Drug Resistance Updates 42 (2019) 12–21

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

KiSS1 in regulation of metastasis and response to antitumor drugs

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ARTICLE INFO

Keywords:
KiSS1
GPR54
Cancer
Metastasis
Drug response

ABSTRACT

Metastatic dissemination of tumor cells represents a major obstacle towards cancer cure. Tumor cells with metastatic capacity are often resistant to chemotherapy. Experimental efforts revealed that the metastatic cascade is a complex process that involves multiple positive and negative regulators. In this respect, several metastasis suppressor genes have been described. Here, we review the role of the metastasis suppressor KiSS1 in regulation of metastasis and in response to antitumor agents. Physiologically, KiSS1 plays a key role in the activation of the hypothalamic-pituitary-gonadal axis regulating puberty and reproductive functions. KiSS1-derived peptides i.e., kisspeptins, signal through the G-protein coupled receptor GPR54. In cancer, KiSS1 signaling suppresses metastases and maintains dormancy of disseminated malignant cells, by interfering with cell migratory and invasive abilities. Besides, KiSS1 modulates glucose and lipid metabolism, by reprogramming energy production towards oxidative phosphorylation and β-oxidation. Loss or reduced expression of KiSS1, in part through promoter hypermethylation, is related to the development of metastases in various cancer types, with some conflicting reports. The poorly understood role of KiSS1 in response to chemotherapeutic agents appears to be linked to stimulation of the intrinsic apoptotic pathway and inhibition of cell defense factors (e.g., glutathione S-transferase-π) as well as autophagy modulation. Deciphering the molecular basis underlying regulation of the metastatic potential is crucial for the establishment of novel treatment strategies.

1. Introduction

Although there has been a revolution in cancer care and treatment driven by the discovery of a multitude of alterations in tumor cells, by the advent of new technologies and by efforts directed at early detection and prevention of cancer, it has failed to lead to a durable control of neoplastic diseases, particularly when metastases develop. Thus, metastatic dissemination of tumor cells represents a major limitation to the cure for different types of cancer (Popper, 2016; Fakih, 2015; Middleton et al., 2018). Frequently, disease outcome remains unsatisfactory due to drug resistance and metastatic dissemination. Drug resistance of tumor cells is often associated to an increase in the metastatic ability (Naiditch et al., 2015; Corno et al., 2017), with the acquisition of mesenchymal features and the occurrence of the so-called epithelial to mesenchymal transition (EMT), a plastic program suppressing epithelial markers while inducing mesenchymal markers (Chaffer et al., 2016).

Metastatic spread is a complex phenomenon consisting of the detachment of tumor cells from the primary tumor towards close or distant sites where tumor cells grow and generate new masses (Lambert et al., 2017). The process occurs in multiple steps starting with local invasion by tumor cells followed by intravasation in blood or lymphatic vessels, arrest in distant organs, extravasation and metastatic colonization (Turajlic and Swanton, 2016). Cells may engage dormancy programs that impair cell proliferative ability as well as pro-angiogenic features (Lambert et al., 2017). Metastatic spread involves several positive and negative regulators, the latter including metastasis suppressor genes often lost in tumors due to reduced transcription. Several metastasis suppressors have been described (Shoushtari et al., 2011; Smith and Theodorescu, 2009) and among them KiSS1 appears to act at the interface of metastasis and drug response. In fact, KiSS1 has been implicated in suppressing the metastatic phenotype of cancer cells as well as in sensitizing them to conventional chemotherapy (Beck and Welch, 2010; Jiffar et al., 2011).

Metastatic spread involves different target organs. Lymph nodes are the most frequently affected organs, whereas liver, lung, brain and bone represent preferential distant target organs. Liver metastases, more frequent than primary hepatic tumors and often symptomless, derive from multiple tumor types such as breast, gastrointestinal cancer and melanoma (Clark et al., 2016). Lung metastases are generated from various extra-thoracic tumors such as breast, colon and skin cancer (Altorki et al., 2018). Brain metastases are the most common type of

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https://doi.org/10.1016/j.drup.2019.02.001
Received 28 December 2018; Received in revised form 3 February 2019; Accepted 6 February 2019

central nervous system (CNS) tumors being more frequent compared to primary brain tumors (Valiente et al., 2018); the most common originate from lung cancer, breast cancer and malignant melanoma (Gavrilovic and Posner, 2005). Skeletal metastases derive mainly from breast, lung, prostate and renal cancer (Weidle et al., 2016). Overall, less attention has been paid to metastasis suppressors than to factors promoting metastasis, likely given to the propensity to hit mechanisms favoring tumor aggressiveness more than to restore mechanisms suppressing the aggressive behavior of tumor cells. However, from the nineties, many studies have implicated KiSS1 in metastasis suppression in different tumor types, and the knowledge on its role in tumor biology has been markedly deepened.

Here, we review the role of KiSS1 in regulating metastatic spread, taking into account its signaling pathway, the recently reported function in regulation of metabolism as well as conflicting biological functions emerging from the literature about its control of the metastatic cell ability. In addition, we examine the poorly understood aspects involving KiSS1 in the regulation of sensitivity of tumor cells to chemotherapeutic drugs. Finally, we discuss the therapeutic potential of KiSS1-derived peptides (i.e., kisspeptins) and how advances in the field of KiSS1 biology may be exploited in the search for new therapies or biomarkers of response.

2. Overview on KiSS1

The KISS1 gene is a metastasis suppressor originally identified in melanoma cells, in which metastatic spread had been suppressed by introduction of human chromosome 6 (Lee et al., 1996).

This chromosome transfer approach led to a misleading assignment of the KISS1 gene to chromosome 6; in fact, further studies clarified that the KISS1 gene mapped to the long arm of chromosome 1 (1q32-q41) as shown by fluorescence in situ hybridization (West et al., 1998) and its expression was likely regulated by a gene localized on chromosome 6 (Miele et al., 2000). Functional approaches indicated that KISS1 was indeed a metastasis suppressor in melanoma cells and incorrectly in breast cancer due to a cell line misidentification (Lee et al., 1996, Lee and Welch, 1997, Korch et al., 2018). The gene was designated KiSS to indicate a suppressor sequence (SS) and because it was discovered in Hershey (Pennsylvania) where the Hershey Chocolate Kisses were produced (Wahab et al., 2016).

