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REVIEW ARTICLE

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Abstract:

Estrogen signaling pathways regulate cellular processes such as proliferation and differentiation, and if deregulated, are involved in several human pathologies. Post-translational modifications (PTMs) play important roles in estrogen signaling pathways. This review focuses on recent findings pertinent to arginine methylation of non-histone proteins and their implications in estrogen signaling. We describe protein arginine methyltransferases and demethylases, the role of methylarginine proteins in estrogen action and cross-talk with other PTMs such as phosphorylation and lysine methylation. The relationships between various PTMs form a specific code which might play an important role in hormone signaling. In addition, deregulations of arginine methylation or of enzymes responsible for these modifications could be key events in estrogen-dependent cancers such as breast cancer.

Arginine methylation was first described as a post-translational modification (PTM) of histones in the late 1960s. However, only recently the responsible enzymes (Box 1) and wide variety of substrates of this modification have been identified. While the addition of each methyl group does not modify the charge of the residue, it does increase its bulkiness and hydrophobicity. Interactions of a methylated protein with its binding partners can therefore be affected by this modification and impact the physiological functions of the substrate protein. Methylarginine substrates include transcription factors, nucleic acid-binding factors, signal transducers, splicing factors and histones. Because of the large number of substrates, protein arginine methyltransferases (PRMTs) and arginine methylation regulate various cellular processes such as cell differentiation, DNA repair, RNA processing, signal transduction, cellular localization, and apoptosis [1-3]. In this review, we describe the recent studies implicating arginine methylation in estrogen transcriptional regulation and likewise, in estrogen-related diseases.

Role of PRMTs and methylarginine proteins in estrogen signaling

Estrogen action – Genomic and non-genomic effects

Nuclear estrogen receptors act mainly as ligand-activated transcription factors [4]. The binding of estrogens such as 17β-estradiol (E2) induces a protein conformation change in the receptor that allows recruitment of coactivator complexes with chromatin-remodeling or histone-modifying activities [5] (Box 2). Steroid-regulated promoters recruit the ligand-bound receptors and regulatory proteins in an ordered, cyclical manner with multiple rounds of coactivator assembly and disassembly [6]. Histone acetylases and some methyltransferases lead to a more open chromatin and increase gene transcription. P300, an acetyltransferase, and PRMT1 and CARM1, two well-characterized PRMTs, cooperate synergistically to regulate

hormone target genes [7]. The activated estrogen receptor can also bind corepressors, such as RIP140 [8], which recruit enzymes with histone deacetylase activity (HDAC) to repress transcription. Anti-estrogen compounds like tamoxifen prevent steroid action by inducing a conformation in the ligand binding pocket of the receptor that fails to bind coactivators and allows the recruitment of corepressor proteins (such as NCoR and SMRT) together with HDACs [9].

In addition to transcriptional regulation, ERα also mediates events through its association with signaling molecules outside the nuclei and independent of its direct influence on the genome [10]. For example, estradiol triggers cell proliferation and cell survival through activation of MAPK kinases and Akt pathways [11]. These non-genomic actions of estrogens occur rapidly and independently of protein synthesis. At the molecular level, ERα palmitoylation anchors a pool of ERα at the plasma membrane [12] where it interacts with Src, PI3K and other scaffold proteins as MNAR (modulator of non-genomic activity of ER). This complex can therefore activate the downstream pathways [13]. Recently, novel non-genomic action of estrogens in breast cancer cells has been described, involving the association of membrane ERα with HDAC6. This association induces tubulin deacetylation, potentially contributing to estrogen-induced cell migration [14].

Methylarginine proteins involved in estrogen action

Arginine methylation affects estrogen-mediated transcription by modifying both histone and non-histone proteins. Since histone methylation has been widely described in a various number of reviews [15-17], this section focuses on methylation of non-histone proteins and their role in estrogen action (Table 1 and Figure 1).

Estrogen receptor

Because PRMT1 [18] and CARM1 (PRMT4) [19] are ERα coregulators (Box 2), ERα could be a target for arginine methylation. Concordant with this, a recent study described ERα as a methylarginine substrate [20]. In this study, Le Romancer *et al.* used *in vitro* methylation assays and showed that PRMT1, but not CARM1, methylated ERα within the DNA binding domain. Mutation of arginine 260 into alanine (R260A) specifically abolished the modification by PRMT1. An antibody specific to methylated R260 confirmed ERα methylation in living cells. Perhaps more interestingly, estradiol treatments of MCF7 cells drastically increased ERα methylation within 5 minutes of treatment. A decrease of the methylated form was observed within less than one hour, suggesting enzymatic removal of the methyl group. Indeed, this disappearance was not due to ERα degradation by the proteasome. Moreover, immunohistochemical experiments performed on human breast tumors showed that the methylated form of ERα was exclusively localized in the cytoplasm of breast epithelial cells. Since rapid effects have been described for non-genomic estrogen actions, the role of methylated ERα in those pathways was investigated. Interestingly, methylated ERα was essential for E2-induced assembly of ERα with Src, the p85 subunit of PI3K and the focal adhesion kinase (FAK), a Src substrate involved in the migration process. E2 activation of Akt was not observed if ERα R260 was mutated to an alanine. Collectively, these results show that ERα methylation is a prerequisite for its association with certain molecules involved in growth factor signaling. Since formation of the ERα/Src/PI3K/FAK complex activates Akt and corresponding downstream pathways, ERα methylation is likely involved in regulating cell proliferation and cell survival.

