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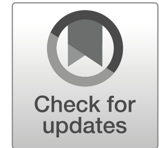

KISS1 in metastatic cancer research and treatment: potential and paradoxes

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Abstract

The significance of KISS1 goes beyond its original discovery as a metastasis suppressor. Its function as a neuropeptide involved in diverse physiologic processes is more well studied. Enthusiasm regarding KISS1 has cumulated in clinical trials in multiple fields related to reproduction and metabolism. But its cancer therapeutic space is unsettled. This review focuses on collating data from cancer and non-cancer fields in order to understand shared and disparate signaling that might inform clinical development in the cancer therapeutic and biomarker space. Research has focused on amino acid residues 68-121 (kisspeptin 54), binding to the KISS1 receptor and cellular responses. Evidence and counterevidence regarding this canonical pathway require closer look at the covariates so that the incredible potential of KISS1 can be realized.

Keywords Metastasis · Metastasis suppressor · KISS1 · KISS1R · Dormancy · G protein-coupled receptor

Abbreviations

DAG	Diacylglycerol
E_2	Estrogen
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
FSH	Follicle-stimulating hormone
GPCR	G protein-coupled receptor
IP3	Inositol trisphosphate
LH	Luteinizing hormone
MAPK	Mitogen activate protein kinase
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol (4,5)-bisphosphate
PLC	Phospholipase C
SNP	Single-nucleotide polymorphism
TNBC	Triple-negative breast cancer

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1 Introduction

1.1 Why care about KISS1?

KISS1 was discovered as a metastasis suppressor in melanoma following microcell-mediated introduction of whole, wild-type chromosome 6 into metastatic melanoma cells followed by subtractive hybridization comparing cells suppressed for metastasis [1]. Early studies identified that KISS1 was highly expressed in the placenta and brain, with lesser expression in the kidney and pancreas, and negligible expression in other tissues [1–4]. Since invasion of trophoblasts during pregnancy resembles tumor invasion, early speculation was that KISS1 inhibits invasion as the explanation for metastasis suppression. While invasion was inhibited in the majority of cancer cell lines tested, the blockage was not complete, and since metastasis only requires some ability to invade (i.e., even weakly invasive cells can metastasize as long as those cells retain the ability to complete the other steps of the metastatic cascade), other processes were thought to be more relevant. Regardless, the capacity to inhibit metastasis garnered some enthusiasm because of the potential to improve cancer patient outcomes. Subsequently, accumulating clinical evidence in

multiple cancer types has reaffirmed KISS1's relevance in cancer and metastasis, highlighting its prognostic value as well as its therapeutic potential.

In this review, we will summarize what is known about KISS1 from multiple disciplines, focusing on its role in cancer. We will also investigate whether the early promise of KISS1 in cancer therapy has been fulfilled, and if the accumulating data warrant further investment in the cancer therapeutics space. Somewhat surprisingly, given KISS1 was originally defined in the context of cancer, most of the current understanding and clinical promise have been realized in physiology. For this review to put KISS1 into perspective, it is critical to explore what is known about its roles outside of cancer. Those data indeed inform concepts related to KISS1 roles in neoplasia.

Central to KISS1 research for the past quarter century are its crucial roles in reproduction, where KISS1 provided evidence for negative and positive feedback regulators of gonadal hormones. Particularly, KISS1-expressing neurons sit at the apex of the hypothalamic-pituitary-gonadal axis to regulate luteinizing hormone, follicle-stimulating hormone, and consequently two key gonadal hormones, estrogen and testosterone. As for completing a regulatory feedback circuit of the reproduction system, KISS1-expressing neurons express gonadal receptors, and indeed, KISS1 expression in those neurons is regulated by gonadal hormones [5]. Besides being highly expressed in the placenta and selected regions of the brain, KISS1 is expressed in lower levels in the liver, pancreas, adipose, and heart. The broader expression compared to initial studies can be attributed to improved methods and has been correlated with physiologic roles in reproduction (ovulation, fertilization, embryo implantation, placentation, etc.), circadian rhythm, adiposity, kidney development, and bone formation [6, 7]. Besides associating with the aforementioned processes, KISS1 is also a regulator of metabolism [8, 9]. Due to KISS1's many physiological roles, disruptions of KISS1 are accompanied with pathologic processes, including hypogonadism [10], polycystic ovary syndrome [11, 12], and preeclampsia [13]. Cumulatively to date, twenty-five KISS1-centric clinical trials have been registered on *clinicaltrials.gov*, targeting reproductive disorders, diabetes, and *in vitro* fertilization. Ultimately, the research on KISS1 during the last three decades agrees unanimously on its importance in many aspects of medicine.

1.2 How is KISS1 regulated?

To critically evaluate how to fully harness KISS1's potential, it is imperative that we fully understand how it is regulated and its mechanism of action. Data to address questions of regulation, as expected, come primarily from the endocrinology literature. Furthermore, KISS1 regulation has been determined to occur at both the RNA and protein expression levels in tissue- and cell type-specific manners.

1.2.1 Gene

KISS1 transcription is selectively regulated based on tissue and cell type.

In the hypothalamus, KISS1 is expressed by select subsets of neurons. Depending upon the neuron subpopulation, estradiol either up- or downregulates KISS1 via a classical or nonclassical ERα pathway, respectively [14]. In addition, the classical ERα pathway may be conserved in the uterus where estradiol also upregulates KISS1 expression [15]. In principle, ERα's classical mechanism of action is exerted through the direct binding of E_2-activated ERα to DNA via estrogen responsive elements (ERE). Nonclassical ERα signaling, on the other hand, regulates gene transcription through the binding of ERα to cofactors such as AP1, SP1, NFκB, etc., which is not dependent on the ERE (reviewed in [16]). In addition, sequences upstream of the *KISS1* promoter and a 3' intergenic region downstream of the last exon appear to act as enhancer regions [17, 18]. As in virtually all endocrine systems, KISS1 expression is determined by the combinations of transcription factors, cofactors, and epigenetic machinery present (or absent).

