[1] Researchers found that more than 95% of melanoma cell metastasis was inhibited. In humans the *KiSS-1* gene encodes the precursor neuropeptide kisspeptin-145, consisting of 145 amino acids in humans with a PEST sequence and has a short half-life.[2] Besides, kisspeptin-145 contains two potential dibasic cleavage sites and one putative site for terminal cleavage and amidation. It can be easily truncated into four shorter peptides with different lengths, namely, kisspeptin-54, kisspeptin-14, kisspeptin-13 and kisspeptin-10 (Fig. 1A, B).[3] In 2001, Ohtaki T *et al* have revealed metastin, the *KiSS-1* encoding peptide product with 54 amino-acid residues, after initially discovering its function of suppressing melanoma and breast cancer metastasis.[4] Kisspeptins belong to the family of RF-amides, all of which contain Arg-Phe-NH2 at the C-terminus and whose structures are crucial in ensuring their biological activity when coupled to the corresponding receptor.

GPR54 (KiSS-1R) is the receptor of the neuropeptide kisspeptin and was initially identified in rat brain tissue by Lee *et al* in 1999. *GPR54*, as a human ortholog found in 2001, is also known by the name *AXOR12* or *hOT7T175*.[5] *GPR54* is mapped to human chromosome 19p13.3 and composed of 5 exons and 4 introns. It contains a 1197 bp-long open reading frame and encodes a protein with 398 amino acid residues.[6] The similarity between the galanin receptor and KiSS-1R is over 45%,[7] which has attracted intense interest in investigating its structural and functional regulation.

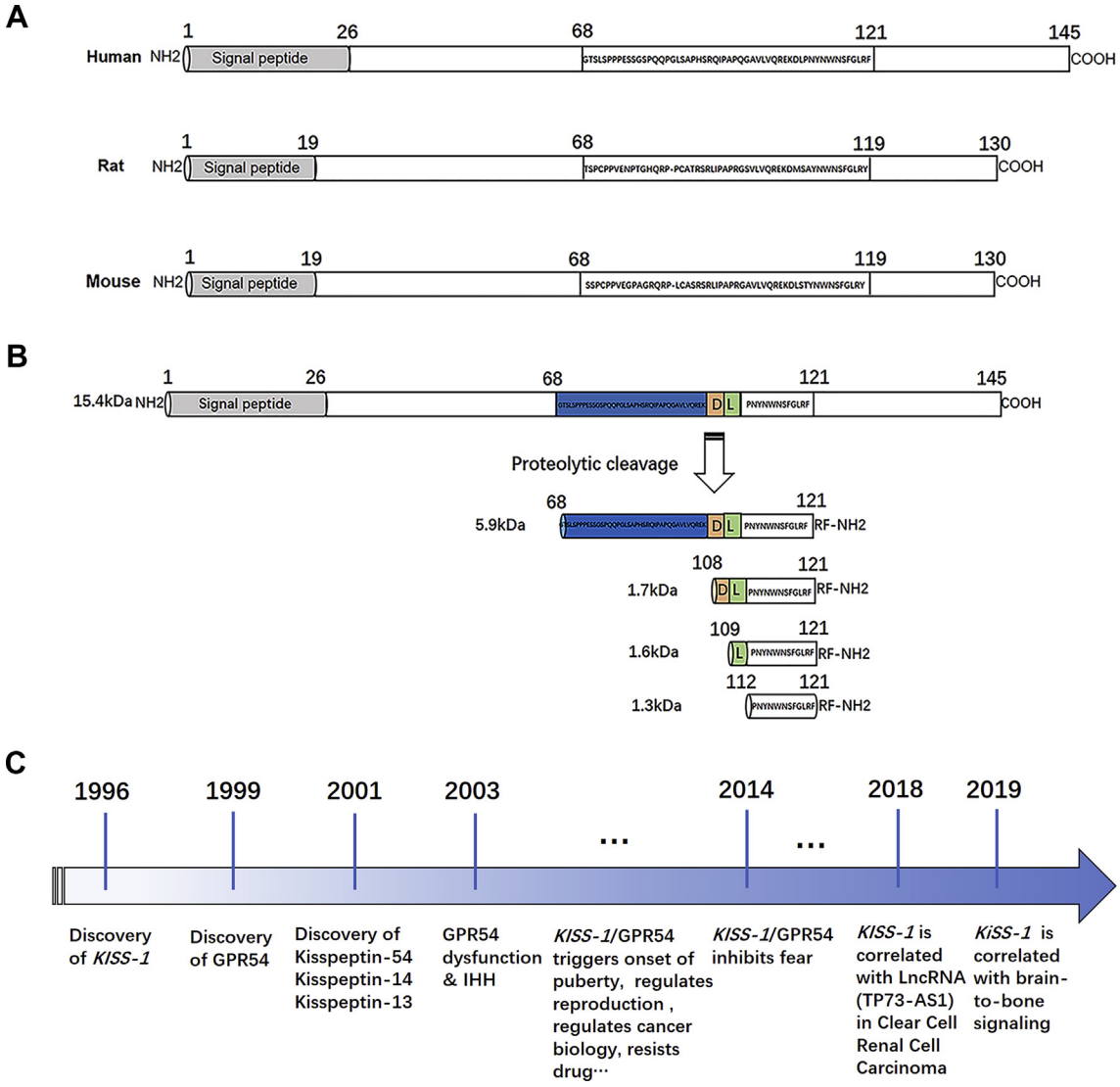

Figure 1 The various structure and sequence of kisspeptin in species. **(A)** Structure and sequence of full length kisspeptin in human, rat and mouse. Sequence of amino acids are gained from NCBI. **(B)** Human kisspeptin size, signal peptide and cleavage points are showed. Precursor kisspepin-145 is easily truncated into kisspeptin-54 (metastin) (5.9 kDa), kisspeptin-14 (1.7 kDa), kisspeptin-13 (1.6 kDa) and kisspeptin-10 (1.3 kDa). **(C)** Evolution history of KiSS-1/GPR54 system researching development.

Since Lee JH *et al* initially discovered the novel tumor metastasis suppressing gene *KiSS-1*,[1] researchers began to explore its corresponding receptor, coding peptides, underlying mechanism and relationships with various carcinomas. Then, in 2003, two independent experiments conducted by de Roux *et al* and Seminara's team showed that the dysfunction of GPR54 contributed to the cause of idiopathic hypogonadotropic hypogonadism (IHH).[8] At this point, subsequent researchers has indicated that kisspeptin played an important role in the mechanisms of reproductive regulation, puberty onset and feedback loop in the hypothalamic-pituitary-gonadal axis (HPG axis), including stimulating gonadotropin-releasing hormone (GnRH), gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[9] The evolution history of KiSS-1/GPR54 system researching development was shown in Fig. 1C.

