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REVIEW ARTICLE

Protein arginine methylation in estrogen signaling and estrogen-related cancers

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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 17 β -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, ER α 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, ER α palmitoylation anchors a pool of ER α 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 ER α 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 ER α coregulators (Box 2), ER α could be a target for arginine methylation. Concordant with this, a recent study described ER α 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 ER α 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 ER α methylation in living cells. Perhaps more interestingly, estradiol treatments of MCF7 cells drastically increased ER α 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 ER α degradation by the proteasome. Moreover, immunohistochemical experiments performed on human breast tumors showed that the methylated form of ER α 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 ER α in those pathways was investigated. Interestingly, methylated ER α was essential for E2-induced assembly of ER α 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 ER α R260 was mutated to an alanine. Collectively, these results show that ER α methylation is a prerequisite for its association with certain molecules involved in growth factor signaling. Since formation of the ER α /Src/PI3K/FAK complex activates Akt and corresponding downstream pathways, ER α 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-1 α : PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) serves as a coactivator for several nuclear receptors, including ER α , as well as other transcription factors, such as nuclear respiratory factor 1 (NRF-1). Expression of PGC-1 α is induced by a variety of physiological stimuli that regulate metabolic activity, such as exposure to cold, exercise, and fasting. PGC-1 α regulates metabolic processes by affecting genes involved in mitochondrial biogenesis, respiration and gluconeogenesis. Teyssier *et al.* showed that the C-terminal region of PGC-1 α is methylated by PRMT1 at one or more sites within a glutamate and arginine rich region. PGC-1 α coactivator activity and the ability of PGC-1 α to induce expression of target genes are both compromised by mutation of modified arginine residues of PGC-1 α or as a result of reduced PRMT1 levels by siRNA. Because inhibition of PRMT1 leads to inhibition of the expression of some PGC-1 α 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-1 α 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 PKC ϵ 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. *Prmt1* and *Fbxo11* expression is up-regulated in high tumor grade breast carcinomas [49, 50] and *Prmt2*, 5 and 10 expression is down-regulated in breast carcinomas [51, 52]. In ovarian adenocarcinomas, *Prmt1*, 2 and 5 expression is down-regulated compared to normal tissues [53, 54].

Prmt1 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 *Prmt1* splicing variants

is altered in breast cancer [55]. Furthermore, this study showed that increased *Prmt1* 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 *Prmt1* 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

ER α 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.

ER α methylation in breast cancer

In MCF-7 cells, E2-induced ER α 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 ER α with a specific antibody showed that ER α is hypermethylated in 50% of human breast tumors [20]. Because ER α 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 ER α methylation may be involved in breast tumorigenesis and resistance to hormonal therapy. Further analyses are needed to consider methylated ER α 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 ER α 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 citrullination 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-1 α 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.

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References

1. Bedford, M.T., and Clarke, S.G. (2009) Protein arginine methylation in mammals: who, what, and why. *Mol Cell* 33, 1-13
2. Bedford, M.T., and Richard, S. (2005) Arginine methylation an emerging regulator of protein function. *Mol Cell* 18, 263-272
3. Aletta, J.M., and Hu, J.C. (2008) Protein arginine methylation in health and disease. *Biotechnol Annu Rev* 14, 203-224
4. O'Malley, B. (2008) The Year in Basic Science: nuclear receptors and coregulators. *Mol Endocrinol* 22, 2751-2758
5. Lonard, D.M., and O'Malley, B.W. (2006) The expanding cosmos of nuclear receptor coactivators. *Cell* 125, 411-414
6. Rosenfeld, M.G. *et al.* (2006) Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev* 20, 1405-1428
7. Lee, D.Y. *et al.* (2005) Role of protein methylation in regulation of transcription. *Endocr Rev* 26, 147-170
8. Augereau, P. *et al.* (2006) The nuclear receptor transcriptional coregulator RIP140. *Nucl Recept Signal* 4, e024
9. Jones, P.L., and Shi, Y.B. (2003) N-CoR-HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors. *Curr Top Microbiol Immunol* 274, 237-268
10. Bjornstrom, L., and Sjoberg, M. (2005) Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol* 19, 833-842
11. Migliaccio, A. *et al.* (2006) Crosstalk between EGFR and extranuclear steroid receptors. *Ann N Y Acad Sci* 1089, 194-200
12. Acconcia, F. *et al.* (2005) Palmitoylation-dependent estrogen receptor alpha membrane localization: regulation by 17beta-estradiol. *Mol Biol Cell* 16, 231-237
13. Greger, J.G. *et al.* (2007) Phosphorylation of MNAR promotes estrogen activation of phosphatidylinositol 3-kinase. *Mol Cell Biol* 27, 1904-1913
14. Azuma, K. *et al.* (2009) Association of estrogen receptor alpha and histone deacetylase 6 causes rapid deacetylation of tubulin in breast cancer cells. *Cancer Res* 69, 2935-2940
15. Litt, M. *et al.* (2009) Histone arginine methylations: their roles in chromatin dynamics and transcriptional regulation. *Biosci Rep* 29, 131-141

