PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 84 (2003) 167–170

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Functional interaction of estrogen receptor α and caveolin isoforms in neuronal SK-N-MC cells☆

Jürgen Zschocke[a,b], Dieter Manthey[a,b], Nadhim Bayatti[a,b], Christian Behl[a,b,*]

[a] *Institute of Physiological Chemistry and Pathobiochemistry, Johannes Gutenberg University, 55099 Mainz, Germany*
[b] *Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 München, Germany*

Abstract

Estrogen receptors (ERs) are expressed in neuronal cells and exhibit a wide variety of activities in the central nervous system. The actions of ERs are regulated in a hormone-dependent manner as well as by a number of co-activators and -repressors. A recently identified co-activator of ERα is caveolin-1 which has been shown to mediate the ligand-independent activation of this steroid receptor. In the present study we have demonstrated that neuronal SK-N-MC cells lacking functional ERα show high levels of caveolin-1/-2 specific transcripts and proteins. Ectopic expression of ERα in SK-N-MC cells leads to the transcriptional suppression of caveolin-1 and -2 genes. This silencing event is accompanied by changes in the methylation pattern of the caveolin-1 promoter. Certain CpG dinucleotides were methylated in the caveolin-1 promoter region of the SK-ERα cells whereas the same sites were non-methylated in control SK-N-MC cells, implicating a gene silencing mechanism including hypermethylation of DNA. In addition, inhibitors of methyltransferases or histone deacetylases, enzymes involved in the establishment and maintenance of silenced chromatin status, partially restored caveolin transcription in SK-ERα cells. In conclusion, our observations provide a possible mechanism of negative feedback regulation of ERα co-activator caveolin by the steroid receptor itself in this cellular model.

Keywords: Estrogen receptor α; Caveolin isoforms; SK-N-MC cells

1. Introduction

The activity of estrogen receptors (ERs) is not only regulated by the endogenous ligand estrogen, but also by a number of co-regulatory factors suppressing or enhancing ER actions [1]. Co-repressors have been shown to associate with steroid receptors which subsequently bind to target genes and suppress gene transcription. In contrast, co-activators enhance transcription of ER target genes. Both classes of regulatory factors are suggested to exert their actions partly by affecting the chromatin remodeling processes. Recently, caveolin-1 has been identified as a new co-activator of ERα [2,3]. Here, we addressed whether a putative relationship between ERα and the caveolin isoforms 1 and 2 exists using a neuronal cell line lacking functional ERα.

2. The caveolin gene family

Caveolin-1 is part of the caveolin gene family consisting of three homologous members designated caveolin-1/-2 and -3 [4]. The isoforms caveolin-1/-2 and -3 show distinct expression patterns. While caveolin-3 is mainly restricted to muscle tissue [5], caveolin-1/-2 are abundantly expressed in endothelial cells, adipocytes and fibroblasts [6]. Caveolin proteins are one of the major components of caveolae, vesicular invaginations of the cell membrane involved in cellular transport and signaling. These structures are enriched in cholesterol and a number of signaling molecules, such as heterotrimeric G proteins, e-NOS, Src tyrosine kinases, which are physically attached to the scaffolding domain of caveolin proteins [7–9]. Through this interaction most of the signaling molecules are held in an inactive conformation, suggesting a role for caveolin as a negative regulator of various signaling pathways.

3. The function of caveolin in the brain

In the recent years, there is accumulating evidence for the presence of caveolin proteins in the central nervous system

☆ Poster paper presented at the 15th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology, "Recent Advances in Steroid Biochemistry and Molecular Biology", Munich, Germany, 17–20 May 2002.

* Corresponding author. Tel.: +49-6131-39-25890; fax: +49-6131-39-25792.
E-mail address: cbehl@uni-mainz.de (C. Behl).

0960-0760/03/$ – see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-0760(03)00026-8

depending on the developmental stage, brain region and cell type analyzed [10,11]. Caveolae like structures have been reported to be potential sites for the localization of the amyloid precursor protein (APP) [12], a protein known to be involved in the pathogenesis of Alzheimer disease and, moreover, appear to participate in the processing of APP [13]. In addition, caveolin is known to play a role in the regulation of signaling through p75NTR and TrkA [14].

4. Expression of caveolin-1 and -2 in neuronal SK-N-MC cells

In contrast to most neuroblastoma cell lines, SK-N-MC cells exhibit robust levels of caveolin-1/-2 isoforms. RT-PCR analysis using caveolin isoform specific primers reveiled PCR products of the expected size for both caveolin isoforms. Western blot analysis performed with caveolin specific antibodies exhibited prominent protein bands for caveolin-1/-2. In addition, SK-N-MC cells showed a strong staining pattern for caveolin-1/-2 proteins as determined by immunostaining, which was characterized by the dotted distribution throughout the cytoplasm and the concentration in micropatches at the cell membrane.