Distribution of the KiSS-1/GPR54 system

The KiSS-1/GPR54 system is widely expressed in different organs in mammals and nonmammal vertebrates. For instance, human KiSS-1 is mostly expressed in the placenta, while it is also distributed in the pancreas, kidney, liver, small intestine, anterodorsal preoptic area and central nervous system, mainly in the arcuate nucleus (ARC) and the anterior ventral periventricular nucleus (AVPV), two different essential constituent regions of the hypothalamus ensuring gonadotrophic hormone release.[1,4,5] GPR54 is highly distributed in the pancreas, placenta, pituitary gland and spinal cord.10 It is relatively abundant in the hypothalamus, limbic system and basal ganglia, as well as in the spleen, peripheral blood leukocytes (PBLs), testis and lymph node. In mice, KiSS-1 was also identified as highly expressed in the ARC and AVPV/PeN (periventricular preoptic nucleus).[11] In addition, there are two or more kisspeptin and receptor types in nonmammalian vertebrates, such as zebrafish. KiSS-1 is mostly expressed in the brain, and KiSS-1R is expressed only in the habenula, an evolutionarily conserved epithalamic structure. Whereas KiSS2 is expressed in the preoptic-hypothalamic area, KiSSR2 is widely distributed in the brain.[12] In addition, for amphibians such as Xenopus, three forms of kisspeptin (KiSS-1a, KiSS-1b, and KiSS-2) and corresponding receptors (GPR54-1a, GPR54-1b, and GPR54-2) were widely expressed in the hypothalamus.[13]

Signaling pathways activated by the KiSS-1/GPR54 system

The underlying mechanisms by which the KiSS-1/GPR54 system regulated in the hypothalamus have been identified: the system coupled to Gαq/11 activates phospholipase C (PLC) and then leads to the hydrolysis of phosphatidylinositol-4, 5-bisphosphate. In this way, inositol-1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG) act as two different potentials 'second messengers'. IP3 is a small molecule that can contribute to the increase in intracellular Ca^{2+} by activating IP3R, which is sufficient for inducing tumor cell apoptosis and differentiation. Additionally, the activation of DAG leads to the activation of protein kinase C (PKC). PKC could also activate mitogen-activated protein kinase 2 (MAPK2) that suppresses metastasis and/or proliferation of tumor cells, such as extracellular signal-regulated kinase 1/2 (ERK1/2) and p38, which also involved in this signaling cascade. Additionally, KiSS-1 prevents degradation of IκB, reduces p50/p65 NF-κB interaction with the matrix metalloproteinase 9 (MMP-9), while other binding transcription regulatory factors of MMP-9, such as AP-1, Sp1 and Ets are not involved in KiSS-1/GPR54 system.[9] Besides, β-arrestins could desensitize G-protein signaling and participate in activation of signaling routes as molecular scaffolds, for example, activation of ERK 1/2, p38, PI3K/Akt, and cJun N-terminal kinase 3 (JNK3). Activation of ERK 1/2 caused by the combination of the KiSS-1/GPR54 system could also be triggered by G-protein independent pathway through recruiting and activating arrestin β2, while arrestin β1 might inhibit this activation.[14] Moreover, kisspeptin-10 might suppress the chemokine stromal cell-derived factor 1 (SDF-1), the ligand of CXC chemokine receptor 4 (CXCR4) that is highly expressed in malignant tumors. Thus, the response to prometastatic chemokine receptor in tumor cells and phosphorylation of PI3K/Akt is inhibited. Recently, a kisspeptin-involved pathway was reported to inhibit Akt, activate p38 and upregulate arachidonic acid. This kisspeptin-involved pathway is not involved in arrestin.[15] However, its specific pathway has not been clarified clearly so far Fig. 2.

Influential regulators of the KiSS-1/GPR54 system

To date, the regulators and functions of KiSS-1/GPR54 system have not been reviewed systematically. In this review, we collected studies on the KiSS-1/GPR54 system and identified multiple factors among diverse animal models (Table 1), including zebrafish, mouse, rat, hamster, ewe, pig, brand's voles and frog, *etc*.

For example, researchers found Tributyltin (TBT), an organotin compound used in various industrial materials, downregulated KiSS-1 and took part in inhibiting non-reproduction of female zebrafish. Moreover, TBT disturbed the reproductive behaviors, probably by suppressing cyp19a1b expression in the brain and inhibiting the expressions of reproduction-related regulators such GnRH-3 and kiss2.[16] In goldfish, anti-androgen flutamide (Flu) and Vinclozolin (VZ), a kind of pesticide, promoted mRNA of mid-brain *KiSS-1* and GnRH3. VZ was similar to Flu and acted as anti-androgen to impair fish reproduction. Combination exposure of testosterone and either VZ or Flu resulted in increases in the brain *KiSS2*, GnRH3, and AR (androgen receptor) mRNA.[17]

When offspring female mouse exposed to 15 mg/L arsenic, mRNA and protein level of kisspeptin, GnRH1, Oct2 and Ttf1 in the hypothalamus could be upregulated, leading to precocious puberty.[18] Moreover, as a weak estrogen agonist, Bisphenol A (BPA) caused reproductive dysfunction via interfering feedback regulatory mechanism of the HPG-axis in mice. In both male and female pups which exposed to BPA, the expression of *KiSS-1*, GnRH and FSH mRNA could be upregulated at the hypothalamic-pituitary level.[19]

