Minireview

Identification of RIP140 as a nuclear receptor cofactor with a role in female reproduction

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Abstract Nuclear receptors function as ligand-dependent transcription factors by recruiting cofactors that remodel chromatin and recruit the transcription machinery. RIP140 (receptor interacting protein with a molecular weight of 140 kDa) is a widely expressed corepressor that has the potential to inhibit the transcriptional activity of most, if not all nuclear receptors. Mice devoid of RIP140 indicate that it plays a crucial role in female fertility and in adipose biology. It is essential in the ovary for ovulation, specifically oocyte release but not luteinisation of mature follicles. Our goal is to identify the key nuclear receptor(s) and their target genes responsible for ovulation.

Key words: Steroid hormones; Corepressor; RIP140; Ovary

1. Introduction

Nuclear receptors comprise a superfamily of ligand-dependent transcription factors that range from 21 members in Drosophila, 48 members in mouse and human to greater than 250 in *Caenorhabditis elegans*. Their function as transcriptional activators or repressors depends on the recruitment of cofactors to target genes where they play distinct roles. Gene activation depends on remodelling chromatin to allow the subsequent recruitment of transcription machinery. This process can be reversed to repress gene transcription transiently or ultimately to silence genes completely when they are packaged into heterochromatin. These alterations are achieved by the recruitment of coactivators and corepressors that reorganise chromatin templates for the recruitment of basal transcription factors and RNA polymerase II.

Two distinct types of chromatin remodelling activities have been well characterised. Firstly, there are enzymes that catalyse modifications of histones that make up the nucleosome core particle, including acetylation [1], methylation [2–4] and phosphorylation [5]. These modifications result in a change in the association of a nucleosome with DNA. For example, histone acetyltransferases (HATs), which acetylate lysine residues in the N-terminal tail of histones are proposed to unfold chromatin structure, thereby facilitating the binding of transcriptional regulators to promoters [1]. The second class of enzymes consists of adenosine triphosphate (ATP)-dependent chromatin remodelling complexes [6,16], which use the energy of ATP hydrolysis to locally disrupt the association of histones with DNA.

The process of transcriptional activation can be reversed by the recruitment of corepressors, including enzymes such as histone deacetylases (HDACs) or phosphatases, which remove the histone modification. Presumably chromatin remodelling activities are recruited to target promoters in a highly regulated manner by mechanisms involving complex protein–protein interactions but these have yet to be fully elucidated. Biochemical and genetic studies have identified a large number of receptor interacting proteins but whether they are all bona fide coactivators or corepressors remains to be established.

We exploited a far-Western blotting procedure to identify proteins that could interact with the ligand binding domain of the oestrogen receptor in the presence of oestrogen (Fig. 1). This analysis led to the discovery of two families of receptor interacting proteins with molecular weights of 140 and 160 kDa, RIP140 and RIP160, that were subsequently found to interact with most if not all nuclear receptors in a ligand-dependent manner [7,15]. This review will focus on the function of RIP140.

2. Properties of the RIP140 corepressor

RIP140 is a nuclear protein of 128 kDa encoded by a single gene on chromosome 21 that is highly conserved in vertebrates (Fig. 2). Binding to nuclear receptors in a ligand-dependent manner is mediated by 10 LXXLL (peptide sequence where L is leucine and X is any amino acid) or LXXLL-like motifs [8] found in many cofactors including the RIP160/p160 family of coactivators. A so-called 'charge clamp' found on the surface of the ligand binding domain of receptors induces the motif to form a helical structure that docks into a groove formed within the activation domain of the receptor called activation function 2 (AF2) [9]. Upon binding, RIP140 inhib-

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Abbreviations: RIP140, receptor interacting protein with a molecular weight of 140 kDa; HDACs, histone deacetylases; LXXLL, peptide sequence where L is leucine and X is any amino acid; CtBP, carboxy-terminal binding protein; LH, luteinising hormone; p.c., post-coitum; COX-2, cyclooxygenase 2

