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Coexpression of CAV-1, AT1-R and FOXM1 in prostate and breast cancer and normal cell lines and their influence on metastatic properties

Karolina Kowalska[1]*, Magdalena Nowakowska[2]*, Kamila Domińska[1] and Agnieszka W. Piastowska-Ciesielska[1]✉

[1]Department of Comparative Endocrinology and [2]Department of Molecular Cancerogenesis, Faculty of Biomedical Sciences and Postgraduate Training, Medical University of Lodz, Łódź, Poland

The aim of this study was to evaluate the coexpression of caveolin-1 (CAV-1), angiotensin II type 1 receptor (AT1-R) and forkhead box MI (FOXM1) in prostate and breast cancer cell lines, in comparison with normal cell lines. CAV-1, AT1-R and FOXM1 expression was determined by reverse transcription-quantitative polymerase chain reaction and western blot analysis in the prostate cancer cell lines PC3, DU145 and LNCaP; prostate normal cell line PNT1A; breast cancer cell lines MCF-7 and MDA-MB-231; and the normal breast cell line 184A1. A correlation between the expression levels of the investigated genes and their metastatic properties was determined by the Spearman's rank test ($P<0.05$) and Aspin-Welsch *t*-test, respectively. In prostate cell lines, a significant correlation was noted between CAV-1 and AT1-R expression and between FOXM1 and CAV-1 expression. A correlation between the expression levels of the investigated genes and their metastatic potential was also observed, with relatively high expression of all the investigated genes in the normal prostate cell line PNT1A. In comparison to prostate cancer cell lines, an adverse dependency between CAV-1, AT1-R, FOXM1 expression and metastatic potential was observed in the breast cancer cell lines. Relatively high expression of all tested genes was observed in the normal breast cell line 184A1, which was decreasing respectively with increasing metastatic potential of breast cancer cell lines. The results obtained here indicate that CAV-1, FOXM1 and AT1-R may be potential markers of tumorigenesis in certain types of cancer *in vitro*.

Key words: angiotensin II receptor type 1; caveolin-1; breast adenocarcinoma; forkhead M1 transcription factor; prostate adenocarcinoma

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INTRODUCTION

The process of cancerogenesis is a multistep process that comprises various complex changes at the genomic, transcriptomic and proteomic levels (Mahmoud *et al.*, 2015). The last event of this process is metastasis, which leads to cancer cell dissemination and development of novel tumours at different sites. The leading cancer types in epidemiological statistics are those associated with the reproductive tract, particularly breast and prostate cancer. It is of interest to elucidate the molecular mechanisms responsible for the development and progression of these tumours and to identify novel molecular markers that enable early diagnosis and characterization of tumour features (Siegel *et al.*, 2015).

The expression of caveolin-1 (CAV-1) and angiotensin II (Ang II) appears to be associated with cancer progression and metastatic potential (Piastowska-Ciesielska *et al.*, 2013a). CAV-1 is a 22 kDa hairpin membrane protein that forms part of a caveolae, the non-clathrin subdomains of lipid rafts (Goetz *et al.*, 2008; Yang *et al.*, 2012). CAV-1 is known to participate in cell signalling, cellular transformation, metastasis, cell death, survival and angiogenesis *via* interactions with growth factor receptors, cytokines, oncoproteins and metalloproteinases (Bouras *et al.*, 2004; Burgermeister *et al.*, 2008; Han & Zhu, 2010; Trimmer *et al.*, 2011). The expression levels of CAV-1 may vary depending on the type and stage of the tumour. For example, high levels of CAV-1 have been reported in metastatic prostate cancer (Piastowska-Ciesielska *et al.*, 2013a), while in breast cancer CAV-1 has been reported to act as a tumour suppressor, regulating the expression of breast cancer 1, early onset (BRCA1), epidermal growth factor receptor (EGFR), the E3 ubiquitin-protein ligase mouse double minute 2 homolog (Mdm2) and the oncoprotein signal transducer and activator of transcription 3 (Stat3) (Glait *et al.*, 2006; Agelaki *et al.*, 2009; Bartholomew *et al.*, 2009; Chiu *et al.*, 2011).