Some of the earliest understanding of *KISS1* regulation came from metastatic melanoma studies right after its discovery. As the original quest for a metastasis suppressor gene on chromosome 6 ended up with a gene identified on chromosome 1, *KISS1*, it stood to reason that *KISS1* regulators resided on chromosome 6 (detailed in [19–21]). Indeed, subsequent experiments revealed that the essential regulator for the *KISS1* in melanoma – the transcription factor CRSP3/DRIP130 – resides on chromosome 6 [21]. CRSP3/DRIP130 also regulates a key cofactor TXNIP/VDUP1 [21]. Subsequently, binding studies using CRSP3, together with TXNIP/VDUP1 and a basal transcription factor SP1, identified SP1 binding sites at nucleotides - 93 to - 58 bp of the *KISS1* promoter, the binding results in active gene transcription [22].

In breast cancer cell lines MCF7 and MDA-MB-435, transcriptional activator protein AP-2α binds to SP1 at nucleotides - 288 to - 188 bp in the *KISS1* promoter [23]. Using another breast cancer cell line, MDA-MB-231, de Roux's group suggested that E_2 downregulates *KISS1* via a nonclassical ERα pathway (like in the hypothalamic subpopulation) but independent of SP1 [24]. As ER happens to be important in *KISS1* regulation in the hypothalamus and breast cancer subtypes, an association between hormonal status and *KISS1* regulation in breast cancer may exist. Concomitantly, SP1 regulation of *KISS1* may also depend on the hormonal status. However, data from two independent ER-negative breast cancer cell lines (MDA-MB-435 and MDA-MB-231) complicate

interpretations. It appears that *KISS1* regulation in breast cancer cells may depend on additional undetermined features rather than exclusively relying on hormonal status.

Though not directly interacting with the gene region, many other proteins, long noncoding RNA, and miRNA are emerging as important regulators of *KISS1* gene expression in diverse cancer types as well (Table 1). Together, these results highlight the complexity and tissue-specific nature of *KISS1* regulation.

1.2.2 Protein

As a typical secreted protein, KISS1 has a signaling sequence that targets the nascent protein to the endoplasmic reticulum for transport to the plasma membrane via the Golgi and secretory vesicles. Either at the outer leaflet of the plasma membrane or outside the cell, a proprotein convertase, furin, cleaves full-length KISS1 at dibasic sites into multiple fragments called kisspeptins (KP) [37]. Of note and somewhat unexpected, furin had previously been believed to be catalytic intracellularly [38]. Despite this, the majority of KISS1 research has focused on KISS1/KP after secretion. The most well-studied KP, a 54 amino acid polypeptide, spans residues 68-121 called KP54. Other smaller peptides derived (by still relatively ill-defined mechanisms) from KP54 have been detected and named based upon the number of amino acids: KP14 (aa 108-121), KP13 (aa 109-121), KP10 (aa 112-121). Early data strongly agree that all of these peptides belong to the family of RF-amides [3, 4, 39]. Their C-termini are amidated, which contributes to their binding to the receptor KISS1R which, in turn, triggers multiple signaling cascades (see Sect. 1.3). Because the above-referenced KP equivalently bind to KISS1R, researchers refer to KP-54, KP-14, KP-13, and KP-10 all as kisspeptin for short. As we will discuss below, there are other polypeptides derived from KISS1, which can also legitimately be referred to as KP. We, therefore, recommend that KP be defined by relative position from KISS1 rather than length to avoid confusion. Until a naming consensus is reached, we use the common conventions in this review and will attempt not to be ambiguous about which KP is being discussed.

Besides furin, other enzymes are also associated with KISS1 cellular processing. The most well associated enzymes are the matrix metalloproteinases (MMP), whose expression patterns overlap significantly with KISS1. MMP-16 and MMP-24 are expressed specifically in the brain; MMP-2, MMP-9, and MMP-14 are highly expressed in placenta. These two tissues most highly express KISS1. All of these MMPs (MMP-16, MMP-24, MMP-2, MMP-9, MMP-14) cleave KP at Gly118 and inactivate KP/KISS1R signaling [40].

Though less studied than KP, another fragment of KISS1, named kissorphin (KSO), generated from the cleavage of KP10 at Gly118 by MMP, also has physiologic functions. The 6-residue KSO (aa 112-117) shares sequence with neuropeptide FF (NPFF); can be amidated at the C-terminus; binds and activates the RFamide receptor NPFFR [41]; binds to Alzheimer's amyloid-β peptide, prion protein, and amylin peptides [42]; and, possibly, possesses an anti-opioid character [43–45]. Linkages between KSO and cancer, if any, are not yet clear.