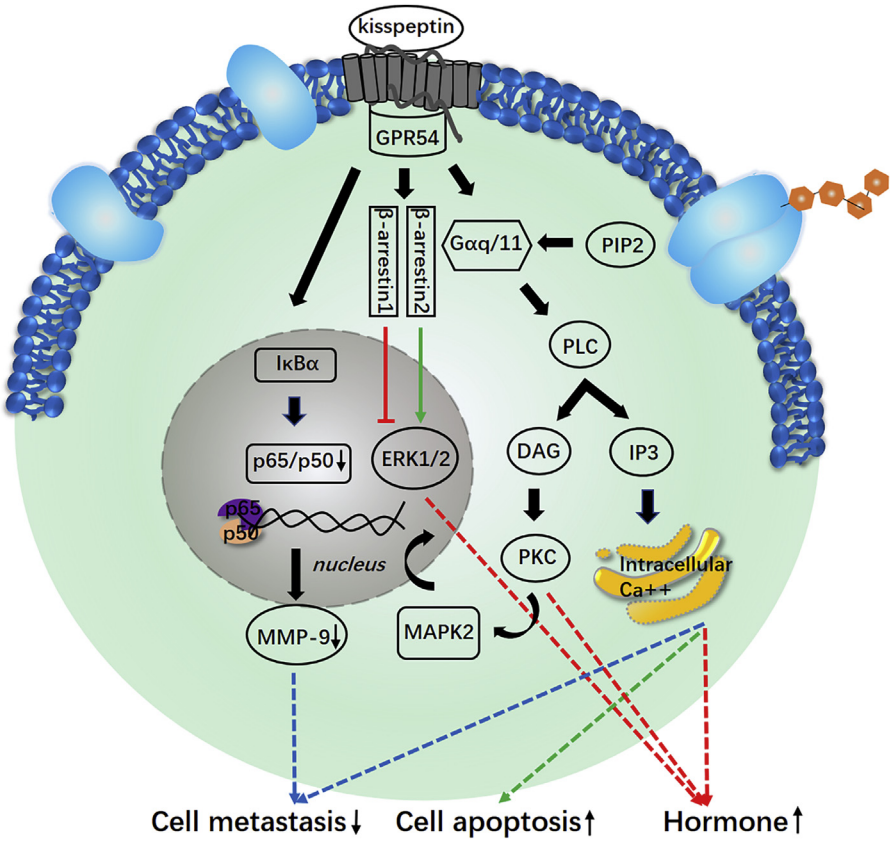

Figure 2 Confirmed schematic mechanism of the KiSS-1/GPR54 system. The KiSS-1/GPR54 system coupled to Gαq/11 activates PLC hydrolysis. IP3 and DAG act as two different potentials 'second messengers'. IP3 contributes to upregulate intracellular Ca^{2+}, and DAG activates PKC, leads to activation of MAPK2. Activated GPR54 could also recruit arrestin-1 and arrestin-2, arrestin-1 suppresses but arrestin-2 upregulates phosphorylation of ERK1/2. Additionally, NF-κB pathway and MMP-9 is downregulated. Dotted arrows are associated with cell metastasis, cell apoptosis and hormone-releasing.

In AVPV cell models, E2 promoted *KiSS-1* at 24 h while in ARC cell models inhibited gene expression at 4 h. In AVPV kisspeptin neurons, over a 24-h period, E2 diffused into the cell and bound to ERα nuclear receptors, ERα receptors dimerized and bound to ERE sites in the *KiSS-1* promoter, causing upregulation of *KiSS*-1 mRNA. While in ARC kisspeptin neurons, after 4 h E2 exposure, Gpr30 was activated and suppressed expression of *KiSS*-1 mRNA. Researchers also found the downstream effectors of Gpr30-*KiSS-1* pathway consisted of ERα and/or ERβ and CREB1.[20]

Pituitary adenylate cyclase-activating polypeptide PACAP, an essential peptide activating adenylate cyclase in anterior pituitary cells, could regulate HPG axis by upregulating *KiSS-1* in hypothalamus, corticotropin-releasing hormone and neurotensin.[21]

In rat, researchers found Di-(2-ethylhexyl) phthalate (DEHP), a toxic substance used in plastic products, downregulated *KiSS-1* but upregulated kisspeptin, causing dysfunction of hypothalamus in pubertal female rats. This may be *KiSS-1* neurons in the hypothalamus are sensitive to DEHP and promoted the transition of *KiSS*-1 mRNA, resulting in downregulation of *KiSS*-1 mRNA but elevation of protein.[22] Classical antipsychotic drugs such as chlorpromazine and haloperidol greatly suppressed kisspeptin.[23] Prepubertal rats promoted secretion of GnRH as well as prepubertal development when exposed to low level of Mn.[24] Besides, increased sucking intensity and Anti-GnRH immunization also could inhibit *KiSS-1* with the use of qRT-PCR.[25,26] Adiponectin and activator of its downstream targeted AMPK, globular adiponectin or AICAR, downregulated *KiSS-1* in an immortalized hypothalamic *KiSS-1* gene-positive neuron.[27] Ghrelin significantly decreased the *KiSS-1* and *KiSSR* mRNA transcription in rat islets and CRI-D2 cells revealed the essential role KiSS-1/GPR54 system in metabolic activities.[28] Additionally, two anorexigenic hormones Glucagon-like peptide (GLP1) as well as leptin upregulated *KiSS*-1 mRNA in the embryonic rat hypothalamic cell line rHypoE-8 cells, then influenced secretion of GnRH mRNA rather than act directly on the GnRH neurons. However, combined treatment with GLP-1 and leptin failed to enhance their individual effects on *KiSS-1* as investigated.[29,30] Adversely, exposed to a common anesthetic called isoflurane chronically downregulated *KiSS-1* through suppressing androgen-receptor and induced disorder of HPG axis.[31]

In hamster, short day lengths and pinealectomy downregulated *KiSS-1* and postponed reproduction, suggesting that these effectors of photoperiod-induced reproductive quiescence were not controlled by melatonin directly but decoded melatonin-signal durations to control seasonal rhythms of reproduction.[32]

Table 1 The regulators of KiSS-1 *in vivo* and *in vitro*.

Animal models	Regulators	*KiSS*-1 mRNA	kisspeptin	Function	References
Zebrafish	[a]Tributyltin (TBT)	Down	ND	Non-reproductive behaviors	16
Goldfish	[a]Vinclozolin (VZ)/[a]Flutamide (Flu)	Up/Up	ND	Reproduction	17
Mouse	[a]Arsenic	Up	Up	Precautious puberty	18
	[a]Bisphenol A (BPA)	Up	ND	Reproduction	19
	Estradiol (E2)	Up/Down	ND	Reproduction	20
	Pituitary adenylate cyclase-activating polypeptide (PACAP)	Up	ND	Reproduction	21
Rat	[a]Di-(2-ethylhexyl) phthalate (DEHP)	Down	Up	Precocious puberty	22
	[a]Classical antipsychotic drugs	ND	Down	Anxiety-related behaviors	23
	[a]Mn	Up	ND	Reproduction	24
	[a]Increased sucking intensity	Down	ND	Reproduction	25
	[a]Anti-GnRH immunization	Down	ND	Reproduction	26
	[a]Globular adiponectin or AICAR	Down	ND	Puberty onset and reproduction	27
	Ghrelin	Down	ND	Diabetes	28
	Adiponectin	Down	ND	Metabolic disorders	29
	Glucagon-like peptide (GLP1)	Up	ND	Reproduction	30
	Leptin	Up	ND	Metabolic disorders	30
	[a]Isoflurane	Down	ND	Reproduction	31
Hamster	[a]Short day lengths/pinealectomy	Down	ND	Reproduction	32
Ewe	[a]Food, energy, protein and trace minerals	Up	ND	Reproduction	33
Pig	[a]Soyabean Isoflavones (SIF)	Down	ND	Puberty	34
Brandt's voles	[a]6-methoxybenzoxazolinone (6-MBOA)	Down	ND	Reproduction	35
Frog	Endocannabinois	ND	Down	Reproduction	36

[a] Regulators of KiSS-1 i*n vitro* and others *in vivo*. Up, expression of *KiSS*-1 mRNA or kisspeptin is upregulated after influenced by various factors; Down, *KiSS*-1 mRNA or kisspeptin is suppressed. ND, expression has not been determined.

