Regulation of hormone signaling by nuclear receptor interacting proteins

Vanessa DUONG, Patrick AUGEREAU, Eric BADIA,

Stéphan JALAGUIER and Vincent CAVAILLÈS[1]

INSERM, U540, Montpellier, F-34090 France ;

Université Montpellier I, Montpellier, F-34000 France.

[1] Corresponding author : Dr V Cavaillès

INSERM, U540, 60 rue de Navacelles, Montpellier, F-34090 France ;

Phone 33 4 67 04 37 68 - Fax 33 4 67 54 05 98

E-mail v.cavailles@montp.inserm.fr

Non-standard abbreviations:

RIP140, receptor interacting protein of 140 kDa; ER, estrogen receptor; AR, androgen receptor; AhR, aryl hydrocarbon receptor; ERR, ER-related receptor ; ERE, estrogen response element; LBD, ligand binding domain ; AF, activating function; HDAC, histone deacetylase; TSA, trichostatin A; CtBP, Carboxyl-Terminal Binding Protein ; TIF2, transcriptional intermediary factor 2; Mdm2, mouse double minute 2; UV, ultraviolet.

Keywords:

Transcription repression, nuclear receptors, RIP140, Mdm2, HDAC.

Abstract

Nuclear receptors are ligand-activated transcription factors which regulate the expression of genes critical for the growth of hormone-dependent cancers. Their expression and activity are controlled by various cofactors which are important players in hormone-dependent carcinogenesis. RIP140 is a negative transcriptional regulator which is recruited by agonist-liganded receptors. Its strong repressive activity involves four silencing domains which interact with histone deacetylases (HDACs), carboxyl-terminal binding proteins (CtBPs) and additional partners. RIP140 positively regulates transactivation when nuclear receptors are recruited to target promoters through interaction with the Sp1 transcription factor. In human breast cancer cells, RIP140 expression is upregulated at the transcriptional level by various ligands of nuclear receptors revealing the existence of regulatory loops. The Mdm2 oncogenic ubiquitin-ligase is another protein which directly interacts with nuclear receptors. It is involved in a ternary complex with ERα and p53 and regulates ERα turn-over. In MCF-7 human breast cancer cells, various p53-inducing agents (such as UV irradiation) abolished E2-dependent turn-over of ERα without affecting its transactivation potential. Altogether, our results show that RIP140 and Mdm2 are two important regulators of ERα expression and activity and could therefore play major roles in hormone-dependent breast carcinogenesis.

1- Introduction

1.1 Control of nuclear receptor transactivation

Nuclear receptors are ligand-activated transcription factors which subsequently bind to specific responsive elements located in the regulatory region of target gene promoters (1). They stimulate transcription using both a constitutive amino-terminal and a ligand-dependent carboxyl-terminal activation function (AF1 and AF2, respectively), the latter being associated with the ligand-binding domain. These activation functions act independently or synergistically depending on the cell type and promoter context, by recruiting a number of cofactors (2).

1.2 Role of coregulators in hormone-related cancers

Transcription cofactors function usually as part of multimolecular complexes, acting either by stabilisation of the basal transcription machinery or by chromatin remodelling which implicates various enzymatic activities such as histone acetyltransferases and deacetylases (3). Different types of post-translational modifications (phosphorylation, acetylation…) also regulate their activity, localization or interaction with receptors (4). The physiological roles of these cofactors begin to be investigated by invalidation of the corresponding gene in mice. They are also implicated in several pathologies such as hormone insensitivity syndromes or hormone-dependent cancers (5). Clinical studies have investigated the potential interest of these molecules as prognosis or diagnosis markers. They have also

described quantitative or qualitative alterations of these genes in tumours. Finally, several approaches are underway to define whether steroid receptor transcription cofactors could be valuable targets in cancer treatment (modulation of their binding to receptors, modification of their expression or inhibition of their enzymatic activity).

2- Negative regulation of nuclear receptor activity by RIP140

RIP140 is a widely expressed protein of 1158 residues which was isolated through its recruitment by ERα AF2 in the presence of ligand (6). Subsequently, RIP140 was shown to interact with many nuclear receptors such as ERα, TR, RAR and RXR (7), AR (8), VDR (9), PPARα/LXRα (10), GR (11), SF1 and DAX-1 (12). More recently, gene knockout in mice indicated that it is an essential protein for female fertility and energy homeostasis (13, 14).