Though preliminary, some posttranslational modifications of KISS1 have also been reported. Yan et al., using thyroid cancer cell lines, suggested that an E3 ubiquitin ligase SMURF1 might be associated with the ubiquitination of KISS1, leading to KISS1 degradation [46]. In two screenings for studying the cellular distribution of phosphorylated proteins, KISS1 appears to be phosphorylated at Ser134 in both Jurkat and MEF cells (https://www.phosphosite.org/siteAction.action?id=11169746). Phosphorylation at Tyr112

Table 1 Upstream regulators of KISS1

Regulator		Cancer type	Cell Line(s)	Reference(s)
Protein	WASF3	Breast	MDA-MB-231, SkBr3, BT474	[25]
	Melatonin/GATA3		MDA-MB-231, HCC70	[26]
	DNAJB6		MDA-MB-231, -435	[27]
	Wnt5a	Melanoma	UACC903, UACC1273	[28]
	Wnt5a	Prostate	PC3	[25]
	Notch1		LNCaP	[29]
	UHRF1	Bladder	RT4	[30]
	SIRT1	Colorectal	SW620, SW480	[31]
ncRNA	TP73-AS1	Renal	A498, 786O	[32]
	LUCAT1	Prostate	PC3	[33]
	TC0101441	Ovary (epithelial)	SKOV3, CAOV3	[34]
	MNX1-AS1	Osteosarcoma	SOSP-9607, Saos2	[35]
	miR-345	Breast	MDA-MB-231Br	[36]

in Jurkat cells has also been reported [47]. We found no independent verification of these modifications; however, they posit an intriguing alternative mechanism of KISS1 regulation.

1.3 How does KISS1 mediate cellular responses?

Shortly after the discovery of KISS1, an orphan G protein-coupled receptor which shares significant sequence homology with galanin receptors, GPR54, was identified [48]. In 2001, three labs independently and nearly simultaneously discovered that KP are the ligands of GPR54 [2–4]. GPR54 was subsequently named KISS1 receptor (KISS1R).

KISS1R is a 7 transmembrane G protein-coupled receptor. Heterotrimeric G proteins, consisting of subunits α, β, and γ, initiate signals depending upon the α subunit $G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, or $G_{\alpha 12/13}$ (reviewed in [49]). KISS1R is typically coupled with $G_{\alpha q/11}$ but can also associate with another $G_{\alpha q}$ member, $G_{\alpha 15/16}$, in hematopoietic organs [3, 50]. Accordingly, KP/KISS1R signaling fits well under a prototypical $G_{\alpha q}$ model. Briefly, a ligand-activated receptor activates the effector protein PLC-β which, in turn, hydrolyzes PIP_2 into two second messengers, IP_3 and DAG. IP_3 diffuses into the cytosol triggering Ca^{2+} efflux from the endoplasmic reticulum, while membrane-associated DAG activates PKC. KP/KISS1R signaling in specific cell types diverges from here. The main route for downstream PKC signal is through MAP kinases (ERK1/2 and p38-related pathways) [2–4, 51–54].

KISS1R also reportedly activates another small G protein, RhoA [55, 56], transactivates EGFR [57], and associates with β-arrestin [58]. There exists crosstalk with KISS1/KISS1R signaling and other cancer-associated signaling pathways as well, including EGFR [57], CXCL12/CXCR4 [59–61], TNFα [55], NFκB [62], PI3K [62], and TGFβ [51]. Therefore, downstream pathways of KISS1R signaling are numerous and have the potential to affect multiple cellular processing and phenotypes. When coupled with knowledge that KISS1 and KISS1R are differentially expressed and differentially regulated in a cell type-specific manner, one must be careful not to extrapolate findings from one cell type to another.

The list of cellular responses of KP/KISS1R signaling continues to expand. Each of the pathways in the previous paragraph regulate cancer-associated phenotypes, such as migration and invasion [52–55, 61–64], stress fiber formation [3], proliferation [53, 62], cell cycle arrest [65], apoptosis [62, 65, 66], autophagy [36, 66, 67], and angiogenesis [56, 68]. Therefore, each represents a viable explanation for how KISS1/KISS1R signaling mediates metastasis suppression.

To further understand KISS1 mechanisms of action, several labs have overexpressed KISS1. Yan et al. overexpressed KISS1 and observed repression of NF-κB translocation to the nucleus which, in turn, reduced MMP9 expression in HT1080 cells [69]. Complicating interpretation, many studies (including our own), express KISS1 in cells which do not have detectable expression of KISS1R. Yet, re-expression or overexpression of KISS1 results in phenotypic changes, including stabilizing the master of mitochondrial biogenesis PGC1α, inhibition of AMPK, and downregulation of PPARα [70, 71]. Likewise, Jiang et al. demonstrated that KISS1 suppresses metastasis in ovarian and prostate cancer cells that do not express KISS1R through PKCα [72].

While intriguing, challenges still exist in ascribing particular signaling cascades to the anti-metastatic functions of KISS1. These challenges include the following: (i) many reports utilize non-metastatic cells from multiple tissue origins; (ii) some studies either over-express KISS1R or use cancer cell lines in which the KISS1R is not expressed; and (iii) drug (i.e., KP or KP mimic) concentration and exposure time vary widely. Taken together, these results present a highly complex situation in which canonical KP/KISS1R signaling is called into question as the exclusive mechanism by which KISS1 mediates functions.

2 The relationship of KISS1 and KISS1R

Whereas KISS1 and KISS1R function to regulate many aspects of development (see [6, 73, 74] for comprehensive review), from a cancer perspective and possibly normal physiological perspective, it is intriguing to critically evaluate the discrepancies observed in which KISS1 and KISS1R may be independent of each other. Knockout models for *Kiss1* and *Kiss1r* in mice provided an early indication for potential independent roles, as whereas mice from either knockout background do not undergo normal sexual maturation resulting in infertility, $Kiss1^{-/-}$ are less severely affected than $Kiss1r^{-/-}$ mice [75]. Some potential explanations for the observed differences are as follows: (i) unknown, yet independent functional roles for both Kiss1 and Kiss1r, (ii) genetic polymorphism(s) that subtly affect Kiss1 or Kiss1r function or penetrance, or (iii) an incomplete knockout of *Kiss1* (either from a technical limitation or unknown biology such as transmission of kisspeptin from the placenta) [75–77]. While no data yet exists to back up the last two hypotheses, accumulating evidence support the hypothesis that KISS1 and KISS1R can function independently. Therefore, this section aims to dissect the multidisciplinary evidence for independent functional roles for the ligand KISS1 and the receptor KISS1R.