Ang II is the major element of the renin-angiotensin system, which is involved in oxidative stress, maintenance of the water balance in the body and development of hypertension (Uemura *et al.*, 2008a; Jethon *et al.*, 2012). It is well documented that Ang II has a potential role in carcinogenesis by activating the mitogen-activated protein kinases (MAPKs) (Uemura *et al.*, 2008a), growth factors (Uemura *et al.*, 2005b) and angiogenesis (Uemura *et al.*, 2005a). Thus, overexpression of Ang II type 1 re-

✉e-mail: agnieszka.piastowska@umed.lodz.pl
*These authors contributed equally

Abbreviations: Ang II, angiotensin II; AT1-R, angiotensin II type 1 receptor; ATCC, American Type Culture Collection; BRCA1, breast cancer, early onset 1; CAV-1, caveolin-1; ECACC, European Collection of Authenticated Cell Cultures; EGFR, epidermal growth factor receptor; FOXM1, forkhead box M1; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; H3F3A, H3 histone, family 3A; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer; MAPKs, mitogen-activated protein kinases; Mdm2, E3 ubiquitin-protein ligase mouse double minute 2 homolog; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; mRNA, messenger RNA; RIPA, radioimmunoprecipitation assay extraction buffer; RPS17, ribosomal protein 17; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; Stat3, signal transducer and activator of transcription; TE, Tris-ethylenediaminetetraacetic acid; VEGF, vascular endothelial growth factor

ceptor (AT1-R) has been described to correlate with invasiveness of prostate, breast and ovarian cancers (Dominska & Lachowicz-Ochedalska, 2008).

Forkhead box M1 (FOXM1) is a transcription factor that is overexpressed during development (Laoukili *et al.*, 2007). In adult tissues, its expression is suppressed in resting or terminally differentiated cells, with the exception of actively dividing cells in the testis and thymus (Glait *et al.*, 2006; Laoukili *et al.*, 2007; Kalin *et al.*, 2011). Furthermore, increased expression of FOXM1 and amplification of its gene region have been associated with various types of human cancer, including glioblastoma (Liu *et al.*, 2006), non-small cell lung cancer (Kim *et al.*, 2006), prostate adenocarcinoma (Kalin *et al.*, 2006) and breast cancer (Millour *et al.*, 2010). In addition, it has been shown that this member of the forkhead family of transcription factors also participates in the regulation of cancer-associated processes, including invasion, angiogenesis and metastasis (Chandran *et al.*, 2007; Wang *et al.*, 2008; Park *et al.*, 2011; Bergamaschi *et al.*, 2014), through direct transcriptional regulation of vascular endothelial growth factor (VEGF) (Zhang *et al.*, 2008), matrix metalloproteinase 2 (MMP2), MMP9 (Dai *et al.*, 2007; Wang *et al.*, 2008) and CAV-1 (Huang *et al.*, 2012). A strong correlation between FOXM1 and CAV-1 expression has been demonstrated in pancreatic cancer, indicating that these proteins are associated with cancer development and progression (Huang *et al.*, 2012). As previously reported, CAV-1 may modulate the expression of AT1-R by controlling its transport to the plasma membrane in prostate cells as a part of lipid rafts (Piastowska-Ciesielska *et al.*, 2013a). Since FOXM1 and CAV-1 expression are correlated, FOXM1 may also participate in the regulation of cancerogenesis of prostate and breast cells. Thus, the study presented here aimed to compare the expression of AT1-R, CAV-1 and FOXM1 in prostate and breast cancer cell lines, which exhibit a different metastatic potential to that of normal prostate and breast cell lines, in order to identify any significant pattern of their coexpression and participation in the acquisition of cancer progression features. To the best of our knowledge, this study is the first report on differences in the expression levels of AT1-R in cancer cell lines exhibiting distinct metastatic properties.

MATERIALS AND METHODS

Cell culture. Metastatic human prostate adenocarcinoma cell lines LNCaP and PC3 were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (Brunswick, Germany), whereas the DU145 cells and normal adult prostatic epithelial PNT1A cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and the European Collection of Authenticated Cell Cultures (ECACC; Salisbury, UK), respectively. Mammary gland adenocarcinoma cell lines MCF-7 and MDA-MB-231, and normal mammary gland 184A1 cells were obtained from ATCC. Cell lines were cultured in RPMI or Dulbecco's modified Eagle's medium with 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES) and antibiotics (penicillin, 50 U/ml; streptomycin, 50 μg/ml; and neomycin, 100 μg/ml), in a humidified atmosphere of 5% CO_2 at 37°C. Cells were cultured in 6-well culture dishes for RNA and protein isolation. All cell culture media, reagents and cell dishes were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA).

Isolation of protein and RNA. Total protein extracts were isolated using a radioimmunoprecipitation assay extraction buffer (RIPA; Sigma-Aldrich, St. Louis, MO, USA), supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich) and 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich). Protein concentration was determined by the Bradford method using a commercially available kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), as previously described (Piastowska-Ciesielska *et al.*, 2011).