In prepubertal Tibetan sheep ewes, food, energy, protein and trace minerals supplements during the cold season upregulated *KiSS-1*, therefore activated HPG axis, secretion of GnRH, FSH and LH in promoting uterine and follicular development. Researchers recognized that higher intakes of Cu, Mn, Zn and Fe potentially changed the circulating concentrations of metabolic hormones.[33]

Soyabean Isoflavones (SIF) is the major class of phytoestrogen that combined with estrogen receptor in advance. And researchers found it downregulated *KiSS-1* and postponed puberty startup in female Bama miniature pigs. Mechanically, downregulation of *KiSS-1* may suppress crucial regulators of the steroid hormones synthesis, including StAR and 3β-HSD.[34]

Researchers found that a high dose of 6-methoxybenzoxazolinone (6-MBOA), the secondary plant metabolite originated from the Gramineae family, could inhibit expression of *KiSS-1* in ARC of brand's voles under the long-day photoperiod.[35] Conversely, under short photoperiod, 6-MBOA improved the concentration of testosterone, reproductive activity of Brandt's voles and mRNA levels of *GPR54* and GnRH through elevating the mRNA levels of *StAR* and *Cyp11a1* that encoding the key enzymes for synthesis.[35]

It was widely recognized that endocannabinoids suppressed reproduction in vertebrates, regardless of gender. In testis of amphibians which underwent anandamide (AEA) treatment, such as frog, Vincenza Ciaramella *et.al* found kisspeptin, as well as *GPR54* mRNA and protein were decreased via cannabinoid receptor 1 (CB1). AEA regulated the reproductive activity through modulating the KiSS-1/GPR54 system and via the biosynthesis of estradiol in testis. AEA treatment could also decrease the expression of Ccyp17 and Cyp19, thus promoted estradiol biosynthesis and Faah protein, the direct target of estrogens in mammalian testis.[36] While in mice, CB1 and Faah were found inhibited by BPA and resulted in food intake constraint of mice. Additionally, exposure to BPA accompanied by decreased total plasma cholesterol levels, which was consistent with the expression status of endocannabinoid system-related components.[37] Obviously, a majority of the reported regulators of the KiSS-1/GPR54 system interfered with reproductive activities in multiple animal models, it informed us that we should avoid exposure to these harmful factors as far as possible.

Essential role of the KiSS-1/GPR54 system in physiological homeostasis

Influence on the reproduction and puberty onset

In mammals, *KiSS-1* was identified as an essential regulator in hormone release. Combination of kisspeptin and GPR54 actively stimulated the secretion of gonadotropin-releasing hormone (GnRH) by stimulating the median preoptic area GnRH neurons. In mammals, biosynthesis and secretion of kisspeptin ensure the stability of GnRH and gonadotropin secretion, thus strongly upregulate the release of LH and

FSH.[38] In this way, the KiSS-1/GPR54 system regulates the menstrual cycle and promotes reproduction. Moreover, the secretion of sex steroids, in response to gonadotropins stimulated by GnRH, could feedback to regulate kisspeptin neurons, participate in advance vaginal opening, promote uterus weight, somatic and germ cell development and improved sperm functions in testis.[39] In the AVPV, estradiol (E2) could bind to the estrogen receptor (ERα) to induce dimerization. ER is indispensable in this feedback regulation, and KiSS-1 cannot be expressed in the hypothalamus without its existence. Moreover, the influence of KiSS-1 expression activated by sex hormone is totally opposite in the AVPV and arcuate nucleus (ARC). Sex hormone positively regulates the expression of KiSS-1 in the AVPV and shows a negative feedback mechanism in the ARC.[40]

The alterations in the *KiSS-1* gene gives rise to the pathogenesis of GnRH-dependent disorders in human. Researchers discovered more than 294 single nucleotide polymorphisms (SNPs) in *KiSS-1*. There are 42 mutations in the untranslated region (UTR) and 30 variations in exons, the rest mutations are located on intronic regions.[41] Loss of function of *GPR54* mutations are associated with hypogonadotropic hypogonadism, infertility or even with absence of pubertal development. Additionally, activating mutations could ultimately lead to precocious puberty as prevention of desensitization of the KiSS-1/GPR54 pathway was caused.[41] Kisspeptin was initially found correlated with the puberty onset in two independent researches regarding *GPR54* gene mutations in patients with IHH.[8,42] Subsequently, heterozygous and homozygous *KiSS-1* mutations were identified in patients with IHH.[43] Interestingly, women with heterozygous *KiSS-1* or *GPR54* mutations deliver homozygous *KiSS-1* or *GPR54* mutations to their babies.[44] In 2008, researchers found the first monogenic defect, a heterozygous activating mutation (p.Arg386Pro) of *GPR54* gene in a patient with central precocious puberty (CPP) due to premature activation of the HPG axis.[45] Furthermore, activating heterozygous mutation (p.Pro74Ser) in *KiSS-1* was found to have a greater capacity to stimulate signal transduction and lead to kisspeptin bioavailability than the wild type in a boy with CPP.[46] In Chinese Han girls, it was discovered that SNP rs5780218 was positively correlated with the risk of CPP.[47] However, the molecular mechanism by which the KiSS-1/GPR54 system regulates CPP is too limited to be reported.[48] Additionally, loss-of-function mutations of *GPR54* or *KiSS-1* is closely related to normosmic congenital hypogonadotropic hypogonadism (CHH) (nCHH), the disease with defection of GnRH secretion or gonadotrope cell dysregulation in the pituitary.[49] Researchers found the functional loss of GPR54 only affects gonadotrope axis and might occur in the hypothalamus exclusively.[49,50] It is reported that *KiSS-1* or *GPR54* heterozygous mutations seem unable to suppress functions of uterine and placental in human.[43] Indeed GnRH pulsatile treatment has been applied to therapy in patients with *KiSSR* mutations, while no impact on the LH surge was found during this treatment.[50]