Before elaborating upon putative alternative functions, it is important to recognize that nature has done some of the experiments for us. Critically, germline mutations of *Kiss1* or *Kiss1r* have been observed in patients [10, 78–83], but also in multiple species. In the majority of cases, hypogonadism or reproductive deficiencies have been observed. However, the severity of the pathologies is variable and obviously affected by polygenic signaling and covariates.

2.1 Evolutionary history

KISS1 phylogenetics collectively show that, throughout evolution, KISS1/KISS1R biology contributes to genetic fitness in species rather than conferring an essential unique biological function [84, 85]. For example, kisspeptin appears to be dispensable for reproduction in teleost while necessary in placental mammals. In addition, kiss and kiss receptor genes are missing altogether in chickens. Furthermore, phylogenetics offers insights into the relationships between KISS1 and KISS1R throughout evolution.

It stands to reason that if KISS1 and KISS1R function together exclusively, they should coevolve. But do they coevolve? Synteny analysis has identified 3 paralogs of *KISS* (*Kiss1, 2, 3*) and 4 paralogs of *KISSR* (*KissR1, 2, 3, 4*) in vertebrates and 2 paralogs of *KISS* and 2 paralogs of *KISSR* in mammals. Importantly, the annotated number does not indicate a one-to-one pairing relationship between the ligand and receptor (e.g., *kiss1* pairs with *kissR1*); rather, it is based on the order of their discovery. Interestingly, early research led to the hypothesis that there is a conservation of kiss/kissR pair, i.e., in select species the pairing appeared to match (in primates, rodents, and cattle, kiss1 and kissR1; in platypus, kiss1 and 2 and kissR1 and 2; in lizard, kiss2 and kissR2 [85]).

Accumulating evidence challenges the coevolution hypothesis. First, *in vitro* studies show that both kiss1 and kiss2 can activate both kissR1 and kissR2. In other words, there is no unique selectivity for the pairing. Second, later studies - using more complete genome databases and expanded species analyses - showed that some species have more kiss receptors than kiss (ligand) genes (e.g., in spotted gar, kissR1–4 and kiss1 and 2; in European eel, kissR1–3 and kiss1 and 2; in coelacanth, kissR1–4 and kiss1–3 [86]). Third, the presence of a pseudo-kiss2 gene (translated into KP10 that is nonamidated, inactive) was reported in primates including human [87]. While challenging the coevolution hypothesis, the above evidence suggests that a high degree of conservation between the paralogs circumvents the need for two paralogs to coexist. Pasquier et al. suggest that this may be attributed to differential physiological roles, which may include tissue specificity, differential regulation, and/or differential mechanisms of action, e.g., differential regulation in the hypothalamic subpopulations [86]. In that same line, alternative splicing of different isoforms of *KISS1R* has been identified in a modern teleost species, implicating differential tissue expression [88].

Since the discovery of its pairing to GPR54, *KISS1* has long been classified under the RF-amide peptide family based on its RF-amide motif (other members include ligands NPFF, QRFP, NPVF, PrRP). Due to the diversity in *KISS* paralogs, they form their own branch in the RF-amide peptide family. Different ligand branches within the family promiscuously bind with receptors in other branches. Accordingly, *in vitro* studies show that KP binds to NPFFR1 (GPR147) and NPFFR2 (GPR74) [89, 90]. However, the classification under the RF-amide peptide was recently questioned partly because *KISS-KISSR* evolutionary history is distinct from other members in the family. Instead, assuming coevolution with their cognate receptor, *KISS* may deserve their own group called KISS/galanin/spexin family based on their cognate receptor (KISS1R is mostly homologous to galanin receptor) [91].

Altogether, despite inconclusive data, phylogenetic studies suggest that other receptors for KISS1 exist and the action mode of KP/KISS1R is tissue-specific. Of note, phylogenetics also provide useful data of which researchers should be aware, such as existent isoforms of genes and the necessity for appropriate model animal selection.

2.2 Differential effect of KP10 in mouse and humans

Alignment of KISS1 protein sequences across species has identified that the majority of KISS1 (precursor) amino acid sequence is highly variable. Despite this variability, KP10 is a highly conserved domain in primates, rodents, cattle, and zebrafish. Despite sequence conservation, KP10 exhibits differential effects within a given species, as illustrated by studies in the pancreas and placenta below, which suggests a genetic conservation, but a physiological divergence.

In the studies of KISS1 regulating pancreatic insulin production, both stimulation vs inhibition have been observed. Initially, discrepancies were attributed to differences in experimental models (whole pancreas *vs* cells, perfused *vs* static tissue culture), forms of KISS1 (KP54, KP13, KP10), and species (mouse, rat, human, monkey, pig). Subsequently, Song and colleagues concluded the discrepancy arose from the spectrum of KISS1 concentration among research groups [92]. At nM concentrations, KISS1 inhibits glucose-stimulated insulin secretion, while at μM concentrations, KISS1 stimulates insulin secretion in mouse pancreas islets in both perfused or static cultures as well as in an *in vivo* mouse model [92]. The conflict seemed to be resolved until a recent trial of KP10 at nM administration in 19 healthy men concluded KP10 stimulated insulin secretion [93]. Interestingly, Lyubimov et al. showed that human KP10 has higher affinity for NPFF2R than murine KP10, resulting in slightly less than 20-fold differential EC_{50} [90]. Their results highlight how different pathways may be activated when utilizing reagents which are not from the same species. Also, the findings illustrate some level of promiscuity for ligand-receptor binding in KISS1 signaling.

