FoxA1 corrupts the antiandrogenic effect of bicalutamide but only weakly attenuates the effect of MDV3100 (Enzalutamide™)

S. Belikov a, C. Öberg a, T. Jääskeläinen b, V. Rahkama b, J.J. Palvimo b, Ö. Wrange a,*

*Department of Cell and Molecular Biology, Karolinska Institutet, SE-17177 Stockholm, Sweden
aInstitute of Biomedicine, University of Eastern Finland, Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland

A R T I C L E   I N F O

Article history:
Received 21 June 2012
Received in revised form 6 September 2012
Accepted 2 October 2012
Available online 11 October 2012

Keywords:
FoxA1
Androgen receptor
Xenopus oocyte
Chromatin presetting
Bicalutamide
MDV3100 (Enzalutamide)

A B S T R A C T

Prostate cancer growth depends on androgens. Synthetic antiandrogens are used in the cancer treatment. However, antiandrogens, such as bicalutamide (BIC), have a mixed agonist/antagonist activity. Here we compare the antiandrogenic capacity of BIC to a new antiandrogen, MDV3100 (MDV) or Enzalutamide™. By reconstitution of a hormone-regulated enhancer in Xenopus oocytes we show that both antagonists trigger the androgen receptor (AR) translocation to the nucleus, albeit with a reduced efficiency for MDV. Once in the nucleus, both AR-antagonist complexes can bind sequence specifically to DNA in vivo. The forkhead box transcription factor A (FoxA1) is a negative prognostic indicator for prostate cancer disease. FoxA1 expression presets the enhancer chromatin and makes the DNA more accessible for AR binding. In this context the BIC-AR antiandrogenic effect is seriously compromised as demonstrated by a significant chromatin remodeling and induction of a robust MMTV transcription whereas the MDV-AR complex displays a more persistent antagonistic character.

1. Introduction

Androgenic hormones and the androgen receptor (AR), a nuclear receptor family member, are driving the growth of prostate cancer cells. Treatment of prostate cancer usually involves androgen deprivation therapy (Huggins and Hodges, 1972) and the use of AR antagonists (Balk and Knudsen, 2008). These compounds compete with endogenous hormone agonists, testosterone and 5α-dihydrotestosterone (DHT), for binding to AR but do not support the formation of productive AR transcription complexes. This is due to a reduced recruitment of coactivators that are otherwise targeted to the androgen responsive enhancers by the agonist-AR complex (van de Wijngaart et al., 2012). These regimes often lead to cell death or cell cycle arrest and tumor remission. Unfortunately, the remission is usually transient and tumor usually progresses to castration resistance prostate cancer (CRPC) (Balk and Knudsen, 2008). CRPC cells also tend to depend on the AR for growth (Haendler and Cleve, 2012). Experimental studies of the development of resistance to anti-androgen therapy have indicated that an increased level of AR may shift the effect of the today often used antiandrogen, bicalutamide (BIC), into an agonist (Kelly et al., 1997; Chen et al., 2004; Makkonen et al., 2011). However, the resistance that develops during BIC exposure probably involves also other events than merely an increase in AR concentration (Amaral et al., 2012). As an example, hormone resistant prostate cancer cells were recently shown to selectively promote the expression of M-phase cell cycle genes (Wang et al., 2009). One possible route for the development of hormone resistance is the increased expression of other transcription factors (TFs) that may enhance the AR-mediated effect (Sahu et al., 2011 and see below). The mixed agonist/antagonist properties of BIC and other AR antagonists have stimulated the search for a second-generation of antiandrogens with more purely antagonistic properties. One such example is MDV3100 (MDV) (Tran et al., 2009; van de Wijngaart et al., 2012), also dubbed Enzalutamide™. MDV was reported to have several promising properties in comparison to BIC; higher AR affinity, reduced nuclear AR translocation and impaired DNA binding and recruitment of coactivators (Tran et al., 2009).

Recent studies showed other transcription factors (TFs) to be required to assist DNA binding by the nuclear receptors at their respective target enhancers. The occurrence of a DNase I hypersensitive site (DHS) over the receptor binding site prior to hormone

© 2012 Elsevier Ireland Ltd. All rights reserved.
administration and ligand-induced nuclear translocation argues for that these TFs are involved in the formation of a preset, i.e. more open, chromatin environment at the enhancer. This results in increased access of the nuclear receptor to the chromatinized DNA. An increasing body of evidence argues for that this TF-mediated chromatin presetting is required for the receptor to bind its target DNA (Belikov et al., 2004a,b, 2009; John et al., 2008, 2011; Siersbaek et al., 2011). A common feature of these chromatin-presetting TFs is their tendency to bind constitutively to the DNA sites. Forkhead, GATA and Oct families of TFs fill this criterion and tend to cluster in composite binding sites in the vicinity of ARE (Wang et al., 2007). One of these TFs that was shown to be involved in chromatin presetting, and thereby assist the DNA binding of several steroid receptors is FoxA1 (forkhead box A1). Incidentally, the level of FoxA1 expression in prostate cancer correlates with the tendency of metastatic growth (Gerhardt et al., 2012). Moreover, the FoxA1 gene is often mutated in such tumors (Grasso et al., 2012), and the expression of wild type FoxA1 or these mutant FoxA1 variants caused an increase in tumor proliferation. During embryogenesis, FoxA1 participates in specification of liver- and other gut-specific cell types as well as prostate, lung, and mammary gland cells (Kaestner, 2010). It was dubbed a pioneer TF since FoxA1 can bind to its target site within a nucleosome and open condensed chromatin, thereby facilitating the binding of other TFs nearby (Cirillo et al., 2002; Zaret and Carroll, 2011). FoxA1 acts as a licensing factor for several steroid hormone receptors. It enhances glucocorticoid hormone action in the liver (Rigaud et al., 1991), it is enriched near glucocorticoid response elements in putative AT-20 cells (John et al., 2011), it is required for estrogen receptor (ER)-mediated gene induction in breast cancer (Eckhoute et al., 2006; Hurtado et al., 2011), for AR action in the prostate (Gao et al., 2003) and in prostate cancer cells (Lupien et al., 2008; Wang et al., 2009) and it is required together with FoxA2 to maintain gender specific AR and ER-mediated gene expression in the liver (Li et al., 2012).