Transcriptional coregulators

Transcriptional coregulators play critical roles in controlling ER-mediated transcription. They function through protein-protein interactions, by facilitating or inhibiting recruitment of other coregulators or specific components of the transcription machinery (Box 2). Methylation of

these cofactors may influence complex formation, enzymatic activity, subcellular localization, and stability, leading to a subtle regulation of ER-mediated transcription (Figure 1).

SRC-3: p/CIP/AIB1/SRC-3 is a member of the p160 coactivator family involved in CARM1 and PRMT1 recruitment to ER target genes [7]. SRC-3 (steroid receptor coactivator-3) protein levels are amplified in breast cancer and associated with poor prognosis [21, 22]. In fact, studies classified SRC-3 as an authentic oncogene [23, 24]. Two independent groups found that CARM1 modifies SRC-3 [25, 26], methylating it at the C-terminal region which contains the p300 and CARM1-binding sites. SRC-3 methylation, which is induced by estradiol, leads to the dissociation of CBP and CARM1 from SRC-3. Moreover, SRC-3 arginine methylation reduces its stability and causes an increase in its turnover. These results show that arginine methylation is implicated in the regulation of coregulator stability in response to estradiol. In addition to this role, arginine methylation also regulates the balance between coactivator complex assembly and disassembly. Studies suggest that repetitive association and dissociation of steroid receptors and coactivators from their target promoters may be required to maintain an activated state of transcription [6]. Altogether, these results highlight coactivator methylation as an important regulatory mechanism in hormonal signaling.

CBP/p300: Recently, Lee *et al*. reported that p300 is methylated by CARM1 at R2142 which is located within the C-terminal GRIP1 binding domain. Interestingly, methylation of R2142 inhibits the interaction between p300 and GRIP1 whereas PADI 4 removes this methylation mark, thereby enhancing the p300–GRIP1 interaction. These methylation and demethylation events alter the conformation and activity of the coactivator complex and regulate estrogen receptor-mediated transcription [27]. This provides another example of arginine methylation regulating coactivator complex assembly, conformation and function.

PGC-1α: PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) serves as a coactivator for several nuclear receptors, including ERα, as well as other transcription factors, such as nuclear respiratory factor 1 (NRF-1). Expression of PGC-1α is induced by a variety of physiological stimuli that regulate metabolic activity, such as exposure to cold, exercise, and fasting. PGC-1α regulates metabolic processes by affecting genes involved in mitochondrial biogenesis, respiration and gluconeogenesis. Teyssier *et al.* showed that the C-terminal region of PGC-1α is methylated by PRMT1 at one or more sites within a glutamate and arginine rich region. PGC-1α coactivator activity and the ability of PGC-1α to induce expression of target genes are both compromised by mutation of modified arginine residues of PGC-1α or as a result of reduced PRMT1 levels by siRNA. Because inhibition of PRMT1 leads to inhibition of the expression of some PGC-1α target genes involved in mitochondrial biogenesis, it is tempting to speculate a role for PRMT1 in this regulatory pathway [28]. However, the binding partners of methylated PGC-1α remain to be determined.

RIP140: RIP140 (Receptor Interacting Protein of 140 kDa) is a well-known ER hormone-dependent binding corepressor [8]. *In vitro* and *in vivo* arginine methylation of RIP140 by PRMT1 has been described in 3T3-L1 adipocytes [29], and liquid chromatography-tandem mass spectroscopy identified that arginine methylation occurs on R240, R650, and R948 as a mono-methyl mark, suppressing RIP140 repressive activity by two mechanisms. First, methylation of R240, located in the HDAC3 interaction domain of RIP140, impairs its interaction with HDAC3, reducing its repressive function. Second, arginine methylation of the three sites increases RIP140's interaction with CRM1, a component of the export machinery; this leads to RIP140 export to the cytoplasm, thereby reducing its nuclear repressive function. So far, RIP140 arginine methylation links to adipocyte differentiation in a physiological context. RIP140 expression enhances fat accumulation in differentiated adipocytes cells by

inhibiting lipolysis enzyme expression, whereas a constitutive hypermethylated mutant (where arginine is replaced by phenylalanine) failed to exert an effect on fat accumulation because of reduced repressor activity [29]. Because RIP140 repressive activity is inhibited by arginine methylation and because RIP140 is a key factor in estrogen signaling, it will be of interest to verify whether RIP140 arginine methylation plays a role in estrogen genomic and non-genomic pathways.