Lastly, the conserved physiology of KISS1 in placentation and pregnancy between mice and humans has been questioned as well. Whereas compelling evidence suggests that KISS1 plays significant roles in regulating human placentation, $Kiss1^{-/-}$ mice still delivered litters that were not significantly different from $Kiss1^{WT}$ [94]. Taken together, these results imply that important considerations need to be taken into account

when translating KISS1 findings from model systems to humans.

2.3 Constitutive receptor activity of KISS1R

Direct evidence supporting KISS1-independent functions of KISS1R stem from studies showing KISS1R desensitization [95] via intracellular internalization of KISS1R [58]. This observation implicates a constitutive receptor activity [96]. Briefly, after prolonged KP exposure, GRK2 rapidly uncouples KISS1R from $G_{\alpha q/11}$ (desensitization) and facilitates KISS1R binding to β-arrestin. β-arrestin then sequesters membrane KISS1R via clathrin-coated vesicles. A small portion of "used" KISS1R undergoes degradation, while the rest is recycled back to the cellular membrane. Even in the absence of KP, KISS1R internalization (regardless of $G_{\alpha q/11}$-coupling) displays dynamic turnover, with a high degree of internalization (~ 60–70% of the total receptors) [58]. This helps to maintain a sufficient pool of signaling-competent KISS1R on the cell surface. Under chronic KP stimulation, provided that the cytosolic Ca^{2+} pool can sustain the $G_{\alpha q/11}$-coupled-KISS1R pathway and that KISS1R is retained on the cell surface, signaling continues. The proposed mechanism fits well in the case of KISS1R-expressing neurons, which quickly respond to cues without new cycles of transcription or translation. Here, an interesting observation is that the internalized KISS1R may trigger signaling on its own without KP stimulation (constitutive receptor activity) [58]. Subsequently, Zajac et al. showed that KISS1R is directly associated with EGFR and stimulation of ER-negative breast cancer cells with EGF can regulate the endocytosis of both receptors, regardless of KP10 treatment [57]. Moreover, knocking-down KISS1R in an MDA-MB-231 variant cell line that does not express KISS1 [Note: other variants of MDA-MB-231 express KISS1 and KISS1R.] reduces cell migration, even with no KP treatment [97]. Thus, it appears that the mode of action of KISS1R probably expands beyond the prototypical $G_{\alpha q}$-coupled receptor.

In addition, MMTV-PyMT/Kiss1r$^{+/-}$ mice develop tumors later than MMTV-PyMT/Kiss1r^{WT} mice. Particularly, subcutaneously implanting primary MMTV-PyMT/Kiss1r$^{+/-}$ cancer cells into immunocompromised mice shows reduced primary tumor growth, suggesting KISS1R has a role in tumorigenicity [98]. This could be explained by the tumorigenicity promoting role of KP/KISS1R but also could implicate the involvement of KISS1R in cancer, with or without KISS1. These transplantation experiments could have just as easily been done in syngeneic FVB mice which has an intact immune system and could be more amenable to dissecting any purported immune functions. Unpublished data from our group show KISS1R in macrophage populations, implicating an immune paracrine crosstalk in addition to autocrine or endocrine functions (Ben Beck, Warren Denning and Danny Welch, unpublished observations).

2.4 KISS1 function independent of KISS1R

Strong supporting evidence for an independent function of *KISS1* comes from *Kiss1r*$^{-/-}$ mice studies where KP shows subtle effects. First, KP at μM concentrations stimulates insulin secretion in response to glucose in *Kiss1r*$^{-/-}$ mice [92]. Second, KP still regulates neuronal excitability in *Kiss1r*$^{-/-}$ mice. Similar excitation is observed when activating NPFFR1, suggesting that KP effect may be exerted through NPFFR1 instead of Kiss1r [99]. A role for KISS1 that is independent of both KISS1R and NPFFR is also a possibility as shown by a study in neurotoxicity by Chilumuri and colleagues [100]. Particularly, knocking-down *KISS1* in human neuronal cells shows increased amyloid toxicity. In contrast, KISS1 overexpression induces neuroprotection. Intriguingly, their initial hypothesis that the neuroprotective effect is exerted through either KP or KSO has been experimentally refuted, as administering antagonists of either receptors, KISS1R and NPFFR1, shows the same effect. Taken together, the data do not preclude an as yet unidentified receptor as well.

3 Why study KISS1 in cancer?

3.1 Clinical evidence of KISS1 relevance in metastasis

Most clinical evidence supports, or is at least consistent with, KISS1 metastasis suppressor roles as observed in preclinical models, i.e., expression is lost as tumors progress toward metastasis and/or increased expression is associated with better prognosis (Table 2). Data vary depending upon whether KISS1 is measured at the protein or RNA level, mostly likely because protein and RNA expression do not directly correlate [133, 134].