Apart from its action in cancer cells, KiSS1 plays a relevant physiological role in the neuroendocrine control of reproduction (Pineda et al., 2010), with an established role in secretion of gonadotropins and other hormones (Gahete et al., 2016), as initially shown by studies linking the KiSS1 system and the reproductive function (de Roux et al., 2003; Seminara et al., 2003) (Fig. 1). The KiSS1R/GPR54 (also known as hOT7T175, AXOR12) was discovered in 1999 as an orphan receptor and in 2001 its natural ligand was identified (Kotani et al., 2001) (see below). KiSS1R/GPR54 is expressed in placenta, pituitary gland, pancreas, brain and spinal cord; the binding with its ligand leads to the activation of phospholipase C, Ca^{2+} mobilization and phosphorylation of Extracellular signal-regulated kinases 1/2 (ERK1/2) and p38. Physiologically, this signaling pathway is essential to trigger the secretion of gonadotropins at puberty (Seminara and Kaiser, 2005). In tumor cells, KiSS1 signaling has been linked to suppression of metastasis with evidence of requirement of secreted KiSS1 for suppression of organ metastasis and maintenance of dormancy of disseminated malignant cells (Nash et al., 2007). However, in this model system, the expression of the receptor appears be unnecessary, because exogenous KiSS1 was shown to be able to suppress metastasis from a melanoma cell line lacking the expression or expressing very low levels of GPR54. This would imply the occurrence of a non-canonical signaling independent of GPR54 for KiSS1 to exert a metastasis suppressive activity in such a model. Conversely, KiSS1 secretion is needed for metastasis suppressor activity as exogenous expression of a non-secreted KiSS1 does not display anti-metastatic activity (Nash et al., 2007). The requirement of KiSS1R expression in the metastasis suppressive effect of KiSS1 has been reported in several studies in different tumor types such as renal cell carcinoma, pancreatic cancer and colon cancer (Shoji et al., 2009; Uno et al., 2016; Kim et al., 2018).

3. KiSS1 signaling in physiology and cancer

Canonical signaling of KiSS1 occurs trough the GPR54 receptor (Wahab et al., 2016). The ligand of this G-protein coupled receptor is peculiar because it is not a single protein, but consists of multiple peptides (Kotani et al., 2001). In fact, the KISS1 gene encodes for a 145-aa hydrophobic polypeptide, from which the 54-amino acid protein kisspeptin-54 (KP54, also known as metastin) is cleaved (Kotani et al., 2001; Harihar et al., 2014). The KiSS1 protein which harbors an N-terminal secretion signal peptide and KiSS1 processed by furin or possibly by additional members of the pro-protein convertase family of proteases, has been proposed to occur outside the cell (Harihar et al., 2014; Nash et al., 2007; Beck and Welch, 2010). Peptides derived from the KiSS1 polypeptide even shorter than KP54, are collectively named kisspeptins (consisting of 10, 13 and 14 amino acids) and share a common RF-amidate motif (Arg-Phe-NH_2) with metastin (Ohtaki et al., 2001). In the full-length protein, the amino acid sequence of KP54 contains pairs of basic residues with the R66-R and GK123-R acting as cleavage sites for furin or other convertases. The proteolytic processing of KiSS1, occurring with modes similar to that of neuropeptides (Yang et al., 2016), was already predicted upon identification of the kisspeptins-GPR54 interaction due to the presence of basic residues in the full amino acid protein sequence leading to KP54; the shorter peptides were probably the result of metastin instability leading to KP10, KP13 and KP14 (Kotani et al., 2001). In a recent study, an exclusive role for furin in KiSS1 processing has been demonstrated based on the fact that upon treatment of KiSS1 overexpressing cells with pro-protein convertase inhibitors and based on the evidence that knockdown of furin but not of other proteases (i.e., PCSK5 or PCSK7), abolished KiSS1 processing (Harihar et al., 2014).

Following binding of kisspeptins to the GPR54 receptor, hydrolysis of phosphoinositides (i.e., phosphatidylinositol 4,5-bisphosphate), calcium mobilization and arachidonic acid release were observed, apart from activation of the Mitogen Activated Protein Kinases (MAPK) ERK1/2 and p38, stress fiber formation and inhibition of cell proliferation. For the ligand-receptor interaction, the peptides need to be amidated as non-amidated synthetic kisspeptins do not bind GPR54 (Kotani et al., 2001). In this regard, although KiSS1 has been proposed to be a member of the RF-amide peptide family, phylogenetic analysis suggests that KiSS1 belongs to the KiSS1/galanin/spexin family (Yun et al., 2014). Notably, spexin which is a ligand for galanin receptor 2 (GLR2) and galanin receptor 3 (GLR3) is involved in regulation of metabolism as it inhibits long fatty acid intake in adipocytes and hepatocytes (Kolodziejski et al., 2018).

The comparison of the phenotypes of mice with a targeted disruption of the Kiss1 gene and *Gpr54* null mice was instrumental to the discovery of KiSS1 as the gene encoding the GPR54 ligands. In fact, the phenotypes were identical, thereby strongly suggesting that KISS1 is the gene encoding for GPR54 ligands. The characterization of the mice led also to the discovery that the physiological role of KiSS1 was the activation of the hypothalamic–pituitary–gonadal axis (d'Anglemont de Tassigny et al., 2007).

The KiSS1/GPR54 axis has been reported to play an essential role in mammalian fertility, with production of kisspeptins by neurons located in the hypothalamus (AVPV and ARC regions) (Colledge, 2009). Kisspeptin binding to KiSS1R stimulates hypothalamic neurons to produce the gonadotropin releasing hormone (GnRH) which leads to secretion of pituitary gonadotropins (i.e., LH and FSH) and sexual steroids (Trevisan et al., 2018). A role for KiSS1 and its receptor in human reproduction is supported by evidence that mutations of these genes are associated with hypogonadotropic hypogonadism, with infertility or even with absence