2.1 Effects on nuclear receptor activity

Our recent work has highlighted both the interaction and transrepression activity of RIP140 on different members of the nuclear receptor superfamily *i.e.* ERRs (15) and AR (16).

- Estrogen-receptor-related receptors

Estrogen-receptor-related receptors (ERRα, β and γ) exhibit strong sequence similarity to ERα (17, 18) and interfere with estrogen signaling (reviewed in (19)) in part through binding to the same DNA binding elements, namely the ERRE for ERR-response element or the classical estrogen response element (ERE) (20). As expected from

results obtained with other nuclear receptors, we have shown that different regions of RIP140 interact *in vitro* with the three ERRs (15). Indeed, we have observed a significant binding of radiolabelled ERRα, β and γ with chimaeric GST-RIP140 proteins encompassing the amino-terminal (residues 27-439), the central (residues 429-582) or the carboxyl-terminal region of the molecule (residues 683-1158). In transient transfection experiments, RIP140 inhibits ERRα, β and γ activity on ERE- or ERRE-containing reporter constructs (artificial reporter plasmids and natural promoters harbouring these binding sites such as pS2, ERRα or osteopontin) (15).

- Androgen receptor

The androgen receptor (AR) is a cytoplasmic protein which undergoes nuclear translocation upon hormone binding (21). We have recently evidenced the interaction between AR and RIP140 and delineated the interaction between the LBD of AR and several domains of RIP140 which contain LxxLL motifs (16). In R1881-treated LNCaP prostate cancer cells, RIP140 is recruited to AR-target genes such as the PSA gene and completely relocalized from small nuclear foci to a diffuse pattern. Interestingly, the antagonist-bound AR which also translocates to the nucleus is not able to induce such a redistribution of RIP140. Our results indicate that RIP140 is a *bona fide* AR repressor since it inhibits AR-mediated transactivation and reverses the TIF2-induced overactivation of AR. Moreover, in mouse embryo fibroblasts lacking the RIP140 gene, AR activity is significantly increased as compared to the wild type counterpart cells (22).

2.2 Mechanisms of transrepression

The strong transcriptional repressive activity of RIP140 which was initially attributed to competition with coactivator binding to nuclear receptors (23) also involves an intrinsic inhibitory effect due to several domains which recruit various repressive effectors. We have identified two evolutionary conserved CtBP (Carboxyl-terminal Binding Proteins) binding motifs (PIDLS and PINLS motifs located respectively between residues 440 to 444 and 565 to 569) which explain partly the repressive action of RIP140 (24). RIP140 also interacts with class I and II HDACs. We show that RIP140 directly binds HDACs through a sequence comprised between residues 115 and 199 and using fluorescent chimaeric proteins, we have evidenced its colocalization with HDAC5 in intact cells (24). However, our results indicate i) that the repressive activity of RIP140 could be partially affected by inhibition of HDAC enzymatic activity and ii), that two additional domains in the C-terminal region of the protein support strong repressive activity but do not require HDAC activity or CtBPs (24), thus suggesting that the global repressive activity of RIP140 may depend on the interplay among several negative regulatory modules.

2.3 Effect on HRE-independent transactivation

RIP140 also regulates HRE-independent transactivation by nuclear receptors which involves their indirect recruitment on target genes through protein-protein interaction in particular with AP1 or Sp1 factors

(25). We previously reported the inhibition by RIP140 of estradiol-induced AP-1-dependent transcription of ERα (26). RIP140 antagonizes the stimulatory effect of GRIP1 and competes for binding to c-Jun and ERα both *in vitro* and in intact cells. More recently, we have demonstrated that overexpression of RIP140 strongly increases ERRα- and ERRγ-mediated transactivation *via* Sp1-response sites, on both isolated sites and natural promoters (15). This positive regulation exerted by RIP140 involves HDAC either directly or indirectly as suggested by overexpression of HDAC1 or treatment with TSA.

