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Review

Review

How Protein Methylation Regulates Steroid Receptor Function

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Abbreviations: AR, androgen receptor; BC, breast cancer; ER, estrogen receptor; GAR, glycine and arginine-rich motif; GC, glucocorticoid; GR, glucocorticoid receptor; IGF, insulin-like growth factor; JHDM, JmjC domain-containing histone demethylase; KMT, lysine methyltransferase; LBD, ligand binding domain; MS, mass spectrometry; NLS, nuclear localization signal; NR, nuclear receptor; NTD, aminoterminal domain; PC, prostate cancer; PR, progesterone receptor; PRMT, protein arginine methyltransferase; PTM, post-translational modification; SR, steroid receptor; TF, transcription factor.

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Abstract

Steroid receptors (SRs) are members of the nuclear hormonal receptor family, many of which are transcription factors regulated by ligand binding. SRs regulate various human physiological functions essential for maintenance of vital biological pathways, including development, reproduction, and metabolic homeostasis. In addition, aberrant expression of SRs or dysregulation of their signaling has been observed in a wide variety of pathologies. SR activity is tightly and finely controlled by post-translational modifications (PTMs) targeting the receptors and/or their coregulators. Whereas major attention has been focused on phosphorylation, growing evidence shows that methylation is also an important regulator of SRs. Interestingly, the protein methyltransferases depositing methyl marks are involved in many functions, from development to adult life. They have also been associated with pathologies such as inflammation, as well as cardiovascular and neuronal disorders, and cancer. This article provides an overview of SR methylation/demethylation events, along with their functional effects and biological consequences. An in-depth understanding of the landscape of these methylation events could provide new information on SR regulation in physiology, as well as promising perspectives for the development of new therapeutic strategies, illustrated by the specific inhibitors of protein methyltransferases that are currently available.

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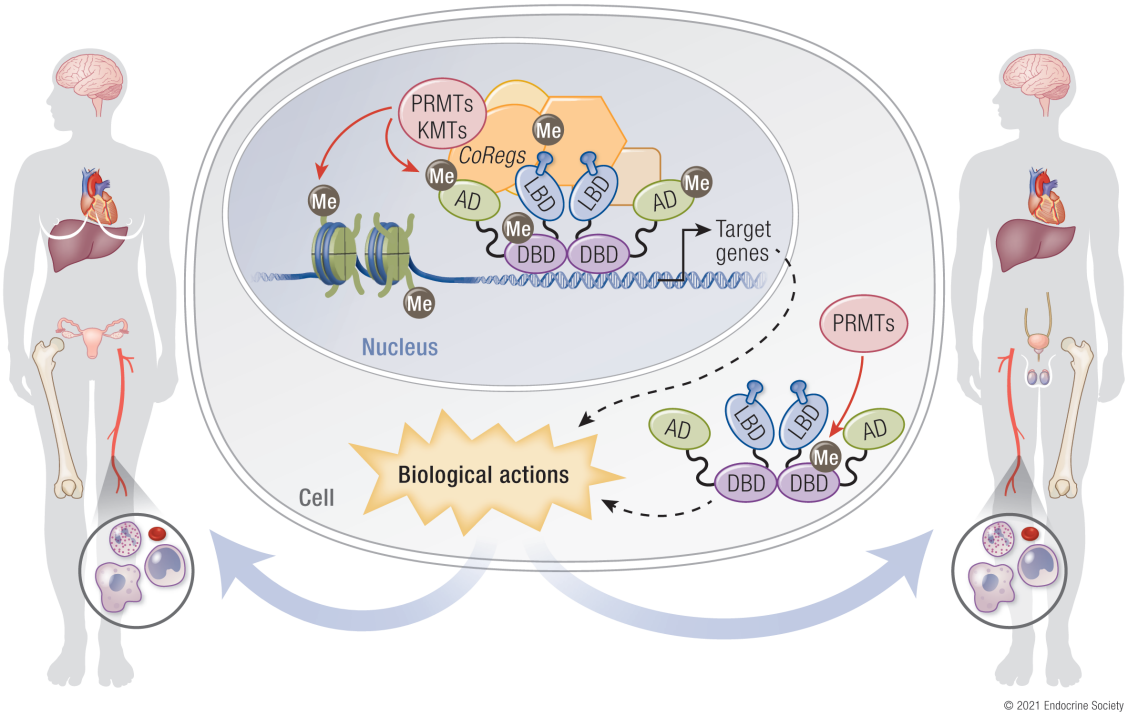
Key Words: ERα, PR, AR, GR, protein arginine methyltransferases, lysine methyltransferases, protein demethylases, coregulators, steroid receptors, methylation

Graphical Abstract



ESSENTIAL POINTS

- Steroid hormones and their receptors play many critical roles in human physiology and pathology through specific gene regulation and modulation of cytoplasmic signaling pathways.
- In addition to other types of post-translational modifications, methylation of lysine and arginine is recently shown to regulate steroid receptor activity.
- Methylation of histones and nonhistone proteins also participate indirectly in the activities of steroid receptors.
- Protein methyltransferases and demethylases play important roles in physiology and their dysregulation contributes to human pathologies.
- Increased understanding of the biology of steroid receptor methylation, along with specific methylation enzyme inhibitors, will result in new potential therapeutic options.

Introduction

Steroid hormones play critical roles in various target tissues regulating body homeostasis. Their ability to easily transit through cell membranes aroused interest of scientists for their therapeutic potential. Glucocorticoid (GC) was the first type of steroid to be used in the clinic. The physician Philip Hench successfully treated rheumatoid arthritis symptoms with cortisone, a discovery for which the Nobel Prize in Medicine was awarded in 1950 (1). Thereafter, a plethora of experiments documented the influence of steroid hormones on many biological processes throughout the lifespan. The female hormone estrogen is well known for its effects on mammary gland and reproductive tract development. Progesterone, the other female hormone, plays a vital role during pregnancy. In males, the androgen hormone is important for sexual development and reproductive function. The mediators of these hormones remained elusive until the early 1960s, when radiolabeled lipophilic ligands were developed by Jensen and Jacobson (2). Their innovative experiments led to the identification of "radioligand-binding proteins," now known as steroid receptors (SRs). Interestingly, these receptors were able to migrate from the

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cytoplasm to the nucleus, implying that ligand-bound receptors could influence transcription. This hypothesis was confirmed by Ashburner, who showed that the addition of the ecdysteroid hormone was responsible for the activation of a specific subset of genes in drosophila salivary glands (3). This "Ashburner hormonal model" was the foundation for our current knowledge on the regulation of transcription by these receptors and is still, nowadays, unexpectedly relevant.

Since then, numerous clinical therapies have been developed based on this hormone–receptor association. Among them, mifepristone targets the progesterone receptor (PR) to treat endometriosis (4); dexamethasone acts on the glucocorticoid receptor (GR) to induce anti-inflammatory and immunosuppressive effects (5), and is for instance currently used in the treatment of SARS-Cov2 viral infection (6, 7); tamoxifen blocks the estrogen receptor (ERα), thus inhibiting estrogen binding and consequently reducing the progression of hormone-dependent breast cancers (BCs) (8); antiandrogens targeting the androgen receptor (AR) are frequently associated with chemical or rarely used surgical castration for improving the overall survival of prostate cancer (PC) patients (9).

The nuclear receptor (NR) superfamily consists of evolutionarily related DNA-binding transcription factors (TFs), encoded by 48 genes in humans. Within this superfamily, a subgroup of 5 ligand-regulated receptors, the SRs, has been extensively exploited for the development of selective modulators and raised promising expectations for novel treatment strategies to address diverse medical conditions (10, 11). We will herein focus on SRs for which methylation events have been well documented, namely ERα, PR, AR, and GR.

Methylation has emerged as a major post-translational modification (PTM) of proteins, with wide-ranging consequences on their activity, in modulating their properties, such as subcellular localization, stability, or interactions with partners. Most protein methyltransferases preferentially methylate arginine and lysine residues. Based on the residue targeted, these enzymes are classified as lysine methyltransferases (KMTs) or protein arginine methyltransferases (PRMTs). Protein methylation was initially identified on histone proteins, a process deeply involved in local chromatin remodeling and then in gene regulation. However, a growing number of studies have reported methylation events on nonhistone proteins, revealing that methylation is involved in a large variety of biological effects. Currently, hundreds of methylated substrates have been identified, in various cellular processes including RNA metabolism, DNA repair, or gene transcription. Indeed, SR-dependent transcription is strongly influenced by histone methylation (12, 13), as well as by the methylation of SRs themselves and their associated coregulators. Consistent with other PTMs (ie, phosphorylation, acetylation, etc.), protein methylation is a dynamic and reversible process controlled by the activity of protein demethylases, which are able to remove methyl marks.

It is particularly relevant to note that KMTs and PRMTs are frequently overexpressed in cancer cells compared with normal cells, and that their upregulation is often associated with poor prognosis. In line with this, specific inhibitors targeting the methyltransferase activities have recently been developed to assess the involvement of this PTM in tumorigenesis, making these enzymes interesting targets to modulate SR properties (14, 15).

The aim of this review is to summarize current and emerging knowledge about the influence of protein methylation in the biology of SRs. As these receptors and their signaling are involved in numerous pathologies, targeting protein methyltransferase activities could provide promising new therapeutic tools.

Steroid Receptors

Structure

Phylogenetic studies in eukaryotic organisms have placed the emergence of SRs a long time before the divergence between vertebrates and invertebrates (16). This universality has been a powerful tool for scientists, allowing them to study and to decrypt the regulation of crucial hormone receptor–dependent physiological processes, such as development, cell growth, reproduction, and homeostasis, in simple organisms (compared with humans). Since the 1980s, many NR cDNAs have been cloned and characterized, including GR (16), ERα (17), PR (18), and AR (19).

The cloning of the first SR genes (ie, GR and ERα) was a major breakthrough in the field, highlighting a surprising homology between them. This observation provided the first chemical evidence that distinct hormones bind structurally related receptors. Indeed, SRs share a common organization with 5 functional domains (A/B, C, D, E, F), with varying degrees of structural homology (20, 21) (Fig. 1A). The central DNA binding domain (DBD or C domain) is the most conserved region and is crucial for the association between the receptor and specific hormone-response elements (HREs) (22, 23). The ligand binding domain (LBD) is the second domain with considerable structural homology among NRs (24) (Fig. 1A). It is essential for ligand binding, but its role is much broader. The LBD structure allows the LBD itself to undergo conformational changes leading to the recruitment of a SR partner for dimerization (25), as well as coregulators (26) through a ligand-dependent transactivation domain (also called AF-2 for activation function-2) (27). Between these 2 highly conserved regions, a less-conserved interdomain linker is present in all members of the superfamily (28) (Fig. 1A), called the hinge region

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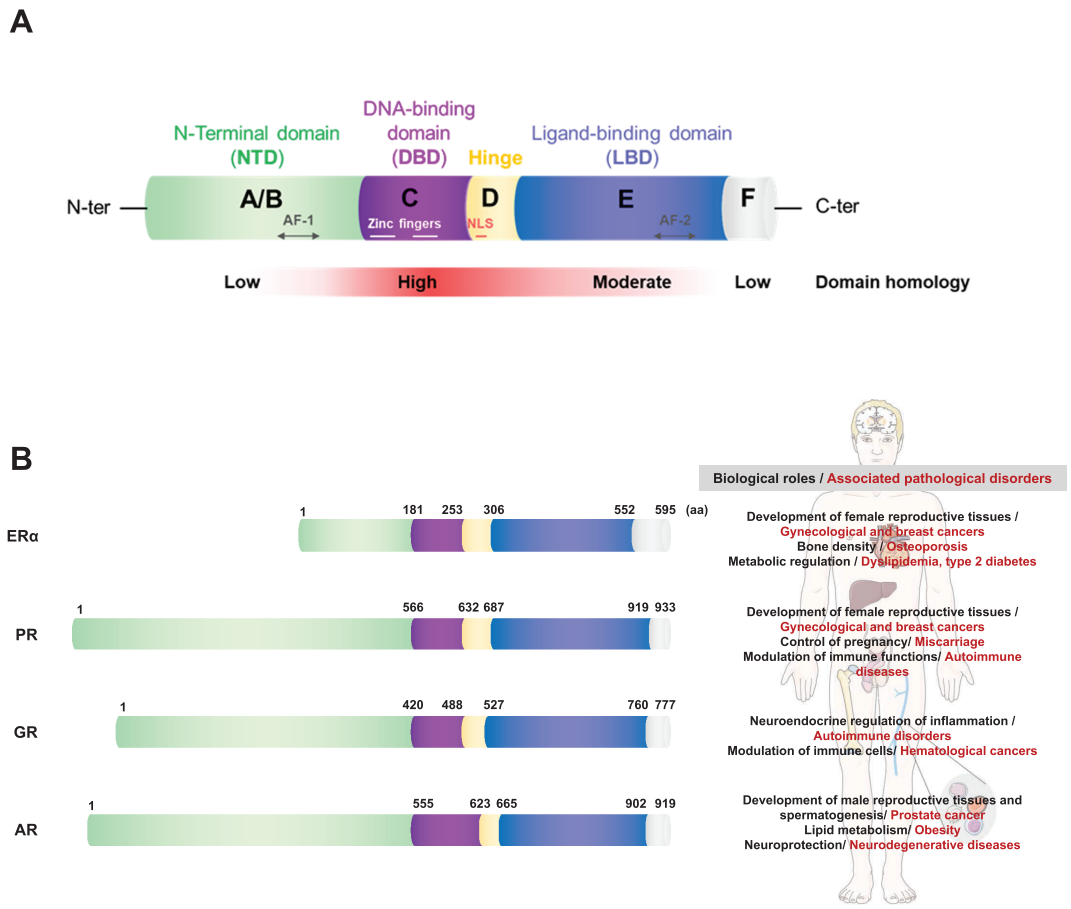

Figure 1. Common structural organization of steroid receptors. (A) Schematic representation of steroid receptors (SRs) structure. The amino-terminal domain (NTD) is variable in size and composition and contains a ligand-independent transactivation domain (AF-1). The DNA binding domain (DBD) is the most conserved region, in which 2 zinc fingers maintain the core of the domain and bind to DNA. A less-conserved hinge region is present between the DBD and the ligand binding domain (LBD) and contains a nuclear localization signal (NLS). The ligand associates with the receptor through the LBD, which also contains a ligand-dependent transactivation domain (AF-2). The functions associated with the F domain are still not clearly understood. (B) The members of the steroid receptor (ie, ERα, PR, GR, and AR) subgroup share a deeply conserved structure of functional domains with some specificities. The main biological roles of these SRs, and the associated pathological disorders when SR signaling are dysregulated, are pointed out on the right.