5. Effect of ERα on caveolin expression in SK-N-MC cells

As caveolin-1 is able to potentiate ERα activity, a putative reciprocal relationship between caveolin and ERα expression is put forward in this study. So far, there are only a few studies dealing with the effects of estrogen on caveolin expression in endothelial cells [15] and smooth muscle cells [16] offering controversial results. We investigated the effect of ectopically expressed ERα in SK-N-MC cells on the expression pattern of caveolin. SK-N-MC cells lack endogenous functional ERα as determined by RT-PCR, Western blot and luciferase assay analysis. Therefore, SK-N-MC cells are a useful model to study the effects of ectopically expressed ERα (SK-ERα) on caveolin expression. Intriguingly, we observed an almost complete abrogation of caveolin-1 and -2 gene expression induced by the presence of ERα in SK-ERα cells. The expression silencing event occurs at a transcriptional level since RT-PCR performed with caveolin isoform specific primers did detect only very low amounts of caveolin transcripts compared to SK-N-MC mock transfected cells. The caveolin gene silencing in SK-ERα cells is an ER ligand-independent process. Apart from Lisanti and co-workers showing that caveolin-1 promotes ligand-independent activation of ERα [2,3], other studies also have been demonstrated that ERs may be activated in the absence of ligand in vitro and in vivo (for review [17]). Common mechanisms underlying these effects involve the trafficking of ERs to the nucleus as well as altered phosphorylation of the steroid receptors. Moreover, ERs show the capability of DNA binding in a hormone independent-manner [18]. In summary, the results provide a number of evidences that ERs mediate certain effects in the absence of ligand, also depending on the cellular paradigm tested.

The silencing process of caveolin expression in ERα-overexpressing SK-N-MC cells is unaffected by the ER antagonist ICI 182 780, suggesting a permanent caveolin silencing mechanism in SK-ERα cells.

6. Involvement of DNA methyltransferases and histone deacetylases in caveolin repression

The caveolin genes are localized in close neighborhood on chromosome 7q31.1 [19], a chromosomal locus often affected by loss of heterozygosity in several epithelial carcinomas [20]. The promoters of the caveolin genes are embedded within CpG islands [21] which are frequently found in promoter regions of housekeeping genes. Hypermethylation of CpG islands correlates in most cases with the transcriptional inactivation of the associated genes [22]. Inhibition of DNA methyltransferases and therefore the methylation of certain cytosine residues within CpG dinucleotides with 5-aza-deoxycytidine (10–100 μM) for 4 days resulted in a re-accumulation of caveolin-1 and -2 transcripts in SK-ERα cells as determined by RT-PCR. Moreover, inhibition of histone deacetylases with Trichostatin A (10–20 ng/ml) for 2 days led to re-activation of only caveolin-1 transcription. In general, histone deacetylases are involved in chromatin remodeling processes, preceding or following DNA methylation, which subsequently lead to chromatin condensation and impaired access of transcription factors to DNA binding sites [23]. Results of both experiments suggest a methylation-dependent gene silencing event occurring in the cases of caveolin-1/-2 in SK-ERα cells.

7. Changes in the methylation pattern of caveolin-1 promoter region accompanied with caveolin-1 silencing

Hypermethylation of distinct regions of the caveolin-1 promoter was observed so far in two breast cancer cell lines MCF-7 and T-47D, both of which exhibit no detectable levels of caveolin-1 proteins [21]. We could demonstrate by bisulfite genomic sequencing, that equivalent cytosine residues were methylated within the caveolin-1 promoter derived from SK-ERα cells. In contrast, caveolin-1 expressing SK-N-MC cells have been non-methylated in all CpG sites analyzed. These results enhance the assumption of a gene silencing mechanism which involves hypermethylation. Interestingly, MCF-7 and T-47D cells endogenously express relatively high levels of ERα [24] suggesting a correlation between ERα expression and caveolin silencing via methylation in these cellular models. Further studies have to clarify whether caveolin transcription is either directly targeted by