- 446 16. Wysocka, J. *et al.* (2006) Histone arginine methylation and its dynamic regulation.
447 *Front Biosci* 11, 344-355
- 448 17. Pal, S., and Sif, S. (2007) Interplay between chromatin remodelers and protein arginine
449 methyltransferases. *J Cell Physiol* 213, 306-315
- 450 18. Koh, S.S. *et al.* (2001) Synergistic enhancement of nuclear receptor function by p160
451 coactivators and two coactivators with protein methyltransferase activities. *J Biol Chem* 276,
452 1089-1098
- 453 19. Chen, D. *et al.* (1999) Regulation of transcription by a protein methyltransferase.
454 *Science* 284, 2174-2177
- 455 20. Le Romancer, M. *et al.* (2008) Regulation of estrogen rapid signaling through arginine
456 methylation by PRMT1. *Mol Cell* 31, 212-221
- 457 21. Lahusen, T. *et al.* (2009) The role and regulation of the nuclear receptor co-activator
458 AIB1 in breast cancer. *Breast Cancer Res Treat* 116, 225-237
- 459 22. Harigopal, M. *et al.* (2009) Estrogen receptor co-activator (AIB1) protein expression by
460 automated quantitative analysis (AQUA) in a breast cancer tissue microarray and association
461 with patient outcome. *Breast Cancer Res Treat* 115, 77-85
- 462 23. Torres-Arzuayus, M.I. *et al.* (2004) High tumor incidence and activation of the
463 PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. *Cancer Cell* 6, 263-274
- 464 24. Kuang, S.Q. *et al.* (2004) AIB1/SRC-3 deficiency affects insulin-like growth factor I
465 signaling pathway and suppresses v-Ha-ras-induced breast cancer initiation and progression in
466 mice. *Cancer Res* 64, 1875-1885
- 467 25. Feng, Q. *et al.* (2006) Signaling within a coactivator complex: methylation of SRC-
468 3/AIB1 is a molecular switch for complex disassembly. *Mol Cell Biol* 26, 7846-7857
- 469 26. Naeem, H. *et al.* (2007) The activity and stability of the transcriptional coactivator
470 p/CIP/SRC-3 are regulated by CARM1-dependent methylation. *Mol Cell Biol* 27, 120-134
- 471 27. Lee, Y.H. *et al.* (2005) Regulation of coactivator complex assembly and function by
472 protein arginine methylation and demethylation. *Proc Natl Acad Sci U S A* 102, 3611-
473 3616
- 474 28. Teyssier, C. *et al.* (2005) Activation of nuclear receptor coactivator PGC-1alpha by
475 arginine methylation. *Genes Dev* 19, 1466-1473
- 476 29. Mostaqul Huq, M.D. *et al.* (2006) Suppression of receptor interacting protein 140
477 repressive activity by protein arginine methylation. *Embo J* 25, 5094-5104
- 478 30. Kouzarides, T. (2007) Chromatin modifications and their function. *Cell* 128, 693-705
- 479 31. Taverna, S.D. *et al.* (2007) How chromatin-binding modules interpret histone
480 modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol* 14, 1025-1040
- 481 32. O'Malley, B.W. *et al.* (2008) Cracking the coregulator codes. *Curr Opin Cell Biol* 20,
482 310-315
- 483 33. Mostaqul Huq, M.D. *et al.* (2008) Post-translational modifications of nuclear co-
484 repressor RIP140: a therapeutic target for metabolic diseases. *Curr Med Chem* 15, 386-392
- 485 34. Huq, M.D. *et al.* (2009) Lysine methylation of nuclear co-repressor receptor interacting
486 protein 140. *J Proteome Res* 8, 1156-1167
- 487 35. Gupta, P. *et al.* (2008) PKCepsilon stimulated arginine methylation of RIP140 for its
488 nuclear-cytoplasmic export in adipocyte differentiation. *PLoS ONE* 3, e2658
- 489 36. Berthet, C. *et al.* (2002) Interaction of PRMT1 with BTG/TOB proteins in cell
490 signalling: molecular analysis and functional aspects. *Genes Cells* 7, 29-39
- 491 37. Lin, W.J. *et al.* (1996) The mammalian immediate-early TIS21 protein and the
492 leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. *J*
493 *Biol Chem* 271, 15034-15044