The enhancer and promoter of the mouse mammary tumor virus (MMTV) is a useful model system for studies of hormone regulation by glucocorticoids (Buetti and Diggelmann, 1981) as well as androgens (Li et al., 2006). Transcription from the MMTV promoter is induced by these hormones via specific DNA binding of the hormone-activated glucocorticoid receptor (GR) or AR to a cluster of receptor response elements (Payvar et al., 1981) (Fig. 4, lower part). The reconstitution of this signal transduction pathway in Xenopus oocytes revealed hormone- and receptor-induced trans- lational nucleosome positioning in the MMTV long terminal repeat (LTR) and DNase I hypersensitivity over hormone response elements (Belikov et al., 2000), similar to that found in tissue culture cells (Zaret and Yamamoto, 1984; Richard-Foy and Hager, 1987). Furthermore, FoxA1-binding sites were defined at three separate locations within the MMTV LTR, two double sites at positions –360/–332 and –51/–39, and a single site at position –225 relative to the transcription start site (Holmqvist et al., 2005; Belikov et al., 2009) (Fig. 4, lower part). Binding of FoxA1 to the MMTV LTR rendered a more accessible chromatin structure detectable as a cluster of DHSs. FoxA1 binding also correlated with a robust enhancement of the hormone activated GR-DNA binding and increased transcription from the MMTV promoter (Belikov et al., 2009, 2012). We previously used this system to study the effect of a glucocorticoid antagonist RU486 (mifepristone™). This GR-ligand induces a weak GR-DNA binding, but fails to recruit any chromatin remodeling activity and thus also fails to induce transcription (Belikov et al., 2001, 2004a,b). However, in the presence of FoxA1 the antagonist RU486 is converted into a partial agonist in terms of induction of the MMTV transcription (Belikov et al., 2009). This resides on the capacity of RU486 to induce nuclear transcription of GR and DNA binding. These two effects and the capacity of FoxA1 to open the chromatin structure around the GR-binding sites enables the weakly activating N-terminal domain of GR to recruit coactivators and elicit a transcriptional response as demonstrated with a GR deletion mutant lacking the C-terminal ligand-binding domain (LBD) (Belikov et al., 2009). This mimics the situation in the full length GR-RU486 complex where the activa- tion capacity of the LBD is corrupted by the bound antagonist. Since this finding may be relevant for other steroid hormone recep- tor antagonists, we decided to analyze this further and to focus on the functionally related AR that is also regulating transcription at the MMTV promoter (Li et al., 2006).

To this end, we exploit the special features of the Xenopus oocyte system to compare the anti-androgenic properties of BIC and MDV in the presence or absence of FoxA1. An advantage of the Xenopus oocyte system is that protein(s) may be expressed in variable amounts by injection of corresponding in vitro transcribed mRNAs (Belikov et al., 2012). The DNA reporter is introduced by intracellular injection of circular single-stranded (ss) DNA, which in our case yielded approximately 600 million gene copies of the MMTV LTR. Importantly, the injection of ssDNA leads to second- strand DNA synthesis coupled to chromatin assembly (Almouzni and Wolffe, 1993). The so obtained chromatin shares characteristics of the chromatin of stably transfected DNA in tissue culture cells (Li et al., 2006). All injected DNA copies participate in the hormone response after chromatin assembly (Belikov et al., 2000). Because of the high copy number of injected DNA, the specific TF-DNA interactions can be quantified with high precision by DMS in vivo footprinting (Belikov et al., 2004a,b, 2012).

Here, we show that the antiandrogenic effect of the BIC-AR complex is corrupted in the presence of FoxA1. This forkhead TF also reduces the AR antagonistic capacity of the MDV-complex, albeit only to a minor extent. We confirm previous findings that the MDV-AR complex is translocated less efficiently to the nuclear compartment than BIC-AR (Tran et al., 2009). However, an increased AR expression results in a correspondingly increased intranuclear uptake of MDV-AR. Importantly, the nuclear MDV-AR complex binds with a similar efficiency to the AREs as the agonist-AR complex, provided that FoxA1 is also present. However, the MDV-AR has a considerably reduced capacity to recruit coactivators, seen as a reduced chromatin remodeling and a reduced MMTV transcription.

2. Materials and methods

2.1. Reagents, plasmids and constructs

AR ligands used were R1881 (Perkin-Elmer Inc, Waltham, MA), MDV3100 (Selleck Chemicals Co. Ltd., Houston, TX) and bicalutam- ide (Bidragon Pharmservice LLC, Burlingame, CA). The reporter plmMTV:M13 contains the 1.2 kb MMTV LTR fused to the HSV TK gene and its transfer to M13 was described (Belikov et al., 2000), as well as the insertions into RN3P vectors of cDNA coding for mouse FoxA1 (Holmqvist et al., 2005) and the production of mRNAs. The human androgen receptor cDNA was kindly provided by Dr. Jiemin Wong and was described in (Li et al., 2006). The hu- man AR cDNA was cloned between the BamH1/Not I sites in the vector used for mRNA production that was described (Zernicka-Goetz et al., 1996). The clone was confirmed by DNA sequencing (Supplement 1). The variable poly-Q region harbored 20 Q resi- dues, the variable poly-G region harbored 16 G residues, the total length was 910 amino acid residues. The plasmids were linearized with Sfi I and in vitro transcribed using the mMESSAGE mMACHINE kit (Ambion) and purified on a spin column (MEGAclear, Ambion); the mRNA stocks were stable for years when saved in small ali- quotes at –83°C. In initial experiments the hAR was expressed from
a CMV driven expression vector (Thompson et al., 2001). 5 ng of this pCMV-hAR vector was then coinjected with the reporter DNA into the oocyte nuclei.

2.2. Oocyte injections

The animal experiments were conducted according to a defined protocol approved by an ethical committee. DNA and mRNA injections into *Xenopus laevis* oocytes have been described previously (Belikov et al., 2009). Indicated amount of AR mRNA and/or FoxA1 mRNA were injected into the cytoplasm, and 3 ng of the MMTV ssDNA reporter were injected in the nucleus ~6 h after the mRNA injection. Agonist, 5 nM R1881 or either of the two antagonist: 10 μM bicalutamide (BIC) or 10 μM MDV3100 (MDV) was added immediately after mRNA injection to certify the antagonistic effect, i.e. to block the effect of endogenous androgens present in *Xenopus* oocytes. Oocytes were routinely harvested ~24 h after mRNA injection.

2.3. DNase I digestion

This was described before (Belikov et al., 2009). DNase I treated samples were purified and developed as described (Belikov et al., 2000). For development of DNasel digestion pattern with indirect end-labeling assay DNA was cleaved with EcoRV, resolved in 1.5% agarose gel, vacuum transferred and hybridized with 32P-labeled Sac I-EcoRV fragment.

2.4. Quantification of the MMTV transcription by S1-nuclease protection and specific DNA binding by DMS in vivo footprinting

Usually pools of ten injected oocytes were analyzed by S1 nuclease protection assay, as described (Holmqvist et al., 2005), and/or DNA binding by DMS methylation protection. The methylation pattern was developed by primer extension as described (Belikov et al., 2001). In general, two pools of oocytes were analyzed for each experimental condition, and the average values presented as columns or a curve with the two individual sample values presented as black dots. Only one visible dot means that the two values were too close to be resolved in the diagram.