Clinical data in some cancer types provide contradictory evidence, most notably in liver, breast, and thyroid cancers. A common denominator for these cancer types is that the primary sites are highly hormonally active but direct connections have not yet been established. Considering that KISS1 is regulated (both negatively or positively) by estradiol depending on the hypothalamic subpopulation neurons and that KISS1 is widely associated with other hormones (e.g., insulin, leptin, prolactin, etc., all of which have been described to associate with cancer to some extent), it stands to reason that tumor hormonal status and the secondary microenvironmental physiology could influence the capacity of KISS1 to suppress metastasis. Moshmi Bhattacharya's group has most extensively explored such relationships in different breast cancer cell lines. Overall, they find that presence of ER_α in luminal subtypes is associated with KP/KISS1R suppression of invasion and metastasis using MCF7 [59]. In contrast, ER_α-negative cells exhibit promotion of metastatic phenotypes in MCF10A [97], Hs578T, and MDA-MB-231 cells [135, 136]. The

Table 2 Clinical correlations of KISS1

Improved prognosis/improved survival/metastasis suppressing	
Bladder	[101, 102]
Breast	[103–107]
Colorectal	[108–110]
Endometrial	[111]
Esophageal	[112]
Gastric	[113, 114]
Liver	[109, 115–117]
Lung	[118, 119]
Melanoma	[120]
Ovarian	[121–123]
Pancreas	[124, 125]
Metastasis promoting	
Breast	[126, 127]
Liver	[128, 129]
Osteosarcoma	[130]
Thyroid	[131]
No correlation with clinical outcome	
Lung	[132]

KISS1 expression was (semi)quantified using mRNA or protein. Readers are cautioned that KISS1 activity presumably requires posttranslational processing; so mRNA data may not be most informative or potentially misleading

situation is not entirely clear, however. Using MDA-MB-231, other groups find KISS1/KISS1R anti-metastatic roles [67, 137]. The discrepancy may arise from distinctive epigenetics of the cell lines. Liu's group proposes that in breast cancer, KP/KISS1R signaling has dual roles: to initially promote tumorigenesis and then to suppress the invasion in the early stage of metastasis [137]. Also, the different laboratories studied metastasis formation in different tissues (i.e., the lung and brain, respectively). Perhaps KISS1/KISS1R effects on metastasis have organ-specific effects on tumor cells. Besides molecular mechanisms studied in breast cancer, these unexpected observations that KISS1 promotes metastasis in liver and thyroid cancer have not been followed up.

Also, although molecular studies have focused on KISS1/KISS1R underlying cancer suppression, many clinical studies do not take KISS1R into account. Interestingly, in those that do, KISS1 and KISS1R expression levels do not correlate. The following section will address these mechanisms of KISS1 loss in more detail.

3.1.1 Epigenetic silencing or downregulation of KISS1-regulating transcription factors

The *KISS1* gene was discovered based on the clinical observation that the deletion of long arm of chromosome 6 (6q) occurs in > 80% of late-stage cases of metastatic melanoma. As discussed above, the long arm of chromosome 6 consists of the positive transcription factor CRSP3/DRIP130 that regulates KISS1 promoting transcription factors TXNIP/VDUP1 (on chromosome 1q) and disruption of the cascade can lead to KISS1 suppression [21]. An alternative route for losing KISS1 expression in melanoma is hypermethylation of another positive transcription factor TCF21 [138].

In bladder cancers, loss of *KISS1* expression occurs through hypermethylation in the gene promoter [101]. Mechanistically, the overexpression of UHRF1 increases methylation of CpG in the *KISS1* promoter repressing its expression [30]. Likewise, hypermethylation was described in colorectal cancer [108]; however, conflicting evidence exists as another study presented an inconclusive role of hypermethylation in colorectal cancer [139]. Lastly, hypermethylation in the *KISS1* promoter was also described but not associated with the downregulation of *KISS1* mRNA in pancreatic ductal adenocarcinoma [140].

Overall, the loss of expression of KISS1 due to epigenetic silencing aligns well with what is commonly observed in most other metastasis suppressors (i.e., there are relatively few mutations observed, but gene expression is silenced [141]). The predominance of epigenetic changes in cancer does not preclude mutations or structural modifications of KISS1 or KISS1R in other pathogenic states.

3.1.2 Single-nucleotide polymorphism (SNP)

In the latest update of the human genome (GRCh37.p13 (Dec 2019)), the *KISS1* gene region includes 2014 SNP. Among them, the most clinically significant, rs587777835, results in an inactivating KISS1 mutation and, ultimately, hypogonadotropic hypogonadism [10]. There are several studies of *KISS1* SNP association in cancer. In the study of breast cancer in Mexican populations, Quevedo et al. specifically chose to focus on 2 SNP, rs12998, and rs5780218 and reported that the latter correlates with higher risk for developing breast cancer [142]. Collectively, however, the majority of *KISS1* SNP studies do not find a statistically significant association between SNPs or mutations of KISS1 with cancer development or disease prognosis [143, 144]. Instead, the loss of KISS1 expression is more commonly explained via epigenetic silencing or downregulation of the transcription factors discussed above. Nevertheless, several studies of *KISS1* SNP in cancer offer intriguing implications, provided that study cohorts are statistically sufficient, and the biology of SNP is characterized. To illustrate these implications, 2 cases are discussed below.

Dova et al. used carcinoma of unknown primary tumor samples and found that 49 out of 50 tumor samples presented wildtype *KISS1*, similar to *KISS1* in the peripheral blood lymphocytes of healthy controls. In contrast, only 1/50 tumor samples displayed a point substitution in the last exon, resulting in P81R *KISS1* [143]. Intriguingly, P81R *KISS1* was independently reported by Pentheroudakis et al. as well

[144]. In the latter study, the mutation resulting in P81R was detected in the cell lines MCF7 and A549 and in 5/50 breast adenocarcinomas samples (3/5 present germline mutation). Regarding the phenotype, P81R *KISS1* tumors have less KISS1 immunoreactivity, 20% vs 50% in wildtype, and account for higher rate of axillary node involvement, 80% vs 55% in wildtype. Although both studies conclude no significant association between P81R *KISS1* mutation and disease, P81R *KISS1* detection in two independent studies (ranged from 2 to 10% and 2 out of 3 studied cancer cell lines) suggests that the mutation could be significant in a larger cohort.