2.4 Regulatory loops involving RIP140

Cloning of the RIP140 gene and analysis of transcriptional regulatory mechanisms revealed that it is involved in several feed-back loops. RIP140 mRNA levels are rapidly and directly increased by 17β-estradiol (E2) in MCF-7 human breast cancer cells (27). This estrogenic regulation which is preferentially mediated by ERα and not restricted to mammary cancer cells, involves a consensus ERE which allows efficient binding of ERα, both *in vitro* and in intact cells (28). Very interestingly, the regulatory feed-back loop that we have demonstrated for ERα also exists for several other nuclear receptors (such as retinoid (29) and androgen (16) receptors) or for the dioxin receptor (AhR) (28, 30).

3- Effect of Mdm2 on ER signaling

Several studies have shown that binding of E2 to ERα significantly decreases its stability. This shorter half-life in the presence of hormone appears to implicate the ubiquitin/proteasome pathway since ERα is ubiquitinated (31) and its ligand-dependent down-regulation is blocked by proteasome inhibitors (32-34).

Previous studies have suggested that ubiquitin-conjugating enzymes or ATPase subunits of the proteasome complex bind nuclear receptors and modulate their functions. In a recent study, we have shown that the Mdm2 oncoprotein is also involved in the ligand-dependent decrease of ERα stability (35).

3.1 Regulation of ERα expression and activity

The Mdm2 oncogene is overexpressed in a wide variety of human cancers (36) and its role in tumorigenesis is linked to its ability to act as an E3 ubiquitin-ligase which is required for the ubiquitination and proteasome-dependent degradation of several growth regulatory proteins including p53 (37-39).

Our data indicate that Mdm2 regulates ERα expression as a ternary complex with p53 (35). Using a modified mammalian two-hybrid system and an *in vitro* protein-protein interaction assay, we have shown that Mdm2 coexists with ERα and p53 within the same protein complex in intact cells. Using transient transfection into p53/Mdm2-/- cells, we have demonstrated that p53 and Mdm2 are required for ligand-dependent ERα turn-over. By chase experiments using cycloheximide,

we have found that Mdm2 overexpression decreases the apparent stability of the ERα protein thus confirming its role in the post-translational regulation of ERα expression. Finally, a mutant of Mdm2 (Mdm2ΔRING) deleted in the carboxyl-terminal part of the protein which contains the RING domain required for its ubiquitin-ligase activity (40) still interacts with ERα in GST-pull down experiment but does not decrease ERα accumulation as compared with the effect of its wild-type counterpart suggesting that the E3 ubiquitin-ligase activity of Mdm2 is directly involved in ERα degradation.

3.2 Effect of stress inducing agents

Since cellular stress results in an increased accumulation of p53 due mainly to the inability of Mdm2 to degrade the protein, we have tested the effect of various stress-inducing agents (which stabilize p53) on ERα turn-over (35). We show that treatments that increase p53 levels in MCF-7 human breast cancer (such as UV irradiation or treatment with RITA which inhibits the interaction of p53 with Mdm2) concomitantly suppress the hormone-dependent down-regulation of ERα. In the case of UV irradiation, our data demonstrate that the effect results from an increase in ERα stability.

3.3 Ligand-dependent turn-over and transactivation

Previous studies proposed that the E2-dependent decrease of ERα accumulation was required for transcriptional activity of the receptor (41). However, our data provide several lines of evidence showing that

the E2-dependent turnover of the receptor is not necessary for ERE-mediated transactivation (35). Indeed, we have found (using p53/Mdm2-/- cells or UV irradiation of MCF-7 cells) that ERα strongly activates transcription in cells where E2 up-regulates receptor levels thus dissociating the effect of E2 on ERα degradation and activity.

4- Conclusions

The data presented herein highlight the complexity of the regulatory mechanisms that control nuclear receptor expression and activity. RIP140 appears as an unconventional transcriptional regulator acting as an anti-coactivator. Several negative regulatory modules are involved in transcriptional repression and post-translational modifications might be key regulatory events controlling this activity. RIP140 gene expression appears also finely tuned and the different regulatory loops and cross-talks that take place could be of importance in the regulation of breast cancer proliferation by hormones and environmental contaminants. Finally, our unpublished data suggest that RIP140 could be involved in the regulation of other transcription factors which control cell cycle progression and this obviously reinforces the relevance of RIP140 as a key factor in breast carcinogenesis.