Correlation with metabolic events

KiSS-1 plays an essential role in inhibiting insulin secretion in the liver of mouse. When the blood glucose level downregulated in the body, the secretion of glucagon is increased and acts on the glucagon receptor in the liver. The cAMP-PKA-CREB pathway is triggered and the transcription of *KiSS-1* gene is activated, resulting in suppression of insulin secretion.[51] Furthermore, Kolodziejski *et al* found serum KiSS-1 was overexpressed in overweight women attendees compared with other women investigated. Additionally, suppressed serum expression of KiSS gene was consistent with the malignant degree of insulin resistance progression in human.[52] A fuel-sensing deacetylase, sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK) were identified as molecules that suppressed puberty in mammals by inhibiting the expression of the KiSS-1, additionally, influenced metabolic disorders such as subnutrition or obesity.[53] Kisspeptin 10 inhibits cell proliferation of fibroblast and the expression of adipogenesis-related genes, such as PPAR-γ and CEBPβ. In addition, kisspeptin 10 inhibits glucose uptake and lipogenesis.[54] In adipocytes, kisspeptin 10 delivers the signal of lipid storage status to the hypothalamus, from which triggered by leptin, thus controls food intake and decreases adiponectin secretion.[54] Similarly, the availability of glucose to start *denovo* lipogenesis in adipocytes was limited by kisspeptin 10. In rat testicle tissue, kisspeptin is capable of enhancing superoxide dismutase activity and mRNA levels and stabilizing the methionine-associated catalase. Thus kisspeptin 10 contributes to cell-protection in lipid peroxidation induced by methionine.[10] Morelli A *et al* found physical exercise contributed to upregulate *KiSS*-1 mRNA in high fat rabbits, thus normalized effect on HPG axis and secretion of GnRH.[55] It provided us with a better understanding that exercise might attenuate metabolic syndrome and precocious puberty, avoid idiopathic central precocious puberty (ICPP) and accelerate recovery of patients with cancers.

Cell metabolism is closely correlated with the proliferation, survival and metastasis of tumor cells. Aerobic glycolysis is generally recognized as a critical feature in tumor metabolism.[56] Tumor cells exhibit Warburg effect as increasing glucose uptake and prefer to glycolysis rather than oxidative phosphorylation (OXPHOS) for ATP.[57] Notably, KiSS-1 takes part in reversing the Warburg effect in tumor cells, including attenuating the acidification of extracellular media, glucose uptake and lactate secretion. Additionally, KiSS-1 could upregulate OXPHOS and promote mitochondrial biogenesis.[57] Interestingly, full-length kisspeptin rather than peptide in lack of the N-terminal signal peptide triggered these metabolic alternations,[57] suggesting a possible interplay between the tumor cells and the microenvironment. Besides, KiSS-1 is related to upregulation of the glucose transporter GLUT1 and decrease of hexokinase II (HK2) occasionally.[57] Moreover, reduced acidification by KiSS-1 is cause by inhibiting the selected subunits (V0d2, V1g3) of the vacuolar H + -ATPase(V-ATPase), a key proton pump the regulating cellular pH.[58] It is identified that KiSS-1 promotes mitochondrial biogenesis and upregulates mitochondrial genes, including chaperone factors, membrane polarization and small molecule import and export factors. Additionally, KiSS-1 contributes to upregulation of mitochondrial nuclear respiratory factors (NRF1) and mitochondrial transcription factor A (Tfam), two crucial transcription factors in regulating critical mitochondrial genes and in mitochondrial genome replication, was upregulated.[57]

In tumor cells expressing *KiSS*-1 mRNA, peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) is a ligand-activated transcriptional factor, taking part in regulating diverse metabolism activities, such as the expression and oxidation of genes involved in β−oxidation of fatty acids, citric acid cycle and OXPHOS.[10] Moreover, PGC1α could activate the transcription and duplication of mitochondrial genome by interacting with NRF1 and promoting the transcription of Tfam.[57] Interestingly, researchers found PGC1α was possibly indispensable for antimetastatic effect of KiSS-1, because KiSS-1 stabilized the protein level of PGC1α through avoiding proteasomal degradation.[57] Indeed, studies showed KiSS-1 promoted fatty acid conjugation to acyl-CoA, fatty acid acetyl-CoA transport into mitochondria and β-oxidation, and activated short chain fatty acid catabolism.[59]

In summary, in cells expressing KiSS-1, mitochondrial mass was increased, utilization of glucose was decreased and aerobic glycolysis was converted into mitochondrial respiration and OXPHOS and secretion of lactic acid was suppressed probably due to the elevated pH in the extracellular microenvironment.[60] Metabolic pathways significantly correlate with dormant cells activation, cell proliferation, survival and metastasis. Future investigation into metabolic mechanisms by which tumor cells interact with tumor environment (TME) will be substantially directive to prevent tumor metastasis.

Effects on other physiological activations

Apart from reproductive and endocrinological regulations, the KiSS-1/GPR54 system also takes part in modulating fear and attenuating negative mood. Ogawa *et al* found that in the presence of KiSS-1, zebrafish appeared less anxious, even in the circumstance of alarm substance (AS) stimulation.[61] Also, kisspeptin in the habenula may contribute to avoiding fear, via decreasing c-fos mRNA levels in the ventral habenula (vHb) and the median raphe (MR) and increased mRNA levels of 5-HT-related genes (pet1 and slc6a4a).[61] Comninos and coworkers discovered that kisspeptin activated sexual promotion and promoted the interviewee's positive emotions, contributing to ensuring healthy structural and functional development in the nervous system.[62] These two studies highlighted the potential therapeutic of *KiSS-1* in nervous system regulation. In human, the vasculature KiSS-1/GPR54 system is localized in smooth muscle of vessels with the same developmental origins, umbilical vein, coronary artery, aorta and coronary atherosclerosis. Furthermore, kisspeptins are recognized as vasoconstrictors from isolated rings of the coronary artery and umbilical vein,7 suggesting that the KiSS-1/GPR54 system might be related to cardiovascular paracrine signaling. However, too few studies have explored the inner relationship between KiSS-1/GPR54 system and metabolic regulations in circulatory system so far.