Arginine methylation cross-talks with other modifications

A code for protein PTMs

This concept has been initially proposed for histone tails which are heavily modified by methylation, in addition to other modifications like acetylation, phosphorylation, and ubiquitination. These epigenetic marks (defined as the "histone code") extend the genetic message beyond DNA sequences. They can be recognized or "read" by non-histone proteins containing for instance bromo- or chromodomains and participate in chromatin remodelling and transcriptional regulation [30, 31]. An important point deals with cross-talks that exist between the different PTMs targeting histone tails, i.e. the modification of one residue influencing the modification of neighbouring amino acids. Finally, recent publications extended these observations to non-histone proteins, with nuclear receptor and coregulator PTM coding emerging as a major level of regulation [32].

Lysine methylation of RIP140

RIP140 has been described as a substrate for several PTMs, including phosphorylation, acetylation and arginine methylation [33]. PTMs affect its subcellular distribution, protein-

protein interaction, and biological activity in adipocyte differentiation. Huq *et al*. found recently that endogenous RIP140 is also modified by lysine methylation in differentiated 3T3-L1 cells [34]. Using mass spectrometry, they found three lysine residues (K591, K653, and K757) as potential methylation sites. The loss of lysine methylation by mutation of the target sites enhances arginine methylation, suggesting a communication between lysine and arginine methylation. This study unraveled a potential code of modifications between lysine and arginine methylation, which regulates the functionality of a non-histone protein.

Phosphorylation regulates arginine methylation

The proximity of SRC-3 methylation and phosphorylation sites suggests potential cross- talk between methylation and phosphorylation of SRC-3. Indeed, Naeem *et al.* showed that phosphorylation of SRC-3 decreased its methylation by approximately fivefold, indicating that prior phosphorylation antagonizes methylation at least *in vitro* [26]. By contrast, RIP140 phosphorylation by PKCε triggers its arginine methylation by inducing subsequent recruitment of the chaperone 14-3-3 necessary for PRMT1 recruitment and therefore RIP140 methylation [35]. The combination of these PTMs stimulates RIP140 nuclear export and decreases its repressive activity. Altogether, these studies show that arginine methylation of a non-histone protein can be influenced by phosphorylation, enhancing the arguments in favor of a code of modifications extended to coregulators.

Regulation of PRMT activity: effects on estrogen signaling

Protein-protein interactions

Recent studies demonstrate that the methyltransferase activity of PRMTs can be modulated by protein–protein interactions**.** PRMT1 was initially identified as an interactor of the antiproliferative proteins BTG1 (B-cell translocation gene 1) and TIS21/BTG2, stimulating its

activity towards selected substrates [36, 37]. PRMT1 activity is also modulated in a substrate-dependent manner by the BTG protein partner, hCAF1 (CCR4 associated factor 1) [38], which specifically inhibits Sam68 and histone H4 Arg3 methylation [39]. Notably histone H4, when methylated by PRMT1 at Arg3, becomes a better substrate for p300, whereas acetylation of H4 by p300 inhibits its methylation by PRMT1 [40, 41]. This cross-talk has been described to contribute to the complex "histone code" in hormone signaling. Because both hCAF1 [42] and PRMT1 [18] have been described as transcriptional regulators of the nuclear receptor response, these results suggest a putative mechanism for hCAF1 in estrogen-stimulated transcription through participation in PTM coding.

CARM1 activity is also regulated by protein-protein interactions. Indeed, CARM1 is a component of a nucleosomal methylation activator complex (NUMAC) and interacts with BRG1 (brahma-related gene 1), among others [43]. Once CARM1 interacts with BRG1, it can then methylate histones. Moreover, CARM1 and BRG1 are both recruited to ER-target genes and cooperatively activate ER-dependent transcription [43]. Therefore, modulation of PRMT1 and CARM1 activities by protein-protein interaction can be considered an important component of regulation in estrogen signaling pathway.