(or D domain). This domain supports the DBD in the transcriptional activity of SRs (29, 30) by ensuring their nuclear localization with a nuclear localization signal, and by cooperating with the LBD to mediate SR dimerization (31) and its interactions with certain coregulators (32). At the N-terminal end, each member exhibits an aminoterminal domain (NTD or A/B domain), highly variable in size, amino acid composition, and PTMs (33) (Fig. 1B). This A/B domain notably includes a ligand-independent transactivation domain (or AF-1), which can act independently, although optimal receptor activity generally requires a synergistic cooperation between AF-1 and the ligand-dependent transactivation domain AF-2 (34). At the other extremity, the F region is highly variable and not present in all members of the superfamily. Although little is known about it, mutations or complete deletions can strongly alter the transcriptional activity of the receptor, ligand binding, as well as interactions with coregulators (35).

Biological and Pathological Roles

SRs are ligand-inducible TFs playing crucial roles in the regulation of diverse and essential aspects of mammalian physiology, by controlling the expression of genetic programs to achieve synchronized and accurate functional responses (36–38). Responding to endocrine hormones, they trigger adaptive signals and serve as a potent regulatory interface between the cellular and organismal environment and the genome. However, they also drive pathological states when their signaling pathways become dysregulated. This feature has led to the development of numerous treatments to control endocrine-associated diseases, including neurological disorders, chronic inflammatory diseases, or cancers (39).

ERα acts in concert with PR to regulate the physiological development of female reproductive tissues and mammary glands. Variable concentrations of ovarian progesterone and estrogen hormones regulate decisive phases

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of human female life (puberty, pregnancy, and menopause). ERα knockout in pubescent mice totally impairs mammary gland development (40), whereas in mature women, ERα is a crucial modulator of breast cell proliferation and survival, strongly influencing their risk of developing BC. In bone tissue, ERα modulators such as tamoxifen maintain bone mineral density in postmenopausal women and prevent osteoporosis. A set of studies has also reported that ERα signaling is deeply involved in metabolic homeostasis and metabolic disorders, such as diabetes, dyslipidemia, or obesity, and influences numerous biological systems, including the immune and cardiovascular systems, due to the presence of ERα in all these sites (41).

Progesterone is the "hormone of pregnancy" in postpubescent women, as it is essential for the establishment and maintenance of pregnancy (42). Hormone-associated PRs target genes in multiple organs or systems to prepare the body for pregnancy, including the endometrial epithelium, the venous walls or in mammary glands (43). Another notable role for PR is the modulation of immune functions, with deep and distinct consequences in autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (44, 45). Recent studies have also highlighted the pleiotropic protective effects in brain cells exerted by progestin-liganded PR, making PR a key mediator of neuroprotection after stroke, in both sexes (46, 47).

The effects of GCs, including the natural human GC cortisol, are mediated by intracellular GR, which is expressed throughout the body, but with a substantial heterogeneity in GC sensitivity and biological responses across tissues. Once released into the bloodstream, GCs affect all of the major body systems, including cardiovascular, musculoskeletal, immune, nervous, and reproductive systems (48). GCs exert anti-inflammatory and immunosuppressive effects, and have thus been widely used in the clinic for treating autoimmune diseases, inflammatory diseases, and hematological cancers (49). Moreover, their ability to trigger apoptosis of immature B and T lymphocytes (50), has been exploited by clinicians to treat cancers, including leukemia and lymphoma.

The most remarkable role of AR is the maintenance of male reproductive tissues and spermatogenesis, as demonstrated by the complete ablation of the male reproductive tract (seminal vesicles, vas deferens, epididymis, and prostate; small inguinal testes with arrested spermatogenesis) in male AR-knock out (KO) mice (51). Not surprisingly, androgen-associated AR is strongly involved in several aspects of PC, the second most common solid cancer in men (52). Beyond the reproductive activity, AR signaling controls several homeostatic processes, such as bone growth, and glucose and lipid metabolism. It seems that the receptor acts as a negative regulator of adipocyte development in adult males, resulting in late-onset obesity when knocked out (53). Similarly to other SRs, the field of action of AR is vast, and a decisive role for androgen-activated receptors in neurodegenerative processes, such as spinal bulbar muscular atrophy, has been depicted (54, 55). Interestingly, in vivo studies highlighted underestimated and essential roles for AR and androgen signaling in female reproduction, including ovarian and breast physiology, such that dysregulation leads to dysfunctions and cancers (51, 56–58). In contrast, a recent work showed that AR acts as a tumor suppressor in ERα-positive BC (59).

Signaling Pathways

The binding of steroid hormones to their receptors triggers a conformational change within the LBD and functions as a critical step, shifting the receptor function from an inactive to an active state (60, 61). Steroid-bound receptors generally dimerize as homodimers and translocate to the nucleus (Fig. 2). They target specific HREs, generally palindromic, which fully or partially resemble 2 consensus half-sites (5′-AGGACA-3′ for GR, or 5′-AGGTCA-3′ for ERα), arranged as inverted repeats and typically separated by 3 nucleotides (20). The ligand-dependent conformational change also primes the receptor for the recruitment of coregulators and chromatin-modifying complexes, 2 major components of NR signaling (27, 62, 63). Indeed, ligand binding ultimately turns the receptor into a potent transcriptional regulator, which then assembles huge multiprotein complexes, also called coregulator complexes, to activate or repress the expression of target genes (Fig. 2). Alternatively, ligand-stimulated SRs can regulate gene expression by an indirect binding on chromatin, whereby they associate with other DNA-bound TFs, such as AP-1 or SP-1 (64–66) (Fig. 2).

In addition to these agonist-induced transcriptional effects, most SRs are able to act on chromatin in a ligand-independent fashion. This "unliganded form" (ie, receptor without ligand), first demonstrated for the nonsteroid thyroid hormone receptors and later for PR, GR, and ERα, maintains gene silencing prior to hormonal activation (67–69) (Fig. 2). In spite of solid evidence demonstrating that ligand-independent SRs resort to a passive repression, either by competition for binding sites with coactivators or through the formation of inactive heterodimers with liganded homodimers (70, 71), recent reports suggest that inhibition of gene transcription is brought about by a more active mechanism. Advances in high-resolution and comparative techniques, such as cistrome analysis, clearly

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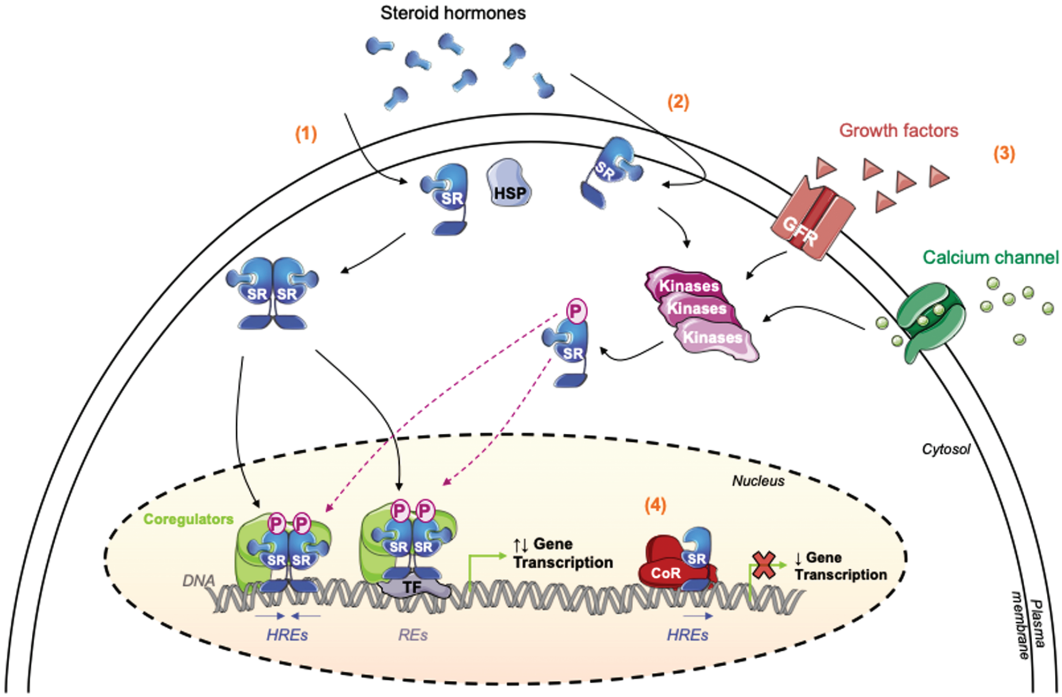

Figure 2. Steroid receptor signaling pathways. The steroid hormone enters into the cell by passive diffusion through the plasma membrane and binds with high affinity to its specific receptor. (1) Classical steroid hormone nuclear signaling. The ligand–receptor complex undergoes conformational changes triggering its dissociation from the chaperone heat-shock protein (HSP), receptor dimerization, and its translocation into the nuclear compartment. Inside the nucleus, the ligand-associated SR binds to specific DNA sequences that serve as enhancer or silencer elements, recruits coregulators and enzyme-modifying chromatin complexes to locally perturb the chromatin organization and regulate assembly or disassembly of an active transcription complex. SR-dependent multiprotein complexes target selective hormone-response elements (HREs) on target promoters, or indirectly interact with chromatin through transcription factors (TFs) on their response elements (REs). This could affect the level of growth factor receptors (GFRs), calcium signaling actors, or cellular proliferation effectors, among many other cellular pathways regulated by SR target genes. (2) Nongenomic signaling. The steroid ligand binds to SRs located at the plasma membrane or in the cytoplasm and triggers rapid post-translational modifications, often dependent on activating kinase cascades (MAPK, PI3K, Src), that in turn result in the transcriptional activation of the receptor. Conversely, SR genomic effects can regulate rapid nongenomic events, highlighting a potent crosstalk dependent on ligand-bound SRs. (3) Nonclassical steroid hormone nuclear signaling. Apart from the binding of the specific steroid hormone, SRs can also be indirectly activated by growth factors, leading the recruitment and the activity of cytoplasmic phosphorylation cascades, the same involved in the classical signaling, namely MAPK and PI3K/Akt kinases. (4) Unliganded-receptor nuclear signaling. More recent data revealed that unliganded forms of SRs play critical roles on chromatin and deeply take part in gene repression of a subset of target genes after recruitment of corepressors (CoR).

showed that unliganded receptors can bind their own targets, resulting in a proper and optimal expression of singular genetic programs (growth, apoptosis, development, etc.) (72). They act as active repressors and target the basal transcription machinery. How these unliganded forms are addressed to the nucleus rather than to the membrane/cytosol is still unknown, but some mechanistic studies have revealed that the presence of specific marks on chromatin (ie, histone methylation marks) most of the time associated with collaborating epigenetic silencers definitely could play a role in the targeting and the binding of native receptors to chromatin (72). Interaction with the ligand reverses the epigenetic landscape, consistent with the hypothesis that liganded SRs undergo a conformational change that enables the loading of coactivators needed for chromatin remodeling and gene activation. Further investigations are required to fully understand this regulation, certainly dependent on kinase-inducing regulatory phosphorylation and growth factor signaling, similarly to ligand-induced SRs (73, 74). Nevertheless, it is not surprising that SRs can exert strong and decisive effects independently of ligands, as they evolved from an ancestral SR unable to bind ligands (75).

In addition to these nuclear effects, SRs exhibit nongenomic regulatory properties (76) (Fig. 2). Generally, too rapid to be dependent on gene transcription and protein synthesis, and insensitive to mRNA or protein synthesis inhibitors, they involve SRs at the cell surface and/or in the cytoplasm. In human mammary cancer cell lines for instance, the addition of hormones (ie, estradiol and progesterone) leads to the formation of cytoplasmic multiprotein complexes in which SRs coexist with protein kinases and adaptor proteins. The steroid ligand, as well as some decisive growth factors acting through their receptors, like epidermal growth factor (77), activate a membrane-associated receptor and downstream-associated pathways, such as the tyrosine kinase/p21ras/mitogen-activated protein kinase, Src, and PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) (78–80).

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Importantly, the final and overall biological effects of the hormone in the target tissue correspond to the convergence of both cytoplasmic and nuclear effects. Indeed, cytoplasmic kinase-dependent phosphorylation of SRs converts them into a transcriptionally active form, suggesting a convergence between classic genomic and rapid, nongenomic signaling pathways (81) (Fig. 2). While many studies have revealed a strong impact of the nongenomic effects on SR nuclear signaling, recent data illustrated how, inversely, hormone-mediated gene activation could affect nongenomic responses. The regulation of calcium homeostasis, playing a crucial role in tumorigenic progression, is a highly relevant illustration. SRs broadly regulate the transcription of many genes encoding components of the calcium pathway (calcium channels, calcium receptors, etc.), which once translated into proteins and activated, propagate nongenomic signals into the cell, contributing to cancer progression and survival (82, 83).