Role of the KiSS-1/GPR54 system in cancer biology

According to various clinical data, the functional potential of the KiSS-1/GPR54 system depended on different cell types.[63] We herein reviewed the function of KiSS-1/GPR54 signaling according to cancer biology, which provided us with a better understanding of its diagnostic and therapeutic roles in cancers. In most cases, downregulation of KiSS-1 and/or GPR54 were/was associated with poorer prognosis in cancer patients. Nevertheless, protumorogenic role was also exhibited in a minority of cancers, such as hepatocellular cancer and breast cancer (Table 2).

Osteosarcoma

Yin *et al* found that the proliferation rate of MG-63 osteosarcoma cells in the low-expression KiSS-1 group was higher than that in the other group. Overexpressed *KiSS-1* increased caspase-3 and Bax mRNA, and suppressed level of Bcl-2. Thus, they concluded that *KiSS-1* suppressed the progression and metastasis of osteosarcoma cells by promoting cancer cells apoptosis and autophagy. This finding provoked us to consider *KiSS-1* as a novel target for osteosarcoma drug treatment.[64] Moreover, Herber and coworkers recently found that the density of the trabecular and cortical bones was promoted in the absence of ERα in arcuate KiSS-1 cells.[65] It is noteworthy that KiSS-1 expression correlates with the brain-bone axis, bringing us a new understanding of female bone homeostasis and metabolic regulation.

Gastric cancer

Dhar DK *et al* first reported that the *KiSS-1* gene was strongly correlated with suppressing tumor progression in gastric cancer. Subsequently, the underlying mechanism by which *KiSS-1* regulates gastric carcinoma cells was discovered by upregulating p38 MAP kinase and therefore inhibited the MMP-9 pathway, thus suppressed motility, chemotaxis and invasion of tumor cells.[66] Consistently, Li *et al* transfected a *KiSS-1* vector into BGC-823 cells and evaluate MMP-9, in concert with the effect of *KiSS-1* initially found in melanoma, they found that this gene downregulated MMP-9 and thus inhibited proliferation and invasion of gastric carcinoma cells.[67]

Hepatocellular carcinoma

Interestingly, the function of the *KiSS-1* gene went far beyond anti-metastasis in hepatocellular carcinoma (HCC) cells, possibly due to disorders of hormonal environment. Interestingly, Ikeguchi *et al* discovered KiSS-1 expression was upregulated in HCC, especially in higher levels of malignant HCC.[68] For elevated secretion of estrogen in a majority of HCC patients, KiSS-1 might be aberrantly overexpressed and exhibited the contradictory role when compared to melanoma cancer *etc*. Contradictorily, Shengbing *et al* revealed that KiSS-1 expression was negatively related to MMP-9 expression and HCC metastasis, from which they hypothesized that KiSS-1 repressed intrahepatic metastasis and distant metastasis by inhibiting MMP-9 expression and then cell−matrix adhesive regulation.69 Rather than polyclonal anti-human KiSS-1 antibody corresponding to kisspeptin-54, the major protein product of *KiSS-1*, in this study, researchers focused on kisspeptins with 1−145 amino acids length in patients with HCC. It is credible to investigate biology of HCC because the antibody used in this study included peptides with greatly receptor binding activity such as kisspeptin-14 and kisspeptin-13. Interestingly, these controversial observations of *KiSS-1* in

Table 2 Variation of KiSS-1 and KiSS-1R and corresponding effects on different cancer cells types compared with controlled cells.

Cancer types	KiSS-1	KiSS-1R	Regulations	Pathways	References
Osteosarcoma	Down		progression, metastasis		64
Gastric cancer	Down		proliferation, invasion	MMP-9	66,67
Hepatocellular carcinoma	Up/Down		metastasis	MMP-9	68,69
Esophageal squamous cell carcinoma	Down	Down	progression, metastasis		70
Thyroid cancer	Up/Down	Up	metastasis, proliferation, migration, apoptosis	ERK, NF-κB, Akt	72—74
Colorectal cancer	Down	Down	metastasis, invasion, survival	ERK, NF-κB, MMP-9	76,77
Head and neck cancer	Down		drug resistance	GST-π, NF-κB	79
Oral squamous carcinoma	Down		metastasis, survival		80
Gallbladder cancer	Down		growth, differentiation, metastasis		81
Cholangiocarcinoma	Down		metastasis, survival		82
Renal cell carcinoma	Down		progression, metastasis	MMP-2	85,86
Bladder cancer	Up/Down	Up	metastasis, survival	PLC	93—95
Urothelial cancer	Not detected		metastasis	NF-κB, MMP-9	96
Ovarian cancer	Down	Down		NF-κB, MMP-9	97,98
Endometrial cancer		Down	invasion, metastasis	SDF-1/CXCR4	99,102
Breast cancer	Up/Down	Up/Down	metastasis		103—106

Up, upregulated when compared with controlled cells; Down, downregulated when compared with controlled cells. Not detected, expression has not been detected.

HCC provoked us to detect the intricate mechanism by which different cleavage product of KiSS-1 regulating tumor progression.

Esophageal squamous cell carcinoma

Previous studies concerning the KiSS-1/GPR54 system in esophageal squamous cell carcinoma (ESCC) are limited. Among 71 ESCC patients, Ikeguchi *et al* showed that *KiSS-1* and/or *hOT7T175* gene expression were/was along with lymph node metastasis, could be identified as a specific marker in ESCC progression. However, no effects on tumor size or tumor invasive degree and absent mechanical pathways were shown in this study.70 Moreover, overexpression of TCF21 was discovered inhibited invasion and metastasis in ESCC,[71] In view that transcription factor 21 (TCF21) was regulated by miRNA-21, it is significant to explore how did miRNA influenced KiSS-1 at the post-transcriptional level.

Thyroid cancer

Ringer *et al* first reported the overexpression of kisspeptin receptor in papillary carcinomas when compared to adjacent normal tissues. It demonstrated the crucial role of *KiSS-1* in biology of thyroid cancer. Additionally, KiSS-1/GPR54 system activated ERK but not p38MAPK or Akt in anaplastic thyroid carcinoma cells.[72] The anti-metastasis role of KiSS-1/GPR54 was found in a novel GPR54 overexpression model of thyroid cancer through activating PKC, p42/44 MAPK, and Akt. Moreover, researchers discovered that KiSS-1/GPR54 system upregulated a gene involved in calcineurin inhibition—myocyte-enriched calcineurin-interacting protein 1 (MCIP-1), key roles in inhibiting cancer metastasis.[73] Additionally, Yan *et al* discovered that increasing the ubiquitin-dependent degradation of KiSS-1 by Smurf1 promoted cancer cell viability, migration, invasion and metastasis of thyroid cancer cells via activating NF-κB pathway.[74]