Phosphorylation of CARM1

The methyltransferase activity of CARM1 is negatively regulated by phosphorylation. Two groups describe phosphorylation of CARM1 at two different serine residues (S229, S217) during mitosis [44, 45]. Both phosphorylations abolish CARM1's ability to bind the methyl donor adenosyl-methionine and subsequently inhibit CARM1 methyltransferase activity. In both cases, CARM1 transactivation of estrogen receptor-dependent transcription is reduced. Moreover, phosphorylation at S217 promotes CARM1 cytoplasmic localization, which occurs mainly during mitosis, suggesting that the CARM1 methyltransferase activity is turned off

during mitosis when gene transcription is silent and turned on in G1 phase when gene transcription becomes active. Deregulation of this precise switch of CARM1 activity may affect progression of the cell cycle in breast cancer cells. Indeed, it was shown that CARM1 is involved in estrogen-induced cell cycle progression of MCF-7 breast cancer cells [46].

Arginine methylation and estrogen-dependent cancers

Prmt knockout mice have been developed, providing interesting findings on the relevance of arginine methylation *in vivo* [1]. For instance, *Prmt1* and *Carm1* gene disruptions result in an embryonically lethal phenotype and neonatal death respectively, confirming a fundamental role for these enzymes in cellular metabolism [47, 48]. However, until now, these genetically modified animals have not provided information regarding roles for PRMT in estrogen-dependent physiological processes. By contrast, recent studies using microarray and quantitative PCR-based approaches described the aberrant expression of arginine methylation enzymes in estrogen-dependent cancers (Table 2).

PRMT expression in estrogen-related cancers

Several recent papers analyzed PRMT expression in estrogen-dependent cancers. P*rmt*1 and F*bxo*11 expression is up-regulated in high tumor grade breast carcinomas [49, 50] and P*rmt*2, 5 and 10 expression is down-regulated in breast carcinomas [51, 52]. In ovarian adenocarcinomas, P*rmt*1, 2 and 5 expression is down-regulated compared to normal tissues [53, 54].

P*rmt*1 isoforms exist as a result of alternative mRNA splicing, and amino acid sequence comparison indicates that they are all enzymatically active, but with different N-terminal hydrophobic regions. Goulet *et al*. found that the expression profile of P*rmt*1 splicing variants

is altered in breast cancer [55]. Furthermore, this study showed that increased P*rmt*1 expression was detected in human breast tumor samples compared with adjacent normal breast tissue, confirming the studies mentioned previously. Strikingly, increased arginine-methylated protein levels were also observed in breast cancer cell lines. Therefore, an altered P*rmt*1 isoform expression profile correlates with a differential pattern of arginine methylation in breast cancer cell lines, suggesting that misregulation of arginine methylation could contribute to the propagation of breast cancer.

Increased PADI4 expression in breast tumors

Immunohistochemistry detected significant PADI4 expression in various malignancies including breast carcinomas, endometrial carcinomas and uterine adenocarcinomas, with no detectable PADI4 expression in benign and healthy tissues [56]. Quantitative PCR and western blot analyses also showed higher PADI4 mRNA and protein levels in malignant tissues compared to benign and non-tumor tissues [57]. Interestingly, in MCF7 breast cancer cells, *PADI4* mRNA expression gradually increased with time after estradiol stimulation through both classical and non-classical ER-mediated pathways [58]. Altogether, these results suggest increased PADI4 expression in breast cancer tissues, probably in response to estradiol.

CARM1/E2F1 breast cancer growth induced by estrogens

ERα controls the expression of cell cycle genes which in turn mediate breast cancer proliferation. Frietze *et al*. showed that CARM1 is essential for estrogen-induced cell cycle progression in MCF-7 breast cancer cells. Upon silencing of CARM1 by siRNA, the E2-mediated stimulation of MCF-7 cell cycle progression was strongly reduced. This silencing resulted in decreased expression of E2F1 and E2F1-target genes (cyclin E1, cyclin A,

cdc25A), providing a direct link between CARM1 and cell cycle regulation and identifying CARM1 as a potential new target in the treatment of estrogen-dependent breast cancer [46]. Aberrant expression of CARM1 has also been linked to human breast cancer [59], with elevated CARM1 levels found in aggressive breast tumors that also express high levels of the oncogenic coactivator AIB1 (amplified in breast cancer 1). Compiled high levels of CARM1 and AIB1 could work in synergy to enhance target gene expression and thereby cell proliferation.

ERα methylation in breast cancer

In MCF-7 cells, E2-induced ERα methylation is transitory suggesting the involvement of a not yet identified arginine demethylase whose expression or activity could be deregulated in breast cancer [20]. The evaluation of methylated ERα with a specific antibody showed that ERα is hypermethylated in 50% of human breast tumors [20]. Because ERα methylation is necessary for estrogen-induced cellular kinase pathways, this deregulation in breast cancer could lead to sustained activation of those kinases which in turn would activate estrogen signaling. This bidirectional crosstalk appears to play a critical role in breast cancer development by maintaining the activation of signaling pathways and survival of breast cancer cells even in the presence of tamoxifen [60]. It is then tempting to speculate that the deregulation of ERα methylation may be involved in breast tumorigenesis and resistance to hormonal therapy. Further analyses are needed to consider methylated ERα a new prognostic marker.