Mechanisms of Regulation

Many different signals can influence the adaptive responses mediated by SRs in cells, and, among them, PTMs trigger subtle but potent adjustments in SR signaling. Indeed, PTMs introduce structural constraints into functional domains that, in turn, alter their properties.

For a long time, phosphorylation was the main modification identified to regulate SR functions. More recently, several other PTMs, such as acetylation, methylation, palmitoylation, ubiquitylation, and SUMOylation, that mostly target alkaline residues (ie, lysine and arginine) have been described (84, 85). They are involved in every step of the receptor signaling pathways and contribute to the overall process of transcription. For instance, receptor stability, hormone binding and sensitivity, subcellular localization, and interactions with partners or DNA are affected by PTMs (86). Most of the time, these modifications are both dynamic and reversible, dependent on enzymes that deposit the marks and enzymes that remove them. This tightly controlled dynamic is crucial for an optimal regulation of SR activity. Concerning transcriptional regulation, receptor-modifying enzymes, like methyltransferases or deacetylases, act as influential SR coregulators, allowing a tight regulation of gene expression by directly modifying receptors (87). Moreover, their interactions with SRs can be direct or indirect, being recruited to enhancers or promoters by upstream coregulators (88, 89). They also remodel chromatin structure by modifying histones, and they promote (coactivator) or inhibit (corepressor) the recruitment and activation of RNA polymerase II, depending on the specific genes and cellular environment (89-92). Depending on which genes need to be activated (or repressed), specific coregulators are preferentially required for hormonal regulation of selected physiological responses that are dependent on a given TF (89). Since the first SR (ie, GR) was described, over 300 coregulators have been discovered (93). Different combinations of these coregulators are required for hormonal regulation of different target genes of the same SR in a given cell type, enabling the independent regulation of different subsets of the SR target genes to control different physiological response pathways (89).

Protein Methyltransferases/Demethylases

Protein methylation is a common covalent PTM that consists in the addition of methyl groups from a donor, S-adenoslyl-L-methionine (AdoMet, or SAM) to specific substrates (94). These substrates are preferentially methylated on either a lysine residue, and thus are modified by enzymes, KMTs, or an arginine residue, by PRMTs.

This PTM is a complex phenomenon, as lysine residues can be mono-, di-, or tri-methylated and arginine residues can be mono- or dimethylated, symmetrically or asymmetrically (95) (Fig. 3). The addition of methyl groups on both residues hardly affects the positive charge of the amino acids but deeply influences the bulkiness and hydrophobicity of the targeted protein. As such, protein methylation mostly functions as a potent signal for the recruitment of effector partners, called readers (because they distinguish the modified from the unmodified form of the target protein and translate that into a function) (96). As for other PTMs, methylation is a reversible process, involving enzymes able to remove methyl marks on lysines (lysine demethylases or KDMs) or on arginines (arginine demethylases) (97, 98). Both protein methyltransferases and protein demethylases are strong actors in transcription, as they influence the histone methylation landscape and consequently the opening or compaction of chromatin (99). However, growing evidence highlights that methylation of nonhistone proteins plays important roles in cells and sharply affects crucial homeostatic cellular processes. Inevitably, dysregulation of these processes can lead to diverse pathologies, including cancer.

KMTs, or Lysine (K) Methyltransferases

The first KMT was uncovered on a bacterial flagellar protein in 1959 (100). Numerous other enzymes were discovered since then, making this family of proteins 1 of the largest class of epigenetic enzymes (95). KMTs catalyze the transfer of 1 or more methyl group(s) from the AdoMet donor to the ε-nitrogen of a lysine residue, generating mono-, di-, or trimethyllysine (Kme1, Kme2, and Kme3, respectively) (Fig. 3A) (101). KMTs methylate a wide variety of substrates: it has been predicted that human cells contain more than

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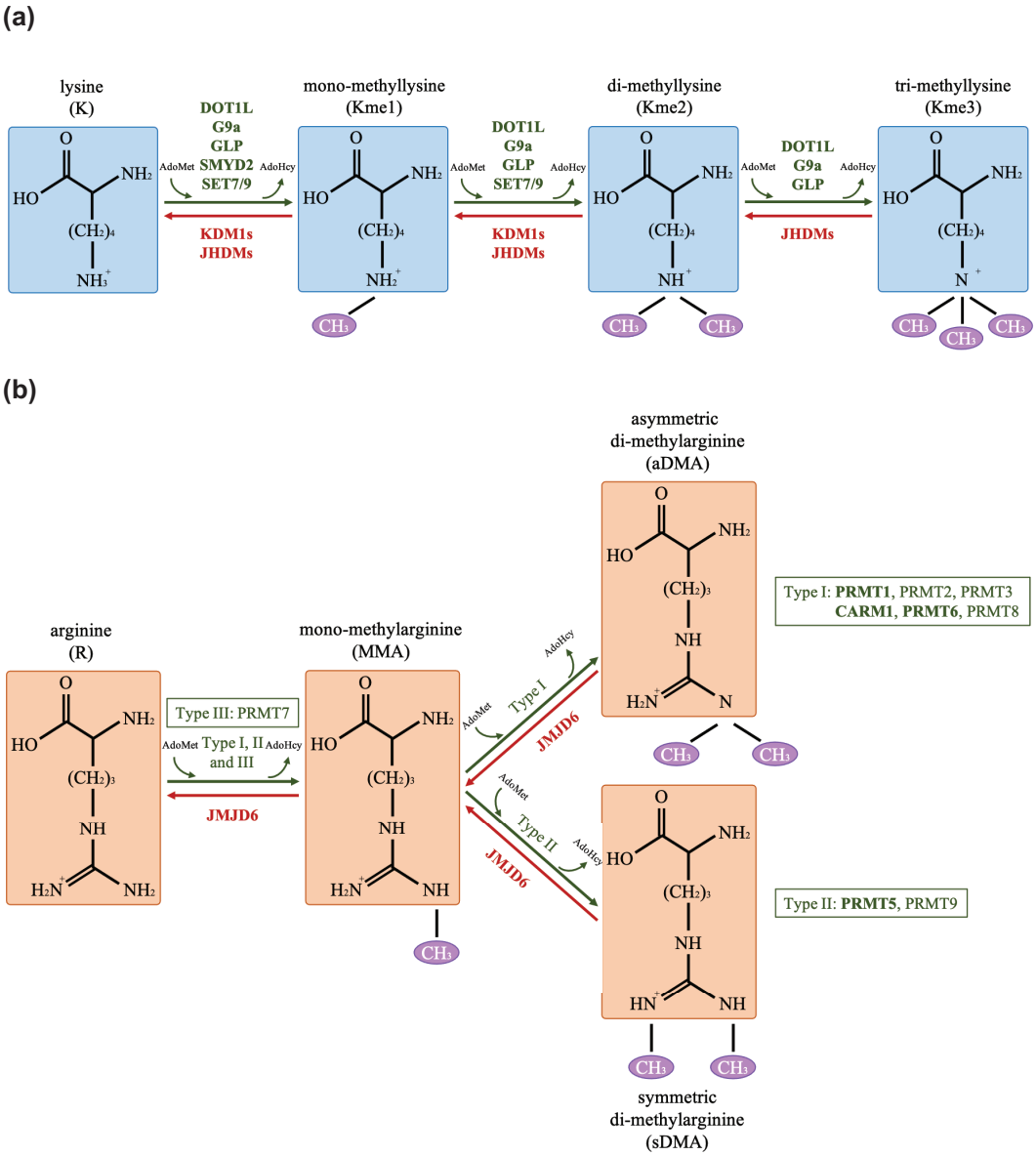

Figure 3. Process of protein methylation. Lysine residues are methylated by lysine methyltransferases (KMTs, green arrow) to generate, mono- (Kme1), di- (Kme2), or tri-methyllysines (Kme3). (A) KMTs use the methyl donor S-Adenosylmethionine (AdoMet) to add methyl (-CH_3) groups on targets and produce S-adenosylhomocysteine (AdoHcy) in addition to methyllysines. This process is highly dynamic and can be reversed by lysine demethylases (KDMs, red arrow). (B) Arginine methylation is catalyzed by a family of 9 PRMTs, divided into 3 subgroups (type I, II, or III, green arrows). All use the methyl donor AdoMet to add methyl (-CH_3) groups on targets and produce AdoHcy in addition to methylarginines. PRMTs that promote monomethylation (MMA), symmetric dimethylation (sDMA), or asymmetric dimethylation (aDMA) lead to the production of monomethylarginine, asymmetric dimethylarginine, or symmetric dimethylarginine respectively. JMJD6 is currently the only enzyme identified with an arginine demethylase activity (red arrow). PRMTs on which we focus in this article are highlighted in bold.

1000 proteins with 1 form of methyllysine (102). To date, 2 KMT families have been described: the SET KMT family, containing the majority of KMTs (103), and the seven β-strand methyltransferases (7βS) or class I family (104).

The SET KMT family is characterized by the presence of the evolutionarily conserved Su(var)3-9n Enhancer-of-zeste and trithorax (SET) domain. This is the catalytic core of the enzymes, flanked by the pre- and post-SET domains, each displaying a defined substrate and product specificity (105). In their structure, SET KMTs also express the peptide binding and the AdoMet binding pockets (95). The SET KMT family is classified into subfamilies, based on the sequence of the pre- and post-SET domains.

The 7βS family differs from the SET KMTs family by the catalytic domain. It is composed of 4 motifs, designated I, post-I, -II, and -III. Motifs I and post-I play an important role in the interaction with the methyl donor AdoMet.

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However, many KMTs in this family do not possess all of the motifs but may harbor different ones. Although this family of KMTs is smaller than the SET family, a proteomic study predicted that the human genome encodes more than 100 7βS KMTs (106).

KMT substrates are involved in various cellular pathways including transcription, cell proliferation, DNA damage repair, inflammation and immune response (Table 1) (107). They were first described as histone KMTs, because numerous KMTs methylate lysine residues in histone N-terminal tails (169). However, recent technical advances in mass spectrometry (MS)–based proteomics have highlighted that numerous nonhistone proteins are modified by lysine methylation, revealing that KMT substrates extend far beyond histones (170).

In this review, we will focus on the enzymes involved in SR signaling, namely G9a, GLP (G9a-like protein), SMYD2, SET7/9 (SETD7), and DOT1L (DOT1 like histone KMT).

G9a/GLP

G9a and GLP are SET KMTs belonging to the Suv39h family (171). They cooperatively play a predominant role during early embryonic development of mice, as G9a or GLP knockout induced growth retardation and early lethality (171, 172). G9a and GLP were first described as histone methyltransferases, performing Kme1 and Kme2 at lysine 9 of histone H3 (H3K9) (171). Once methylated, H3K9me1/2 becomes a docking site for effectors, especially the heterochromatin proteins HP1α, HP1β, and HP1γ that strongly influence gene silencing (173). Since then, nonhistone substrates have been identified including trimethylation of substrates such as G9a itself (124) or ATF7IP (110). Most of their substrates are involved in DNA damage response, cell cycle regulation, cell proliferation, and chromatin modulation, but also in skeletal muscle differentiation (Table 1). Although the role of G9a in the initiation and progression of cancer is well known (174), it also appears to be implicated in other diseases, such as Alzheimer's disease (175). These enzymes preferentially methylate a nonexclusive motif such as ARKS/T (124).

SMYD2

SMYD2 is a protein methyltransferase that is capable of performing Kme1 and Kme2 (Table 1) (125). Initially described to methylate histone H3 at lysine 36 (H3K36) (176), it seems that this KMT can also methylate diverse nonhistone proteins (Table 1) (177). For instance, SMYD2 was reported to monomethylate p53 at K370 and pRb at K860 (127, 128), to inhibit their activities. Large-scale analysis revealed a low level of specificity for SMYD2 towards its substrates, as only the LF-K motif has been identified (178).

SMYD2-deficient zebrafish show malformation of both the atrium and ventricle, and a reduced heart rate and cardiac function (179). Mechanistically, in the cytoplasm of cardiomyocytes, SMYD2 monomethylates the heat shock protein 90 (HSP90) at K616 and interacts with the sarcomeric I-band region at the titin N2A domain via its N-terminal and extreme C-terminal regions to influence cardiac contraction (180). Since aberrant SMYD2 expression and its dysfunction are often closely related to cardiovascular diseases and cancer, SMYD2 is a promising candidate for the treatment of these pathologies.

SET7/9

SET7/9 is a SET KMT that performs mono- and dimethylation (127). It was first identified to monomethylate H3K4, leading to the recruitment of RNA polymerase II and thus maintains the transcription of target genes and the structure of active or potentially active euchromatin (181). As other KMTs, SET7/9 also has nonhistone substrates involved in DNA damage response, cell cycle regulation, cell proliferation, chromatin modulation and cell differentiation (Table 1). SET7/9 preferentially methylates proteins containing the K/R-S/T/A sequence (105). Interestingly, SET7/9 knockout mice are viable and develop normally (182). SET7/9 seems to have different effects on carcinogenesis depending on the cancer type (183). SET7/9 also appears to play a role in diabetes and atherosclerosis by activating inflammatory genes (184).

DOT1L

DOT1L is a 7βS KMT that performs mono-, di-, and trimethylation. For a long time, its unique substrate was H3K79, the methylation of which contributes to activating the transcription of genes involved in DNA damage response and cell cycle regulation (185), but also in immune response (186, 187). In addition, DOT1L is involved in neointimal hyperplasia development, as it is upregulated in the rat injured artery wall (188). Contrary to the other KMTs presented before, DOT1L has no identified nonhistone substrate, aside from AR which we will described later in this review (189). Interestingly, the loss of DOT1L in mice induces developmental problems (ie, growth retardation, cardiac dilatation) and death in utero (190). Furthermore, DOT1L seems to be implicated in diseases such as obesity and cancer. Indeed, inhibition of DOT1L increases adiposity, making it a potential therapeutic target for obesity (191), and DOT1L mislocalization in leukemia promotes H3K79 methylation and activation of the leukemic transcriptional program (185).