Colorectal cancer

KiSS-1 acted as an anti-tumor role in CRC progression. Moya *et al* demonstrated that *KiSS-1* was a negative predictor of CRC aggressive potential and that its methylation contributes to the clinical evaluation of CRC patients' diagnoses and prognoses.[75] Moreover, the high expression of KiSS-1R might correspondingly predict high disease-free survival in CRC patients, predicting a clinically valuable biomarker of KiSS-1/GPR54 system in CRC.76 Mechanistically, Ji *et al* concluded that KiSS-1 inhibited tumor invasion and metastasis potential by suppressing ERK, degraded the NF-κB pathway and therefore reduced MMP-9 expressive activity. Similarly was found that overexpression of KiSS-1 could inhibit the PI3K/Akt/NF-κB pathway, by which KiSS-1 limited CRC distant metastasis potential.77 In colon cancer cell lines, it has been identified that the anti-metastatic effect of tumor-elevated kisspeptin in colon cancer patients may be mediated by the expression of endothelial monocyte activating polypeptide II (EMAP-II), a cytokine that is specifically induced by apoptosis and could render the tumor-associated vasculature sensitive to tumor necrosis factor.[78]

Head and neck cancer (HNC)

Few studies have reported the functional effect of KiSS-1/GPR54 system in HNC. In head and neck squamous cell carcinoma (HNSCC), Jiffar *et al* discovered that *KiSS*-1 mRNA and protein levels were downregulated among cisplatin-resistant tumors. Suppressed KiSS-1 could upregulate glutathione S-transferase (GST)-π and activate the NF-κB pathway, therefore mediated cisplatin (CDDP) resistance in HNSCC. Additionally, compensating KiSS-1 in CDDP

resistance cells inhibited tumor proliferation as well as metastasis,[79] providing as a promising target for overcoming chemotherapy-resistant. In oral squamous cell carcinoma (OSCC), Shin *et al* investigated 99 primary OSCC samples, 51 metastatic LN samples and 12 normal oral mucosal tissues through IHC, showed the suppressed KiSS-1 in metastatic tumors and positively associated with better clinical outcome. Hence, *KiSS-1* was considered as a reliable prognostic predictor of clinical outcomes in OSCC.[80] However, the underlying mechanism by which KiSS-1 regulating OSCC progression still needs to be elucidated in the near future.

Gallbladder cancer

To date, only one study has reported the expression status of KiSS-1 in gallbladder cancer (GBC), the most popular primary biliary cancer. Wang and colleagues used in situ hybridization and clearly revealed the suppressed KiSS-1 expression in gallbladder adenocarcinoma tissues. Moreover, they highlighted the negative correlation between KiSS-1 and differentiation, tumor size, invasive and metastasis ability of adenocarcinoma.[81] It implied that *KiSS*-1 might represent a vital biomarker of gallbladder adenocarcinoma development.

Cholangiocarcinoma (CCA)

Investigations in regard to significance of *KiSS-1* and its molecular signal pathways were relatively limited in CCA so far. Uthaisar and coworkers previously reported that *KiSS-1* was suppressed in KKU-213L5 cells, which were isolated from an original human CCA cell line and possessed a great metastasis ability. They also discovered that upregulation of KiSS-1 predicted to a poorer overall survival of patients with CCA.[82] These results were similar to findings in various other cancer tissues.

Non-small-cell lung cancer (NSCLC)

Expression of KiSS-1 was downregulated in advanced-stage NSCLC (III-IV) as compared to early-stage disease (I-II),[83] controversially, Karapanagiotou *et al* showed that metastin was not a useful metastasis predictor for NSCLC, as investigated the serum metastin level in 96 NSCLC patients and 49 healthy participants with ELISA.[84] Researchers supposed that low distributive expression of KiSS-1 and GPR54 in lung, additionally, neutralization by multiple cytokines in cancer environment might be influencing factors of this result.[84] However, mRNA expressions of *KiSS-1* and *GPR54* were not proclaimed in this study and the detailed mechanism of KiSS-1/GPR54 system in NSCLC remains to be elucidated.

Renal cell carcinoma

It is reported that metastin (45—54) suppressed MMP-2 expression, therefore inhibited the motility and invasion of renal cell carcinoma (RCC) cells. The *KiSS-1* gene might emerge as a probable therapeutic target in RCC.[85,86] LncRNAs have been characterized as involved in various processes such as proliferation, invasion, apoptosis of tumor cells. Liu *et al* found that the upregulated lncRNA P73 antisense RNA 1T (TP73-AS1) triggered the downregulation of *KiSS-1* in ccRCC tissues, therefore inhibited the PI3K/Akt/mTOR pathway, inhibited apoptosis and promoted progression of cancer cells.[87] To date, upcoming studies have tried to elucidate regarding mechanism by which LncRNA and miRNA regulating *KiSS-1* in cancers. For example, it was reported that upregulation of miRNA-3648 overexpression targeted to TCF21, downregulated protein level of TCF21, afterward downregulated its downstream effector KiSS-1 protein, thus promoted migration and invasion in bladder cancer.[88] Similar in renal cancer, miRNA-21 downregulated TCF21 to inhibit *KiSS-1*.[89] In breast cancer, downregulation of *KiSS-1* inhibited nuclear factor NF-κB and thus triggered ZEB1/2 upregulated by WASF3. Moreover, ZEB1/2 could suppress miR-200a/200b/429 and suppress the metastasis of cancer cells.[90] In addition, invasion of the brain-localized circulating breast cancer was found enhanced by upregulating autophagy signaling pathways via the CXCL12-miR345-*KiSS-1* axis.[91] Furthermore, *KiSS-1* was targeted by overexpressed LncRNA LUCAT1 in prostate cancer. Thus, deregulated expression of mRNA and protein promoted migrative and invasive ability of prostate cancer.[92] Although multiple non-coding RNAs corresponding with the KiSS-1/GPR54 system are still uncertain, activated pathway networks have deepened our understanding of kisspeptin's intricate roles in different cancers. Besides, these non-coding RNAs may offer novel potential targeting therapies in various diseases.