The study by Brunet et al. is especially interesting [145]. Particularly, rs71745629 *KISS1* was associated with prolonged latency of metastatic colorectal cancer. In the studied cohort (*N*=172), although colorectal cancer patients with the *KISS1* rs71745629, T/* genotype do not have better overall survival, they do have a significantly better progression-free survival, 12 months *vs* 4 months for those with the homozygous T/T genotype. Mechanistically, *KISS1* rs71745629, T/* genotype results in the deletion of adenosine 417 (417delA) in the terminal exon of *KISS1* gene; this creates a frameshift and a downstream STOP codon, translated into a 145-aa KISS1 protein. In contrast, in the homozygous T/T genotype (417A), the protein is 138 aa long. This implicates an isoform of KISS1 protein more likely to suppress metastasis. Of note, the 145-aa isoform was reported in the discovery of KISS1 as a metastasis suppressor in 1996 [1]. Through multiple updates of the genome reference consortium database, the 138-aa KISS1 seems to be more prevalent. Nonetheless, multiple intriguing questions arise from this study, including how the genotype affects KISS1 production and whether the isoforms have different effects on cellular response.

3.2 A model to study cancer dormancy

3.2.1 The clinical relevance of cancer dormancy and where KISS1 fits in

Metastasis is the major cause of cancer-related deaths. After treatment of primary cancer, despite being considered disease-free, a substantial cohort of cancer patients relapse in a type-specific manner. The time between the disease-free announcement and relapse is called metastatic latency. Particularly, long metastatic latency (years), or metastatic dormancy, has been clinically observed in breast, prostate, melanoma, renal, lung, and head and neck cancer [146]. Metastatic latency represents a promising window of opportunity to screen, intervene, and prevent a relapse [147]. Metastatic latency can vary significantly in patients. Further identification of the mechanisms that promote residual cells dormancy may provide the necessary framework for the development of novel therapeutics to prevent progressive disease [148–150].

Cancer dormancy research has evolved from captivating interest to recent mechanistic studies, and we will hopefully see its clinical applications in the future [151]. Molecular pathways in cancer dormancy have been compiled extensively in the last decade [146, 152]. However, challenges remain which include a lack of robust study models and limited study material for statistical analyses. We predict that *KISS1* can help fill in this gap. The direct evidence comes from experiments in which introducing *KISS1* gene in highly metastatic cancer cells keeps them dormant in secondary site [72, 153, 154]. Though currently there is no direct data for the molecular mechanism underlying this observation, accumulating data of KISS1 both in physiology and cancer signaling highly overlap with the molecular pathways described in cancer dormancy (Table 3). Whereas the predominant approach in the study of KISS1 in cancer is to either utilize endogenously expressing KISS1R or overexpressed KISS1 cancer cells, in our model showing KISS1-induced dormancy, cancer cells do not express KISS1R. Altogether, KISS1-induced dormancy is likely attributed to multiple molecular players in both cancer cells and the tumor microenvironment.

3.2.2 *in vitro* models do not recapitulate KISS1 dormancy effects

Metastasis is a stepwise process in which a single step is necessary but insufficient to lead to the end point: secondary outgrowth [173]. Pinning down the exact step(s) in which a metastasis suppressor is involved will inform development as a therapeutic. Unfortunately, *in vitro* studies have not always led to unequivocal definition of KISS1's mechanism of action. For example, KISS1-expressing cancer cells can form primary tumors, circulate, and seed in the lung, but do not grow out. In other words, KISS1 suppresses metastasis at the last step, the outgrowth of cancer cells at the secondary site. To study the secondary outgrowth in lung, an *ex vivo* pulmonary metastasis assay (PUMA) has been proposed and shown to be an appropriate model [174]. Accordingly, Young et al. utilized the PUMA with the goal to model the metastasis suppressor effect of KISS1 and study the underlying mechanism [175]. GFP-labeled, KISS1-expressing cancer cells were injected into the tail vein; 20 min later when cells are lodged in the lung capillaries, lungs were harvested, cut into small sections, and *ex vivo* cultured up to 3 weeks. After 3 weeks, whereas modest fluorescent puncta were detected *in vivo*, the fluorescent signal increased dramatically in the PuMA lung. Something essential for KISS1 to suppress outgrowth may have been altered in the PUMA. The result once again emphasizes that *in vivo* models most faithfully recapitulate the metastasis process.

Table 3 Signaling pathways/molecules potentially linking KISS1/KISS1R and cancer cell dormancy [146, 155, 156]

	Physiology/ endocrinology	Cancer
FAK	[2, 3]	[157]
SDF1/CXCL12/CXCR4		[59–61]
TGFβ	[158]	
BMP4		[159]
BMP7		[160, 161]
Wnt5A		[28]
LIF/LIFR	[94]	[162]
eIF2α/p38		[163]
ERK/p38/PKC	[3, 164–167]	[53, 60, 65, 124, 168]
MMP9/angiogenesis	[56]	[53, 69, 169, 170]
TNFα/immune evasion	[171]	[55]
Autophagy	[172]	[66, 67, 70]

3.3 KISS1 potential clinical application for cancer: diagnosis, prognosis, and therapy

3.3.1 Diagnosis

Dotterweich et al. demonstrated the use of KP10 conjugated with a fluorophore for the diagnosis of multiple myeloma (malignant plasma cells homing in the bone marrow). Conventional detection methods for myeloma are MRI and measurement of excess serum or urinary IgG; however, downsides exist regarding the accessibility and specificity. Experiments show that when myeloma cells homing to the bone marrow interacts with mesenchymal and osteoprogenitor cells, these stromal cells significantly upregulate KISS1R. Using fluorophore-conjugated KP10, the group further showed that the bone where tumor is injected fluoresces compared to no signal observation in the un-injected bones, suggesting the specific binding of fluorophore-conjugated KP10 to tumor site. Subsequent mechanistic studies will further the exciting potential of this application [176].