ERα turn-over is also under complex regulation and, among other factors, the Mdm2 oncogene plays an important role. It regulates concomitantly the stability of two major proteins in breast cancer *i.e.* ERα and p53, which are both stabilized upon cellular stress. Although it does not seem to be required for hormone-dependent transcriptional

activity, ligand-induced turn-over of ERα could target ER-associated factors to the proteasome and as a consequence indirectly regulate cell proliferation, apoptosis or invasion.

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References

1. Aranda A and Pascual A (2001) Nuclear hormone receptors and gene expression. Physiol Rev 81: 1269-1304.
2. Hall JM and McDonnell DP (2005) Coregulators in nuclear estrogen receptor action: from concept to therapeutic targeting. Mol Interv 5: 343-357.
3. Lonard DM and O'Malley BW (2005) Expanding functional diversity of the coactivators. Trends Biochem Sci 30: 126-132.
4. Smith CL and O'Malley BW (2004) Coregulator function: a key to understanding tissue specificity of selective receptor modulators. Endocr Rev 25: 45-71.
5. Kumar R, Gururaj AE, Vadlamudi RK, Rayala SK (2005) The clinical relevance of steroid hormone receptor corepressors. Clin Cancer Res 11: 2822-2831.
6. Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ, Parker MG (1995) Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. EMBO J 14: 3741-3751.
7. L'Horset F, Dauvois S, Heery DM, Cavailles V, Parker MG (1996) RIP-140 interacts with multiple nuclear receptors by means of two distinct sites. Mol Cell Biol 16: 6029-6036.
8. Ikonen T, Palvimo JJ, Janne OA (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen

receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. J Biol Chem 272: 29821-29828.

9. Masuyama H, Brownfield CM, St-Arnaud R, MacDonald PN (1997) Evidence for ligand-dependent intramolecular folding of the AF-2 domain in vitamin D receptor-activated transcription and coactivator interaction. Mol Endocrinol 11: 1507-1517.
10. Miyata KS, McCaw SE, Meertens LM, Patel HV, Rachubinski RA, Capone JP (1998) Receptor-interacting protein 140 interacts with and inhibits transactivation by, peroxisome proliferator-activated receptor alpha and liver-X-receptor alpha [In Process Citation]. Mol Cell Endocrinol 146: 69-76.
11. Subramaniam N, Treuter E, Okret S (1999) Receptor interacting protein RIP140 inhibits both positive and negative gene regulation by glucocorticoids. J Biol Chem 274: 18121-18127.
12. Sugawara T, Abe S, Sakuragi N, Fujimoto Y, Nomura E, Fujieda K, Saito M, Fujimoto S (2001) RIP 140 modulates transcription of the steroidogenic acute regulatory protein gene through interactions with both SF-1 and DAX-1. Endocrinology 142: 3570-3577.
13. Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, So PW, Medina-Gomez G, Vidal-Puig A, White R, Parker MG (2004) Nuclear receptor corepressor RIP140 regulates fat accumulation. Proc Natl Acad Sci U S A 101: 8437-8442.

14. White R, Leonardsson G, Rosewell I, Ann JM, Milligan S, Parker M (2000) The nuclear receptor co-repressor nrip1 (RIP140) is essential for female fertility. Nat Med 6: 1368-1374.
15. Castet A, Herledan A, Bonnet S, Jalaguier S, Vanacker JM, Cavailles V (2006) Receptor-interacting protein 140 differentially regulates estrogen receptor-related receptor transactivation depending on target genes. Mol Endocrinol 20: 1035-1047.
16. Carascossa S, Gobinet J, Georget V, Lucas A, Badia E, Castet A, White R, Nicolas JC, Cavailles V, Jalaguier S (2006) Receptor-interacting protein 140 is a repressor of the androgen receptor activity. Mol Endocrinol 20: 1506-1518.
17. Giguere V, Yang N, Segui P, Evans RM (1988) Identification of a new class of steroid hormone receptors. Nature 331: 91-94.
18. Horard B and Vanacker JM (2003) Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand. J Mol Endocrinol 31: 349-357.
19. Giguere V (2002) To ERR in the estrogen pathway. Trends Endocrinol Metab 13: 220-225.
20. Vanacker JM, Pettersson K, Gustafsson JA, Laudet V (1999) Transcriptional targets shared by estrogen receptor- related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. EMBO J 18: 4270-4279.
21. Pratt WB and Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 18: 306-360.