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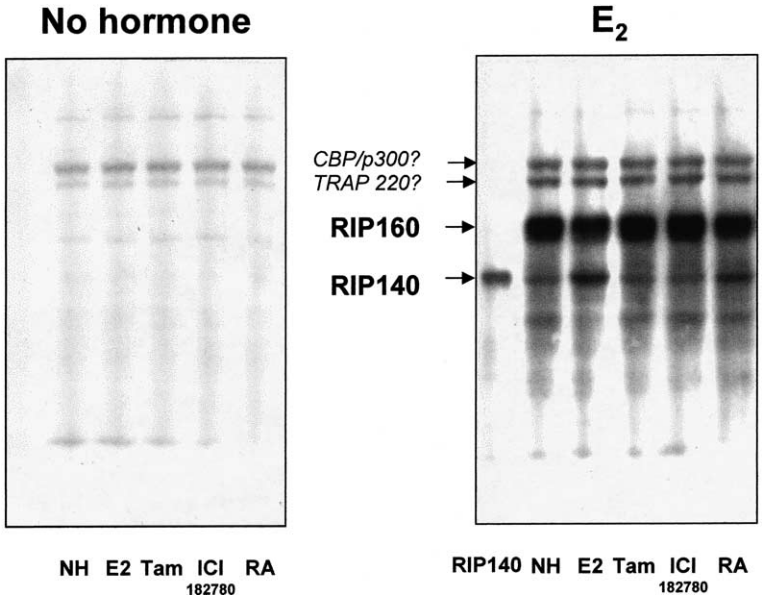

Fig. 1. Far-Western blotting to identify oestrogen receptor interacting proteins. Nuclear extracts were prepared from ZR-75 breast cancer cells, separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto nitrocellulose. Blots were probed with [^{32}P]-labelled glutathione-S-transferase-ligand binding domain (GST-LBD) in the presence or absence of 10 nM 17β-oestradiol of hormone. Prior to the preparation of nuclear extracts the ZR-75 cells were grown in 10% foetal calf serum (FCS) devoid of oestrogens (NH), 10 nM oestradiol (E2), 10 nM 4-hydroxy tamoxifen (Tam), 10 nM pure anti-oestrogen ICI 182780 and retinoic acid (RA).

its the transcriptional activity of nuclear receptors and functions as a corepressor. Transcriptional repression seems to depend on the recruitment of HDACs [10] and/or carboxy-terminal binding protein (CtBP) [11]. CtBP was identified by its interaction with adenoviral E1a repressor and shown to interact with a so-called PXDLS motif which is also found in RIP140. Recent work in our laboratory confirms the presence of these two binding sites in RIP140 but further suggests that there are additional repression domains at the C-terminus of the protein. Thus RIP140, in common with the RIP160/p160 coactivators, seems to function primarily as a platform for the recruitment of additional proteins and enzymes required for chromatin remodelling.

3. Biological role of RIP140

The role of RIP140 has been investigated by generating mice devoid of the gene [12]. Replacement of the RIP140 coding sequence with that of *lac Z* enabled us to monitor β-galactosidase activity produced under the control of the RIP140 promoter as an indication of the cellular distribution of RIP140 expression. A survey of tissues indicates that it is