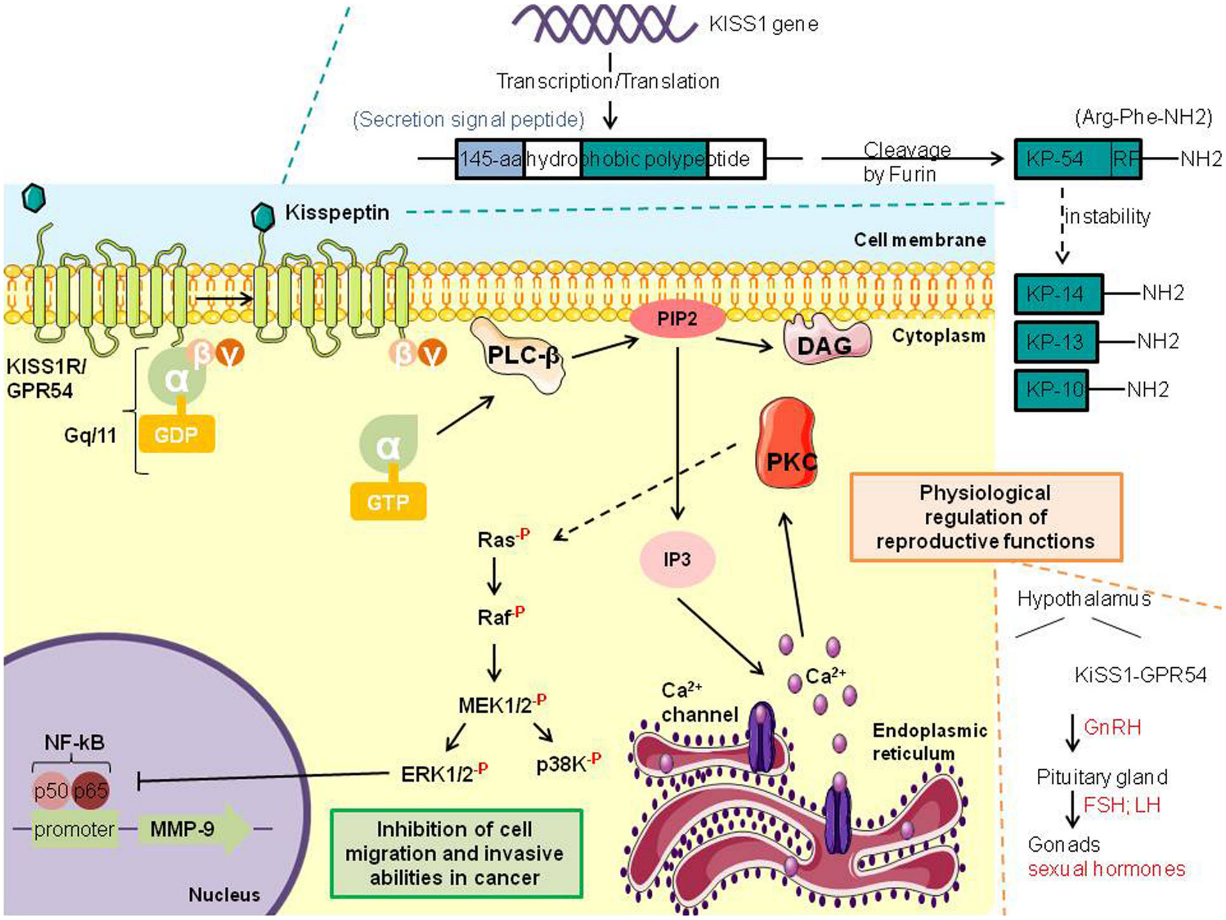

Fig. 1. Schematic representation of the role of KiSS1 in physiology and cancer. KiSS1 processing is shown together with the major components of KiSS1 signaling.

of pubertal development for loss of function GPR54 mutations (Topaloglu et al., 2012).

In cancer, the role of KiSS1/GPR54 signaling has not been fully elucidated, although KiSS1 and/or its receptor appear to be involved in metastasis formation and disease development sometimes with opposite effects (Nash et al., 2007; Blake et al., 2017). In fact, Nash et al., have provided evidence that KiSS1 blocks metastatic colonization in melanoma, being necessary for suppression of metastasis and – when secreted – to maintain dormancy in disseminated cells (Nash et al., 2007). KiSS1/GPR54 signaling increases intracellular Ca^{2+} levels and suppresses motility as shown in chemotaxis and wound healing assays (Hori et al., 2001; Castano et al., 2009). In addition, KiSS1 decreases the binding of nuclear factor kappa B (NF-kB) to the promoter of matrix metalloproteinase-9 (MMP-9), thereby decreasing MMP-9 expression; no modulation of the binding of other transcription factors (i.e., AP-1, Sp1, Ets) known to regulate MMP-9 was observed (Yan et al., 2001). MMPs, in particular MMP-9, can degrade the extracellular matrix (ECM) and favor tumor cell dissemination. Therefore, the decrease of this protease level induces inhibitory effects on the motility and invasive capability of cancer cells.

Other studies have confirmed the metastasis-suppressive role of KiSS1 in additional tumor types apart from melanoma (Nash et al., 2007), with an inverse correlation between KISS1 expression and metastases in lung, pancreatic, gastrointestinal, gynecological, prostate and bladder cancer (Beck and Welch, 2010; Cao et al., 2016; Wang et al., 2012; Kim et al., 2018). The mechanism underlying the ability of KiSS1 to suppress metastasis, besides being dependent on KiSS1 receptor (Kim et al., 2018; Shoji et al., 2009; Uno et al., 2016), has also been linked to negative regulation of CXC chemokine receptor 4 (CXCR4) (Navenot et al., 2005).

The KiSS1-KiSS1R axis has been documented to play a pro-metastatic role in breast cancer. In fact, the receptor stimulates migration and invasiveness of estrogen-receptor (ER) negative mammary epithelial and breast cancer cells, which therefore acquire EMT-like features. Since invasion was not stimulated in ER positive cells, ER appears to negatively regulate KiSS1R-mediated invasion in this context (Cvetkovic et al., 2013). Besides, KiSS1R signaling has been implicated in inducing pro-invasive cell abilities through stimulation of invadopodia formation with activation of MMP-1, cofilin and cortactin (Goertzen et al., 2016). The mechanism leading to this behavior appears to involve β-arrestin 2 and the canonical Erk1/2 MAP kinase, independently of Src (Goertzen et al., 2016).

Recently, KiSS1-mediated autophagy has been proposed as a mechanism used by astrocytes to promote breast cancer metastases to the brain (Kaverina et al., 2017). Specifically, stroma cell-derived facto r 1 (SDF1)/ CXC motif chemokine 12 (CXCL12) produced by astrocytes was shown to downregulate KiSS1 levels by inducing microRNA-345; conversely, exogenous expression of KiSS1, besides reverting the invasive phenotype of breast cancer cells, downregulated the autophagy-related genes ATG5 and ATG7 (Kaverina et al., 2017). Regarding the cross-talk with the SDF1/CXCR4 pathway, a recent study supports that KP10 is capable to reduce SDF1-induced increase of cell invasion by downregulation of CXCR4 expression (Grundker et al., 2015).

Additional evidence supports that selected kisspeptins are endowed with biological activities counteracting cancer cell dissemination. For instance, kisspeptin-10 inhibits tumor angiogenesis by suppressing Sp-1 mediated VEGF expression (Cho et al., 2009).

4. The role of KiSS1 in metabolism

Altered metabolism is a key feature of tumor cells which use glycolysis to produce energy also in the presence of oxygen, therefore carrying out poorly efficient metabolic reactions (Hanahan and Weinberg, 2011; Cairns et al., 2011). In fact, incomplete oxidation of glucose to lactate generates less ATP than complete oxidation through respiration in the mitochondria (Liberti and Locasale, 2016). High glycolytic rates promote cell proliferation through the production of glycolytic intermediates, which are shunted into subsidiary pathways to fuel metabolic pathways *de novo* generating nucleotides, lipids, amino acids and NADPH (Lunt and Vander Heiden, 2011). Although metabolic changes have not been systematically related to metastasis, various pathways including glycolysis, oxidative phosphorylation, amino acid and lipid metabolism have been implicated in cancer metastasis (Teoh and Lunt, 2018). For instance, reduced glucose oxidation enhances tumor metastasis and reactive oxygen species (ROS), hence generating mitochondrial DNA mutations can regulate tumor cell metastasis (Kamarajugadda et al., 2012; Ishikawa et al., 2008). Besides producing energy and biosynthetic intermediates, cell metabolism can fuel tumor cell capability to disseminate as well as to survive and proliferate in various environments with different metabolic substrates (Teoh and Lunt, 2018).