2.5. Analysis of intracellular amounts of expressed AR and FoxA1 protein

*Xenopus* oocytes were injected with mRNAs coding for AR and FoxA1 proteins followed by DNA injection and placed in oocyte medium, OR2, sometimes containing [14C]-lysine (Belikov et al., 2007). Whole oocytes or nuclei, isolated by dissection under the microscope, were extracted, the former in buffer (10 mM Hepes pH 7.8; 10% glycerol (v/v); 5 mM DTT; and protease inhibitor, complete mini, EDTA-free (Roche) one tablet/10 ml), the latter in 1x SDS sample buffer, 30 μl/five nuclei. Protein extracts were separated by 7% SDS PAGE followed by autoradiography of the dried gels or Western blot. Estimation of the relative amounts of expressed AR were described (Belikov et al., 2012). In brief, the relative amounts of different proteins were calculated based on the intensity of each protein band in an autoradiogram and quantified with a Fuji BAS-7000 bioimaging analyzer and Image Gauge 4.1 software. The same software was used to estimate the relative amounts of AR in nuclei and cytosol based on the ECL signal from Western blots using a FujiFilm LAS-1000 camera and Image Gauge 4.1 software. Several exposures were collected to certify quantification of signal intensity within the linear range.

2.6. Antibodies and siRNAs

FoxA1 was probed with an antibody from Abcam (#23738), an antibody against actin (A2103, Sigma) was used as a loading control for oocyte experiments and anti-GAPDH (Santa Cruz Biotechnology sc-25778) for mammalian cell experiments. AR antibody was described before (Makkonen et al., 2008). ON-TARGET plus SMARTPool human FOXA1 (3169) and ON-TARGET plus Non-targeting Pool were from Thermo Fisher Scientific Inc. (Lafayette, CO).

2.7. RNA interference, RT-qPCR and Western blotting

RNA interference and RT-qPCR were performed as described (Jaaskelainen et al., 2012) except that the siRNA treatment lasted 72 h, and after that, the ligands were added for the next 18 h. Western blotting of mammalian cell samples was performed as described (Rytinki et al., 2012).

2.8. Colocalization studies of AR and FoxA1 by laser confocal microscopy

HEK293 cells (Flp-In-293, from Invitrogen) on ibidi 8-well chambers (Integrated Biodiagnostics) were co-transfected with pEGFP-AR and pmCherry-FoxA1 (10 ng/well each) for 22 h. Subsequently, the cells were exposed to R1881 (10 nM), bicalutamide (BIC, 10 μM), MDV3100 (10 μM), or vehicle (ethanol) 2 h prior to microscopy. Images of live cells were captured separately by using Zeiss Axio Observer microscope equipped with Zeiss LSM 700 confocal module using 63 × magnification (EGFP at 488-nm excitation and mCherry at 555-nm excitation).

3. Results

3.1. Expression of androgen receptor (AR) in *Xenopus* oocytes

When expressing various proteins in *Xenopus* oocytes by cytoplasmic injection of *in vitro* transcribed mRNA we routinely keep the oocytes at 19°C (Belikov et al., 2012). However, when using this temperature for AR expression we recovered little if any AR protein (see Supplement 1 for the AR amino acid sequence). By increasing the temperature to either 22°C or 25°C the AR expression was robust and correlated with the amount of injected mRNA maintaining stable AR protein levels at 6–30 h (Supplement 2A). Since *Xenopus* oocytes are more stable at the lower temperature we found it optimal to use 22°C (Supplement 2A and B). The analysis of the relative AR protein levels, either by Western blot or by autoradiography of oocyte protein extract resolved by SDS-PAGE (Supplement 2A–C), rendered similar results.

3.2. FoxA1 stimulates AR-DNA binding and MMTV transcription of both agonist- and antagonist-bound AR

Several experiments were conducted to optimize the injection regime in order to address the effect of FoxA1 on the agonist/antagonist balance of the AR complexes formed with BIC or MDV. A concern is that *Xenopus* oocytes contain endogenous androgens, such as dehydroepiandrosterone (Yang et al., 2003) and testosterone, the latter at <3 nM concentration in absence of activation by hCG (Lutz et al., 2001). These hormone levels were sufficient to activate the AR when expressed by mRNA injection, since a robust MMTV transcription occurred in the absence of added exogenous androgen (c.f. Fig. 1A, second column “-“), here set to 100%. Importantly, we detected only minor level of basal MMTV transcription (0.9%) by injection of the reporter DNA alone, (Fig. 1A, first column, “-“), thus indicating that endogenous
Fig. 1. FoxA1 stimulates AR-DNA binding and MMTV transcription of agonist- and antagonist-AR complexes. Oocytes were injected with 4.6 ng AR mRNA ± 1.4 ng FoxA1 mRNA and then incubated without or with the indicated ligands: 5 nM R1881, 10 μM BIC or 10 μM MDV. 6 h later 3 ng ss MMTV DNA reporter was injected in the nuclei. Oocytes were harvested for analysis ~24 h after mRNA injection. Two independent analyses for each ligand were done. The average values are presented as columns with the two individual sample values presented as black dots. (A) Pools of 12 injected oocytes were analyzed for MMTV RNA by S1 nuclease protection assay. (B) Same pools of oocytes were assayed by DMS in vivo footprinting. Autoradiogram of primer extension is to the right with AREs and FoxA1 sites indicated as boxes to the right and protected band as empty circles to the left. Black circles signify reference bands unaffected by protein binding. The diagrams on the left side show quantification of the indicated bands, the upper diagram the average of three AREs and lower diagram the average of two proximal FoxA1 sites. (C) Nuclei from five Xenopus oocytes of each pool were manually dissected, nuclear and cytosol extracts were prepared and analyzed by Western blot as described in Section 2. Amount of sample per lane correspond to one nucleus and half a cytosol. Filters were probed with antibodies to AR, FoxA1 and actin. Estimation of total AR protein amounts in relative terms were based on the Western blot and used to estimate the nuclear fraction of AR (%) as shown in the diagram below.
Xenopus AR, that have been reported to be expressed in oocytes (Lutz et al., 2001), either did not bind to the MMTV promoter DNA or was not present at sufficient levels to elicit a transcriptional response. The addition of agonist, R1881 (5 nM), resulted in a minor (~1.3-fold) increase in MMTV transcription provided that AR mRNA had been injected (Fig. 1A, third column).

Since our aim was to compare the effects of the antiandrogens on AR activity it was crucial to minimize the influence of endogenous androgens. Hence, the antiandrogens were added to the oocyte medium, at 10 μM final concentration, immediately after injection of the mRNA(s) coding for AR ± FoxA1 thus providing large excess of antiandrogen from the start of AR expression. This approach worked, since a robust inhibition of the MMTV transcription was seen by either BIC or MDV in the absence of FoxA1 (i.e. 8% and 0.4%, respectively, relative to the MMTV transcription in absence of any exogenous ligand here set to 100%) (Fig. 1A, columns 4 and 5). Importantly, the expression of FoxA1 together with AR caused a ~2-fold stimulation of MMTV transcription seen in the absence of exogenously added agonist and a 1.7-fold stimulation of the R1881-driven transcription (Fig. 1A, compare left and right half ± FoxA1). A FoxA1 dependent 10-fold increase (from 8.0% to 83.4%) in the MMTV transcription in BIC treated oocytes indicated that the antiandrogenic effect of this compound was almost lost in the presence of FoxA1. Transcription in the presence of MDV is also strongly increased by FoxA1 (from 0.4% to 12.5%), however, BIC-driven transcription is 6.7-fold higher (83.4% vs. 12.5%) thus confirming the considerably more robust AR antagonistic properties of MDV as compared to BIC (Tran et al., 2009). In agreement with our previous results, there is only a minimal effect on MMTV transcription in the presence of FoxA1 alone as compared to basal transcription, 2% and 0.9%, respectively (Belikov et al., 2012) (Fig. 1A, compare columns 6 and 1).