For RNA isolation, cells were suspended in the TRIzol reagent (Life Technologies; Thermo Fisher Scientific, Inc.), and processed according to the manufacturer's protocol. Isolated RNA was diluted in 50μl of sterile deionised water. The RNA concentration was determined spectrophotometrically with Spectometer Lambda 25 UV/VIS (PerkinElmer, Waltham, MA, USA) in Tris-ethylenediaminetetraacetic acid (TE) buffer at 260 and 280 nm.

Western blot analysis. Protein samples were mixed with the Laemmli sample buffer (Sigma-Aldrich) and heated at 100°C for 3 min. Total of 60 μg of protein for FOXM1, and 30 μg of protein for CAV-1 and AT1-R, were separated electrophoretically on 12.5% sodium dodecyl sulphate-polyacrylamide gels (Bio-Rad Laboratories, Inc.), and then transferred to polyvinylidene fluoride membranes (Sigma-Aldrich) by semi-dry blotting (Whatman, Biometra GmbH, Göttingen, Germany). Membranes were visualized prior to blocking with a Ponceau S dye 0.1% solution in 5% CH_3COOH (Sigma-Aldrich). Subsequently, the membranes were blocked in 5% fat-free milk (Sigma-Aldrich) for 1 h prior to overnight incubation at 4°C with primary rabbit anti-CAV-1 polyclonal antibody (dilution 1:200; catalogue no. SC-894 Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit anti-FOXM1 polyclonal antibody (dilution 1:500, catalogue no. ABN286; Merck Millipore, Darmstadt, Germany) and rabbit anti-AT1-R polyclonal antibody (dilution 1:200; catalogue no. SC-1173; Santa Cruz Biotechnology, Inc.), which were diluted in 1% fat-free milk. As a reference, mouse anti-glyceraldehyde 3-phosphate dehydrogenase monoclonal antibody (GAPDH; dilution 1:1000; catalogue no. SC-59540; Santa Cruz Biotechnology, Inc.) was used. Next, membranes were washed three times for 15 min with Tris-buffered saline-Tween 20 (TBST) buffer, and incubated for 1h with secondary antibodies conjugated with alkaline phosphatase: anti-rabbit IgG (catalogue no. A3687), anti-mouse IgG (catalogue no. A3562) (dilutions 1:15000; Sigma-Aldrich). Following three washes in TBST buffer for 15 min, the bands were visualized using Novex® AP Chromogenic Substrate (BCIP/NBT) (Life Technologies; Thermo Fisher Scientific, Inc.). Densitometric analysis of protein expression levels was conducted as previously described, using ImageJ version 1.34 software (http://rsb.info.nih.gov/ij/; National Institutes of Health, Bethesda, MD, USA) (Piastowska-Ciesielska *et al.*, 2014). The results of western blot analysis were calculated as the ratio of CAV-1, FOXM1 and AT1-R expression, compared to the GAPDH expression.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Complementary DNA was transcribed from 5 μg of total RNA using ImProm RT-II™ Reverse Transcription System (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. RT was conducted in a LightCycler® 480 instrument (Roche Diagnostics, Basel, Swit-

Table 1. Sequences of primers used in this study.

Gene	Sequence of primers:	Product size [bp]
AT1-R	sense primer: 5′ATTCGACCCAGGTGATCAAA3′ antisense primer: 5′CCACCAAGCTGTTTCCAAAT3′	168
CAV-1	sense primer:5′AGTGCATCAGCCGTGTCTATTCCA3′ antisense primer: 5′TCTGCAAGTTGATGCGGACATTGC3′	102
FOXM1	sense primer: 5′ TGCCCAGCAGTCTCTTACCT 3′ antisense primer: 5′ CTACCCACCTTCTGGCAGTC 3′	139
H3F3A	sense primer: 5′AGGACTTTAAAAGATCTGCGCTTCCAGAG3′ antisense primer: 5′ACCAGATAGGCCTCACTTGCCTCCTGC3′	76
RPS17	sense primer: 5′AAGCGCGTGTGCGAGGAGATCG3′ antisense primer: 5′TCGCTTCATCAGATGCGTGACATAACCTG3′	87

AT1-R, angiotensin II type 1 receptor; CAV-1, caveolin-1; FOXM1, forkhead box M1; H3F3A, H3 histone, family 3A; RPS17, ribosomal protein S17

zerland), as previously described (Piastowska-Ciesielska *et al*., 2013b). Primers were designed using Primer3 106 version 0.4.0 software (http://frodo.wi.mit.edu/). qPCR was performed using a LightCycler® FastStartDNA Master 108 SYBR Green I kit (Roche Diagnostics), according to the manufacturer's protocol. Universal Human reference RNA (Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA) was used as a calibrator for each reaction. The relative expression of CAV-1, FOXM1 and AT1-R was normalized to the expression of the reference genes: ribosomal protein S17 (RPS17) and H3 histone, family 3A (H3F3A). The sequences of the primer pairs used are listed in Table 1. In order to avoid detection of non-specific products, melting curve analysis was performed for each reaction, and the qPCR data were analysed according to the Roche algorithm (Pfaffl *et al*., 2002). Samples were evaluated from ≥ 4 replicates.