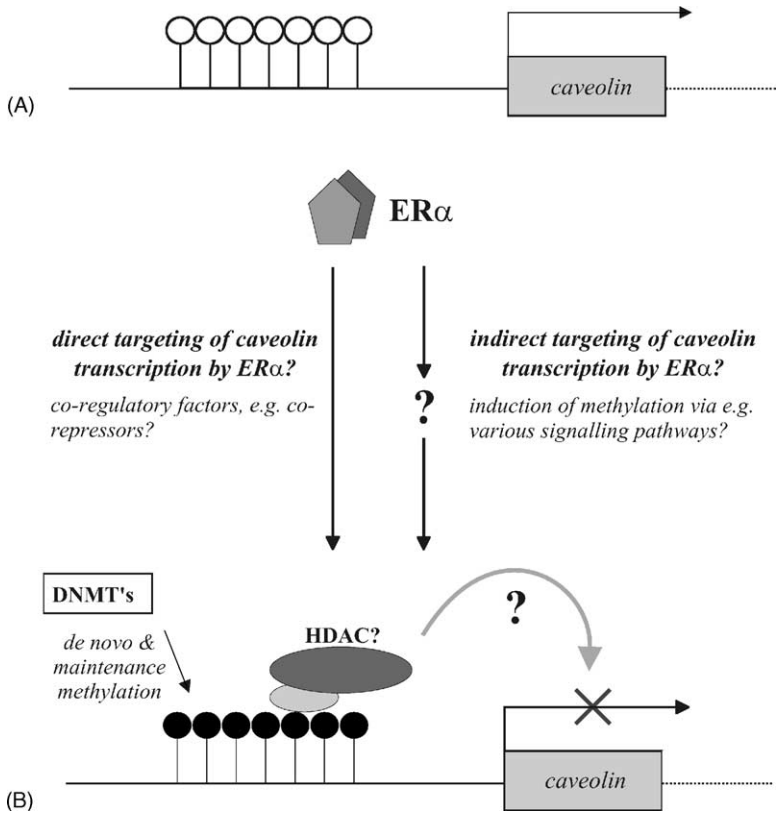

Fig. 1. A stretch of genomic DNA including parts of the caveolin-1 promoter as well as the first exon is shown (A). Particular CpG dinucleotides found in the caveolin-1 promoter are non-methylated in caveolin-1 expressing SK-N-MC cells, which is indicated by the white circles. Ectopic expression of estrogen receptor α (ERα) in SK-N-MC cells correlates with an either direct or indirect targeted down-regulation of caveolin transcription (B). This down-regulation is accompanied by a de novo methylation of the analyzed CpG dinucleotides, indicated by the black circles. The observed silencing event, which could be reversed by the use of specific inhibitors of DNA methyltransferases (DNMT's) and histone deacetylases (HDAC), supports a possible involvement of a methylation-dependent mechanism of gene regulation.

ERα or suppressed by an indirect ERα induced mechanism. A detailed analysis of the functional interaction of ERα and caveolin isoforms has been recently published [25].

8. Conclusions

We showed for the first time that the ectopic expression of ERα can lead to epigenetic alterations by means of changes in methylation patterns of the caveolin promoters resulting in altered gene activity (Fig. 1). Our studies provide a possible mechanism of negative feedback regulation of the ERα co-activator caveolin by the steroid receptor ERα. The results might also have important implications for the role of ER in tumorgenesis. In further studies we have to determine whether these effects occur in vivo in brain tissue [25].

Acknowledgements

This work is supported by grants from the Deutsche Forschungsgemeinschaft and by the European Commission in the framework of a EU-project organized by Bart van der Burg (Utrecht).

References

[1] N.J. McKenna, R.B. Lanz, B.W. O'Malley, Nuclear receptor coregulators: cellular and molecular biology, Endocr. Rev. 20 (3) (1999) 321–344.
[2] A. Schlegel, C. Wang, B.S. Katzenellenbogen, R.G. Pestell, M.P. Lisanti, Caveolin-1 potentiates estrogen receptor α (ERα) signaling. Caveolin-1 drives ligand-independent nuclear translocation and activation of ERα, J. Biol. Chem. 274 (47) (1999) 33551–33556.
[3] A. Schlegel, C. Wang, R.G. Pestell, M.P. Lisanti, Ligand-independent activation of oestrogen receptor α by caveolin-1, Biochem J. 359 (Part 1) (2001) 203–210.
[4] B. Razani, M.P. Lisanti, Caveolins and caveolae: molecular and functional relationships, Exp. Cell Res. 271 (1) (2001) 36–44.
[5] Z. Tang, P.E. Scherer, T. Okamoto, K. Song, C. Chu, D.S. Kohtz, I. Nishimoto, H.F. Lodish, M.P. Lisanti, Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle, J. Biol. Chem. 271 (4) (1996) 2255–2261.
[6] P.E. Scherer, T. Okamoto, M. Chun, I. Nishimoto, H.F. Lodish, M.P. Lisanti, Identification, sequence, and expression of caveolin-2 defines a caveolin gene family, Proc. Natl. Acad Sci. U.S.A. 93 (1) (1996) 131–135.