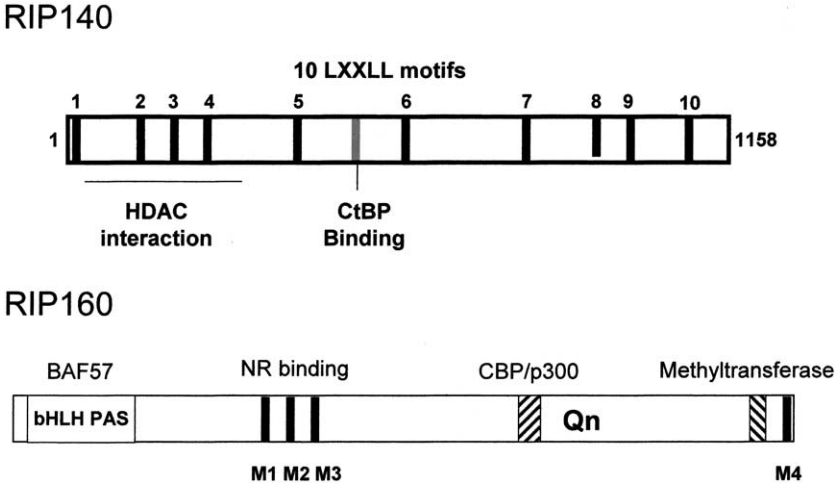

Fig. 2. Schematic organisation of RIP140 and RIP160. RIP140 comprises 1158 amino acids with nine perfect LXXLL motifs 1–9 and an LXXLL-like motif 10 together with binding sites for HDACs and CtBP. RIP160 proteins comprise a family of proteins of approximately 1400 amino acids with three conserved LXXLL motifs M1–M3 and a fourth motif M4 present in certain isoforms. In addition there is a basic helix-loop-helix region capable of interacting with BAF57, a binding site for CBP/p300, a glutamine-rich region Qn, implicated in binding androgen receptors and a binding site for a protein methyltransferase.

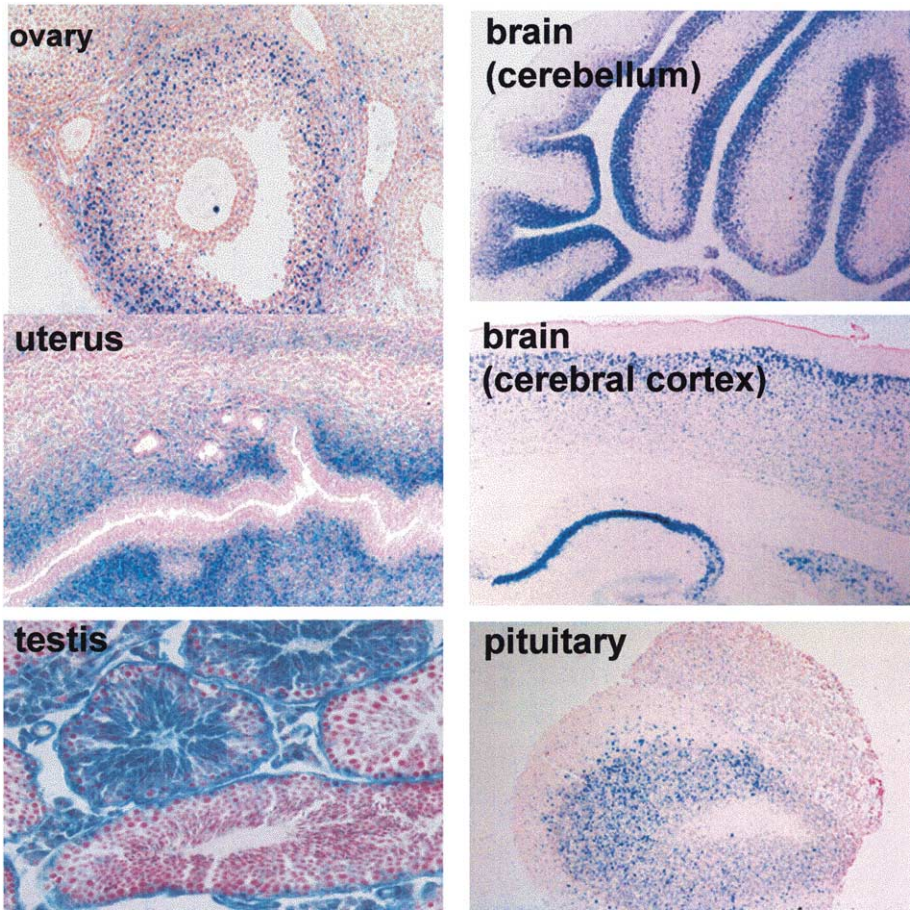

Fig. 3. Tissue distribution of RIP140. The RIP140 coding sequence has been replaced by the *lac Z* gene so that β-galactosidase activity represents a marker of RIP140 promoter activity. Expression was detected in numerous tissues including the ovaries, uterus, testes, brain and pituitary. It is noteworthy that the expression in the ovary is predominantly in mural granulosa cells with some expression in thecal cells while that in the uterus is predominantly in the stromal compartment.