Statistical analysis. Data were expressed as the mean $\pm$ standard error. Statistical differences between the samples were analysed according to their metastatic potential using the Aspin-Welsch *t*-test. *P*-values were calculated using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference. Correlations between all the investigated genes were calculated by the Spearman's rank test ($P<0.05$).

RESULTS

CAV-1, FOXM1 and AT1-R expression in prostate cancer cell lines

In prostate cell lines, a strong and significant correlation was noted between the messenger (m)RNA expression levels of CAV-1 and AT1-R [Spearman correlation coefficient (rs)=0.6290; $P=0.0067$], and between the mRNA expression levels of FOXM1 and CAV-1 (rs=0.6719; $P=0.0016$). The correlation between AT1-R and FOXM1 was also positive, but not significant (rs=0.3143; $P>0.05$). The mean mRNA expression levels of the investigated genes and their statistical association with metastatic properties are presented in Tables 2 and 3, respectively.

A correlation between the expression levels of the investigated genes and their metastatic potential was noticed, with a relatively high expression of all the genes tested in the normal prostate cell line PNT1A. When considering only cancer cell lines, the lowest expression levels of all the investigated genes was observed for the LNCaP cell line, which also had the lowest invasiveness capacity. CAV-1 expression was the highest in the DU145 cell line, while the expression of AT1-R and FOXM1 was comparable between PC3 and DU145 cells.

The protein expression profile of AT1-R suggests the influence of post-transcriptional mechanisms, resulting in relatively high protein expression levels in the LNCaP cell line ($P<0.01$). The remaining cell lines expressed comparable levels of AT1-R, which were in general consistent with its mRNA expression profile. Significant differences were observed between the DU145 and LNCaP cell lines ($P<0.01$) (Fig. 1A). CAV-1 protein expression was the highest in DU145 cells, while it was the lowest

Table 2. Mean expression levels of CAV-1, AT1-R and FOXM1 obtained by reverse transcription-quantitative polymerase chain reaction.
Metastatic potential was determined based on the well-documented properties of these genes, geometrical mean of H3F3A and RPS17 were used as control.

		Mean expression level		
Cell line	Metastasis potential	AT1-R	CAV-1	FOXM1
PNT1A	0	9.49	4.28	6.80
PC3	medium	30.00	3.60	8.06
DU-145	high	29.89	24.45	6.50
LNCaP	low	3.25	0.05	0.69

CAV-1, caveolin-1; AT1-R, angiotensin II type 1 receptor; FOXM1, forkhead box M1.

Table 3. Statistical significance of the expression levels of various genes in respect to their metastatic potential, as obtained by reverse transcription-quantitative polymerase chain reaction analysis in prostate normal and cancer cell lines.

Metastasis potential	*P*-value		
	CAV-1	AT1-R	FOXM1
0 *vs*. low	0.004	ns	0.005
0 *vs*. high	0.007	0.027	ns
Low *vs*. high	0.0035	0.01	0.031
Medium *vs*. low	ns	ns	0.019
Medium *vs*. 0	ns	ns	ns
Medium *vs*. high	0.0058	ns	ns

ns, not significant; CAV-1, caveolin-1; AT1-R, angiotensin II type 1 receptor; FOXM1, forkhead box M1.