Concluding remarks

Arginine methylation impacts various levels of regulation in estrogen signaling, and thereby appears to be a key regulatory event in genomic and non-genomic estrogen actions. In

genomic events, arginine methylation of transcriptional coregulators influences coregulators complex formation, activity, subcellular localization, and stability. Methylation of histones participates in chromatin remodeling in concert with other PTMs according to the histone code. In non-genomic events, estrogen receptor methylation is necessary for the formation of a transduction signaling complex and may participate in downstream events. Although, recent findings strongly enhanced our knowledge of the role of arginine methylation in estrogen signaling, a complete understanding will only be realized through answering fundamental questions (Box 3). For instance, since expression of other PRMTs beside PRMT1 and CARM1 is deregulated in estrogen-dependent cancers (Table 2), could other PRMTs be also involved in genomic and non-genomic estrogen regulatory mechanisms? Are other substrates implicated in estrogen signaling?

Deregulation of arginine methylation, methyltransferases and demethylases are described in estrogen-related cancers, strengthening the notion of a connection between arginine methylation patterns and cancer progression. Moreover, hypermethylation of ERα in human breast cancers may indicate that methylarginine proteins represent novel interesting prognostic biomarkers. Moreover, inhibiting methyltransferase activity, in particular that of PRMT1 and CARM1, by selective molecules appears as a potential therapeutic tool. Recently, using an approach based on a protein virtual screen, Spannhoff *et al*. identified specific PRMT1 inhibitors. These compounds operate as a brake on steroid hormone actions, suggesting their potential for future drug development in cancer therapy [61]. There is little doubt that detailed insights into the function and regulation of arginine methylation will unravel the pathogenesis of various diseases, in particular hormone-dependent cancers, and eventually contribute to the discovery of novel biological markers or therapeutic targets.

Box 1. Arginine methylation enzymes

The enzymes responsible for arginine methylation are called protein arginine methyltransferases (PRMTs). So far, eleven members have been identified in the PRMT family [62, 63]. PRMT-encoding genes are well-conserved through evolution [63], sharing common structural and functional domains (Figure I). Although circumstantial evidence over the past 40 years depicted arginine methylation as an irreversible PTM, some data suggest that this modification could be reversed. For instance, histone H4 is transiently and cyclically methylated on arginine 3 [64] and estrogen receptor α methylation also appears transient [20]. While enzymes capable of removing or preventing such methylation have been identified, their roles are still controversial. Peptidylarginine deiminase 4 (PADI4) has been described to convert monomethylated arginine to citrulline by deimination [27, 65, 66]. However, a full reversion would then need an enzyme to convert citrullines to arginine residues. More importantly, PADI enzymes do not deiminate methylated arginine residues *in vitro* [67, 68], and it seems rather that histone citrunillation simply interferes with methylation of arginine residues. The first histone demethylase removing asymmetrical dimethylation at arginine 2 of histone H3 and symmetrical dimethylation at arginine 3 of histone H4 is the Jumonji domain-containing 6 protein (JMJD6) [69]. However, no publication has confirmed these results and, very recently, the Bottger's group demonstrated that JMJD6 is a lysine hydroxylase involved in RNA splicing indicating that demethylation of methylarginine residues is not its major activity [70]. Altogether, this suggests that additional arginine demethylases remain to be identified.

Box 2. Estrogen receptor and coregulator complexes

Estrogens or anti-estrogens induce ER ligand binding domain (LBD) conformational change, DNA binding to specific response elements in promoter regions of target genes and recruitment of coregulator complexes [4]. Agonist-induced LBD conformational change allows recruitment of coactivator complexes composed of p160 coactivators or PGC-1α and of histone-modifying enzymes (p300, CARM1 and PRMT1), whereas antagonists permit recruitment of corepressors complexes containing SMRT, NCoR and HDAC enzymes. Recruitment of coactivator complexes helps to pull down chromatin remodeling ATPase complexes (e.g. BRG1, SWI/SNF1), which participate in chromatin remodeling. This event facilitates the recruitment of the Mediator complex and thereby of the transcription machinery (which contains among others the RNA Polymerase II and the TATA Binding Protein, TBP) to the initiation start point [6]. Enzymes associated with coactivator complexes are acetyltransferases (p300, SRC-1, SRC-3) and methyltransferases (CARM1, PRMT1). They modify histone tails inducing the open state of chromatin and gene expression. On the contrary, HDACs deacetylate histones leading to the closed conformation of chromatin and gene repression. In the presence of estradiol, ER can also bind corepressors, such as RIP140 which interacts with HDACs, leading to gene repression (Figure I).