KiSS1 has been reported to play a role in glucose metabolism and mitochondrial biogenesis in melanoma cells, in which the expression of full length KiSS1 is associated with a decrease of aerobic glycolysis with prevalent occurrence of oxidative phosphorylation (Liu et al., 2014) (Fig. 2). Aerobic glycolysis is instead the predominant metabolic pathway acting if the exogenously expressed KiSS1 lacks the secretion N-terminal signal peptide, and the metastasis-suppressing capability of KISS1 is lost (Liu et al., 2014). Of note, enhanced mitochondria biogenesis is only found in wild-type KiSS1-expressing cells. KiSS1 was shown to be capable of modulating pH, leading to extracellular inhibition of acidification in association with lower glucose uptake and lactate secretion, occasionally linked to increase of the glucose transporter GLUT1 and decrease of hexokinase II (HK2) levels (Liu et al., 2014). Inhibition of acidification was related to reduced expression of selected subunits (V0d2, V1g3) of the vacuolar H^+-ATPase (V-ATPase), a proton pump which is key for control of extracellular and intracellular pH (Pamarthy et al., 2018). An interesting feature of KiSS1 expressing cells was the enhanced expression of nuclear encoded mitochondria genes (e.g., AIFM2 apoptotic protein, HSP60 and HSP90AA1 chaperones) as well as of the mitochondrial transcription factors Nuclear respiratory factor (NRF1) and Transcription factor A (Tfam) in association with mitochondrial genes (i.e., ND2, RNR2). Besides, KiSS1 induced the expression of peroxisome proliferator-activated receptor (PPAR)-α (PGC1α), a ligand-activated transcriptional factor that belongs to the family of nuclear receptors and regulates the expression of genes involved in $\beta-$oxidation of fatty acids (Tan et al., 2016), known to interact with NRF1 and to activate the transcription of Tfam, leading to activation of transcription and duplication of mitochondrial genome (Liu et al., 2014). PGC1α seems critical for KiSS1 anti-metastatic effect because KiSS1 stabilized the protein likely *via* protection of proteasome-dependent degradation by Ubiquilin-1, and KISS1 knockdown resulted in decreased nuclear PGC1α protein levels; interestingly, KiSS1 upregulated the expression of Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), both genes implicated in fatty acid synthesis, whereas it down-regulated the expression of PGC1α (Liu et al., 2014).

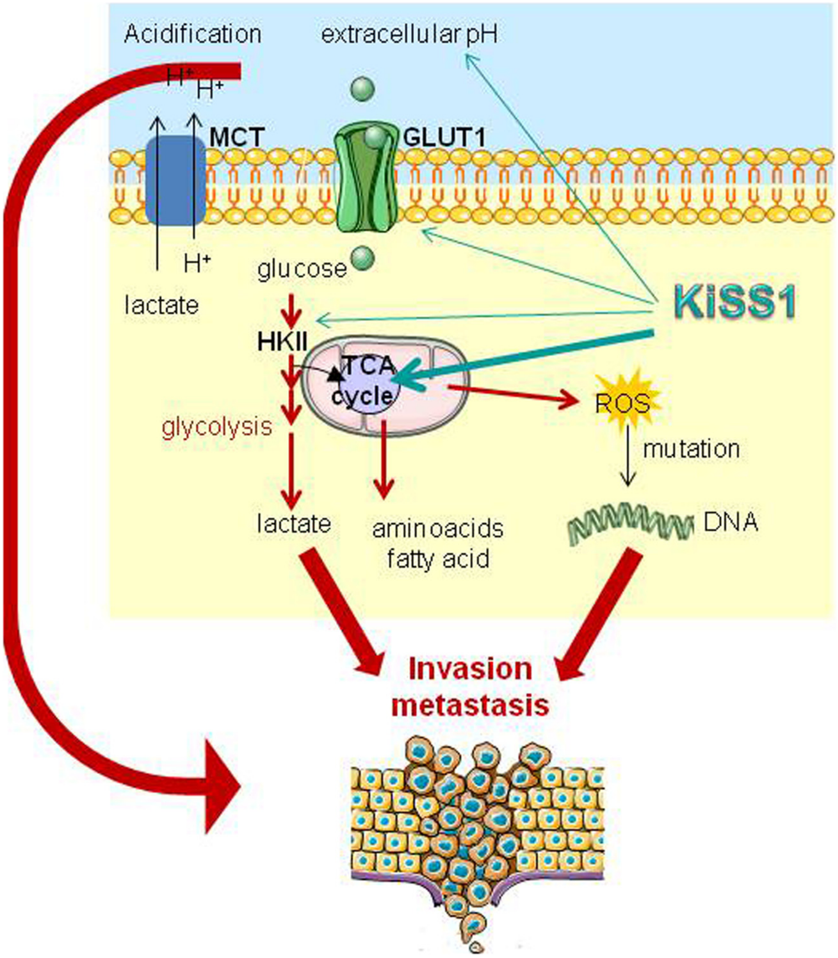

Fig. 2. KiSS1 and regulation of metabolism. Selected metabolic pathways are shown with the indication of KiSS1 effects.

In a recent study, the expression of KiSS1 was shown to result in down-regulation of glycolysis in highly metastatic melanoma cells, with KiSS1 expressing cells being less glycolytic than control cells (Manley et al., 2017). When the metabolic cell plasticity was examined by measuring mitochondrial adaptation in the presence of glucose (i.e., Crabtree effect), KiSS1 expressing cells failed to switch toward glycolysis and to suppress mitochondrial respiration (Manley et al., 2017). This study supports that KiSS1 does not confer upon cells the capability to rely only on glucose as the major energy source, but primarily to use mitochondrial metabolism. Besides, KiSS1 was shown to decrease the expression of the glucose transporter gene SCL2A2 and of the mitochondrial hexokinase II (HKII), but not of other relevant enzymes of the glycolytic pathway (Manley et al., 2017). In this regard, it has to be noted that expression of HKII has been linked to tumor aggressiveness in various cancer types (Ogawa et al., 2015). Thus, by attenuating aerobic glycolysis and sustaining mitochondrial metabolism, KiSS1 reprograms energy production towards oxidative phosphorylation and β-oxidation. Indeed, KiSS1 promoted fatty acid conjugation to acyl-CoA, fatty acid acetyl-CoA transport into mitochondria and β-oxidation and stimulated short chain fatty acid catabolism, as shown by gene expression studies. In addition, KiSS1 enhanced the expression of ACC, but not of other lipogenic enzymes, differently from what previously observed (Liu et al., 2014). Interestingly, increased β-oxidation appeared to be mediated by persistent activation of Adenosine Monophosphate-Activated Protein Kinase (AMPK) *via* KiSS1-induced inhibitory phosphorylation of ACC. PGC1α, previously shown to be critical for the anti-metastatic effect of KiSS1 (Liu et al., 2014) was also shown to be necessary for KiSS1-mediated up-regulation of β-oxidation genes. Of note, the regulatory role of PGC1α on KiSS1 metastatic function was evident *in vivo* both in the experimental and spontaneous metastasis setting (Manley et al., 2017).