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Table 1. Lysine methylated substrates

KMT	Substrate	Site	Effect of lysine methylation	References
GLP	HIF-1α	K674me1/2	Represses HIF-1α transcriptional activity	(108)
	p53	K373me2	Negatively regulates p53 activity	(109)
	ATF7IP	K16me3	Stimulates formation of ATF7IP / MPP8 complex	(110)
	DNMT3A	K47me2	Induces the formation of Dnmt3a–MPP8–GLP/G9a inactive complex	(111)
	LIG1	K126me2/3	Induces LIG1-mediated recruitment of UHRF1 to replication foci	(112)
G9a	CDYL1	K135	Negatively regulates its binding to chromatin	(113)
	C/EBPβ	K39	Represses C/EBPβ transactivation	(114)
	HIF-1α	K674me1/2	Represses HIF-1α transcriptional activity	(108)
	MEF2D	K267	Inhibits its chromatin recruitment and transcriptional activity	(115)
	MTA1	K532	Positively regulates its corepressor activity in NuRD complex	(116)
	MyoD	K104	Inhibits MyoD transcriptional activity	(117)
	Pontin	K265, K267, K268, K274, K281, K285	Enhances p300 recruitment and increases HIF1 transcriptional activity	(118)
	Reptin	K67me1	Negatively regulates transcription of hypoxia genes	(119)
	RUNX3	K129me1/2, K171me1/2	Suppresses its transcriptional activity	(120)
	p53	K373me2	Negatively regulates p53 activity	(109)
	PLK1	K209me1	Supports DNA damage repair	(121)
	ATF7IP	K16me3	Stimulates formation of ATF7IP/MPP8 complex	(110)
	DNMT3A	K47me2	Induces the formation of Dnmt3a–MPP8–GLP/G9a inactive complex	(111)
	FOXO1	K273me1/2	Decreases FOXO1 stability	(122)
	G9a	K165me2/3	Induces G9a interaction with HP1γ	(123)
		K239me3		(124)
	LIG1	K126me2/3	Induces recruitment of UHRF1 to replication foci	(112)
SMYD2	EZH2	K307me1/2	Represses transcription	(125)
	GFI1	K8	Promotes GFI1-mediated transcriptional repression though LSD1 recruitment	(126)
	p53	K370me1	Negatively regulates p53 activity	(127)
	pRb	K860me1	Regulates RB Binding to the Transcriptional Repressor L3MBTL1	(128)
		K810me1	Promotes E2F transcriptional activity	(129)
	PARP1	K528me1	Enhances PARP1 activity in response to DNA damage	(130)
	β-catenin	K133me1	Activates Wnt signaling	(131)
	MAPKAPK3	K355me1	Activates MAPKAPK3	(132)
	PTEN	K313me2	Activation of the phosphatidylinositol 3-kinase-AKT pathway	(133)
	HSP90AB1	K531, K574me1	Enhances its polymerization and the chaperone complex formation	(134)
SET7/9	FoxO3	K271me1	Decreases FoxO3 protein stability and increasing transcriptional activity	(135)
	FXR	K206	Supports the transactivation of FXR target genes	(136)
	HIV Tat	K51me1	Activates HIV transcription	(137)
		K71me1		(138)
	LIN28A	K135me1	Modifies transcription of c-myc target genes	(139)
	PGC-1α	K779	Allows transcription of PGC-1α target genes	(140)
	pRb	K873	Supports Rb-dependent transcriptional repression	(141)
	RelA	K314me1, K315me1	Negatively regulates NF-κB transcriptional activation	(142)
		K37me1	Stabilizes the DNA-RelA complexes and induces the transcription of a subset of NF-κB-regulated genes	(143)
	RORα2	K87	Enhances its target gene transcription	(144)
	YY1	K173me1, K411me1	Positively regulates YY1 DNA-binding activity	(145)
	YY2	K247me1	Positively regulates YY2 DNA-binding activity	(146)
	p53	K372me1	Stabilizes p53 chromatin-bound fraction	(147)
	PARP1	K508	Stimulates ARTD1 mediated ADP-ribosylation	(148)
	SIRT1	K333, K235, K236, K238	Enhances p53 acetylation in response to DNA damage	(149)
	SUV39H1	K105me1, K123me1	Negatively regulates it activity in response to DNA damage	(150)
	UHRF1	K385me1	Enhances the formation of UHRF1–PARP1 complex at DNA damage site	(151)
	ATG16L1	K151me1	Inhibits autophagy by impairing the formation of the ATG12–ATG5-ATG16L1 complex	(152)

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Table 1. Continued

KMT	Substrate	Site	Effect of lysine methylation	References
	β-catenin	K180me1	Decreases β-catenin stability	(153)
	DNMT1	K142me1	Facilitates DNMT1 ubiquitin-dependent degradation	(154)
	E2F1	K185me1	Promotes E2F1 ubiquitin-dependent degradation	(155)
	eL42	K53me1, K80me1, K100me1	Enhances translation	(156)
	HIF-1α	K32	Enhances HIF-1α stability	(157)
	IFITM3	K88me1	Reduces IFITM3 antiviral activity	(158)
	MYPT1	K442me1	Increases MYPT1 stability	(159)
	PLK1	K191me2	Promotes dynamic kinetochore-microtubule attachments	(160)
	RIOK1	K411me1	Promotes ubiquitin-dependent degradation of RIOK1	(161)
	Rpl29	K5me2	Facilitates Rpl29 nuclear localization	(162)
	Sam68	K208	Positively regulates Sam68 protein stability	(163)
	Smad7	K70me1	Induces Smad7 ubiquitination and proteasomal degradation	(164)
	Sox2	K119me1	Induces Sox2 ubiquitination and proteasomal degradation	(165)
	STAT3	K140me2	Promotes STAT3 binding to SOCS3 promoter	(166)
	TAF10	K189me1	Increases TAF10 interaction with RNA polymerase II	(167)
	Yap	K494me1	Promotes Yap cytoplasmic sequestration by the Hippo pathway	(168)

Abbreviations: K, lysine; Kme1, monomethyllysine; Kme2, dimethyllysine; Kme3, trimethyllysine.

KMTs targeting

In view of these data, it has become evident that members of the KMT family constitute attractive new therapeutic targets. BIX-01294, the first G9a inhibitor, was identified in 2009, and numerous inhibitors targeting G9a have since been produced (192). Targeting this enzyme gave rise to promising results in bladder cancer, especially with the selective inhibitor CM-272 that leads to cancer regression in vivo (193). Similar results were obtained in non–small cell lung cancer, where the selective inhibitor UNC0638 prevents tumor growth in vitro and in vivo (194).

The first SMYD2 inhibitor was identified in 2011, and the same year the SMYD2 crystal structure was reported (195). Since then, increasingly potent and selective SMYD2 inhibitors have been produced (196). Inhibition of SMYD2 was shown to increase the sensitivity of ovarian cancer cells to the PARP inhibitor olaparib (197) and to sensitize non–small cell lung cancer cells to the anticancerous agent cisplatin (198).

For SET7/9 inhibitors, cyproheptadine was demonstrated to selectively inhibit SET7/9 activity (199), and many derivatives of this inhibitor have since been studied (200).

Of the KMTs presented in this review, only the DOT1L inhibitor pinometostat is currently undergoing clinical trial according to the clinical trials website (www.clinicltrials.gov). To date, pinometostat is registered in 2 phase Ib/II clinical trials for different hematological malignancies (NCT03724084 and NCT03701295).

KDMs, or Lysine (K) DeMethylases

The discovery of KMTs quickly raised the question of the existence of lysine demethylating proteins. The first KDM was identified in the early 2000s, and numerous other KDMs have since been discovered (201). They are classified into 2 groups based on their structure and the type of lysine demethylation they perform (Fig. 3A).

The first group, called KDM1, includes KDM1A (LSD1) and KDM1B (LSD2). LSD1 contains a flavin adenine dinucleotide–dependent amine oxidase domain and performs demethylation of Kme1 and Kme2 only (202, 203) (Fig. 3A). The second group is larger and includes JmjC domain-containing histone demethylases (JHDMs). The demethylase activity of the JmjC domains requires Fe^{2+}, 2-oxoglutarate and oxygen. JHDMs are capable of demethylating Kme1, Kme2, and Kme3 through hydroxylation (204) (Fig. 3A). JHDMs have been classified into subgroups (KDM2-7), according to their JmjC domain sequence and domain architecture. Indeed, KDMs contain DNA and histone binding domains, such as zinc fingers, Tudor domains, and PH domains. For example, KDM4A, KDM4B, and KDM4C from the KDM4 subgroup have 2 PH domains and 2 Tudor domains in addition to their JmjC and JmjN domains, but no zinc fingers; and KDM4D only has JmjC and JmjN domains (205).

In this section, we will focus on LSD1 and KDM4 family members, which are currently the main KDMs involved in SR regulation.

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LSD1

Similarly to KMTs, the first KDM substrates described were initially histones, and nonhistone substrates were later identified. The first identified substrate for LSD1 was H3K4. LSD1-mediated demethylation of H3K4me1/2 induces a repression of target gene transcription by interacting with TFs harboring a SNAG domain, a N-terminal highly conserved repressive domain (eg, SNAIL1/2 and GFI1/B) (206). In addition, LSD1 demethylates nonhistone proteins, among which p53, DNMT1, and HSP90, as well as ERα, which we will further explore later in this review (207). Nevertheless, LSD1 is also a coactivator of several SRs. The relief of repressive histone marks, such as the demethylation of H3K9me1/2, triggers chromatin and DNA conformational changes that are essential for promoting AR and ERα-dependent transcription (208, 209).

KDM4

Enzymes from the KDM4 subgroup can activate or repress transcription depending on the targeted lysine residue. KDM4A was first identified as a H3K9me2/3 and H3K36me2/3 demethylase (210). By demethylating H3K36me3, KDM4A antagonizes HP1γ and allows cell cycle progression (211). Like LSD1, nonhistone substrates of KDM4A have been identified. Indeed, KDM4A was recently identified in a complex with SCF^{Fbxo22} and methylated p53. Indeed, it was reported that KDM4A-mediated p53 demethylation is necessary for the destabilization of methylated p53 induced by SCF^{Fbxo22} (212).

KDMs targeting

Some KDMs are very attractive therapeutic targets. For instance, LSD1 is dysregulated in many cancer types including small cell lung cancer and acute myeloid leukemia (213). Since the discovery of LSD1, many potent and selective inhibitors have been identified, including GSK2879552, which displays an antitumor activity in small cell lung cancer xenograft mouse models (214). Currently, the clinical trials website (www.clinicltrials.gov), references 5 LSD1 inhibitors undergoing clinical trials mostly in cancer patients (215). Among them, the GSK2879552 clinical trial in myelodysplastic syndrome patients completed phase II in 2019, but the outcome does not advocate for completing such trials (NCT02929498). In addition, the dual LSD1/MAO-B inhibitor ORY-2001 efficiently rescues memory and behavioral alterations in mouse models of Alzheimer's disease. Of note, the observed effects were essentially attributed to the inhibition of LSD1 (216). Interestingly, ORY-2001 is currently undergoing a phase II clinical trial in patients with Alzheimer's disease (NCT03867253).

KDMs from the KDM4 subgroup are dysregulated in several diseases such as cancers, cardiovascular diseases, and mental retardation (217). Despite the therapeutic potential of the KDM4 subgroup, there are few potent and selective inhibitors to date (218).

PRMTs, or Protein Arginine (R) Methyltransferases

Arginine methylation was first described in the 1970s (219–222), though the first PRMT was only identified in 1996 (223). Similarly to KMTs, PRMTs are structurally defined as S-adenosylmethionine (AdoMet)-dependent methyltransferases. PRMT enzymes belong to the class I methyltransferases, which is characterized by a 7-stranded β-sheet structure. They also harbor additional conserved sequences, including the motifs I, post-I, and the Thr-His-Trp (THW) loop that forms the AdoMet binding pocket (224). PRMTs transfer 1 or 2 methyl group(s) from the AdoMet methyl donor to the guanidine nitrogen atoms of the targeted arginine, resulting in S-adenosylhomocysteine (AdoHcy) and methylarginine production (Fig. 3B). Three main forms of methylated arginines exist in eukaryotes: monomethylarginine (MMA), ω-N^G,N^G-asymmetric dimethylarginine (aDMA) in which 2 methyl groups are added to the same guanidine nitrogen, and ω-N^G,N'^G-symmetric dimethylarginine (sDMA) where 1 methyl group is attached to each guanidine nitrogen (225) (Fig. 3B). So far, 9 PRMTs have been characterized as active and structurally conventional arginine methyltransferases in human cells (226). They are classified into 3 fundamental subgroups, based on the type of methylation they catalyze. Type I PRMTs (PRMT1, PRMT2, PRMT3, CARM1 for Coactivator Associated Arginine Methyltransferase 1 [PRMT4], PRMT6, and PRMT8) produce MMA and aDMA, while type II enzymes (PRMT5 and PRMT9) deposit MMA and sDMA marks. Type III contains only PRMT7 and is responsible for MMA only (227) (Fig. 3B).

Here, we will focus on PRMT1, CARM1, PRMT5 and PRMT6 which are currently the 4 main PRMTs involved in SR regulation.