38. Rouault, J.P. *et al.* (1998) Interaction of BTG1 and p53-regulated BTG2 gene products with mCaf1, the murine homolog of a component of the yeast CCR4 transcriptional regulatory complex. *J Biol Chem* 273, 22563-22569
39. Robin-Lespinasse, Y. *et al.* (2007) hCAF1, a new regulator of PRMT1-dependent arginine methylation. *J Cell Sci* 120, 638-647
40. Wang, H. *et al.* (2001) Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* 293, 853-857
41. Huang, S. *et al.* (2005) Methylation of histone H4 by arginine methyltransferase PRMT1 is essential in vivo for many subsequent histone modifications. *Genes Dev* 19, 1885-1893
42. Prevot, D. *et al.* (2001) Relationships of the antiproliferative proteins BTG1 and BTG2 with CAF1, the human homolog of a component of the yeast CCR4 transcriptional complex: involvement in estrogen receptor alpha signaling pathway. *J Biol Chem* 276, 9640-9648
43. Xu, W. *et al.* (2004) A methylation-mediator complex in hormone signaling. *Genes Dev* 18, 144-156
44. Higashimoto, K. *et al.* (2007) Phosphorylation-mediated inactivation of coactivator-associated arginine methyltransferase 1. *Proc Natl Acad Sci U S A* 104, 12318-12323
45. Feng, Q. *et al.* (2009) Biochemical control of CARM1 enzymatic activity by phosphorylation. *J Biol Chem*
46. Frietze, S. *et al.* (2008) CARM1 regulates estrogen-stimulated breast cancer growth through up-regulation of E2F1. *Cancer Res* 68, 301-306
47. Pawlak, M.R. *et al.* (2000) Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable. *Mol Cell Biol* 20, 4859-4869
48. Yadav, N. *et al.* (2003) Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. *Proc Natl Acad Sci U S A* 100, 6464-6468
49. Miller, L.D. *et al.* (2005) An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A* 102, 13550-13555
50. van 't Veer, L.J. *et al.* (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415, 530-536
51. Richardson, A.L. *et al.* (2006) X chromosomal abnormalities in basal-like human breast cancer. *Cancer Cell* 9, 121-132
52. Finak, G. *et al.* (2008) Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 14, 518-527
53. Ivshina, A.V. *et al.* (2006) Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 66, 10292-10301
54. Hendrix, N.D. *et al.* (2006) Fibroblast growth factor 9 has oncogenic activity and is a downstream target of Wnt signaling in ovarian endometrioid adenocarcinomas. *Cancer Res* 66, 1354-1362
55. Goulet, I. *et al.* (2007) Alternative splicing yields protein arginine methyltransferase 1 isoforms with distinct activity, substrate specificity, and subcellular localization. *J Biol Chem* 282, 33009-33021
56. Chang, X., and Han, J. (2006) Expression of peptidylarginine deiminase type 4 (PAD4) in various tumors. *Mol Carcinog* 45, 183-196
57. Chang, X. *et al.* (2009) Increased PADI4 expression in blood and tissues of patients with malignant tumors. *BMC Cancer* 9, 40