22. Christian M, Kiskinis E, Debevec D, Leonardsson G, White R, Parker MG (2005) RIP140-targeted repression of gene expression in adipocytes. Mol Cell Biol 25: 9383-9391.
23. Treuter E, Albrektsen T, Johansson L, Leers J, Gustafsson JA (1998) A regulatory role for RIP140 in nuclear receptor activation. Mol Endocrinol 12: 864-881.
24. Castet A, Boulahtouf A, Versini G, Bonnet S, Augereau P, Vignon F, Khochbin S, Jalaguier S, Cavailles V (2004) Multiple domains of the Receptor-Interacting Protein 140 contribute to transcription inhibition. Nucleic Acids Res 32: 1957-1966.
25. Safe S and Kim K (2004) Nuclear receptor-mediated transactivation through interaction with Sp proteins. Prog Nucleic Acid Res Mol Biol 77:1-36.: 1-36.
26. Teyssier C, Belguise K, Galtier F, Cavailles V, Chalbos D (2003) Receptor-Interacting Protein 140 Binds c-Jun and Inhibits Estradiol-Induced Activator Protein-1 Activity by Reversing Glucocorticoid Receptor-Interacting Protein 1 Effect. Mol Endocrinol 17: 287-299.
27. Thenot S, Charpin M, Bonnet S, Cavailles V (1999) Estrogen receptor cofactors expression in breast and endometrial human cancer cells. Mol Cell Endocrinol 156: 85-93.
28. Augereau P, Badia E, Fuentes M, Rabenoelina F, Corniou M, Derocq D, Balaguer P, Cavailles V (2006) Transcriptional regulation of the human NRIP1/RIP140 gene by estrogen is modulated by dioxin signalling. Mol Pharmacol 69: 1338-1346.

29. Kerley JS, Olsen SL, Freemantle SJ, Spinella MJ (2001) Transcriptional activation of the nuclear receptor corepressor RIP140 by retinoic acid: a potential negative-feedback regulatory mechanism. Biochem Biophys Res Commun 285: 969-975.
30. Kumar MB, Tarpey RW, Perdew GH (1999) Differential recruitment of coactivator RIP140 by Ah and estrogen receptors. Absence of a role for LXXLL motifs. J Biol Chem 274: 22155-22164.
31. Wijayaratne AL and McDonnell DP (2001) The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276: 35684-35692.
32. Alarid ET, Bakopoulos N, Solodin N (1999) Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. Mol Endocrinol 13: 1522-1534.
33. El Khissiin A and Leclercq G (1999) Implication of proteasome in estrogen receptor degradation. FEBS Lett 448: 160-166.
34. Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW (1999) Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci U S A 96: 1858-1862.
35. Duong V, Boulle N, Daujat M, Chauvet J, Bonnet S, Neel H, Cavailles V (2006) Differential regulation of estrogen receptor turn-over and transactivation by Mdm2 and stress-inducing agents, submitted for publication.

36. Momand J, Jung D, Wilczynski S, Niland J (1998) The MDM2 gene amplification database. Nucleic Acids Res 26: 3453-3459.
37. Honda R, Tanaka H, Yasuda H (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett 420: 25-27.
38. Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387: 296-299.
39. Michael D and Oren M (2003) The p53-Mdm2 module and the ubiquitin system. Semin Cancer Biol 13: 49-58.
40. Honda R and Yasuda H (2000) Activity of MDM2, a ubiquitin ligase, toward p53 or itself is dependent on the RING finger domain of the ligase. Oncogene 19: 1473-1476.
41. Lonard DM, Nawaz Z, Smith CL, O'Malley BW (2000) The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol Cell 5: 939-948.