PRMTs are involved in many essential cellular processes and KO of most of them causes embryonic lethality. PRMT1 and PRMT5 KO are lethal (ie, induce embryonic or post-natal death) (228). Moreover, CARM1-KO mice die at birth and display a reduced size (229). In contrast, PRMT6-KO mice are viable (230). Tissue ablation of PRMTs has contributed to determining their involvement in metabolic, immune, muscular, and neurodegenerative disorders and cancers (228, 231). The different PRMTs regulate a wide variety of important cellular processes (eg, DNA repair, transcriptional regulation, immune system

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response, RNA processing and signal transduction) (232) by methylating a growing number of substrates (Table 2). It is well known that proteins harboring glycine and arginine-rich motifs (GARs) are often targets for PRMTs (346). However, even if it is the case for PRMT1, 5, and 6, CARM1 prefers to methylate its substrates within a PGM (proline, glycine, methionine) motif (347).

PRMT1

PRMT1 was initially shown to catalyze the methylation of H4R3, an epigenetic active mark (348). To date, many nonhistones substrates have also been identified (Table 2). For instance, BReast CAncer 1 (BRCA1) methylation by PRMT1 affects its tumor suppressive capacity in BC cells and samples (233). More recently, PRMT1 was shown to dimethylate the KMT EZH2 (236). This event inhibited its ubiquitylation and consequently increased the stability of the protein, which further impaired expression of EZH2 target genes, contributing to a sustained and aggressive phenotype of BC cells (epithelial mesenchymal transition, invasion, and metastasis).

CARM1

CARM1-dependent methylation of various substrates notably contributes to tumorigenesis (347). For instance, methylation of the core subunit BAF155 of the chromatin complex SWI/SNF promotes proliferation, migration and metastasis of BC cells in vivo. Moreover, BAF155 methylation was associated with poor survival of BC patients (282). CARM1 methylation of the lysine demethylase LSD1 stabilizes the protein, activating vimentin transcription through histone demethylation, which triggers invasion and metastasis of BC (286).

PRMT5

PRMT5 was shown to methylate, among other substrates, the HOXA9 protein, a TF that plays a crucial role in hematopoietic stem cell expansion and is commonly dysregulated in acute leukemia (304). This modification is involved in epithelial mesenchymal transition activation, due to induced expression of proinflammatory endothelial–leukocyte adhesion molecules, such as E-selectin. As such, PRMT5 seems to be a critical actor in the induction of the proinflammatory function of HOXA9, which is important in the pathobiology of inflammation and cardiovascular inflammatory diseases. PRMT5 also controls carcinogenesis by methylating the TF E2F1. This PTM increases the stability of the target, promoting cell cycle progression and cell growth (302). Despite its oncogenic role, new reports highlighted that PRMT5 is also involved outside of the cancer field. For instance, PRMT5-induced methylation of SREBP1 (311) and SPT5 (310) participate in regulating lipid metabolism.

PRMT6

Histone H3R2 was thought to be the major histone target site of PRMT6 in cells, and PRMT6 was widely considered to be a transcriptional repressor (349, 350). However, in several cases, PRMT6 was reported to act as a transcriptional coactivator, by depositing the H3R17me2a mark, similarly to CARM1 (351). PRMT6 also methylates nonhistone proteins regulating transcription, DNA repair and cell signaling (Table 2).

PRMTs targeting

The majority of PRMTs and their variants have been shown to be overexpressed in cancer compared with normal tissues (352). PRMT1 overexpression has been reported in breast, prostate, lung, colon, and bladder cancer, as well as in leukemia. Similarly, overexpression of CARM1 and PRMT5 has been observed in PC, colon cancer, and BC, whereas PRMT6 has been shown to be overexpressed in bladder, lung, and BC (15). Moreover, their expression is often associated with poor prognosis (15). In view of these findings, it has become increasingly evident that members of the PRMT family constitute new targets for treating pathologies, including cancer (353-355). The first PRMT inhibitor, AMI-1, was discovered in 2004 by Bedford et al. (356). Many potent and selective inhibitors have since been produced (357). Although none of the inhibitors selectively inhibit PRMT1, several inhibitors specific for other Type I PRMTs are available. TP-064 (358) and GSK3359088 (359) targeting CARM1 are effective in inhibiting tumor growth for multiple myeloma. EPZ2020411 selectively inhibits PRMT6, but this inhibitor is under preclinical development (360). Some inhibitors are currently undergoing clinical trials in patients with different types of cancers. On the clinical trials website (www.clinicltrials.gov), 6 clinical trials for agents that target PRMTs are referenced (1 for type I enzymes, and 5 for PRMT5). Most of the inhibitors in clinical trials are in phase I, assessing their safety, pharmacokinetics, pharmacodynamics, and clinical activity. GSK3368715 is the only type I PRMT inhibitor in clinical trials (361). It was shown to inhibit in vitro tumor cell growth in lymphoma, acute myeloid leukemia, and numerous solid tumor cell lines.

JNJ64619178 is a PRMT5 inhibitor that provokes tumor growth inhibition and regression in patient-derived xenografts. In addition, PF-06939999, PRT811, and PRT543 are PRMT5 inhibitors with antiproliferative and antineoplastic activities in cancer cell lines. GSK3326595 is the only inhibitor currently in a phase II clinical trial. Recently, this inhibitor was shown to circumvent palbociclib (CDK4/CDK6 inhibitor) resistance in melanoma (362).

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Table 2. Arginine methylated substrates

PRMT	Substrate	Site	Effect of arginine methylation	Reference
PRMT1	BRCA1	504–802	Facilitates its binding to promoters	(233)
	C/EBPα	R35, R156, R165	Dissociates from SWI/SNF Mediator complex	(234)
	c-Myc	R299, R346	Activates its transcriptional activity by promoting its binding to p300	(235)
	EZH2	R342	Suppresses EZH2 target transcription	(236)
	FOXO1	R248, R250	Blocks FOXO1 phosphorylation by Akt	(237)
	FOXP3	R48, R51	Enhances its transcriptional activity	(238)
	GLI1	R597	Enhances its transcriptional activity	(239)
	MyoD	R121	Activates its transcriptional activity by promoting its DNA-binding	(240)
	Nrf2	R437	Enhances its transcriptional activity	(241)
	RACO-1	R98, R109	Enhances its binding to c-jun, activates AP1 transcription	(242)
	RelA	R30	Inhibits its binding to DNA	(243)
	RIP40	R240, R650, R948	Decreases its corepressor function	(244)
	RunX1	R206, R210	Abrogates Sin3a binding, promoting its transcriptional activity	(245)
	STAT1	R31	Dissociates from PIAS1 and enhances IFNα/β induced transcription	(246)
	TAF15	R203	Enhances TAF15-depend transcription	(247)
	TLS	R216, R218, R242, R394	Enhances transcription of surviving	(248)
	TOP3B	R833, R835	Involved in interaction with TDRD3, promoting its topoisomerase activity	(249)
	Twist 1	R34	Represses import into nucleus and E-cadherin expression	(250)
	53BP1	1319¯1480	Localizes to DNA breaks	(251)
	APE1	R301	Protects mitochondrial DNA from oxidative damage	(252)
	DNA polβ	R137me1	Inhibits its interaction with PCNA, enhances base excision repair	(253)
	E2F1	R109	Induces PARP cleavage in response to DNA damage	(254)
	hnRNPK	R296, R299	Inactivates caspase 3 after DNA damage	(255)
	hnRNPUL1	R584, R618, R620, R645, R656	Stimulates its recruitment to DNA damage	(256)
	MRE11	GAR domain	Enhances its exonuclease activity	(251)
	ASK1	R78, R80	Negatively regulates ASK1 signaling	(257)
	Axin	R378	Increases Axin stability and inhibits Wnt signaling	(258)
	CaMKII	R9, R275	Suppresses cardiac CaMKII hyperactivation	(259)
	EGFR	R198, R200	Promotes EGFR activation	(260)
	p38 MAPK	R49, R149	Enhances p38α activation	(261)
	Smad4	R272	Activates wnt signaling	(262)
	Smad6	R74, R81	Activates BMP signaling	(263)
		R74, R81	Inhibits NFkB signaling	(264)
	Smad7	R57, R67	Enhances TGF-β signaling	(265)
	TSC2	R1457, R1459	Regulates mTORC1 activity	(266)
	BAD	R94, R96	Inhibits its association with 14-3-3	(267)
	CDK4	R55, R73, R82, R163	Destabilizes CDK4-Cyclin-D3 complex and inhibits cell cycle progression	(268)
	CNBP	R25me1/2a, R27me1/2a	Decreases its RNA binding	(269)
	cTnI	R146me1/2a, R148me1/2a	Inhibits cardiomyocytes hypertrophy	(270)
	EIF4G1	R689me1, R698me1	Contributes to its stability and facilitates translation initiation complex assembly	(271)
	EZH2	R342me2a	Enhances its stability	(236)
	G3BP1	R435 me1/2a, R447 me1/2a	Prevents stress granule formation	(272)
	hnRNP A1	R214, R226, R223, R240	Enhances its RNA binding	(273)
	HSP70	R416, R447	Protects PDAC cells from apoptosis	(274)
	INCENP	R887	Facilitates interaction with AURKB (maintains chromosomal alignment)	(275)
	KCNQ	R333, R345, R353, R435	Facilitates its ion channel activity by PIP2 interaction	(276)
	MYCN	R65	Stabilizes MYCN protein	(277)
	RBM15	R578	Facilitates its degradation by CNOT4 (RNA splicing)	(278)
	rps3	R64, R65, R67	Targets rps3 into ribosomes (translation)	(279)

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Table 2. Continued

PRMT	Substrate	Site	Effect of arginine methylation	Reference
	RunX1	R233, R237	Resists to apoptosis under stress condition	(280)
	TRAF6	R88, R125	Inhibits its ubiquitin ligase activity	(281)
CARM1	BAF155	R1064	Regulates transcription related to c-Myc pathway	(282)
	C/EBPβ	R3	Dissociates from SWI/SNF mediator complex	(283)
	CARM1	R551	Promotes its effect on transcription and mRNA splicing	(284)
	HSP70	R469me1	Activate RA-induced *RARβ2* transcription	(285)
	LSD1	R838	Stabilizes LSD1, enhancing E-cadherin and decreasing vimentin transcription	(286)
	Pax7	R161	Activates *Myf5* transcription via MLL1/2 complex	(287)
	Pontin	R333, R339	Activates Foxo3-induced autophagy gene expression	(288)
	PRMT5	R505	Enhances its enzymatic activity, decreasing γ-globin gene transcription	(289)
	RNA pol II	R1810	Activates the transcription of small nuclear RNAs	(290)
	RUNX1	R223	Induces the repressor complex formation	(291)
	SOX2	R113	Enhances Sox2-mediated transactivation by self-association	(292)
	p300	R754	Promotes BRCA1 recruitment to p21 promoter during DNA damage	(293)
	GAPDH	R234	Inhibits glycolysis by repressing its activity	(294)
	HuD	R236, R248	Decreases p21 stability	(295)
	HuR	R217	Stabilizes mRNAs	(296)
	MDH1	R248me1/2a	Inhibits Gln metabolism	(297)
	PKM2	R445, R447	Enhances its pyruvate kinase activity	(298)
	RPIA	R42	Enhances its enzymatic activity (pentose phosphate pathway)	(299)
PRMT5	Actin	R256me1	Either activates or represses transcription	(300)
	BCL6	R305	Facilitates its transcriptional repressive activity	(301)
	E2F1	R111, R113	Inhibits its transcriptional activity	(302)
	GATA4	R317	Inhibits its transcriptional activity	(303)
	HOXA9	R140	Promotes transcription of E-selectin	(304)
	RelA	R30	Enhances NFKB transcriptional activity	(305)
		R30me1, R35		(306)
		R174		(307)
	RNA pol II	R1810	Controls termination of transcription	(308)
	SHP	R57	Facilitates its transcriptional repressive activity	(309)
	SPT5	ND	Releases SPT5 from Bscl2 promoter (lipid metabolism)	(310)
	SREBP1a	R321	Enhances SREBP1transcriptional activity	(311)
	53BP1	GAR motif (both ADMA and SDMA)	Enhances DNA repair process	(312)
	FEN1	R192	Facilitates DNA repair by binding to PCNA	(313)
	p53	R333me1, R335, R337	stimulates p53-dependent G1 arrest in response to DNA damage	(314)
	RAD9a	R172, R174, R175	Regulates cell cycle checkpoints	(315)
	RUVBL1	R205	Removes 53BP1 from DNA breaks then enhances HR-mediated DSB repair	(316)
	TDP1	R361, R586	Stimulates TDP1/XRCC1 recruitment to DNA breaks	(317)
	ASK1	R89	Inhibits H_2O_2-induced ASK1 activation	(318)
	BRAF	R671	Inhibits ERK activation (EGFR signaling)	(319)
	CRAF	R563		
	DUSP14	R17me1/me2s R38, R45me1	Promotes its ubiquitination, inhibiting TCR signaling	(320)
	EGFR	R1175me1	Inhibits EGF-induced ERK pathway	(271)
	YBX1	R205	Activates NF-κB signaling	(321)
	G3BP1	R460	Prevents stress granule assembly	(272)
	GLI1	R990, R1018	Stabilizes GLI1 protein	(322)
	GM 130	R18, R23	Regulates GA ribbons, maintaining Golgi architecture	(323)
	hnRNP A1	R218, R225	Enhances interaction with IREs RNA to promote translation	(324)
			Facilitates HIV-1 IRES-mediated translation	(325)
	HSP90A	R345, R368	Suppresses the cell apoptosis	(326)

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Table 2. Continued

PRMT	Substrate	Site	Effect of arginine methylation	Reference
	KLF-4	R374, R376, R377	Inhibits its ubiquitination, maintaining genome stability	(327)
	LSH	R309	Decreases stem-like properties	(328)
	PDCD4	R110	Inhibits its tumor suppressive activity	(329)
	RPS10	R158, 160	Facilitates its assembly into ribosome	(330)
	ZNF326	R175	Regulates alternative splicing	(331)
PRMT6	FOXO3	R188, R249	Activates transcriptional activity	(332)
	HIV-1 Tat	R52, R53	Inhibits Tat transcriptional activation	(333)
	HIV-1 nucleocapsid	R10, R32	Inhibits reverse transcription	(334)
	RFX5	R466, R468	Down-regulates transcription	(335)
	TOP3B	R833, R835	Promotes transcription	(249)
	DNA pol β	R83, R152	Promotes Polβ activity in DNA strand break repair	(336)
	CRAF	R100	Diminishes MEK/ERK signaling	(337)
	PTEN	R159	Inhibits PI3K–AKT signaling	(338)
	BAG5	R15, R24	Represses cell autophagy	(339)
	GPS2	R323	Prevents GPS2 degradation	(340)
	HIV-1 Rev	R38	Inhibits viral RNA export to the cytoplasm	(341)
	p21	R156me1/me2a	Enhances cytoplasmic localization of p21	(132)
	p16	R22, R131, R138	Weakens p16-mediated apoptosis	(342)
	PRMT6	R35	Stabilizes PRMT6 protein level	(343)
	RCC1	R214	Induces its association with chromatin and activation of RAN	(344)
	SIRT7	R388me1/me2a	Inhibits its deacetylase activity (mitochondria biogenesis)	(345)

When the type of methylation is not specified it is Rme2a for PRMT1, CARM1, and PRMT6, and Rme2s for PRMT5.