We quantified the sequence-specific binding of AR and FoxA1 at the MMTV enhancer by dimethylsulphate (DMS) methylation protection analysis. DMS-dependent methylation at the N7 position of guanines localized in the major groove of DNA occurs in the intact oocytes (Belikov et al., 2001) and the methylation pattern is developed by primer extension. Analyses were done in duplicates and were highly reproducible (Fig. 1B, left, the two black dots shown for each column). It revealed a 3.4-fold stimulation of specific AR-DNA binding (from 93% to 76% methylation) by the addition of FoxA1. Note that a reduced methylation is the readout of stronger DNA binding. (Fig. 1B, compare lanes 2 and 3 to lanes 7 and 8 in the upper diagram and the autoradiogram). This stimulatory effect of FoxA1 on AR-DNA binding was significant ($p < 0.0002, n = 4$), when comparing the oocytes with or without exogenously added agonist, R1881, in the presence or absence of FoxA1. No detectable DNA binding of AR is observed in the presence of BIC or MDV without FoxA1, but a distinct DNA binding of both these AR-complexes is seen in the presence of FoxA1, 89% and 95% methylation, respectively, i.e. equal to 11% and 5% methylation protection.

DNA binding of an agonist-AR complex is directly correlated to transcription while this is not the case for an antagonist-AR complex since the latter is less prone to recruit coactivators (Tran et al., 2009; Wilson, 2011). Indeed, DNA binding of BIC-AR complex in the presence of FoxA1 resulting in 11% methylation−protection and a robust MMTV transcription of 83% can be compared to the effect of the R1881-AR complex in the absence of FoxA1. For the latter, 7% protection renders an MMTV transcription of 132% (Fig. 1A and B). This demonstrates the stronger capacity of the agonist-ligated AR to mediate transcription, despite weaker DNA binding as compared to that of BIC-AR in presence of FoxA1. The MDV-AR on the other hand, which rendered a ~5% methylation protection in the presence of FoxA1 resulted in only 12.5% of MMTV transcription. Hence, the MDV-AR induced half level of DNA-binding as compared to BIC-AR results in only a seventh of its transcription. This again reflects the more antagonistic properties of MDV as compared to BIC and is probably caused by the lower capacity of MDV-AR complex to recruit coactivators (further addressed below). Similar experiments as in Fig. 1A and B were repeated another three times and produced similar results (data not shown).

The FoxA1 binding sites in the context of exogenously expressed FoxA1 show 10% methylation protection (Fig. 1B, lane 6). In agreement with previous results concerning GR (Holmqvist et al., 2005), FoxA1-DNA binding was strongly enhanced by AR (31% methylation protection in presence of R1881 and 28% and 16% in the presence of BIC and MDV, respectively). Hence, the increased binding of FoxA1 in presence of either the BIC-AR or the MDV-AR complex further indicates that both these AR-ligand complexes indeed binds sequence specifically to the ARE DNA in vivo.

The expression of AR and FoxA1 proteins were monitored by Western blot analysis of the nuclear and cytoplasmic compartments after manual dissection (Fig. 1C). About 25−35% of total AR was located in the nuclear compartment, except for MDV-exposed oocytes where about 15% of AR was recovered in the nuclei (Fig. 1C, diagram). The FoxA1 level was similar for all tested ligands with 50−60% recovered in the nuclear compartment. A conclusion from several experiments was that the nuclear transllocation of AR was reduced in the presence of MDV as compared to R1881 albeit to variable extent. The total AR content in the cell was about the same independently of the ligand used. Hence, there was more of cytosolic AR in the MDV-exposed oocytes which is to be expected, since it caused a reduced nuclear transllocation of AR (see below, Supplement 3). However, the significant intranuclear levels of the MDV-AR complex in combination with its distinct DMS methylation protection implies that this complex is indeed capable of both nuclear transllocation and sequence specific DNA binding, although the latter was only seen in the presence of FoxA1. The transcriptional response of this DNA bound AR complex at the MMTV promoter is however strongly reduced. Conversely, we note that the androgen-antagonistic properties of the BIC-AR complex were almost lost in the presence of FoxA1 and in fact this AR bound ligand in FoxA1 context appears to have significant agonistic properties.

### 3.3. Effect of AR ligands on the localization of AR and FoxA1 in mammalian cells

In order to compare the nuclear/cytoplasmic distribution seen in Xenopus oocytes with that in mammalian cells, we monitored the subcellular localization of AR and FoxA1 by confocal microscopy of EGFP-AR and mCherry-FoxA1 expressed in HEK293 cells and after incubation with various AR-ligands as indicated. As shown in Fig. 2, AR is preferentially cytosolic in absence of ligand and is exclusively nuclear in presence of R1881 or BIC. In agreement with a previous report (Tran et al., 2009), incubation with MDV results in a similar level of AR-signal in cytosol and nucleus. FoxA1 shows preferentially nuclear localization irrespective of the AR ligand used. As seen from the yellow signal in the merged pictures and the scatter plots, there is a ligand-dependent co-localization of AR and FoxA1 in presence of either of the three ligands, while the concentration of co-localized signal is much stronger with the agonist, being the weakest with the MDV. The subcellular localization of AR and FoxA1 in these tissue culture cells are in good agreement with the results described above for Xenopus oocytes. The high degree of co-localization of AR and FoxA1 in the presence of androgen in our analyses is in agreement with the previously reported androgen-dependent physical interaction of AR and FoxA1 in prostate cells (Gao et al., 2003).
3.4. The intranuclear MDV-AR complex has a similar ability to bind the ARE DNA as the R1881-AR complex in vivo

Although the MDV-AR complex displayed a reduced nuclear translocation, our DMS in vivo footprinting experiments strongly indicated its capacity to bind sequence specifically to the chromatinized AREs at the MMTV enhancer (Fig. 1B). In order to investigate this further we expressed increasing levels of AR in the context of a constant level of FoxA1 mRNA in three oocyte pools. These pools were then divided in half and incubated either with R1881 or MDV followed by intranuclear DNA reporter injection. Estimation of the nuclear and cytosolic levels of AR based on the Western blot indicates a 2.7-fold and a 1.6-fold reduction in nuclear translocation in the presence of MDV as compared to R1881 for the two lower levels of AR and for the highest AR level, respectively (Fig. 3D). The nuclear to cytosolic ratio of AR was between 0.95 and 0.26 for the agonist and 0.05–0.08 for the antagonist-AR complex (Fig. 3E) confirming the reduced nuclear translocation of AR in the presence of MDV in comparison to the agonist. However, the absolute amount of intranuclear AR is increased in response to the enhanced AR expression in the cell. This finding was confirmed in another AR titration experiment (data not shown).