3.3.2 Prognosis

Clinical evidence (Table 2) consolidates KISS1's relevance in disease progression toward metastasis. Accordingly, many studies propose the prognostic value for KISS1. The promise of KISS1 as a biomarker for predicting metastasis or survival is context dependent. Additionally, most studies have measured KISS1 expression within the primary tumor and lacked parallel measurement in metastases. As discussed previously, loss of expression in what is likely to be a minority population of metastatic cells within a primary tumor is suboptimal as an expression biomarker. Furthermore, realistically, a single gene is implausible to be a prognostic tool for such a complex multifactorial disease as cancer (though there are exceptions, e.g., CML with the involvement of Philadelphia chromosome, rare cancers where single genes can confer malignancy). As we further categorize cancer into molecular subtypes for precision medicine, the combinations of multiple genes including KISS1 may be useful; nevertheless, taking all variants into account is a statistical and experimental challenge.

3.3.3 Therapy

The relevance of KISS1 to cancer dormancy not only provides a study model for cancer dormancy but also presents a potential therapy to intervene in metastasis (discussed in [19]). As KISS1 is typically downregulated in cancer, it stands to reason that finding a way to re-express or add back a metastasis suppressor will have significant therapeutic potential in preventing metastatic outgrowth. Firstly, KISS1's metastasis suppressor effect is exerted after its secretion, which omits complications of the cellular membrane barrier that impedes many drugs to access the cell genome or other intracellular targets. Secondly, KISS1 is endogenous, thus theoretically less or non-immunogenic. In addition, its limited side effects in clinical trials also demonstrate a strong safety profile. Ultimately, characterizing the mechanism by which KISS1 suppresses metastasis is a must. Immunotherapy breakthroughs have recently revolutionized the cancer therapeutic space. While there is no direct association of KISS1, the fact that KISS1R is more relatively ubiquitously expressed in lymph nodes suggests that KISS1R may have a yet to be defined role in the immune system. In the meantime, early therapeutic progress related to KISS1 is encouraging.

Empirically, natural compounds have demonstrated anticancer effects. Though it is challenging to pin down the exact mechanisms underlying the biological effects of natural compounds, many research groups employ this drug discovery approach. Honokiol, a small biphenolic compound extracted from magnolia bark, exerts its anticancer effect through diverse molecular pathways essential for cancer [177]. Interestingly, a microarray of renal cell carcinoma treated with honokiol (40 μM) for 24 hours identified KISS1 as the top upregulated gene and KISS1R as the third most upregulated gene; further validation and knockdown study confirmed honokiol inhibited renal cancer cell invasion partly via the upregulation of KISS1 [178]. Research on the anticancer effect of honokiol has continued characterizing the detailed pathways and provides intriguing suggestive evidence for further development [177].

After the seminal case reports in 2003 identifying a clinical phenotype (hypogonadism) due to impaired KISS1/KISS1R signaling [76, 179], KP/KISS1R garnered great research interest and thrived beyond cancer, moving to physiology fields. The recognition of the great potential of a short 10-aa peptide

as a drug candidate was illustrated by attempts of multiple research groups to generate the stable synthetic mimetic-KP10 peptides [180–182]; the culmination is a drug called TAK-448 (Takeda Pharmaceuticals) put on clinical trial phase I (NCT02381288) for the effect of downregulating testosterone in healthy and prostate cancer males. This study demonstrated that TAK-448, an agonist of KISS1R, is tolerable and can effectively reduce PSA in cancer patients; however, the effect was not robust and did not go to the next phase for cancer trials [183]. Unfortunately, challenges exist in measuring effective therapeutic indices for anti-metastatic drugs, and thus further development of biomarkers and criteria for measuring efficacy are desperately needed. Follow-up studies of KP-10 in combination with cytotoxic therapies, KP-10 prevention of relapse, and/or KP-10 mediated immune activation/regulation may provide additional opportunities for the advancement of KISS1 cancer therapeutics.

4 Concluding remarks

The review attempts to integrate KISS1 data from multiple fields to make sense of the biology of KISS1 with the goal to realize its clinical potential in metastatic cancer. Unfortunately, what was once thought of as a straightforward exercise has been more difficult than initially expected. Nonetheless, some clear lessons have been learned. Critically, KISS1 is a central node in signaling where its upstream and downstream vary depending on tissue and cell type. As conflicting data arise, researchers should be aware of alternative hypotheses besides the long-standing presumptions and be stringent in including covariates such as status of KISS1, KISS1R, ERα, and polymorphisms.

The existence of, and detection of, KSO shows that additional, similarly sized KISS1-derived polypeptides exist. Many of those peptides do not share functions (e.g., KSO does not interact with nor activate KISS1R); therefore, we recommend a naming convention in which all KISS1-derived peptides (termed kisspeptins) are defined by position, not size. Doing so will reduce confusion in the future. Still, the promise of KISS1 as a metastasis suppressor which could improve cancer patient outcomes remains.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Informed Consent This review summarizes only published results from clinical studies. To the best of the authors' knowledge, all studies were performed in compliance with applicable human subject protection policies, guidelines, and laws.

Animal Studies This review summarizes only published results utilizing animals for experimental studies. All work from the authors' laboratories was approved by relevant Institutional Animal Care and Use Committees. To the best of the authors' knowledge, all other studies were performed in compliance with applicable policies, guidelines, and laws regarding humane housing, handling, and treatment of research animals.

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