A role for KiSS1 in the regulation of lipid metabolism has been investigated in mouse fibroblasts and rat adipocytes (Pruszynska-Oszmalek et al., 2017). In that context, KP10 was found to inhibit fibroblast cell proliferation and expression of genes implicated in adipogenesis (i.e., PPAR-γ and CEBPβ); suppression of glucose uptake and lipogenesis by KP10 were also observed; in adipocytes, KP10 induced stimulation of leptin, which signals to the hypothalamus the status of lipid storage in adipocytes to control food intake, and decreased adiponectin secretion (Pruszynska-Oszmalek et al., 2017). These findings corroborate the view of the contribution of KiSS1 to the regulation of lipid metabolism, particularly of lipid accumulation in a physiological context. Indeed, both KiSS1 and GPR54 were expressed at higher levels in adipocytes than in pre-adipocytes, suggesting a role for this axis in the storage of triglycerides, whose accumulation was inhibited by KP10 which also counteracted differentiation of pre-adipocytes into adipocytes (Pruszynska-Oszmalek et al., 2017). In addition, KP10 modulated glucose re-uptake, thereby limiting the availability of glucose to start *de novo* lipogenesis in adipocytes (Pruszynska-Oszmalek et al., 2017). Recently, in a peculiar setting, i.e., rat testicle tissue, a role for KP10 in protecting cells in lipid peroxidation induced by methionine has been reported in relation to kisspeptin capability to increase superoxide dismutase activity and mRNA levels and to prevent methionine-associated catalase down-regulation (Akkaya et al., 2017). These findings suggest a possible antioxidant role for KiSS1 in a specific biological context. Given the crosstalk among proliferation, survival and metabolic pathways, future efforts to normalize metabolism may be particularly useful in controlling metastasis, although the mechanism identified in cellular studies should be validated with further investigation using *in vivo* preclinical models.

5. KiSS1 expression and its regulation

Multiple mechanisms participate in the regulation of the expression of KISS1, under physiological and pathological conditions. Physiologically, specific transcription factors have been implicated in KiSS1 gene expression. For instance, a hypothalamic network of proteins controlling female puberty has been reported to regulate KISS1 gene expression, with KISS1 transcription starting at a single site (TSS) around 150 bp upstream of the ATG translation initiation codon (Mueller et al., 2011). In detail, the KiSS1 promoter is activated by TTF1 and CUX1-p200, and repressed by EAP1, YY1, and CUX1-p110, all found to be recruited to the promoter in intact cells and expressed in kisspeptins neurons, i.e., hypothalamic neurons expressing the KISS1 gene and kisspeptins (Mueller et al., 2011). Interestingly, sex differences in KISS1 gene expression have been reported in rodents by KiSS1 expressing neurons with higher levels in females not ascribable to sex hormone differences (Semaan and Kauffman, 2013).

Epigenetic modifications have also been involved in the physiological regulation of KISS1 gene expression, particularly in the control of mammalian puberty and reproductive function with studies addressing DNA methylation, or histone acetylation as well as regulation by microRNAs (Semaan and Kauffman, 2013). Histone H3 acetylation plays a role in the regulation of KISS1 gene expression in hypothalamic populations of neurons as evidenced by an association between estradiol-induced histone H3 acetylation of the KISS1 promoter and increased KISS1 gene expression (Tomikawa et al., 2012). KISS1 gene expression was also shown to be regulated through enhancer regions located in the 5’ gene region and 3’ intergenic sequences (Tomikawa et al., 2012).

Among the transcription factors implicated in the regulation of KiSS1 expression in cancer, a role for the transcription factor 21 (TCF21), a member of the class A of basic helix-loop-helix family has been proposed (Arab et al., 2011). In fact, TCF21 can directly bind the KISS1 gene promoter and increase its expression via interaction with TCF12 and the TCF3 isoform E12 (Arab et al., 2011). Of note, in esophageal squamous cell carcinoma TCF21 was down-regulated as compared to normal tissues and its overexpression in cell lines resulted in KiSS1 up-regulation in association with down-regulation of EMT-related proteins (Chen et al., 2018). Moreover, in renal cell cancer, miR-21 has been shown to down-regulate TCF21 in parallel with KiSS1, therefore increasing tumor cell invasive ability (Zhang et al., 2012).

A contribution for KiSS1 methylation to the control of KISS1 gene expression has been reported in different tumor types such as colorectal cancer and bladder cancer, both in preclinical models and in clinical specimens (Moya et al., 2013; Lam et al., 2016; Cebrian et al., 2011). In particular, the demethylating agent azacytidine was shown to increase KISS1 gene expression in cell lines of these tumor types. Low gene expression of KISS1 was associated with hypermethylation of a CpG island close to the TSS of the human KISS1 gene (Cebrian et al., 2011; Moya et al., 2013).

In spite of the acquired knowledge on the down-regulation of positive transcriptional regulators of the KISS1 gene in tumors or about promoter hypermethylation, the mechanisms controlling decreased KiSS1 gene expression or its genomic deletion in different tumor types are still partially understood (Mitchell et al., 2007). Under some circumstances, KiSS1 protein levels may reflect mRNA levels, but this is not a general rule. For instance, in thyroid cancer, KiSS1 levels have been reported to be post-translationally regulated *via* Smad ubiquitination regulatory factor 1 (Smurf1), which controls the ubiquitin-dependent degradation of KiSS1 (Yan et al., 2018). In breast cancer, KiSS1 gene expression has been shown to be regulated by direct interaction of the AP-2α and Sp1 transcription factors, which cooperate to increase KiSS1 promoter activity without direct binding of AP-2α to the KISS1 promoter, as shown by co-transfection experiments (Mitchell et al., 2006). Indeed, AP-2α and Sp1-containing transcription complexes have been evidenced at two Sp1-binding sites of the KiSS1 promoter. In melanoma, loss of the Sp-1 co-activator DRIP-130 is associated with reduced activation of the KISS1 promoter; functional approaches support that co-expression of Sp1 and DRIP-130 in highly invasive cells results in restoration of KISS1 gene expression (Mitchell et al., 2007). Since DRIP-130 is encoded by a gene located at chr 6q16.3-q23, this region is key for regulation of KISS1 gene expression. In particular, the key region for Sp1-DRIP-130-mediated regulation of KISS1 gene expression lies between – 98 and – 58 bp of the KISS1 promoter, a GC rich region that when lost, leads to lack of gene expression regulation.

Besides, it has been recently reported that the expression levels of the epigenetic regulator ubiquitin-like with PHD and RING finger domains 1 (UHRF1) and those of KISS1 are negatively correlated in bladder cancer cells both *in vitro and in vivo* and forced ectopic expression of KISS1 partially abrogates UHRF1-induced cancer cell invasion; exogenous expression of UHRF1 results in enhanced methylation of CpG nucleotides and decreased KiSS1 gene expression (Zhang et al., 2014).

The multiple modes of regulation of KISS1 gene expression identified in tumors suggest the possibility to exploit different approaches to induce KiSS1 re-expression such as the use of epigenetic treatment to control its degradation via proteasome (e.g., proteasome inhibitors). A challenge is the lack of specificity of these strategies that – however – may act as multi-target tools to block metastases and inhibit the growth of primary tumors.