DNA binding monitored by DMS in vivo footprinting revealed a dose-response relationship between the increasing levels of AR expression and DNA binding (Fig. 3A, see protected bands at AREs highlighted with arrowheads, and 3B quantified DMS methylation at the AREs). This was also the case for the AR-MDV complex (Fig. 3A and B) although the methylation protection was reduced to about half of that level seen for the agonist. At the highest AR level the difference was only 1.6-fold when comparing the DNA binding of the agonist- and the antagonist-bound AR (Fig. 3B). Incidentally, this is in agreement with the 1.6-fold higher nuclear content of the agonist-AR complex (Fig. 3D). Since the MDV-AR complex shows a lower nuclear translocation, we decided to compare the methylation protection quantified by in vivo footprinting (Fig. 3B) as a function of the nuclear AR content either in presence of R1881 (black diamonds) or MDV (open diamonds) (see Fig. 3F). Here the double samples of the DMS analysis are displayed as error bars. The diagram reveals a linear correlation between the amount of nuclear AR and sequence specific DNA binding to the chromatinized AREs in the nucleus irrespective of nature of bound ligand: the agonist or the antagonist. This confirms and strengthens our observation (c.f. Fig. 1B) that the MDV-AR complex has a similar capacity as the R1881-AR complex to bind sequence specifically.
to the ARE DNA once it has been translocated to the nucleus and provided that the FoxA1 is present. Importantly, the MMTV transcription analysis showed that the MDV-AR complex rendered a low level of transcription providing only a marginal 1.5-fold increase at the highest level of AR expression (Fig. 3C, +++), while the nuclear MDV-AR complex concentration was increased 5.6-fold (Fig. 3D, compare MDV+ to +++). In comparison, the 3.2-fold increase in agonist-nuclear receptor complex (Fig. 3D compare R1881+ to ++++) rendered a 7.6-fold increase in transcription (Fig. 3C). Hence the DNA bound AR-MDV complex does not seem to recruit the co-activators required for transcription activation in spite of its capacity to bind specifically to the MMTV enhancer DNA.

Is the MDV-AR complex able to mediate a transcriptional response by itself if strongly over expressed or is FoxA1 absolutely required? We addressed this further by repeating the AR titration experiment shown in Fig. 3 but this time in the absence of FoxA1. Also in this case the nuclear translocation of MDV-AR, estimated by Western blot, was reduced compared to the agonist-AR complex but the absolute amounts of nuclear MDV-AR complex were increased positively correlating with AR expression (Supplement 3B). Note also the increased cytosolic level of MDV-AR complex apparently due to the reduced nuclear uptake (Supplement 3B). A difference was seen in the MDV-AR driven MMTV transcription in the absence of FoxA1 as compared to the when FoxA1 is present (compare Supplement 3A to Fig. 3C). In absence of FoxA1 there was a low but proportionally increased level of transcription that correlated to the level of AR expression and thus remained the same in relative terms indicating a linear dose-response (Supplement 3A, lower section of the diagram). Importantly, the
level of MMTV transcription in presence of MDV was much lower in the absence of FoxA1, i.e. only 2–3% of the agonist driven transcription for each of the three levels of AR expression. This is very different from the near constant level of much higher MDV-AR driven transcription seen in the presence of FoxA1, especially at the lowest level of AR expression (Fig. 3C). We conclude that the FoxA1 requirement is not absolute but that very high levels of AR are required for MDV to elicit even a minor transcriptional response in absence of FoxA1.

3.5. Chromatin remodeling over the AREs is strongly enhanced by FoxA1 and correlates with the transcriptional response elicited by the different ligands

The capacity of the different AR-ligand complexes to cause chromatin remodeling was analyzed with respect to DNase I hypersensitivity over the MMTV enhancer. This method was previously shown to be useful for evaluation of the effects of glucocorticoid agonists and antagonists and represents a readout of coactivator recruitment since it correlates with the transcriptional induction (Zaret and Yamamoto, 1984; Belikov et al., 2001, 2009). DNase I hypersensitive sites (DHSs) were determined by Southern blotting and indirect endlabeling assay (Fig. 4). AR was expressed via intranuclear injection of AR expression vector with or without FoxA1 and the oocytes were exposed to the agonist or the two AR antagonists as indicated. Fig. 4 shows no detectable effect of DNase I cleavage in the oocytes where AR was expressed in absence of FoxA1 (lanes 5–12). When FoxA1 was expressed alone, there was a weak DHS peak seen in the –360-region and as an increased and diffusely distributed DNase I sensitivity in the more proximal domain of the MMTV enhancer/promoter (lanes 21–24 and the gray line in the scan). These DHSs correspond to the FoxA1 binding sites as indicated below (see Fig. 4, white squares below the horizontal line for MMTV LTR) and was previously shown to function in chromatin presetting for GR induction (Belikov et al., 2009). The co-expression of AR and FoxA1 ± 5 nM R1881 generates a strong enhancement of the previously seen FoxA1 DHS but also an additional cluster of DHSs that correspond to the location of the AREs and a more proximal segment including the promoter region (Fig. 4, lanes 25–32, light green and dark green scans, respectively). The addition of BIC or MDV reduces the DHS intensity over the AREs, more so with the latter, but still maintains the strong sensitivity in the upstream FoxA1 domain at –360 (lanes 33–40, orange and red scans respectively). This increased sensitivity over the

![Fig. 4. Chromatin remodeling over the ARE is strongly enhanced by FoxA1 and correlates with the transcriptional response. AR was expressed by intranuclear injection of 5 ng CMV-hAR expression vector ± 5 ng FoxA1 mRNA injection into the cytoplasm, 3 ng ss MMTV reporter DNA was co-injected with the AR expression vector. The two pools of oocytes, i.e. ±FoxA1, were divided into four groups, ~27 oocytes each, and exposed to the agonist or either of the two AR antagonists as indicated. 24 h later each group of oocytes was homogenized and divided into four equal pools and digested with increasing concentration of DNase I. The 1.3 kb MMTVLTR DNA is displayed to the left and at the bottom. Arrowhead is transcription start site (+1). Boxes to the left are FoxA1 sites (under the line at the bottom figure) and white boxes to the right are AREs (indicated above the line in the bottom figure). Hinf1 and SacI sites to the left serves as internal markers (lanes M) together with a 100 bp ladder size-marker.](image-url)
FoxA1 binding sites in the presence of expressed AR as compared to FoxA1 alone is due to cooperative binding with AR (c.f. Fig. 1B), indicating that the antagonist-AR complexes can bind to their cognate ARE sites in the MMTV enhancer but are much less prone to recruit remodeling activities at the AREs. Likewise, a cooperative FoxA1 binding was also demonstrated in case of the interaction of agonist or antagonist liganded glucocorticoid receptor (GR) with the MMTV LTR (Belikov et al., 2009, 2012).