Box 3. Outstanding questions

- Arginine methylome and methylarginine target proteins

A fundamental issue is to define the entire arginine methylome in estrogen signaling, i.e. to set up proteomic approaches with high-performance mass spectrometry methods in order to describe all the methylated arginine residues in proteins involved in the estrogen pathway. In addition, although several methyl lysine-binding proteins have been identified [71], effectors for arginine-methylated proteins remain to be found. Actually, only three mammalian proteins have been demonstrated to bind methylarginine motifs through their Tudor domains [72, 73]. Defining the proteins which recognize methylated arginines of estrogen receptors and coregulators will certainly enhance our understanding of the downstream cascades dependent on arginine methylation in estrogen signaling.

- Regulation of arginine methylation

Studies addressing the integration of signal transduction and arginine methylation pathways are also needed. Specifically, how are arginine methylation and PRMT activities regulated by PTMs such as phosphorylation, acetylation, ubiquitination, sumoylation and trans- or automethylation? How are PRMT activity and expression regulated by estrogens or other stimuli? Finally, we also clearly need a better characterization of the enzymes that demethylate arginine residues.

- In vivo function of arginine methylation

Most of the published studies discuss the importance of arginine methylation in a cellular context. It will be of great interest to elucidate further the *in vivo* function of the modifications in estrogen target tissues. To achieve this goal, conditional knock-out approaches targeting specifically breast, ovarian or uterus tissues together with knock-in strategies in mice with unmethylatable mutants will be of a great help.

Acknowledgments

This work was supported by a grant from the "Association pour la Recherche sur le Cancer" (#3169) to L.C. and V.C. and the Ligue Contre le Cancer (L.C.). C.T. is funded by the "Ligue Contre le Cancer"

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Figure legends

Figures inside Boxes:

Box 1. Figure I– The PRMT family

The nine members of the mammalian PRMT family are shown. Recently, two related members, FBXO11 (also called PRMT9) and FBXO10, have been added to the family [63, 76]. The common methyltransferase domain consists of a series of short conserved motifs (blue bars) that are important for binding the methyl donor and for catalysis. This catalytic core region is formed by a Rossman fold and two α helices (black boxes). The less conserved β-barrel structure (gray boxes) folds against the catalytic region to form the protein substrate binding cleft [77]. Additional specific motifs, such as the SH3 domain (SH3), the zinc finger (ZnF), the myristylation motif (Myr), the FBox motif and the tetratricopeptide repeat motif (TPR) are represented in green boxes. CARM1 uniquely contains a substantial C-terminal region which contains an autonomous transcriptional activation domain [78].

Box 2. Figure I- Gene regulation mechanism by estrogen receptor and its coregulators

Estrogen receptor (ER) binds to specific regions called estrogen response element (ERE) represented by a pink box on the DNA target gene drawn as a thick black line wrapped around histones symbolized by yellow plots. The initiation start point is represented by a black arrow. The green and red arrows represent the impacts of coactivator and corepressor complexes on chromatin state and gene transcription.

Figure 1 –Arginine methylation and estrogen signaling

Methylarginine proteins are involved in genomic and non-genomic estrogen actions.

(a) In estrogen genomic action, histones and numerous estrogen receptor α (ER) coregulators are substrates for PRMTs. Arginine methylation regulates their transcriptional activity (PGC-

1α), their subcellular localization (RIP140), their stability (SRC-3) and their complex assembly (p300-GRIP1). The removing methyl mark enzymatic function of PADI4 regulates the assembly of the complex containing GRIP1, CARM1 and p300. This action is represented by a yellow dotted line.

(b) In non-genomic estrogen action, induction of arginine methylation of cytoplasmic ERα by estradiol leads to activation of downstream kinase cascades and corresponding target gene activation. The role of methylated RIP140 in this non-genomic pathway remains to be demonstrated. In both figures, the methyl mark is represented by a pink circle.