6. KiSS1 and response to drugs

Among the mechanisms underlying the efficacy of antitumor drugs, apoptotic cell death is the most well-known and widely investigated; under some circumstances, there is evidence that tumor shrinkage may result from apoptosis, at least in preclinical models. The available evidence suggests that antitumor agents not only display a direct effect on tumor cells resulting in inhibition of tumor growth and/or in apoptosis, but they can also engage immune cells to activate immunogenic cell death. In this context, selected factors involved in the control of metastasis (e.g., KiSS1-derived peptides) may play a role, acting to regulate tumor cell response to treatment. In fact, KiSS1 plays a major role in the regulation of metastasis, but some studies link KiSS1 signaling also to response to antitumor drugs by still poorly characterized mechanisms. In principle, KiSS1 role in response to treatment may be linked to all the pathways activated following binding of kisspeptins to the GPR54 receptor, including kinases with a variable action in different molecular backgrounds (Fig. 3). For instance, MAPK acting downstream of GPR54 in tumor cells have been reported to play both protective and pro-apoptotic roles depending on the molecular context (Cossa et al., 2013). At present, little information is available on KiSS1 and apoptosis induction, with some associations and a few mechanistic insights.

In a recent study regarding thyroid cancer (Yan et al., 2018), KiSS1 degradation has been implicated in sustaining cell proliferation and metastatic behavior, thereby supporting an anti-metastatic role for KiSS1 linked to Smurf1, an E3 ubiquitin-protein ligase highly expressed in such a tumor type. In that context, exogenous expression of KiSS1 that inhibited the NF-kB pathway, besides reducing cell aggressive features (i.e., proliferation, migration and invasion) was found to stimulate apoptosis as shown by the activation of caspase 3 and caspase 9 and by enhanced levels of Bax upon KiSS1 expression. Thus, KiSS1 appears to be capable of affecting the basal susceptibility of tumor cells to apoptosis even in the absence of an apoptotic stimulus provided by a drug. In line with this study is the observation that the expression of KiSS1 was down-regulated in non-small cell lung cancer cells selected for resistance to cisplatin, a finding suggesting an association between platinum sensitivity and KiSS1 expression (Zuco et al., 2015). Of note, drug-resistant cells with reduced KiSS1 levels displayed an enhanced metastatic behavior (Zuco et al., 2015).

A role for KiSS1 in regulation of tumor cell response to cisplatin has been proposed in head and neck squamous cell carcinoma preclinical models, in which a decrease in KiSS1 mRNA and protein levels was found in cisplatin-resistant as compared to sensitive tumors (Jiffar et al., 2011). Knockdown of KiSS1 by RNA interference resulted in inhibition of poly-(ADP)-ribose polymerase (PARP) cleavage in cisplatin-treated cells and up-regulation of glutathione S-transferase (GST)-π, potentially through NF-kB activity, which was also up-regulated (Jiffar et al., 2011). Conversely, exogenous expression of KiSS1 increased sensitivity of cisplatin-resistant cells to the drug, with an enhanced inhibition of cell proliferation of around 35% in cells exposed for 24 h to 10–15 μM cisplatin. Of note, KiSS1 expression abrogated cisplatin-induced GST-π activation. Moreover, because genetic manipulation of KiSS1 was shown to modulate NF-kB levels, it is likely that its activation in the absence of KiSS1 and its suppression upon KiSS1 expression can differentially regulate members of the intrinsic apoptotic pathway. These findings suggest the possibility that KiSS1 contributes to cisplatin response, particularly to sensitization both by favoring cell death *via* apoptosis and by inhibiting cell defense processes implicated in protecting DNA from cisplatin-induced damage. In fact, by conjugating electrophilic compounds such as cisplatin to glutathione, GST-π inactivates the drug, thereby avoiding its interaction with the target DNA.

The potential chemo-sensitizing role of KiSS1 is also supported by a recent study in clear cell renal carcinoma in which it was shown that inhibition of apoptosis by the lncRNA-TP73-AS1 is related to repression of KISS1 expression; suppression of KiSS1 mRNA occurred through the interaction of the lncRNA with enhancer of seste homolog 2 (EZH2) and binding to KISS1 gene promoter (Liu et al., 2018).

An additional link between KiSS1 and apoptosis has been hypothesized when examining the role of KiSS1 in regulation of metabolism - particularly when showing the down-regulating effect of the metastasis suppressor on HKII mRNA levels (Manley et al., 2017) - because the mitochondrial bound enzyme is known to inhibit apoptosis, preventing mitochondrial permeability transition and Bax-dependent cytochrome c release (Pastorino et al., 2002).

In contrast to the reports mentioned above in which KiSS1 seems to play a sensitizing action, a recent study carried out in triple negative breast cancer (TNBC) supports that KiSS1R promotes drug resistance at least through two mechanisms i.e., by enhancing gene expression of the multidrug efflux transporter ABCG2 which encodes for BCRP and by inducing the receptor tyrosine kinase AXL (Blake et al., 2017). The two mechanisms appear to act independently because knockdown of AXL by siRNA – associated with reduced levels of the EMT markers snail, slug and N-cadherin - does not decrease BCRP expression in breast cancer cells (Blake et al., 2017). In addition, KiSS1R regulates AXL transcription *via* Sp1, as documented by an increase in Sp1 binding and recruitment of RNA polymerase II to the Sp1 distal sites of the AXL promoter (Blake et al., 2017). Of note, when mRNA levels of KiSS1 and KiSS1R were examined in 20 clinical specimens of TNBC and compared to those measured in normal breast, increased levels of both genes were observed in cancer samples, at least for the receptor; this finding was in line with results obtained from Western blot analysis of a smaller number of cases and controls (Blake et al., 2017). Cells transfected with a KiSS1R-expressing vector were resistant to doxorubicin and less susceptible to drug-induced apoptosis as shown by decreased PARP cleavage than control cells. Such a behavior was counteracted by a KiSS1R antagonist and KiSS1 overexpressing cells displayed reduced drug accumulation and increased BCRP mRNA levels, also observed in the clinical cancer specimens.