Arginine (R) Demethylases

JMJD6

With regards to arginine methylation, only 1 demethylase has so far been identified, and this role was initially a matter of controversy (363). Indeed, in 2007, JMJD6 (Jumonji domain-containing protein 6) was described as a JmJC-containing iron-and 2 oxoglutarate-dependent dioxygenase, able to remove dimethyl groups from H3R2 and H4R3 (98) (Fig. 3B). However, at that time, Webby et al did not confirm these results and reported that arginine-rich (RS) domains of U2AF65 and LUC7-like2 synthesized with dimethylated arginine residues could be Jmjd6 substrates for hydroxylation (364). JMJD6 also catalyzes the hydroxylation of lysine residues of histones H2A and H2B (365).

Despite this multifaceted role for JMJD6, more recent studies confirmed that this enzyme demethylates arginines of some nonhistone substrates. Among them, ERα (366), RNA helicase A (367), the TF PAX3 (368), HSP70 (285), the Ras-GTPase activating SH3-domain-binding-protein 1 (G3BP1) (369) and the ubiquitin ligase TRAF6 (281). It is now broadly accepted that JMJD6 acts as a dual demethylase and lysyl hydroxylase, able to modify proteins on both arginine and lysine residues. Interestingly, JMJD6 is upregulated in a large spectrum of cancers and its enzymatic activities have been associated with tumorigenic roles, making JMJD6 a promising novel therapeutic target (370). However, so far only 1 inhibitor has been developed and its effect on JMJD6 demethylase activity has not been investigated, although it displayed promising antiproliferative effects on ovarian cancer cells (371).

Of note, the fact that JMJD6 is unable to demethylate H3R8, H3R17, H3R26, or H2A sites (98) suggests that other arginine demethylases may play a role in the dynamic regulation of arginine methylation. Indeed, other works demonstrated that some KDMs are also able to remove methyl marks from arginine residues. KDM3A, KDM4A, KDM5, and KDM6B display arginine demethylase activity in vitro on histones and on certain nonhistone peptides (372). JMJD1B (or KDM3B) was recently reported to demethylate H4R3me2s and its intermediate H4R3me1, during the development of hematopoietic stem cells (373). However, its very narrow specificity strongly suggests that other unknown enzymes may display arginine demethylase properties. In addition to true demethylation, arginine methylation levels are further modulated by the conversion of arginine into citrulline by protein arginine deiminase (374).

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Methylation/Demethylation of Steroid Receptors: Biological Implications

Estrogen Receptor α, or ERα

Lysine methylation/demethylation

K302. In 2008, Vertino's team identified the first lysine methylation of a SR (375). They found that SET7/9 catalyzes ERα methylation on lysine 302 (K302), located in the hinge region, to promote ERα transactivation. Moreover, they linked K302 methylation with the stabilization of ERα protein levels (Fig. 4A and Table 3). Indeed, estrogen-induced ubiquitylation of ERα and its subsequent degradation by the proteasome is an important step in the transcriptional activity of the receptor (390, 391). As such, SET7/9 was recognized as a potent modulator of ERα-dependent gene expression. Interestingly, K302 is located in a PTM "hot-spot," where acetylation (K299, K302 and K303), ubiquitylation (K302), and phosphorylation (S305) were previously reported (85). Similarly to what happens on histone tails, the presence of other PTMs in the vicinity of K302 could affect the methylation event. Consistently, previous research reported the existence of connections between acetylation and phosphorylation, which markedly regulates the phenotype of cells. ERα nonacetylated K303 variant (K303R) was detected in primary ductal hyperplasia and aggressive BC (392, 393). K303R promotes phosphorylation on the nearby serine 305 (S305) and promotes high transcriptional activity, even with low estrogen levels. Therefore, the crosstalk between K302 methylation and K303 acetylation can contribute to invasive breast tumors, highlighting that PTMs deeply influence SR signaling in normal and malignant contexts.

K266. Later, K266 was shown to be methylated by SMYD2 (376). In the absence of estrogen, this modification impairs ERα binding to chromatin to prevent gene activation. Upon estrogen stimulation, K266 methylation is diminished, enabling p300-induced acetylation on this lysine residue to activate ERα transcriptional activity (Fig. 4A and Table 3). Moreover, cells with ERα-K266R mutation have a higher capacity to proliferate than wild-type (WT) cells under estrogen-depleted growth conditions, likely indicating that SMYD2-mediated K266 methylation blocks the estradiol-induced (E_2) cellular response. As LSD1 has been shown to remove methyl marks on SMYD2 substrates, including p53 and HSP90 (394, 395), the authors investigated whether it could participate in the regulation of ERα K266 methylation. They showed that LSD1 is able to remove SMYD2-K266 methylation, allowing ERα acetylation on the same residue by p300, activating its transcriptional activity (Fig. 4A). More recently, HSP90 and its cochaperone p23 have been shown to bind SMYD2, inducing an increase in its ability to methylate ERα (396). We can thus speculate that the well-known association between ERα and HSP90 in the cytoplasm before hormonal stimulation could involve SMYD2 in order to maintain ERα in an inactive state in the absence of estrogen.

K235. The latest described ERα methylation on lysine is catalyzed by the KMT G9a, which dimethylates the receptor at K235, in its DBD (Fig. 4A and Table 3). This modification functions as an activator for the expression of some estrogen target genes, activating the growth of ERα-positive BC cells (377). K235 dimethylation is a recognition site for the Tudor domain of PHF20, a member of the MOF histone acetyltransferase complex, which catalyzes the acetylation of histone H4K16, as well as nonhistone proteins involved in transcription. The association of ERα with PHF20 through its K235 methylation site then recruits the MOF complex to deposit acetylation to H4K16 of E_2 target genes, supporting access of ERα to chromatin and improving its transcriptional activity. As previously reported for other methylation events, in the vicinity of K235, S236 was reported to be phosphorylated by protein kinase A. Unlike K235 methylation, S236 phosphorylation is an obstacle for ERα dimerization and its recruitment to DNA. Therefore, these 2 adjacent modifications compete to regulate the transactivation activity of ERα. Aside from K235, K303 in the hinge domain was also revealed to be methylated by G9a in vitro; however, this has not been confirmed in vivo.

Taken together, it seems that ERα activity is regulated by 3 different lysine methylations carried out by 3 different enzymes, in which K302 and K235 methylation strengthens ERα activity, whereas K266 methylation functions as a repressor. Importantly, these 3 ERα residues are located in the same "hot-spot," which undergoes intensive posttranslational modifications, and it is thus fundamental to study the importance of context-dependent effects of methylation events to better understand their interplay with other modification marks within the context of ERα signaling.

Arginine methylation/demethylation

R260. Our team was the first to identify an arginine methylation event for a SR. Indeed, we showed that PRMT1 dimethylates ERα on arginine 260 (R260), located at the junction between the DBD and the hinge domain (Fig. 4A and Table 3). This modification is a crucial event for estrogen nongenomic signaling. Mechanistically, estrogen triggers a rapid and transient ERα methylation, which is required for its interaction with the kinases Src and PI3K. The formation of this complex is a prerequisite for activating the downstream Akt pathway (382). In addition, we demonstrated that insulin-like growth factor (IGF-1) also triggers ERα methylation via PRMT1, an important event for

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IGF-1 signaling in BC, highlighting that targeting PRMT1 activity could be a good strategy to concomitantly impact estrogen and IGF-1 pathways (384).

Together, these results emphasize the importance of PRMT1 as a regulator of both estrogen and IGF-1 signaling, highlighting PRMT1 as a promising target for treating ERα-positive BC patients.

As ERα methylation is a transient event, we investigated whether a demethylase could be involved in the regulation of ERα methylation. We showed that, upon estrogen stimulation, JMJD6 is integrated into the hallmark nongenomic complex metER/Src/PI3K, where it demethylates ERα, causing dissociation of the complex and termination of downstream signaling (366). Moreover, JMJD6 expression is associated with poor prognosis in BC (397), but its enzymatic activities have not yet been fully associated with BC.

Most of the studies on SR methylation have been conducted in cancer cell models; however, metERα expression has been evaluated in human breast tissues concomitantly with ERα/Src and ERα/PI3K complexes, hallmarks of ERα nongenomic signaling. Their expression has been detected at low levels in human breast tissues and high levels in a subset of breast tumors. Interestingly, their high expression is associated with BC aggressiveness (383). More recently, we showed that ERα nongenomic signaling is increased in BC resistant to tamoxifen treatment (398). Later, using a mouse model harboring ERα mutated R264A (equivalent to R260 in human), the role of metERα in physiology was investigated. It was shown that although this arginine is not required for the physiological regulation of the skeleton (399) nor for fertility (385), this residue is involved in ERα activity, such as the rapid dilatation of mesenteric arteries and the endothelial repair of carotid (385).

Altogether, these findings highlight the importance of arginine methylation in ERα nongenomic pathways. Probably because R260 is not conserved among NR, this modification has never been involved in nongenomic signaling triggered by other members of the family.

Progesterone Receptor, or PR

Lysine methylation

K464, K481. PR is largely post-translationally modified, especially by phosphorylation on serine and threonine residues (400). Methylation on lysine residues has also been reported, in the NTD close to the DBD. Chung et al. (378) reported that K464 methylation is essential in PR ligand–independent and –dependent transcriptional activities (Fig. 4B and Table 3). Using MS, they showed that both PR is endogenously monomethylated in T47D BC cells (378). Interestingly, K464 is located in the AF-1 hormone-independent transactivation domain, previously narrowed down to a 91 amino acid sequence preceding the DBD in PRs (Fig. 4B) (401). Site-directed mutagenesis on PR revealed that nonmethylable mutants display a higher ligand-independent activation, in particular perceptible by an increase in PR phosphorylation at S400, a basally phosphorylated site (402). Importantly, K464 mutations have a significant effect on ligand-induced activity of PR, implying that K464 methylation impedes the transcriptional activity of PR.

A more recent study identified K481me1 acting in cooperation with K464 in the ligand-induced transcriptional activity of the receptor (379) (Fig. 4B). Although the KMT is not identified yet, this study is the first to argue in favor of the importance of methylation in regulating PR signaling. These studies suggest that methylation of lysine residues of the AF-1 domain disrupts PR transcriptional activity, supporting the notion that methylation is a modulator of PR signaling in BC cells.

Arginine methylation

R492. Within the same study conducted by Woo et al., R492me1 was shown to synergize with the 2 preceding lysine methylation events (K464 and K481), in the transcriptional activity of PR (379) (Fig. 4B and Table 3). When residues were substituted to neutral polar glutamine (K464Q/K481Q/R492Q), the defective triple-methylation mutant exhibited a strong increase in transcriptional activity in response to progestin, compared with WT PR, suggesting that positive charge due to methylation could act as a brake for transcription efficiency. Moreover, data showed that these key residues provide not only the interaction interface with major PR coregulators, like SRC-1, but also with the AF-2-containing LBD, described as acting with AF-1 to bring PR towards a full transcriptional activation.