Figure

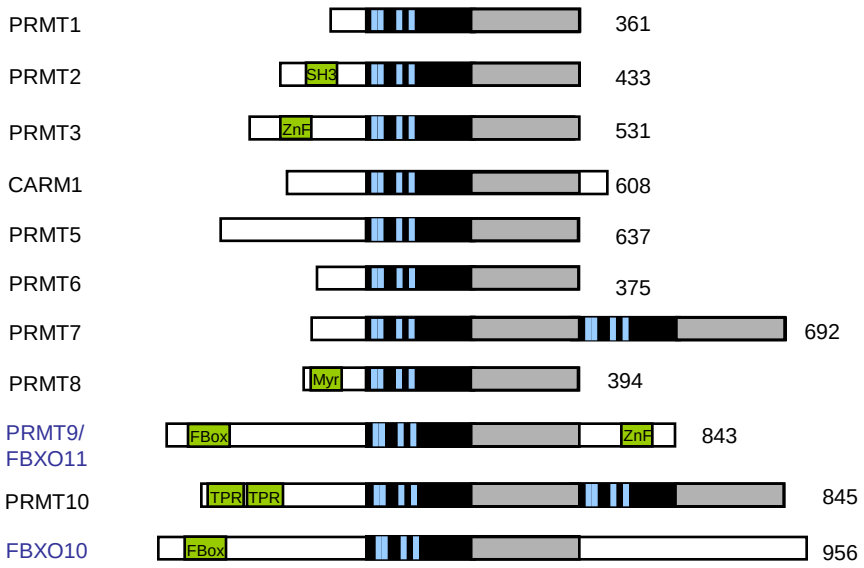

Figure I

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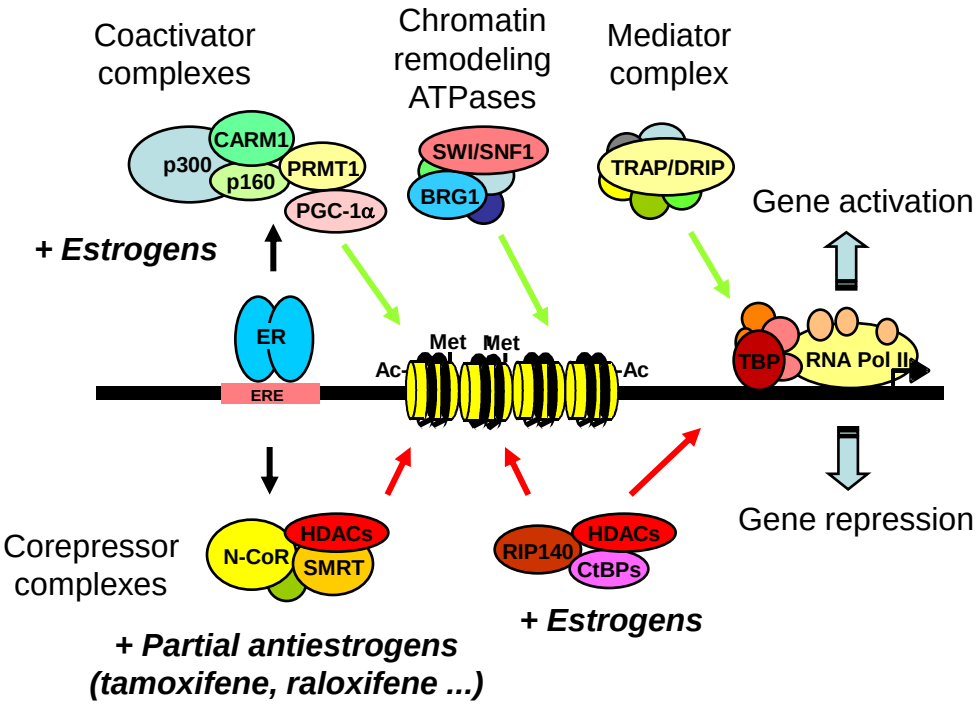