A possible additional mechanism that may link KiSS1 to drug response is modulation of autophagy. Autophagy is a well-characterized adaptive process occurring in response to different forms of stress such as nutrient deprivation and endoplasmic reticulum stress (Dikic and Elazar, 2018). Besides providing nutrients for essential functions under stress conditions, autophagy contributes to the selective elimination of damaged cellular biomolecules and organelles including protein aggregates or damaged mitochondria (Dikic and Elazar, 2018). Both inhibition and promotion of autophagy have been proposed as strategies to improve the efficacy of cancer therapies, mainly based on the assumption that it represents a cytoprotective mechanism activated to maintain cell survival and on the evidence that autophagy-competent dying tumor cells release ATP and HMGB1, both required for the

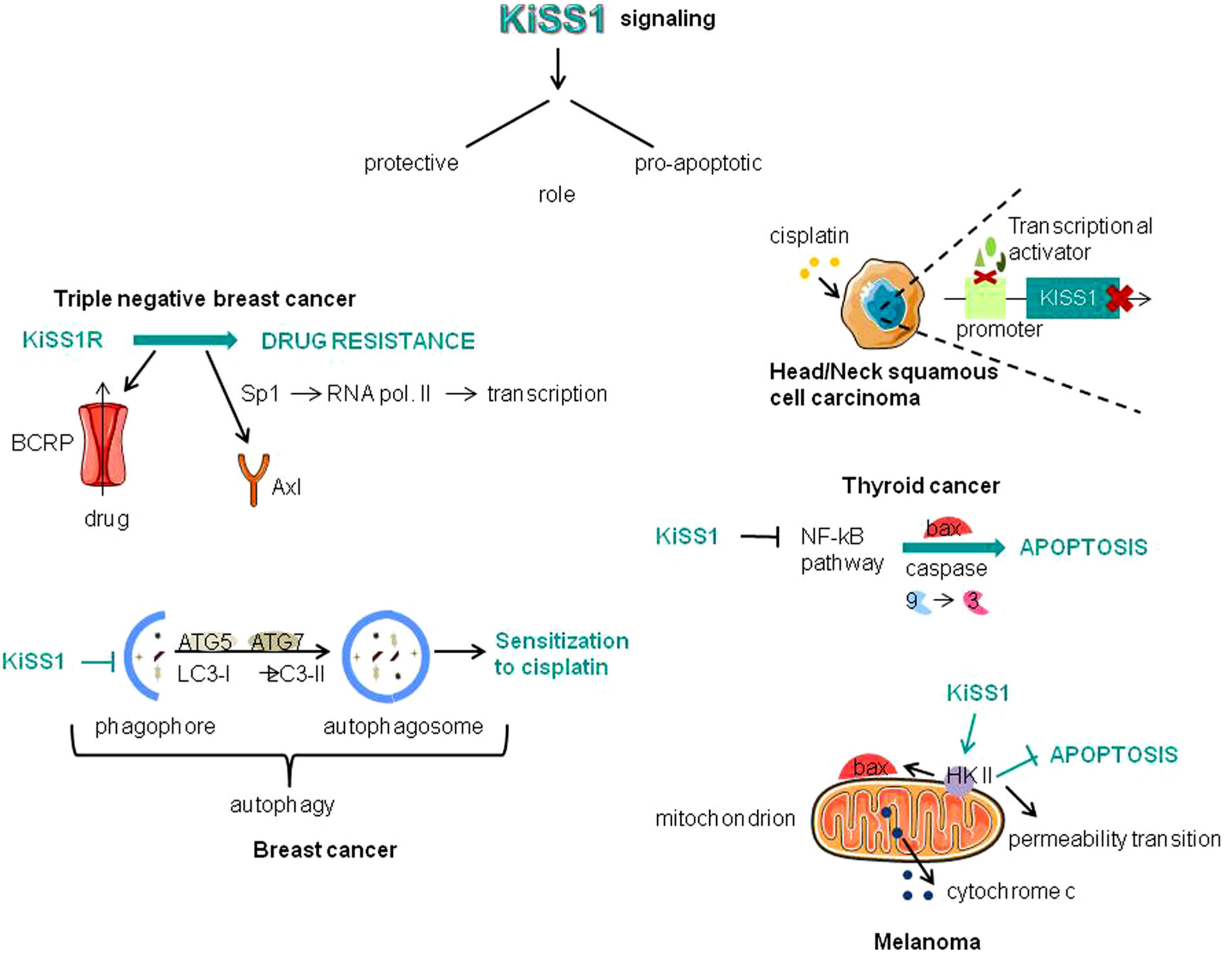

Fig. 3. KiSS1 in response to antitumor drugs. The contribution of KiSS1 to the regulation of apoptosis and autophagy in selected cancer types is schematically represented.

activation of immunogenic cell death by antitumor agents (Martins et al., 2012).

In a study carried out in melanoma, cells expressing KiSS1 were found to be resistant to bafilomycin A1 which inhibits both V-ATPase and autophagy (Liu et al., 2014). When compared to control cells, the abovementioned cells displayed lower accumulation of the autophagic cargo receptor sequestosome 1 (p62) - which binds ubiquitin and acts in the clearance of protein aggregates - a finding that suggests that KiSS1 may lead to a defect in autophagy (Liu et al., 2014). If pharmacological inhibition of autophagy can improve the efficacy of antitumor drugs under specific conditions, a KiSS1-dependent impairment of autophagy might sensitize cells to treatment (Hu et al., 2012; Michaud et al., 2011). Indeed, inhibition of autophagy has been shown to sensitize tumor cells to cisplatin (Sun et al., 2014). Conversely, KiSS1 inhibition has been linked to promotion of autophagy (Kaverina et al., 2017). In fact, in breast cancer cells it has been shown *via* functional approaches that KiSS1 down-regulates two autophagy-related genes (ATG5 and ATG7) and inhibits conversion of LC3-I to LC3-II, whereas KiSS1 down-regulation by RNA interference is associated with reduced cell accumulation of p62 and increased levels of Beclin 1 mRNA. In keeping with the dual role of autophagy in cell response to treatment, modulation of autophagy by KiSS1 appears to be complex and only in part understood. Therefore, additional efforts will be required to clearly define if KiSS1 can contribute to improve cell response to treatment with anticancer agents by affecting autophagy.

7. KISS1 and therapeutic opportunities

Kisspeptins together with kisspeptin analogs and antagonists represent useful tools to investigate physiological aspects of KiSS1 signaling such as endocrinology control of reproductive functions and pathological features of the KiSS1-KiSS1R axis in tumors, with KiSS1 signaling representing a potential therapeutic target (Albers-Wolthers et al., 2017; Roseweir and Millar, 2013; Guzman et al., 2018). Both kisspeptin agonists and antagonists have been reported in the literature and a unique therapeutic role has been ascribed to kisspeptins to treat hormone-dependent disorders of reproduction (Roseweir and Millar, 2013). The effects of kisspeptins on gonadotropin release have been extensively studied in mammals and humans leading to a clear understanding of the role of kisspeptins in reproduction and the possible use in fertility disorders (Chan, 2013; Clarke et al., 2015). The evidence that continuous exposure to kisspeptins results in desensitization to kisspeptin (Chan, 2013), with a stimulation or suppression of reproductive endocrine activity also based on the type of administration leads to the consideration that major drawbacks may be linked to the administration of these agents. Indeed, although all kisspeptins display similar receptor binding affinities *in vitro* (Kotani et al., 2001), different pharmacokinetics have been observed *in vivo*, underlying differential potency (d'Anglemont de Tassigny et al., 2017).

The use of kisspeptins or kisspeptin analogs in cancer treatment has been in part investigated, but no agents are clinically available in cancer. Kisspeptin analogs with agonistic activity have been proposed recently as investigational compounds for potential treatment of androgen-dependent prostate cancer, given their capability to lower prostate specific antigen in preclinical models of androgen-dependent prostate cancer and in patients (Tanaka et al., 2018). A peculiar aspect of this study is the formulation of the analogs that consisted of injectable sustained release depots, due to the need to improve metabolic stability as compared to natural peptides (Asami et al., 2013). Of note, *in vivo* systemic (intraperitoneal) administration of KP10 and KP54 in mice has shown that the two peptides display differential effects on hormone secretion after peripheral delivery, a behavior associated with a shorter half-life for KP10 as compared to KP54 (d'Anglemont de Tassigny et al., 2017).