A similar DNase I experiment was also performed with mRNA expressed AR when using a higher level of AR expression and a lower level of FoxA1 (Supplement 4). This revealed AR and AR-antagonist-dependent DHSSs over the AREs also in the absence of FoxA1 that was reduced by the AR-BIC complex and was barely detectable by the AR-MDV complex. Again the addition of FoxA1 strongly enhanced the DHS intensity, both over the upstream FoxA1 sites but also over the AREs. The presence of FoxA1 also dramatically increased the DHS in the BIC treated oocytes while it only slightly increased the DHS in the MDV-exposed oocytes.

In conclusion, there is a functional segregation of the DHS originating from the FoxA1 binding and the partly overlapping DHS generated by AR bound to its cognate AREs. The latter correlates with the capacity of the three different ligands used here to induce transcriptional response at the MMTV promoter (compare Fig. 4 and Supplement 4 to Fig. 1A). Importantly, the intensity of the DHSSs at the AREs, elicited either by AR agonists or antagonists, is enhanced by the presence of FoxA1 which correlates with the FoxA1-dependent increase in the transcriptional responses (Fig. 1A). However, the DHS over the upstream FoxA1 site did not correlate with the transcriptional response. Instead this FoxA1 DHS is increased in intensity even in the presence of the strongest antagonist, MDV (Fig. 4, compare lanes 21–24 to lanes 37–40). This corroborates the FoxA1 DNA binding data in Fig. 1B, showing that FoxA1 binding to the chromatinized DNA is increased not only in the presence of agonist, but also in the presence of the two AR antagonists. Taken together these findings demonstrate the ability of FoxA1 to preset the chromatin structure, which compromise the antagonistic properties of BIC resulting in enhancement of the AR binding and stimulation of the chromatin remodeling over the AREs which leads to productive transcription elicited by the BIC-AR complex (Fig. 1A). Conversely, the lower DNase I hypersensitivity at AREs in the presence of MDV correlates with the stronger antigenic effect of this compound (Fig. 4, see SCANS below).

3.6. FoxA1 acts as a licensing factor for AR-induced transcription

Previous studies in prostate derived cells showed that FoxA1 may act as a licensing factor for AR-mediated transcription (Gao et al., 2003; Wang et al., 2007). We recently demonstrated FoxA1 to exert this function also on GR-mediated gene induction in Xenopus oocytes (Belikov et al., 2012). This indicates a threshold effect, supposedly mediated by the chromatin structure, to stall the access of AR or GR for DNA. Various combinations of other TFs, including FoxA1, NF1 and Oct1 (Belikov et al., 2004a,b) can reduce this effect by binding nearby. The dependence on these “chromatin-openers” to overcome the chromatin threshold is also determined by the concentration of the nuclear receptor (Belikov et al., 2012). This is also evident when comparing Fig. 4 where a low level of AR absolutely requires FoxA1 to form DHSSs over the AREs while the high AR concentration (c.f. Supplement 4) is able to form such DHSSs also in the absence of FoxA1.

We titrated the FoxA1 protein in order to estimate the amount required to enhance a low level of AR-mediated transcription and DNA binding. We thus injected a constant AR mRNA amount in the absence or presence of increasing concentrations of FoxA1 mRNA (0.09–1.4 ng in five steps) (c.f. Fig. 5). Here the oocytes were not exposed to any exogenous ligand but AR-activation was mediated by the endogenous androgens present in the ovarian tissue that render almost as high expression as with added R1881 (c.f. Fig. 1A). DMS in vivo footprinting (Fig. 5A) demonstrates a weak but enhanced DNA binding both at the ARE and the FoxA1-binding site with increasing concentration of FoxA1. Interestingly, there is no methylation protection over the AREs in the absence of FoxA1 in this experiment and no detectable MMTV transcription (Fig. 5B). This illustrates the absolute requirement for FoxA1 to support the AR-mediated induction event when the concentration of hormone-activated AR is too low to elicit a hormone response on its own. Hence, the FoxA1 is acting as a licensing factor at this low concentration of AR. A control of the total AR in each pool of oocytes by SDS-PAGE and Western blot demonstrated a significant

Fig. 5. FoxA1 acts as a licensing factor for AR-induced transcription. Oocytes, 45 in each group, were injected with 2.8 ng AR mRNA in the absence or presence of increasing amounts of FoxA1 mRNA, 0.09 ng 0.17, 0.35, 0.7 or 1.4 ng followed by DNA injection and analysis as in Fig. 1. No exogenous ligand was added. (A) DMS in vivo footprinting was performed in triplicate samples, otherwise as in Fig. 2B. Columns show the average methylation and error bars signify standard deviations. (B) Quantification of MMTV RNA by S1-nuclease protection of the same oocyte pools as in A.
and equal level of AR in all oocyte pools and an increasing level of FoxA1 as expected (data not shown). We note that the lowest level of FoxA1 used in this experiment renders no detectable increase in the protection over the FoxA1 binding site but a small protection over the AREs (Fig. 5A, compare column 3 in both diagrams). Remarkably, even this minute level of FoxA1 elicits a distinct transcriptional induction (Fig. 5B compare columns 2 and 3). Increasing levels of FoxA1 gradually enhanced DNA binding of both the ARE and FoxA1 response elements while transcription reaches a plateau already at 0.17 ng of FoxA1 mRNA injected oocytes (Fig. 5A and B). Similar results were reported before for FoxA1 titration of the GR-mediated MMTV transcription (Belikov et al., 2012).

3.7. An androgen-responsive gene in LNCaP prostate cancer cells shows a FoxA1-dependent expression to be enhanced by BIC

LNCaP tissue culture cells originating from a lymph node metastasis of a human prostate carcinoma were used to address the effect of FoxA1 on the antiandrogenic activity of BIC and MDV. To that end, we depleted the FoxA1 by using specific siRNAs that were designed in a fashion that minimizes off-target effects (Jackson et al., 2006). Based on quantification of band intensities of western blots using a Li-COR Odyssey Infrared Imaging System (Li-COR Inc.) according manufacturer’s instructions. The numbers below the anti-FoxA1 lanes depict the relative amount of the FoxA1 protein in the samples. (B–E) Effect of FoxA1 depletion on the expression of four select AR target genes as indicated above each diagram. Total RNA levels between samples were normalized using mRNA levels of GAPDH. The figure is a representative of two independent tests with triplicate samples. The columns represent the mean ± SD of three samples.