R637. Our team recently demonstrated a functional crosstalk between arginine methylation, PR transcriptional activity, and progestin-induced proliferation in BC cells (386). We showed that PRMT1 is an important modulator of the transcriptional activity of PR, at least in part through methylation in the hinge region (Fig. 4B and Table 3). Under progestin treatment, PRMT1 dimethylates PR in the nucleus, at R637, within a RGG methylation consensus motif (346, 403). This methylation event modulates PR oncogenic properties, as cells expressing a nonmethylable mutant (R637K) displayed a retarded cell growth and a reduced expression of a subset of genes that promote proliferation and survival of BC cells. R637 methylation facilitates PR degradation, which in turn constitutes a critical stimulatory switch that

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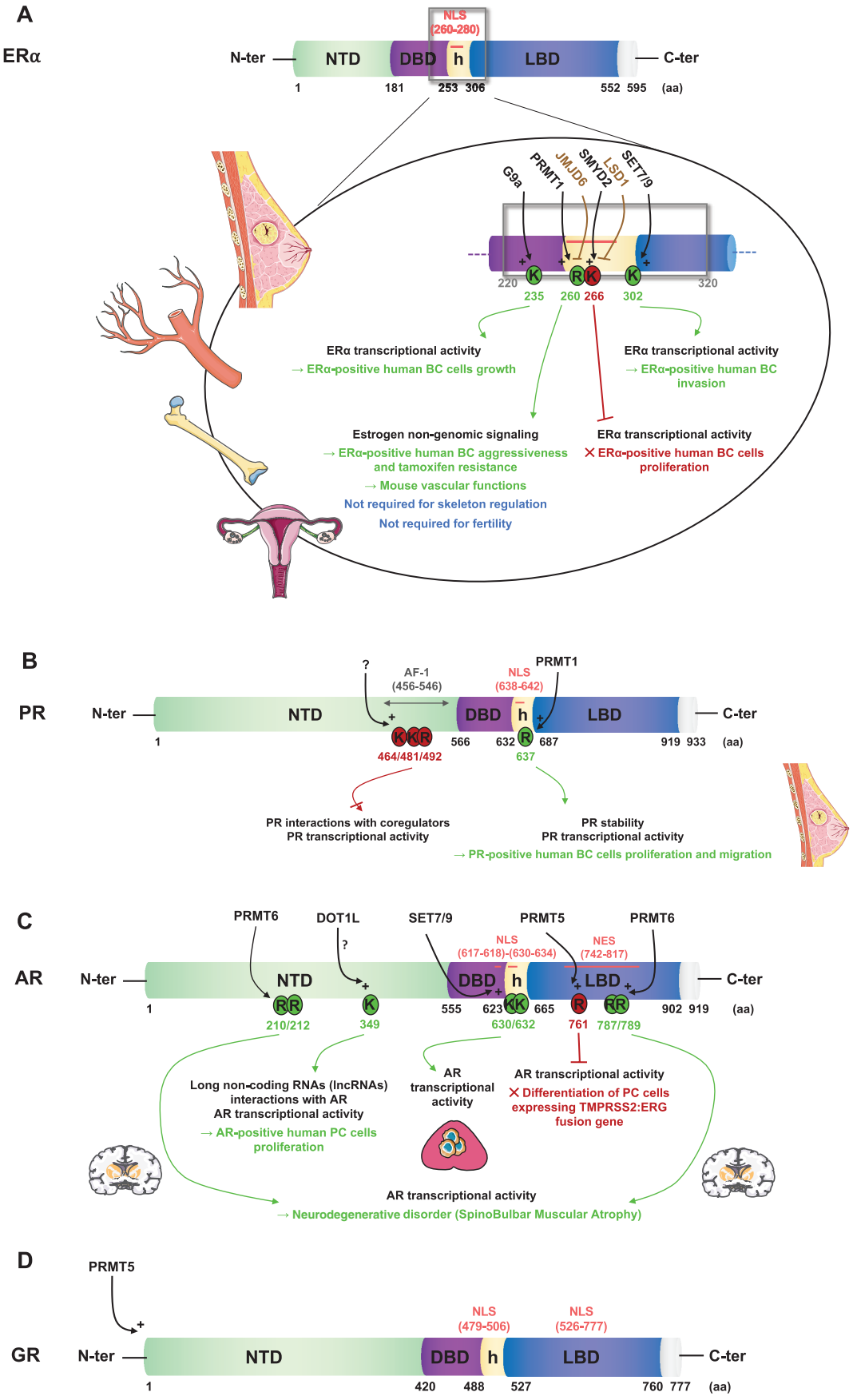

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Table 3. Lysine and arginine methylation of steroid receptors

Receptor	Enzyme	Residue	Biological effect	References
Steroid receptor methylation by lysine methyltransferases				
ERα	SET7/9	K302me1	Promotes transcriptional activity by protein stabilization	(375)
	SMYD2	K266me1	Represses transcriptional activity	(376)
	G9a	K235me2	Promotes transcriptional activity	(377)
PR	ND	K464me1	Decreases ligand sensitivity	(378)
		K481me1	Represses AF1 activity	(379)
AR	SET7/9	K632me1	Promotes its transcriptional activity	(380)
		K630me1		(381)
AR	DOT1L	K349	Activates its transcriptional activity	(189)
Steroid receptor methylation by protein arginine methyltransferases				
ERα	PRMT1	R260me2a	Participates in E2 non genomic signaling	(382, 383)
			Participates in IGF-1 signaling	(384)
			Participates in vascular protective effects	(385)
PR	ND	R492me1	Decreases transcriptional efficiency	(379)
	PRMT1	R637me2a	Regulates stability and transcriptional activity	(386)
AR	PRMT5	R761me1/2s	Represses genes involved in differentiation	(387)
	PRMT6	R210me2a, R212me2a, R787me2a, R789me2a	Activates its transcriptional activity in SBMA, by inhibiting phosphorylation by Akt	(388)
GR	PRMT5	Rme2s	ND	(389)

K, lysine; R, arginine; Kme1, monomethyllysine; Kme2, dimethyllysine; Rme1, monomethyarginine; Rme2a, asymmetric dimethyarginine; Rme2s, symmetric dimethylarginine; ND, nondetermined; IGF-1, insulin-like growth factor; SBMA, spinal and bulbar muscular atrophy. ND, not determined

accelerates the recycling of PR from pre-initiation complexes, which is required for active hormone-dependent transcription (390, 404, 405). Moreover, BC patients with PR-positive tumors expressing high level of PRMT1 show a worse survival than patients with low PRMT1, suggesting that targeting PRMT1 could constitute a new therapeutic option.

Interestingly, SUMOylation of PR on K388 has also been shown to regulate PR stability by competing with ubiquitination. Indeed, the E3 ubiquitin ligase CUEDC2 binding attenuates sumoylation SUMOylation of K388, while promoting ubiquitination (406). As crosstalk between different PTMs have largely been described (85), we can hypothesize that the integration of these different signals tightly regulates PR stability and transcriptional activity.

Taken together, methylation is an indisputable mechanism involved in the regulation of the transcriptional activity of PR, sometimes acting as a repressor or as an activator, once again highlighting the importance of context-dependent effects of this PTM in cells.

Androgen Receptor, or AR

Lysine methylation

K630, 632. In 2011, 2 different teams reported that AR activity can be regulated by SET7/9. The first 1 reported that AR interacts with the methyltransferase, which in turn monomethylates the K632 residue within a KLKK motif, similarly to the sequence methylated in the hinge domain of ERα (407) (Fig. 4C and Table 3). This methylation induced upon ligand binding is necessary for enhancing AR transcriptional activity. As SET7/9 functions as a proproliferative and antiapoptotic factor, highly expressed in PC, it could constitute a potential target to treat these tumor (407). In parallel, a study from another research group demonstrated that K630, and not K632, is methylated by SET7/9, and globally linked with the same functions (381). The discrepancy between these 2 works has so far not been resolved. K632 modification better matches the consensus site of SET7/9, but the identification was performed with a small peptide. MS analyses on the full protein or specific antibodies are needed to clarify this

Figure 4: continued

Figure 4. Biological consequences of SR methylation. All the methylation events targeting the steroid receptors on arginine (R) and lysine (K) residues and reported at this time are represented for (A) ERα, (B) PR, (C) AR, and (D) GR. When identified, the protein methyltransferases involved are noted in black and the demethylases in brown. The methylation events leading to repressive functions are represented in red and the activating functions are in green. For ERα, we enlarged the hinge domain as it is the main region modified by methylation. When decrypted and reported, the biological consequences of the methylation event on the physiology/pathology have been indicated (in green for activating functions, red for repressive functions and blue when no effect). NTD, N-terminal domain; DBD, DNA-binding domain; h, hinge; LBD, ligand binding domain; NLS, nuclear localization signal; NES, nuclear export signal; BC, breast cancer; PC, prostate cancer.

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point. Nonetheless, these 2 methylation sites belong to a rich acetylation target domain. Indeed, K630, 632, and 633 residues are acetylated by p300, p/CAF (408), and TIP60 (409), suggesting, similarly to ERα, a crosstalk between acetylation and methylation.

K349. Lysine methylation is also involved in the regulation of AR transcriptional activity, through the binding of 2 long noncoding RNAs, namely *PRNCR1* and *PCGEM1*, in PC cells. The KMT DOT1L was required for AR binding to *PCGEM1*. Indeed, DOT1L methylates AR on K349, located in the N-terminal region, a critical step for the recruitment of *PCGEM1* to AR (189) (Fig. 4C and Table 3). Moreover, DOT1L is overexpressed in PC and is associated with poor clinical outcome, and this KMT selectively regulates the tumorigenicity of AR-positive PC cells in vitro and in vivo, making a promising therapeutic target (410). However, the results about AR methylation on K349 are matter of debate since they were refuted by Chinnaiyan's team (411).

Arginine methylation

R210, R212, R787, R789. The first link between PRMT6 and AR was unveiled by Sun et al. (412), showing a direct PRMT6/AR interaction in Cos7 cells overexpressing AR. Furthermore, PRMT6 was able to methylate AR, although specific methylation sites were not explored. More recently, this interaction was confirmed with the AR mutant containing polyglutamine stretch in its NTD and implicated in the X-linked transmitted spinal and bulbar muscular atrophy (388). For this interaction, the required regions were the AF-2 domain of AR, the catalytic domain of PRMT6 as wells as a LXXLL motif present in PRMT6. PRMT6 proved to methylate AR and, to a higher extent, polyglutamine-expanded AR. The AR arginine methylation sites were identified in both the NTD and the LBD (R210/212 and R787/789) (Fig. 4C and Table 3), within RXRXXS motifs involved in Akt-mediated AR phosphorylation. Overall, AR transactivation by PRMT6-mediated methylation was regulated by phosphorylation through a mutually exclusive interaction, making PRMT6 a modifier of polyglutamine-expanded AR neurotoxicity. A model is suggested in which arginine methylation of polyglutamine-expanded AR by PRMT6 at the Akt consensus site motif enhances function and toxicity leading to neurodegeneration, whereas phosphorylation by Akt prevents binding to testosterone, thereby protecting neurons from degeneration (388).

Exemplified in this disease model, the PRMT6/AR interaction is likely to play relevant roles in other diseases, such as PC, where PRMT6 is overexpressed in comparison with normal prostate tissue (413, 414) or even physiological conditions. Thus, overexpression of PRMT6 has been observed in the testes of AR-KO mice (51, 415). Results from this study suggest that downregulation of PRMT6 by AR (binding to an androgen response element present in *PRMT6* promoter) is necessary for AR-controlled spermatogenesis (415).

R761. PRMT5 has been shown to methylate AR on R761 in the LBD (Fig. 4C and Table 3). This modification was described in PC cases expressing the TMPRSS2:ERG fusion gene. Mechanistically, the TF ERG recruits PRMT5 to AR target genes involved in prostate differentiation, where the enzyme methylates the receptor (387). This event attenuates AR recruitment to these sites and subsequent transcription from the associated gene promoter. Consistently, R761 methylation supports proliferative functions of the oncoprotein ERG. Thus, R761 methylation could serve as a potent biomarker for AR-dependent proliferation in TMPRSS2:ERG-positive PC, and inhibiting PRMT5 activity could be beneficial for treating this tumor subtype.

Altogether, it appears that AR and its variants are highly methylated on both lysine and arginine residues, participating actively in its full transcriptional activity, even though the interplay between these modifications is still unknown.

Glucocorticoid Receptor, or GR

GR was not known to undergo methylation until recently. Indeed, using pan methyl antibodies recognizing only dimethylated arginines, our team found that GR is methylated by PRMT5 within the nucleus of the ERα-positive BC cell line MCF-7 (Fig. 4D and Table 3) (389). Although the methylated arginine residue(s) and the role of this modification in GR activity is still under investigation, our finding is highly promising as it suggests a new molecular regulation of GR activity involving PRMT5.

Indirect Role of Methylation/Demethylation in Steroid Receptor Transcriptional Activity

Additional indirect methylation events modulate the transcriptional activity of SRs. On the one hand, SR coregulators are also methylated. These methylation events regulate, among other processes, molecular interactions, stability, and subcellular localization of SRs, in order to tightly modulate their transcriptional activity. Specific coregulators are recruited on the promoter/enhancer regions of their target genes to locally remodel the chromatin structure and to orchestrate the assembly or disassembly of an active transcription complex. On the other hand, lysine or arginine methyltransferases, or demethylases, modify residues in histone tails and thereby participate in SR-dependent target gene expression. In this section, we selected key examples of these indirect methylation events to illustrate their impact on SR transcriptional activity.

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Lysine Methylation/Demethylation

G9a/GLP methylation and GR regulation

G9a and its paralogue GLP are interesting illustrations of KMTs involved in SR transcriptional activity through more indirect mechanisms than those previously described. First identified as KMTs methylating the repressive mark H3K9, these enzymes also act as GR coactivators under certain circumstances (90). The self-methylation of G9a/GLP on K185 and K205, respectively, provide a binding site for HP1γ, which facilitates the recruitment of RNA polymerase II, activating the transcription of a subset of GR target genes in specific cell contexts (Fig. 5A). In contrast, phosphorylation of the adjacent threonine (T186 for G9a and T206 for GLP) by Aurora kinase B (AURKB) prevents binding to HP1γ and reduces coactivator functions of G9a and GLP (416), resulting in distinct biological effects in a tissue-specific manner. For instance, the coactivating activity of G9a/GLP regulates migration of the lung cancer cell line A549 (416), and GC-induced cell death in B-acute lymphoblastic leukemia (417) (Fig. 5A). Moreover, a panel of KDM inhibitors identified the JmjC KDM family as demethylases for G9a and GLP. JmjC inhibitors increased G9a methylation, expression of G9a-HP1γ-dependent GR target genes and GC-induced cell death in B-acute lymphoblastic leukemia cell lines (418). In vitro demethylation assays unveiled the KDM4 family as demethylases for G9a and GLP (Fig. 5A). This study was a proof of concept that a methylation/phosphorylation event could influence a coregulator, functioning as a coactivator or corepressor, tightly regulating the transcriptional activity of a SR.