widely expressed but in specific cell types at specific stages of development or hormonal control (Fig. 3). Mice devoid of RIP140 are viable but smaller than their wild-type littermates, partly due to a reduction in white adipose tissue. Strikingly, female mice are completely infertile because there is a complete failure of mature follicles to release oocytes at ovulation. Whereas the pre-ovulatory surge of luteinising hormone (LH) in normal mice normally induces both ovulation and luteinisation, in mice lacking RIP140 the oocytes are retained within the follicle (Fig. 4), while luteinisation proceeds [12]. This phenotype closely resembles that of luteinised unruptured follicle syndrome often associated with infertility in women. Therefore, the ability to suppress the action of nuclear receptors is essential for the normal coordinated control of ovarian function, with the process of oocyte release dependent on the activity of RIP140.

Two approaches indicate that ovulatory failure is due to a primary defect in the ovary itself. Firstly, oocyte release could not be induced by standard exogenous hormone treatments (pregnant mare's serum (PMSG) and human chorionic gonadotropin (HCG)) designed to 'superovulate' mice [12]. This indicates that the defect does not reflect inadequacies in the initial pituitary stimulation of follicle growth or the pre-ovulatory LH surge. Direct evidence that the defect lies at the ovarian level rather than the hypothalamic–pituitary axis comes from ovarian transplantation experiments [17]. Ovaries

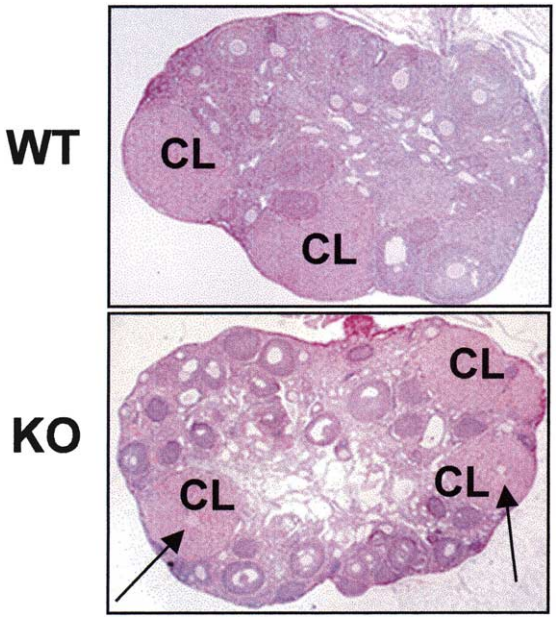

Fig. 4. RIP140 null mice exhibit an anovulatory phenotype. Ovarian sections stained with hematoxylin and eosin demonstrate the presence of retained oocytes (indicated by arrows) in mice devoid of the RIP140 gene.

from 4-week-old immature RIP140 null mice were transferred into wild-type or heterozygous littermates and vice versa. Continuous breeding experiments indicated that the transfer of ovaries from wild-type mice into RIP140 null mice rescued their fertility whereas control mice with RIP140 null ovaries generated very few pups, all of which were dead at birth or died within 72 h. Thus we conclude that RIP140 expression in the ovary is necessary for ovulation and that expression in other tissues is not essential for this process to occur.