[7] S. Li, T. Okamoto, M. Chun, M. Sargiacomo, J.E. Casanova, S.H. Hansen, I. Nishimoto, M.P. Lisanti, Evidence for a regulated interaction between heterotrimeric G proteins and caveolin, J. Biol. Chem. 270 (26) (1995) 15693–15701.
[8] O. Feron, L. Belhassen, L. Kobzik, T.W. Smith, R.A. Kelly, T. Michel, Endothelial nitric oxidesynthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells, J. Biol. Chem. 271 (37) (1996) 22810–22814.
[9] S. Li, J. Couet, M.P. Lisanti, Src tyrosine kinases, Gα subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases, J. Biol. Chem. 71 (46) (1996) 29182–29190.
[10] P.L. Cameron, J.W. Ruffin, R. Bollag, H. Rasmussen, R. Cameron, Identification of caveolin and caveolin-related proteins in the brain, J. Neurosci. 17 (24) (1997) 9520–9535.
[11] T. Ikezu, H. Ueda, B.D. Trapp, K. Nishiyama, J.F. Sha, D. Volonte, F. Galbiati, A.L. Byrd, G. Bassell, H. Serizawa, W.S. Lane, M.P. Lisanti, T. Okamoto, Affinity-purification and characterization of caveolins from the brain: differential expression of caveolin-1, -2, and -3 in brain endothelial and astroglial cell types, Brain Res. 804 (2) (1998) 177–192.
[12] C. Bouillot, A. Prochiantz, G. Rougon, B. Allinquant, Axonal amyloid precursor protein expressed by neurons in vitro is present in a membrane fraction with caveolae-like properties, J. Biol. Chem. 271 (13) (1996) 7640–7644.
[13] T. Ikezu, B.D. Trapp, K.S. Song, A. Schlegel, M.P. Lisanti, T. Okamoto, Caveolae, plasma membrane microdomains for α-secretase-mediated processing of the amyloid precursor protein, J. Biol. Chem. 273 (17) (1998) 10485–10495.
[14] T.R. Bilderback, V.R. Gazula, M.P. Lisanti, R.T. Dobrowsky, Caveolin interacts with TrkA and p75NTR and regulates neurotrophin signaling pathways, J. Biol. Chem. 274 (1) (1999) 257–263.
[15] M. Jayachandran, T. Hayashi, D. Sumi, A. Iguchi, V.M. Miller, Temporal effects of 17β-estradiol on caveolin-1 mRNA and protein in bovine aortic endothelial cells, Am. J. Physiol. Heart Circ. Physiol. 281 (3) (2001) H1327–H1333.
[16] A. Turi, A.L. Kiss, N. Mullner, Estrogen downregulates the number of caveolae and the level of caveolin in uterine smooth muscle, Cell Biol. Int. 25 (8) (2001) 785–794.
[17] N.L. Weigel, Y. Zhang, Ligand-independent activation of steroid hormone receptors, J. Mol. Med. 76 (1998) 469–479.
[18] B.S. Katzenellenbogen, J.C. Reese, Examination of the DNA-binding ability of estrogen receptor in whole cells: implications for hormone-independent transactivation and the actions of antiestrogens, Mol. Cell. Biol. 12 (10) (1992) 4531–4538.
[19] J.A. Engelman, X.L. Zhang, M.P. Lisanti, Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers, FEBS Lett. 436 (3) (1998) 403–410.
[20] J.C. Zenklusen, J.C. Thompson, P. Troncoso, J. Kagan, C.J. Conti, Loss of heterozygosity in human primary prostate carcinomas: a possible tumor suppressor gene at 7q31.1, Cancer Res. 54 (24) (1994) 6370–6373.
[21] J.A. Engelman, X.L. Zhang, M.P. Lisanti, Sequence and detailed organization of the human caveolin-1 and -2 genes located near the D7S522 locus (7q31.1). Methylation of a CpG island in the 5′ promoter region of the caveolin-1 gene in human breast cancer cell lines, FEBS Lett. 448 (2–3) (1999) 221–230.
[22] K.D. Robertson, A.P. Wolffe, DNA methylation in health and disease, Nat. Rev. Genet. 1 (1) (2000) 11–19.
[23] H.H. Ng, A. Bird, Histone deacetylases: silencers for hire, Trends Biochem. Sci. 25 (3) (2000) 121–126.
[24] D.C. Spink, B.C. Spink, J.Q. Cao, J.A. DePasquale, B.T. Pentecost, M.J. Fasco, Y. Li, T.R. Sutter, Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells, Carcinogenesis 19 (2) (1998) 291–298.
[25] J. Zschocke, D. Manthey, N. Bayatti, B. van der Burg, S. Goodenough, C. Behl, Estrogen receptor alpha-mediated silencing of caveolin gene expression in neuronal cells, J. Biol. Chem. 277 (41) (2002) 38772–38780.