Figure I

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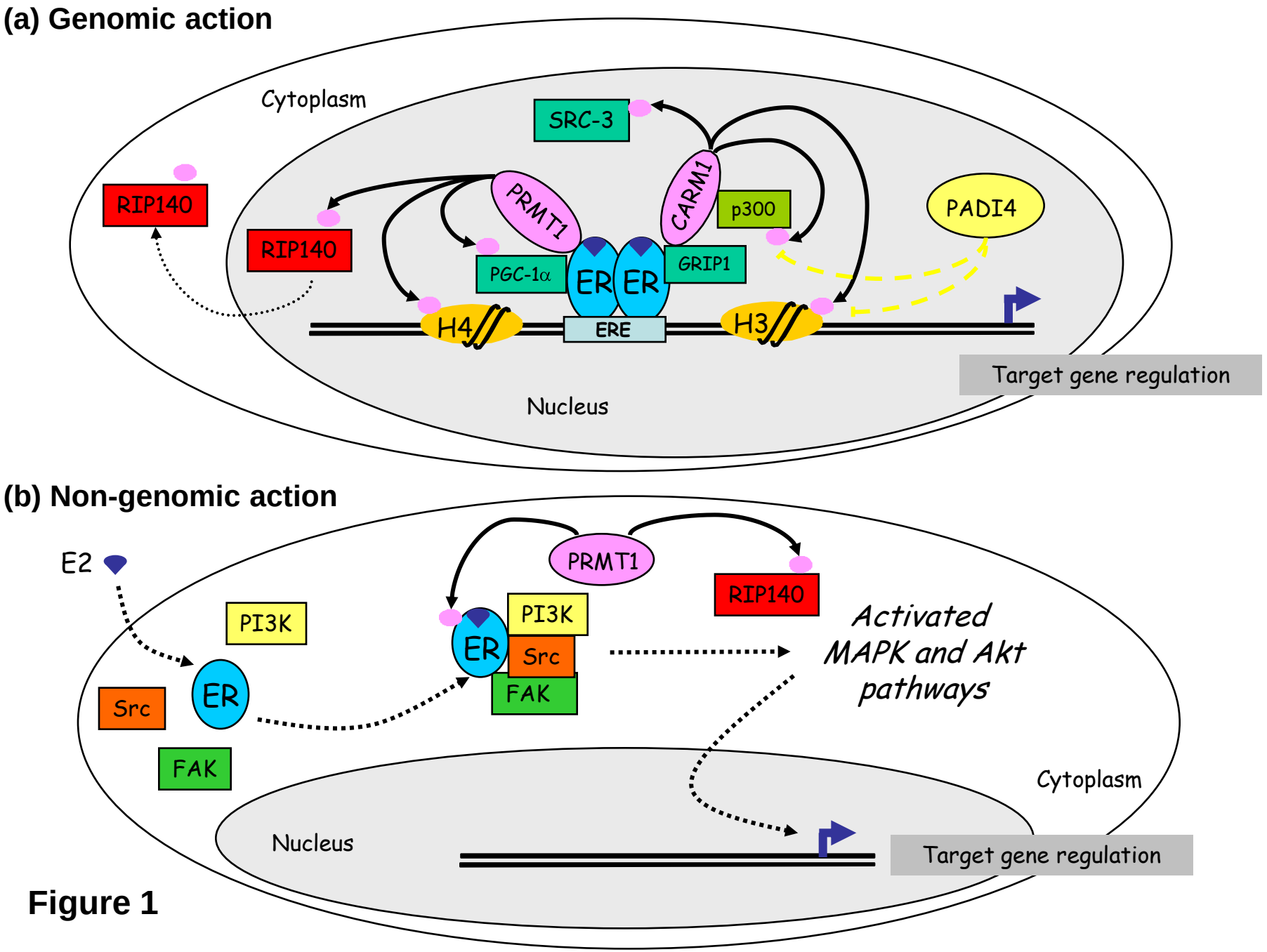

Figure 1

Table 1. Methylarginine proteins involved in estrogen pathway

Enzyme	Substrates	Arginine (R)	Impact	Refs
PRMT1	Histone H4	R3	Gene activation	[40]
	ERα	R260	Interaction with Src and PI3K	[20]
	PGC-1α	R665, 667, 669	Stimulation of coactivator activity	[28]
	RIP140	R240, 650, 948	Inhibition of repressive activity	[29]
CARM1	Histone H3	R2, 17, 26	Gene activation	[19, 74]
	p300/CBP	R2142	Complex assembly regulation	[27]
	SRC-3	R1171	Complex assembly regulation	[25, 26]
		R1178, 1184, 1195	Activity and stability regulation	

Table 2. Arginine methylation enzymes in estrogen-dependent cancers

Enzyme (Locus)	Expression levels	Refs
PRMT1 (19q13.3)	Lower levels in OC	[53]
	Lower levels in p53 mutant BC	[54]
	Expression increases in high tumor grade BC	[49]
	Splicing variants expression altered in BC [b]	[55]
PRMT2 (21q22.3)	Lower levels in ER negative BC	[51]
	Lower levels in OC	[54]
PRMT3 (11p15.1)		
PRMT4 (19p13.2)	Higher levels in aggressive BC [b]	[59]
PRMT5 (14q11.2)	Lower levels in BC	[52]
	Lower levels in OC	[54]
PRMT6 (1p13.3)		
PRMT7 (16q22.1)		
PRMT8 (12p13.3)		
FBXO11 (2p16.3)	Expression increases in high grade BC	[50]
PRMT10 (9p13.2)	Lower levels in BC	[52]
	Lower levels in ER negative and PR negative BC	[52]
PADI4 (1p36.13)	Lower levels in ER negative BC	[75]
	Expression increases in BC [b]	[57]
	Expression increases in MCF-7 cells [b]	[58]
JMJD6 (17q25)	Expression increases in high tumor grade BC	[49, 53]

[a]Abbreviations: OC: ovarian cancer; BC: breast cancer; ER: estrogen receptor
All studies were found in the Oncomine database (www.oncomine.org) but [b].