The precise site of RIP140 action in the ovary required for ovulation has not been determined definitively. As judged by monitoring β-galactosidase activity, the highest levels of RIP140 expression are found in granulosa cells but low levels are also present in thecal and interstitial cells. No expression was detected in the granulosa cells of primary follicles or small follicles, but it became apparent during follicular maturation, with the highest expression occurring in the outer mural cells in pre-ovulatory follicles. Expression levels dramatically decreased after luteinisation but were subsequently regulated in the corpora lutea of pregnant mice (see later).

RIP140 expression was also apparent in the uterus. In non-pregnant females, expression occurred in the glandular epithelium and stroma, and became apparent in primary decidual cells around the time of implantation. By 9.5 days post-coitum (p.c.), expression was primarily detected in differentiating decidual cells on the anti-mesometrial side of the uterus. The role of RIP140 expression in the uterus was examined by performing embryo transfer experiments (thereby bypassing the initial ovulatory defect). The transfer of wild-type embryos into pseudopregnant RIP140 null females resulted in a similar number of implantation sites as for wild-type mice, indicating that uterine RIP140 is not required for implantation. At later stages of pregnancy, however, there was a progressive reduction in the number of surviving embryos in the RIP140 null mice suggesting that uterine RIP140 expression might be required to maintain the pregnancy. In addition, while the majority of pups born from control mothers survived after birth, 75% of the pups from RIP140 null mothers were dead within 24 h.

The high incidence of foetal loss in mid-pregnancy and the death of the majority of pups at or shortly after birth following embryo transfer suggests that some aspects of the maternal expression of RIP140 are suboptimal for the maintenance of pregnancy and for the subsequent survival of offspring. The embryo transfer experiments suggested that the uterine environment and mammary gland function in RIP140 null mice is suboptimal for the complete maintenance of a pregnancy and subsequent suckling of pups. However, the ovarian transfer experiments indicated that uterine and mammary gland RIP140 expression is not essential for pregnancy and survival. We conclude from this apparent disparity that the pregnancy failure and the death of pups may reflect a defect in the RIP140 null ovary which fails to support fully the function of the uterus and the mammary gland.

A continuing role of RIP140 in ovarian function during pregnancy would be consistent with the expression of RIP140 in the ovary during pregnancy. While there is negligible expression at day 6.5 p.c., expression increases at mid-gestation, is maintained until at least day 13.5 p.c. when embryonic death is evident, and then declines to negligible levels at day 17.5 p.c., correlating with initiation of the regression of the corpora lutea. It is noteworthy that while the initial failure of oocyte release did not affect luteinisation and production of progesterone in the early stages of pregnancy, the normal mid-gestation rise in progesterone levels was not as pronounced in embryo-transferred RIP140 null mice. This is despite the fact that the corpora lutea were still large and apparently healthy. Thus although RIP140 is widely expressed in the reproductive tract, its primary role in female reproductive success appears to reflect its function in the ovary.

4. Concluding remarks

The ovulatory failure exhibited by RIP140 null mice resembles that of mice devoid of progesterone receptors [13] and cyclooxygenase 2 (COX-2; [14]), which raises the question as to whether there is a functional interaction between RIP140 and these proteins. Both of these proteins are expressed in the pre-ovulatory follicle following the LH surge: the progesterone receptor is necessary for the expression of a number of proteases involved in follicular disruption, while COX-2 is required for the essential production of prostaglandin E2. It was possible, therefore, that the anovulatory phenotype of RIP140 null mice could be explained by disruption of the normal expression of either the progesterone receptor or COX-2. However this proved not to be the case and the kinetics of induction of the two genes in RIP140 null mice precisely parallels that of wild-type mice. Expression profiling has now indicated a number of other genes whose expression is altered in RIP140 null mice and we are now in the process of determining their role in ovulation.

It is conceivable that RIP140 signalling might serve as a target for contraceptive development since its deletion prevents oocyte release but does not appear to adversely affect the other events associated with the normal ovarian cycle. Therefore our future goals include the identification of the crucial nuclear receptor(s) whose activity is blocked by RIP140 in the ovary and the target genes for these nuclear receptors essential for ovulation.

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