58. Dong, S. *et al.* (2007) Estrogen-enhanced peptidylarginine deiminase type IV gene (PADI4) expression in MCF-7 cells is mediated by estrogen receptor-alpha-promoted transactors activator protein-1, nuclear factor-Y, and Sp1. *Mol Endocrinol* 21, 1617-1629
59. El Messaoudi, S. *et al.* (2006) Coactivator-associated arginine methyltransferase 1 (CARM1) is a positive regulator of the Cyclin E1 gene. *Proc Natl Acad Sci U S A* 103, 13351-13356
60. Tokunaga, E. *et al.* (2006) The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. *Eur J Cancer* 42, 629-635
61. Spannhoff, A. *et al.* (2009) Cancer treatment of the future: inhibitors of histone methyltransferases. *Int J Biochem Cell Biol* 41, 4-11
62. Lee, Y.H., and Stallcup, M.R. (2009) Minireview: protein arginine methylation of nonhistone proteins in transcriptional regulation. *Mol Endocrinol* 23, 425-433
63. Krause, C.D. *et al.* (2007) Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol Ther* 113, 50-87
64. Metivier, R. *et al.* (2003) Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* 115, 751-763
65. Cuthbert, G.L. *et al.* (2004) Histone deimination antagonizes arginine methylation. *Cell* 118, 545-553
66. Wang, Y. *et al.* (2004) Human PAD4 regulates histone arginine methylation levels via demethyl elimination. *Science* 306, 279-283
67. Hidaka, Y. *et al.* (2005) Methylation of the guanidino group of arginine residues prevents citrullination by peptidylarginine deiminase IV. *FEBS Lett* 579, 4088-4092
68. Raijmakers, R. *et al.* (2007) Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases in vitro. *J Mol Biol* 367, 1118-1129
69. Chang, B. *et al.* (2007) JMJD6 is a histone arginine demethylase. *Science* 318, 444-447
70. Webby, C.J. *et al.* (2009) Jmjd6 catalyses lysyl-hydroxylation of U2AF65, a protein associated with RNA splicing. *Science* 325, 90-93
71. Kim, J. *et al.* (2006) Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO Rep* 7, 397-403
72. Cote, J., and Richard, S. (2005) Tudor domains bind symmetrical dimethylated arginines. *J Biol Chem* 280, 28476-28483
73. Cheng, D. *et al.* (2007) The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. *Mol Cell* 25, 71-83
74. Bauer, U.M. *et al.* (2002) Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Rep* 3, 39-44
75. Saal, L.H. *et al.* (2007) Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad Sci U S A* 104, 7564-7569
76. Cook, J.R. *et al.* (2006) FBXO11/PRMT9, a new protein arginine methyltransferase, symmetrically dimethylates arginine residues. *Biochem Biophys Res Commun* 342, 472-481
77. Troffer-Charlier, N. *et al.* (2007) Functional insights from structures of coactivator-associated arginine methyltransferase 1 domains. *Embo J* 26, 4391-4401
78. Teyssier, C. *et al.* (2002) Requirement for multiple domains of the protein arginine methyltransferase CARM1 in its transcriptional coactivator function. *J Biol Chem* 277, 46066-46072

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 Rossmann 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-

1 α), 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 ER α 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 I

Figure

Figure I

Figure

(a) Genomic action

(b) Non-genomic action

Figure 1

Table 1. Methylarginine proteins involved in estrogen pathway

Enzyme	Substrates	Arginine (R)	Impact	Refs
PRMT1	Histone H4	R3	Gene activation	[40]
	ER α	R260	Interaction with Src and PI3K	[20]
	PGC-1 α	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 Lower levels in p53 mutant BC Expression increases in high tumor grade BC Splicing variants expression altered in BC ^b	[53] [54] [49] [55]
PRMT2 (21q22.3)	Lower levels in ER negative BC Lower levels in OC	[51] [54]
PRMT3 (11p15.1)		
PRMT4 (19p13.2)	Higher levels in aggressive BC ^b	[59]
PRMT5 (14q11.2)	Lower levels in BC Lower levels in OC	[52] [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 Lower levels in ER negative and PR negative BC	[52] [52]
PADI4 (1p36.13)	Lower levels in ER negative BC Expression increases in BC ^b Expression increases in MCF-7 cells ^b	[75] [57] [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 .		