Bladder cancer

The expression status of KiSS-1/GPR54 system seemed inconsistent among studies in bladder cancer. Sanchez-Carbayo *et al* found that the downregulation of *KiSS-1* was strictly related to tumor progression and poor clinical outcome of bladder cancer.[93] However, in human bladder transitional cell carcinoma, Nicolle *et al* found that the expression of KiSS-1/GPR54 was increased, and GPR54 showed a greater tendency of abnormity among more aggressive tumors.[94] Mechanistically, on the one hand, KiSS-1 restrained cell migration by inhibiting PKC-α. On the other hand, KiSS-1 coupled to GPR54 and thus activated PLC, an enzyme produced DAG and promoted PKC. Possibly, we suspected that his conflicting effect on PKC might trigger expressive deregulation of GPR54. Furthermore, it is considered that hypermethylation of the *KiSS-1* promoter resulted in overexpression of protein in bladder cancer. The upregulation of Ubiquitin-like with PHD and RING finger domains 1 (UHRF1) enhanced the methylation of CpG nucleotides and silenced expression of *KiSS-1*.95 Notably, it is instructive for us to apply a demethylating agent into clinical treatment to suppress metastasis and improve patient prognosis of bladder cancer. Nevertheless, whether it applies other diseases still needs future prospective investigations.

Urothelial cancer

A total of 151 upper urothelial cancer patients were selected as investigated attendees by Takeda *et al* They found *KiSS-1* in urothelial cancer emerged as a significant biomarker of tumor grade, metastasis and overall survival. Moreover, metastin treatment might be a promising application into inhibiting metastasis of urothelial cancer through suppressing NF-κB pathway and MMP-9. However, GPR54 was not discovered to play an important role in indicating upper tract urothelial carcinoma.[96]

Ovarian cancer

Prentice *et al* first discovered that the KiSS-1/GPR54 system was intimately associated with a better prognosis in ovarian cancer by investigating the expression of kisspeptin and GPR54 in 518 patients with ovarian cancer.[97] Beyond this, expression of tumor-suppressor KiSS-1 was negatively related to vasculogenic mimicry (VM), aldehyde dehydrogenase 1 (ALDH1) and metastasis-associated in colon cancer-1 (MACC1), malignant factors during tumor progression.[98] This finding powerfully avoided the bias predicted and prognosticated by a single biomarker, such as the complex role of KiSS-1 in multiple cancers. function as metastasis suppressors improving the prognosis of ovarian cancer patients.

Endometrial carcinoma

Kang *et al* discovered metastin-10 inhibited invasion and migration of endometrial cancer by combining to its receptor-GPR54.99 Importantly, KiSS-1 and GPR54 expressed in eutopic and ectopic endometrium tissues, might effect on the pathophysiology of endometriosis only for a particular group of patients.[100] In cancer cells lacking the KiSS-1R, it seemed not to be affected when exposed to exogenous kisspeptin-10 *in vitro*. Adversely, the proliferation and metastatic ability of KiSS-1R positive tumor cells was decreased.[101] Mechanically, Schmidt E *et al* reported kisspeptin-10 inhibited chemotactic activities of SDF-1/CXCR4 system, a key role playing in inducing invasion and metastasis of endometrial cancer.[102] This finding offered us a novel understanding of applying metastin-10 into preventing endometrial cancers progression based on disturbing SDF-1.

Breast cancer

The role of the anti-metastasis gene *KiSS-1* in breast cancer was controversial. Lee *et al* first discovered that KiSS-1 suppressed metastasis potential by 95% in the human breast cancer cell line MDA-MB-435.[103] However, a wide range of researches reported the conflicting variation of KiSS-1/GPR54 system. Martin and coworkers reported the unexpected overexpression of KiSS-1 in lymph node distant metastasis breast cancer and the downregulation of GPR54 in patients with a poor prognosis.[104] Furthermore, the upregulated KiSS-1/GPR54 system showed a weak correlation with tumor growth status, lymph node activity, histological type and ER status in breast cancer,[105] upregulation of GPR54 also activated the multidrug efflux transporter ABCG2 and receptor tyrosine kinase AXL (key regulator of drug resistance) thereby acted as a drug resistance enhancer in human primary TNBC cells.106 TNBC is the most aggressive type of breast cancer, lacks the expression of estrogen receptor α, progesterone receptor and human epidermal growth factor receptor 2 (HER2). The drug resistance promoted by KiSS-1R activity is attributed to ERα-deficient in TNBC cells.[9]

KiSS-1 and GPR54 are mostly distributed in placenta and greatly influenced by secretion of hormone. For metabolic disorders resulted from liver cirrhosis in patients with HCC and estrogen disturbance in breast cancer, KiSS-1/GPR54 system was upregulated unanimously when compared to other cancers. So far, it is common to block ERα pathway for patients with breast cancer in systemic therapy, such as application of tamoxifen. Nevertheless, whether accompanied downregulation of KiSS-1/GPR54 system could bring those patients better clinical outcome still needs to be confirmed in the prospective researches. Besides, although *GPR54* is characterized as a promising therapeutic target to restore drug sensitivity in patients with TNBC, detailed mechanisms by which KiSS-1/GPR54 system effects drug resistance in different subtypes of breast tumors remain unclear.

Conclusion and discussion

In this review, we summarized the published studies on KiSS-1/GPR54 signaling in various animal models and its influential factors, provoking concerns of these malignant factors during biological growth and development. Moreover, we analyzed the regulation of KiSS-1/GPR54 signaling in physiological homeostasis, mainly in affecting the puberty onset by activating GnRH secretion as a key endocrine reproductive regulator. In cancer biology, KiSS-1/GPR54 system primarily acted as aggressive and metastatic inhibitor in malignant tumors, in most cases, through the degradation of MMP-9 and inhibition of Akt.[15] However, controversial roles were observed in various tumors. For example, the KiSS-1/GPR54 system played a contradictory role in hepatocellular carcinoma and breast cancer, which was possibly due to disorders of estrogen. Moreover, it is widely accepted that the heterogeneity of tumor cells could cause differences in the tumor growth rate, invasion ability, sensitivity to drugs, and prognosis. For example, Dotterweich J *et al* found kisspeptin and KiSS-1R were upregulated in mesenchymal stem cells and osteoprogenitor cells when co-cultured with multiple myeloma cells. Crosstalk between cancer cells and the bone microenvironment demonstrated that kisspeptin or GPR54 could be a probable biomarker for predicting the change of TME during progression of multiple myeloma.[107] To date, the function of the KiSS-1/GPR54 signaling in different contents of the TME (e.g., fibroblasts, endothelial cells, and tumor-infiltrating lymphocytes) was almost unclear. Thus, it is anticipated that the expression pattern in each component in the TME would be further elucidated, which is conducive for counteracting the complex regulation of KiSS-1/GPR54 signaling and providing therapeutic opportunities for targeting therapy in a wide range of cancers. Furthermore, as an easily measured secreted peptide, expression of kisspeptin together with other factors in liquid biopsies might be helpful biomarkers for disease diagnosis, including dysfunctions of reproduction, metabolic disorders and various malignant cancers.

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Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interests

The authors declare no competing financial interests.

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