Fig. 6. Depletion of FoxA1 modulates the expression of AR target genes in LNCaP prostate cancer cells. Cells were transfected with 40 nM ON-TARGET plus SMARTpool human FOXA1 (siFoxA1) or ON-TARGET plus Non-targeting Pool (siSCR) (Dharmacon) for 72 h and treated as depicted with vehicle, 1 nM R1881, 10 μM BIC or 10 μM MDV alone or together with 1 nM R1881 for the next 18 h. Immunoblotting, RNA extraction, cDNA synthesis, and qPCR were performed as indicated in Section 2. (A) Confirmation of FoxA1 depletion in vehicle-exposed samples by immunoblotting with anti-FoxA1 (1:10000 dilution) and anti-GAPDH (loading control, 1:5000 dilution) antibodies. Detection and quantification of bands were carried out using a Li-COR Odyssey Infrared Imaging System (LI-COR Inc.) according manufacturer’s instructions. The numbers below the anti-FoxA1 lanes depict the relative amount of the FoxA1 protein in the samples. (B–E) Effect of FoxA1 depletion on the expression of four select AR target genes as indicated above each diagram. Total RNA levels between samples were normalized using mRNA levels of GAPDH. The figure is a representative of two independent tests with triplicate samples. The columns represent the mean ± SD of three samples.
similar fashion by FoxA1 as demonstrated above for the MMTV promoter in Xenopus oocytes.

4. Discussion

The reconstitution of the AR-mediated signal transduction in Xenopus oocytes has enabled us to correlate the transcriptional response from an AR responsive enhancer to (i) DNA binding of AR in complex with various ligands, (ii) chromatin remodeling and (iii) nuclear translocation. Our results demonstrate that the antiandrogenic capacity of BIC is markedly corrupted in the presence of FoxA1 while MDV is more persistent in this respect. The strong antiandrogenic activity of MDV was described before (Tran et al., 2009); however, our results reveal for the first time that it can be affected in the presence of FoxA1. Our assays demonstrated that the capacity of MDV to reduce nuclear translocation, as previously demonstrated by Tran et al. (2009), is quite variable and can be overcome by an increased AR expression (c.f. Fig. 3D). Furthermore, our analysis for the first time demonstrates the capacity of the MDV-AR complex to mediate sequence specific DNA binding to the ARE containing enhancer that is strongly enhanced in the presence of FoxA1. In addition, we show that FoxA1 is also able to enhance MDV-AR mediated transcription, albeit to a relatively low extent.

The linear correlation between DNA binding and concentration of intranuclear AR-ligand complex (Fig. 3F) argues for a similar DNA binding affinity for MDV- and R1881-AR complexes in the context of FoxA1. Previous experiments based on transient transfections in Cos-7 cells indicated that MDV-AR was not able to bind to DNA as oppose to BIC-AR and R1881-AR complexes (Tran et al., 2009). Our data are based on direct analysis of sequence specific protein-DNA binding in vivo by DMS methylation protection. The experiment was repeated six times and consistently demonstrated sequence specific MDV-AR dependent methylation protection, however, FoxA1 was required for a significant DNA binding of MDV-AR to occur (Fig. 1B). The use of ss reporter DNA in Xenopus oocyte injection experiments results in chromatin assembly via a replication-like mechanism that results in a chromatin structure that was previously shown to behave as chromatin of stably transfected cells in terms of organization of the MMTV promoter chromatin and the phenotype of an AR mutant (Li et al., 2006). We thus conclude that our in vivo DNA binding results demonstrate that the nuclear MDV-AR complex can bind DNA and that this is strongly enhanced in the context of the preset chromatin environment mediated by FoxA1. The intranuclear colocalization of MDV-AR with FoxA1 in mammalian cells is in agreement with this result (Fig. 2).

Importantly, in spite of its capacity of binding to DNA the MDV-AR complex maintains a potent antiandrogenic activity also in the presence of FoxA1 as demonstrated by its inhibitory effect on transcription (c.f. Fig. 1A) albeit its antiandrogenic strength is reduced in this context. Thus, in the absence of FoxA1, addition of MDV reduces hormone dependent transcription 200 times whereas in the presence of FoxA1 transcription is reduced 16 times (Fig. 1A, compare columns 2 and 5 with 7 and 10). A much stronger effect of FoxA1 is seen on the BIC-AR complex, its antiandrogenic capacity is seriously corrupted by FoxA1, since in its presence transcription is reduced only 2.5 times in comparison to the agonist treated oocytes (Fig. 1A, compare 2 and 4 with 7 and 9).

The distinctly stronger antiandrogenic potency of MDV-AR than of BIC-AR is also illustrated by its lower ability to induce chromatin remodeling over the ARE; this is evident also in the presence of FoxA1-induced chromatin presetting (Fig. 4 and Supplement 4). The presence of DNA-bound MDV-AR complex stimulates FoxA1 to bind stronger, which is seen as enhanced hypersensitivity around the distal FoxA1 binding sites (c.f. Fig. 4 lanes 21–24 and lanes 37–40 and Fig. 1B, compare columns 6 and 10), this has very little effect on the chromatin structure at the AREs (Fig. 4 lanes 37–40 and SCAN) in accordance with a very low transcriptional response (Fig. 1A). This implies that the DNA bound MDV-AR complex at large fails to recruit the coactivators and/or basal TFs that are required to elicit a transcriptional response, i.e. in spite of its capacity to bind sequence specifically to the ARE.

What is the mechanism behind the more persistent antiandrogenic activity of MDV as compared to BIC? The AR protein has the interesting ability to establish a ligand dependent inter- or intra-domain contact between the N- and C-terminal domains, which appears to be required for recruitment of coactivators (Wong et al., 1993; Wilson, 2011). Tran et al. showed that MDV-AR complex is less prone to mediate such an N–C interaction while this capacity is retained in the BIC-AR complex (Tran et al., 2009). These authors also revealed a reduced nuclear uptake of the MDV-AR complex, also confirmed here (see Figs. 1–3 and Supplements 3). This may contribute to the antiandrogenic effect of MDV although an increased AR expression has the potential to compensate for this (see above and Fig. 3D and Supplement 3). The near absence of chromatin remodeling by MDV-AR is in line with its inability to recruit coactivators (Fig. 4 and Supplement 4) (Tran et al., 2009; Wilson, 2011) and is likely due to an altered structure of the AR LBD caused by the bound MDV ligand.