In preclinical models of human mesothelioma, KP10 and a synthetic peptide with a longer half-life (~7 h) were reported to inhibit cell proliferation as well as migratory and invasive ability; the kisspeptin analog displayed anti-metastatic activity *in vivo* as shown by analysis of lung metastasis in an experimental metastases mouse model of human mesothelioma (Ciaramella et al., 2018).

Earlier studies have addressed the possibility to rationally design metastin analogs endowed with high agonistic activity towards GPR54; in this context, shorter KP54 derivatives characterized by a N-terminal basic group and a C-terminal RW-amide motif were found to be strong antagonists (Niida et al., 2006). Such a study also highlighted the relevance of the amino acid stereochemistry, because it was found that the agonistic activity decreased when substitutions with D amino acids were introduced (Niida et al., 2006). A decapeptide analog endowed with KiSS1R agonistic activity has been reported more recently (Asami et al., 2012). The peculiar behavior of this decapeptide characterized by a $N^{\mho}$ methylarginine substitution is an increased stability as compared to KP54 thanks to the resistance to trypsin cleavage (Asami et al., 2012).

A pharmacophore site for metastin proposed a structure-activity relationship study which identified Phe9, Arg12 and Phe13 as crucial for the peptide activity as determined in receptor binging and functional assays (Orsini et al., 2007).

8. Conclusions

From the discovery of KiSS1 in melanoma, an increased body of knowledge has been accumulating over the years with the investigation of the role of KiSS1 in different tumor types and the identification of some relevant aspects of its biological action. Whereas it seems clear that KiSS1 plays a unique role in suppressing metastases in most tumor types by virtue of its ability to suppress colonization and maintain dormancy of disseminated tumor cells, it is evident that an effective suppression of metastatic spread would imply interference with multiple steps of the metastatic cascade.

Although a contradictory action is emerging from an overall consideration of the literature (Marot et al., 2007; Guzman et al., 2018), most studies define KiSS1 as a metastasis suppressor (Ji et al., 2013). From a therapeutic point of view, the epigenetic regulation of KISS1 expression allows to look at exploiting its re-expression by DNA methyl transferase inhibitors or histone deacetylase inhibitors to control metastatic spread (Cebrian et al., 2011; Zuco et al., 2015). Besides, kisspeptin analogs may be developed as therapeutic agents, given that the natural peptides are characterized by multiple drawbacks including poor metabolic stability. An additional layer of complexity for the potential use of kisspeptin analogs as antitumor agents is the well-established role of KiSS1 in the hypothalamic-pituitary-gonadal axis, being the target of relevant regulators, e.g., steroid hormone feedback, nutritional and metabolic regulation (Clarke et al., 2015). The design of kisspeptin analogs targeting tumor cells should therefore consider to prevent delivery to the brain where signals to control reproduction are delivered through kisspeptin neurons with gonadotropin release.

Although the role of KiSS1 in the regulation of response to antitumor agents has been less investigated as compared to its role in suppressing metastases, the generation of KiSS1 mimetics with appropriate pharmacological features may be useful in view of combined treatment with cisplatin or other cytotoxic agents whose action appear to be favored in KiSS1 expressing cells (Jiffar et al., 2011). In this regard, a better understanding of the contribution of KiSS1 to processes leading to sensitization to the effects of antitumor agents is needed, particularly with reference to autophagy whose role in cell response to treatment appears to be dependent on the molecular context (Dikic and Elazar, 2018).

Besides, understanding the role of KiSS1 in regulation of metabolism of cancer cells is still at its infancy (Liu et al., 2014). The contribution of several metabolic genes and specific metabolic products in metastatic dissemination is only partially known. Additional efforts in the investigation of the interplay between metabolism and signaling and of the mechanisms leading to cell adaptation to new environments, together with new knowledge on processes leading to metastasis suppression are expected to provide useful insights to manage metastatic diseases.

KiSS1 also represents a potential biomarker of metastatic spread as suggested by the possibility to monitor – besides the expression in cancer specimens - its levels in blood samples (i.e., plasma and serum). In spite of the fact that several interesting studies have been reported (Jayasena et al., 2012), there is an urgent need to carry out analyses in larger cohorts of patients. In addition, given the complexity of the metastatic process and of tumor response to treatment, it is unlikely that KiSS1 *per se* can be exploited to monitor tumor progression and response but KiSS1 together with other factors might be easily measurable not only in tissues, but also in liquid biopsies likely becoming a helpful biomarker. The advantage of KiSS1 as a possible biomarker is represented by the fact that it is a secreted peptide, whereas – as mentioned above - a disadvantage may be represented by its relevant physiological role in the neuroendocrine system, a feature that should be taken into account to avoid misleading interpretation of results regarding its levels.

A promising field of investigation is represented by the identification of compounds capable of regulating KISS1 gene expression (Li et al., 2018). This approach, besides providing tools for a better understanding of physiological processes (e.g., puberty and fertility) may generate compounds suitable for further development in an attempt to control metastatic spread or facilitate KiSS1-mediated sensitization to chemotherapeutic agents.

In addition to oncogenes and tumor suppressor genes implicated in transformation and tumorigenesis, factors that act in the regulation of metastasis by interfering with specific steps of the metastatic cascade may provide therapeutic opportunities, but a better understanding of specific biological aspects of metastasis regulation is necessary, including those regulated by KiSS1. Particularly, a better understanding of the interaction of disseminated tumor cells with the tumor microenvironment may be useful to counteract metastatic spread (Ghajar, 2015).

9. Future perspectives

Metastatic spread represents an obstacle to the efficient control of most neoplastic diseases. Multiple innovative therapeutic strategies including targeting the tumor microenvironment are currently under investigation. In this context, an increased attention must be paid to mechanisms acting to suppress metastasis. KiSS1 appears to be a key player in metastases regulation, also exploitable as a biomarker to monitor disease progression, and – upon further investigation of its action in modulation of tumor cell response to chemotherapeutic agents – to monitor response to treatment. Because KiSS1 is secreted by tumor cells and generates peptides measurable in biologic fluids, it appears to be a putative biomarker suitable for future applications in routine analyses. In this perspective, it will be still necessary to address key issues, by designing clinical studies with adequate statistical power allowing to draw conclusions regarding the utility of KiSS1 measurement in groups of patients with different stages of tumors and/or different response to treatment. In addition, preclinical pharmacology efforts are expected to generate new KiSS1 mimetics which can be applied as single agents and as modulators of conventional cytotoxic agents.

Conflict of interest

The authors declare no potential conflict of interest

Acknowledgments

This work was in part supported by a grant by Fondazione CARIPLO-Regione Lombardia to PP (grant 2016-1019).

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