LSD1 regulation of histone marks

Among the well-known coregulators that influence SR signaling by altering protein methylation status, LSD1 was the first demethylase described to regulate histone methylation in a hormone-dependent context. Indeed, the recruitment of LSD1 and subsequent H3K4me1/2 demethylation induces repression of androgen-dependent AR target gene expression (including AR itself) (419). In addition to its well-established corepressor functions (203, 420), LSD1 acts as a coactivator for several TFs, including AR and ERα (208, 421-424). This effect was not only observed in PC cells but also in kidney cancer cells, which are usually not considered to be androgen-sensitive. In this study, using pargyline, an inhibitor of LSD1, investigators were able to block demethylation of H3K9 and subsequently AR-dependent transcriptional activation (425). In another recent study, LSD1 demethylated the pioneer TF FOXA1, thereby facilitating its DNA-binding, notably in androgen response element and estrogen response element, where it is a well-known active partner in AR- and ER-mediated transactivation (426). Lastly, LSD1 acts as a coactivator of both AR and its main splice variant, AR-V7, which has been implicated in resistance to androgen deprivation therapy in castration-resistant PC (427). Overall, through enhanced transactivation of AR (and possibly AR variants), LSD1 may favor proliferation and invasiveness of PC cells and impair apoptosis under androgen deprivation therapy (425, 428).

Interestingly, phosphorylation of H3T6 by protein kinase C beta I prevents LSD1 from demethylating H3K4me1/2 and induces a switch in substrate specificity from H3K4me1/2 to H3K9me1/2, orienting LSD1 towards a transcriptional coactivator role (208, 429). Again, this set of experiments clearly underlines that protein methylation can interfere with other PTMs and induce a switch in the functions of SR coregulators, resulting in a fine-tuning of gene expression.

Arginine Methylation/Demethylation

Similarly to lysine residues, methylation on arginine residues of histone tails and coregulators has been reported to indirectly regulate SR transcriptional activities.

Methylation of histone marks by PRMT1 and CARM1

Twenty years ago, numerous studies demonstrated the strong impact of PRMT1 and CARM1 as coregulators that modify histone tails in order to modulate SR-dependent transcription. Historically, CARM1 was characterized as a methyltransferase owing to its capacity to methylate the N-terminal tail of H3 in vitro (430). This finding was then confirmed in vivo on hormone-regulated promoters. Indeed, the transcriptional activities of ERα, GR, and AR are known to be regulated by CARM1-induced methylation of both R17 and R26 of histone H3 (431-433) (Fig. 5A). As CARM1, PRMT1 also acts as a coactivator of SR through histone methylation (12, 13, 434). H4R3 is methylated by PRMT1 and plays a critical role in transcriptional activation, making PRMT1 a potent coactivator of AR transcriptional activity (13). Interestingly, as it was described for lysine methylation on histone tails, cross-talks between arginine methylation and other PTMs, namely lysine acetylation, were reported to be involved in the regulation of SR-dependent transcription. For instance, stimulation by estrogens results in the acetylation of H3K18 by the acetyltransferase CREB binding protein (CBP). This event stimulates CARM1 recruitment to the H3 tail, which methylates H3R17, leading to the implementation of estrogen-regulated gene expression (435). More recently, PRMT5 associated with pICln was described as an epigenetic activator of AR transcription in castration-resistant PC cells, suggesting that targeting its enzymatic activity could represent a novel therapeutic approach (436).

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Methylation of coregulators by CARM1

Aside from direct histone methylation, some transcriptional coregulators are also methylated on arginine residues, making the arginine methyltransferases potent transcriptional modulators, such as CARM1 for ERα- and AR-dependent transcription (437). For example, CARM1 dimethylates CBP on R742 in vivo, playing a role in estrogen-induced gene activation (438) (Fig. 5B). P300 is methylated by CARM1 on R2142, localized in its C-terminal GRIP1 binding domain, inhibiting p300 binding to the coregulator glucocorticoid receptor interacting protein 1 (GRIP1/SRC2), a key coregulator of SRs in vitro and in vivo (439). It was later shown that CARM1-dependent CBP methylation on R742, 768, and 2151 stimulates its histone acetyltransferase activity by increasing its autoacetylation (440). Interestingly, using antibodies specific for the individual methylation sites, they showed that methylation of CBP is required for its recruitment by ERα to specific target genes (Fig. 5B). Using genome-wide analyses, different patterns of binding to ERα target genes were observed for the various methylated CBP species (440). These data suggest that CARM1-dependent CBP methylation induces the expression of specific target genes, diversifying the ERα transcriptional program.

CARM1 was also shown to methylate SR coactivator proteins of the SRC/p160 family, including SRC-3 (441, 442). SRC proteins serve as primary coregulators to recruit secondary coactivators, such as p300/CBP or CARM1, to the promoters of specific estrogen-dependent target genes (443). SRC-3 methylation on R1171 takes place in its C-terminal domain containing its binding sites with p300/CBP and CARM1 (441, 442). Consequently, SRC-3 methylation induced by estrogens triggers its dissociation from CBP and CARM1, a decrease in its stability and a subsequent decrease in ERα-mediated transcription (Fig. 5B). The authors suggested that this methylation event serves as a molecular switch for disassembly of the SRC-3 transcriptional coactivator complex (441).

More recently, 2 new substrates for CARM1 were identified: BAF155 in the SWI/SNF chromatin remodeling complex and MED12, a component of the mediator complex that facilitates RNA polymerase II recruitment (282). CARM1-methylation of MED12 regulates its binding to the chromatin in order to regulate *p21* gene expression, increasing BC cell sensitivity to chemotherapy in vitro and in vivo (282, 444) (Fig. 5B). An additional study confirmed that MED12 is methylated by CARM1 in a cluster of arginine residues located in its C-terminal domain, regulating MED12 recruitment to ERα enhancers (445) (Fig. 5B). These methylated residues are recognized by the coactivator protein TDRD3 to activate ERα target genes. Interestingly, a high-resolution MS analysis of CARM1 substrates identified a list of coregulators involved in ERα transcription (called CARM1 methylome), such as acetyltransferases (P300, P400), KMTs (KMT2C and KMT2D) and components of the SWI/SNF, NuRD, and mediator complexes (232). Of note, JMJD6 regulates also the activation of ERα enhancers by participating in the interaction between MED12 and CARM1 (446) (Fig. 5B).

Aside from CARM1, PRMT1 also dimethylates coregulators to modulate SR signaling. For example, PRMT1 dimethylates arginines on the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a coactivator of many SRs, including ERα (447). PRMT1 and its catalytic activity enhances PGC-1α coactivator activity on ERα reporter genes (Fig. 5B). Endogenously, PRMT1 regulates the expression of PGC-1α target genes that are important for mitochondrial biogenesis (447).

Altogether, these results highlight that methylation of coregulators and histones is heavily involved in the fine regulation of the transcriptional activity of SRs, making these enzymes promising targets to modulate SR functions.

Outlook

There is increasing evidence that SRs are tightly regulated by protein interactions and PTMs targeting the receptors themselves, but also histone tails and coregulatory proteins. Among these PTMs, methylation has assumed an increasingly important role, and given the crosstalk between PTMs, we can extrapolate that the effects extend beyond current knowledge.

Even if ERα appears to be the most post-translationally modified SR (probably because it is the most studied), it is likely that other receptors are also methylated on residues that remain to be identified. This review focused on 4 SRs, but we can easily imagine that other NRs are modified by methylation. It has already been shown for the orphan receptor HNF4, the transcriptional activity of which is regulated by PRMT1 in 2 ways (448): it methylates HNF4-DBD, enhancing its binding to chromatin, and methylates H4R3. In addition, RARα is also methylated on lysine residues, impacting its transcriptional activity (449). Although the thyroid hormone receptor has so far not been shown to be methylated, PRMT1 can regulate its transcriptional activity through H4R3 methylation, contributing to T3-induced metamorphosis of *Xenopus laevis* (450). LSD1 is also suspected to be a functionally important cofactor for the mineralocorticoid (MR) and MR-related disease, such as high blood pressure (451).

In this review, we focused on some methyltransferases though other enzymes may also regulate SR function via methylation. For instance, PRMT2 regulates the transcriptional activities of ERα (452) and AR (453), likely through its enzymatic activity. In addition, PRMT6 enhances ERα ligand–dependent and –independent activity (412), even if the mechanism involved has not been clearly identified.

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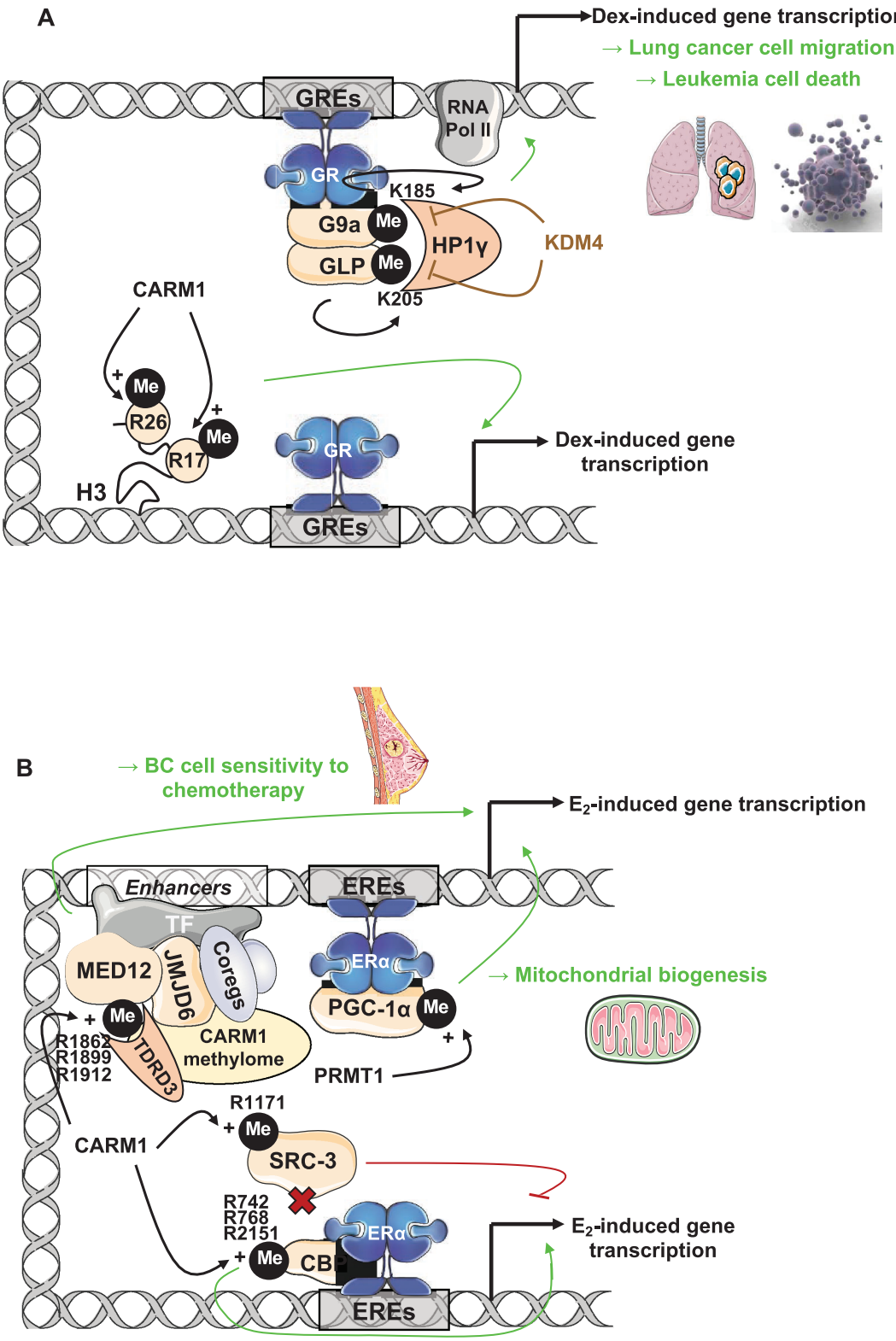

Figure 5. Indirect methylation events regulating SR signaling. Here, we highlight 2 examples, in (A) GR and in (B) ERα, of indirect methylation events (ie, not directly on SRs), regulating the transcriptional activity of these 2 receptors. This concerns the methylation of histone tails on chromatin and/or the methylation of coregulators. When identified, the targeted lysines (K) or arginines (R) and the methyltransferases are noted in black and the demethylases in brown. The methylation events leading to repressive functions are represented with red lines and the activating functions with green arrows. Me, methylation; GRE, GR response elements; ERE, estrogen response elements; H3, histone H3; H4, histone H4; CoA, coactivators; Dex, dexamethasone; E_2, estrogens; BC, breast cancer.

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Most studies have been performed in cancer cell lines, but as the effects of methylation impact key functions of SRs, such as ligand sensitivity and DNA binding, it is likely that methylation is also important for other physiological roles of SRs. For instance, ERα methylation on R260 has been identified firstly in BC, but the role of this methylation has recently been linked in vivo to vascular functions (385). Unfortunately, not all methylation events have been as extensively deciphered, likely due to the difficulty in detecting these modifications in vivo (lack of specific antibodies, lack of mouse models). More investigation is required to identify more thoroughly each methylation event for SRs themselves but also for their coregulators using MS analyses. This may provide a description of the specific pattern of methylation in each tissue and its dysregulation in specific pathologies.

Since SR activities are often linked with cancer, and methylases/demethylases also play a key role in tumorigenesis, future perspectives in cancer treatment could include targeting these enzymes, but their use could be extended to other pathologies where SRs, KMTs, and PRMTs are involved. Compared with other small molecule inhibitors, such as HDAC or RTK, developing small inhibitors of protein methyltransferases is a rising challenge for cutting-edge, drug-designing industrial enterprises.

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