How is FoxA1 reducing the antagonistic activity of the MDV-AR complex? The FoxA1 mediated effect on chromatin is usually referred to as chromatin presetting (or a poised chromatin state). A similar chromatin effect is mediated by NF1 and Oct1 binding at the MMTV promoter in Xenopus oocytes (Belikov et al., 2004a,b; Astrand et al., 2009). As mentioned above (see Introduction), TF-induced chromatin presetting for nuclear receptor DNA binding appears to be required genome-wide (John et al., 2008; Sahu et al., 2011; Siersbaek et al., 2011) and a wide variety of different TFs may exert this function (So et al., 2007; John et al., 2011). Thus, one possible explanation for the FoxA1-mediated effect on the MDV-AR complex is that the activating capacity of the N-terminal domain of AR may trigger transcription in spite of the lack of any detectable C–N interaction. However this activity might be too weak for a transcriptional response to occur unless it is supported by a more open, i.e. preset, chromatin structure here provided by FoxA1. The FoxA1 titration experiment in Fig. 5 shows that AR binding and AR induced transcription are strongly stimulated within a broad concentration range of FoxA1 concentrations. This is in agreement with previous results, demonstrating a licensing function of FoxA1 for the AR signaling in the prostate (Gao et al., 2003), in prostate cancer, (Wang et al., 2007), in liver cancer (Li et al., 2012), and for GR signaling (Belikov et al., 2012) (see also introduction). The latter report demonstrated FoxA1 to recruit H4K16 histone acetyltransferase activity. Others showed this acetylation site to mediate decondensation of the higher order chromatin structure (Shogren-Knaak et al., 2006). This may be the reason for the robust FoxA1–dependent chromatin remodeling revealed as an increased DHS intensity as demonstrated above in Fig. 4 and Supplement 4. It supports the notion that FoxA1 binding lowers the chromatin-implemented threshold, perhaps not only for AR binding to DNA but also with respect to the level of coactivator activity required to be recruited by AR in order to induce transcription. Incidentally, ligand-independent AR variants lacking the LBD have been reported to occur in hormone-refractory prostate cancer (Hu et al., 2009). In agreement with our results for the GR and RU486 (see Section 1 and Belikov et al., 2009), it is conceivable that such constitutively active AR mutants will require assistance from chromatin presetting TFs such as FoxA1 in order to elicit a transcriptional response. We note that a recent analysis of the clinical outcome of a large cohort of prostate cancer patients revealed that
FoxA1 promotes tumor progression (Gerhardt et al., 2012). Importantly, the strongest effect of FoxA1 on tumor progression was seen in tumors with low AR expression levels. This is in agreement with our results demonstrating a much stronger stimulatory effect of FoxA1 at low AR expression in Xenopus oocytes (Fig. 4, Supplement 4, Fig. 5 and data not shown).

In conclusion, our work offers a possible mechanistic explanation for how a prostate cancer cell becomes insensitive to BIC and perhaps even stimulated by this drug (Kelly et al., 1997). The endogenous androgens present in our experiment may mimic a clinical situation where the tumor tissue contain or produce androgenic ligands and where an increased level of FoxA1 expressed in the tumor cells might compromise the therapeutic effect of BIC. In addition, our experiments shed light on how MDV-AR functions in the context of FoxA1. This finding is of potential clinical relevance since FoxA1 was shown to be a bad prognostic factor for prostate tumors (Jain et al., 2011; Sahu et al., 2011; Gerhardt et al., 2012). The dramatic improvement of the antiandrogenic activity of MDV as compared to BIC is hopefully the beginning of better therapeutic prospects in the treatment of prostate cancer. The fact that even MDV displays a slightly compromised antiandrogenic activity in the presence of FoxA1 argues for a continuation of the search for even more potent antiandrogens. In this context it might be beneficial if a more efficient inhibition of the AR nuclear translocation could be achieved (Kim et al., 2012).

5. Disclosure statement

The authors have nothing to disclose.

Acknowledgements

This work was supported by the Swedish Cancer Foundation (Project Number 10 0500) and the Swedish Research Council – Medicine (Project Number K2008-66X-15337-04-3). CÖ was supported by Karolinska Institutet’s grant for PhD students (KID). JJP was supported by the Academy of Finland, the Finnish Cancer Organisations, the Sigrid Juselius Foundation, and the strategic funding of the University of Eastern Finland. We are grateful to Dr. Jiemin Wong, East China Normal University, Shanghai, for kindly providing the cDNA coding for the human androgen receptor.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mce.2012.10.002.

References


Astrand, C., Belikov, S., Wrange, O., 2009. FoxA1 binding directs chromatin structure in the context of FoxA1. This finding is of potential clinical relevance since FoxA1 was shown to be a bad prognostic factor for prostate tumors (Jain et al., 2011; Sahu et al., 2011; Gerhardt et al., 2012). The schematic improvement of the antiandrogenic activity of MDV as compared to BIC is hopefully the beginning of better therapeutic prospects in the treatment of prostate cancer. The fact that even MDV displays a slightly compromised antiandrogenic activity in the presence of FoxA1 argues for a continuation of the search for even more potent antiandrogens. In this context it might be beneficial if a more efficient inhibition of the AR nuclear translocation could be achieved (Kim et al., 2012).

5. Disclosure statement

The authors have nothing to disclose.

Acknowledgements

This work was supported by the Swedish Cancer Foundation (Project Number 10 0500) and the Swedish Research Council – Medicine (Project Number K2008-66X-15337-04-3). CÖ was supported by Karolinska Institutet’s grant for PhD students (KID). JJP was supported by the Academy of Finland, the Finnish Cancer Organisations, the Sigrid Juselius Foundation, and the strategic funding of the University of Eastern Finland. We are grateful to Dr. Jiemin Wong, East China Normal University, Shanghai, for kindly providing the cDNA coding for the human androgen receptor.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mce.2012.10.002.

References


Astrand, C., Belikov, S., Wrange, O., 2009. FoxA1 binding directs chromatin structure in the context of FoxA1. This finding is of potential clinical relevance since FoxA1 was shown to be a bad prognostic factor for prostate tumors (Jain et al., 2011; Sahu et al., 2011; Gerhardt et al., 2012). The schematic improvement of the antiandrogenic activity of MDV as compared to BIC is hopefully the beginning of better therapeutic prospects in the treatment of prostate cancer. The fact that even MDV displays a slightly compromised antiandrogenic activity in the presence of FoxA1 argues for a continuation of the search for even more potent antiandrogens. In this context it might be beneficial if a more efficient inhibition of the AR nuclear translocation could be achieved (Kim et al., 2012).

5. Disclosure statement

The authors have nothing to disclose.

Acknowledgements

This work was supported by the Swedish Cancer Foundation (Project Number 10 0500) and the Swedish Research Council – Medicine (Project Number K2008-66X-15337-04-3). CÖ was supported by Karolinska Institutet’s grant for PhD students (KID). JJP was supported by the Academy of Finland, the Finnish Cancer Organisations, the Sigrid Juselius Foundation, and the strategic funding of the University of Eastern Finland. We are grateful to Dr. Jiemin Wong, East China Normal University, Shanghai, for kindly providing the cDNA coding for the human androgen receptor.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mce.2012.10.002.

References


Astrand, C., Belikov, S., Wrange, O., 2009. FoxA1 binding directs chromatin structure in the context of FoxA1. This finding is of potential clinical relevance since FoxA1 was shown to be a bad prognostic factor for prostate tumors (Jain et al., 2011; Sahu et al., 2011; Gerhardt et al., 2012). The schematic improvement of the antiandrogenic activity of MDV as compared to BIC is hopefully the beginning of better therapeutic prospects in the treatment of prostate cancer. The fact that even MDV displays a slightly compromised antiandrogenic activity in the presence of FoxA1 argues for a continuation of the search for even more potent antiandrogens. In this context it might be beneficial if a more efficient inhibition of the AR nuclear translocation could be achieved (Kim et al., 2012).