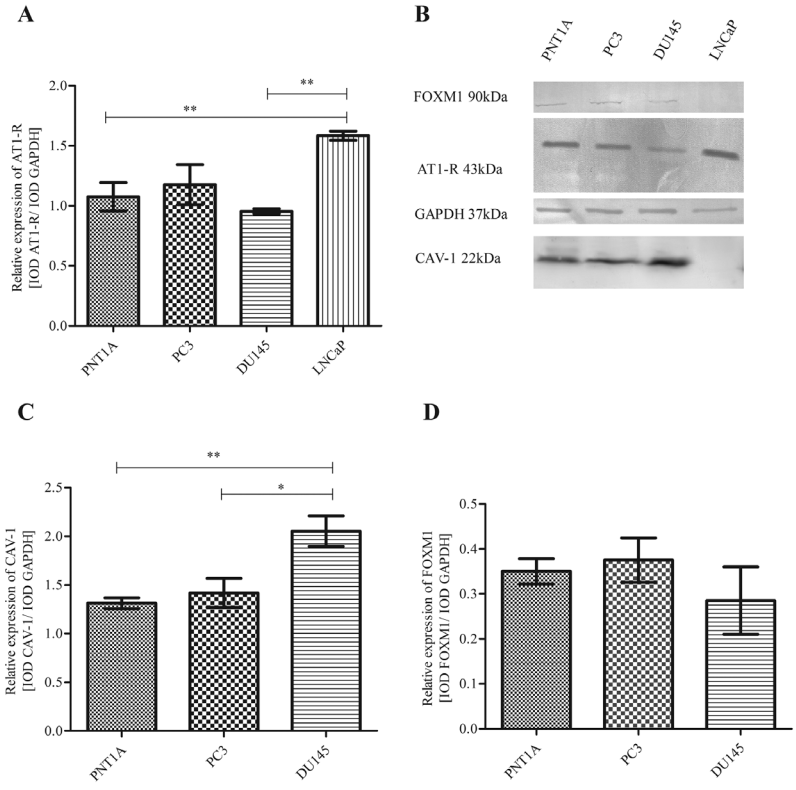

Figure 1. Expression of AT1-R, CAV-1 and FOXM1 in normal prostate and prostate cancer cell lines, as obtained by western blotting. (**B**) Representative images of western blot analysis. The relative expression of (**A**) AT1-R, (**C**) CAV-1 and (**D**) FOXM1 was determined by comparing their protein expression levels to those of GAPDH. **P*<0.05; ***P*<0.01. The experiment was conducted in triplicate. AT1-R, angiotensin II type 1 receptor; CAV-1, caveolin-1; FOXM1, forkhead box M1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

in the PC3 and PNT1A cells. High expression of CAV-1 was also observed in the DU145 cells.

Significant differences between the PNT1A and DU145 cells ($P<0.01$), and between PC3 and DU145 cells ($P<0.05$) were observed (Fig. 1C). FOXM1 protein expression was consistent with its mRNA expression pattern (Fig. 1D).

CAV-1, FOXM1 and AT1-R expression in breast cancer cell lines

In breast cell lines, a significant and positive correlation between the mRNA levels of FOXM1 and CAV-1 (rs=0.4900; $P=0.0500$) was observed, in addition to a tendency in correlation between the mRNA levels of FOXM1 and AT1-R (rs=0.5175; $P>0.05$), and those of CAV-1 and AT1-R (rs=0.3290; $P>0.05$). The mean expression levels of the investigated genes are presented in Table 4. However, in terms of invasiveness and metastatic potential, negative correlations were noticed. Thus, the normal cell line 184A1 exhibited the highest expression of CAV-1, FOXM1 and AT1-R (mean expression levels, 56.1, 9.4 and 44.0, respectively). The decrease of CAV-1 expression in the MDA-MB-231 and MCF-7 cells, compared with normal breast cells, was significant. The difference in expression of all the investigated genes between the two cancer cell lines was inversely correlated with their metastatic potential. Thus, the MDA-MB-231 cell line, which is characterised as having a low metastatic potential, exhibited a significantly higher expression of CAV-1 and FOXM1, in addition to 2-fold increased expression of AT1-R, although this was not significant (Table 4).

At the protein level, AT1-R expression was the highest in the normal breast cell line 184A1, and lower in MDA-MB-231 and MCF-7 cells. Significant differences in expression between 184A1 and MDA-MB-231 cells ($P<0.05$), and between 184A1 and MCF-7 cells ($P<0.01$) were observed (Fig. 2A). The apparent discrepancies observed

Table 4. Messenger RNA expression levels of AT1-R, CAV-1 and FOXM1 in normal breast and breast cancer cell lines.
Differences in expression were statistically analysed according to the metastatic potential of these cell lines, which was determined based on their well-documented properties.

		Mean expression level			Metastasis potential in normal breast and breast cancer cell lines	*P*-value		
Cell line	Metastasis potential	CAV-1	AT1-R	FOXM1		CAV-1	AT1-R	FOXM1
184A1	0	56.1	44	9.4	0 *vs.* low	0.03	ns	ns
MDA-MB-231	low	24.7	45.5	17.2	0 *vs.* high	0.002	ns	0.007
MCF-7	high	0.5	27.1	4.2	low *vs.* high	0.02	ns	0.03

ns, not significant; CAV-1, caveolin-1; AT1-R, angiotensin II type 1 receptor; FOXM1, forkhead box M1

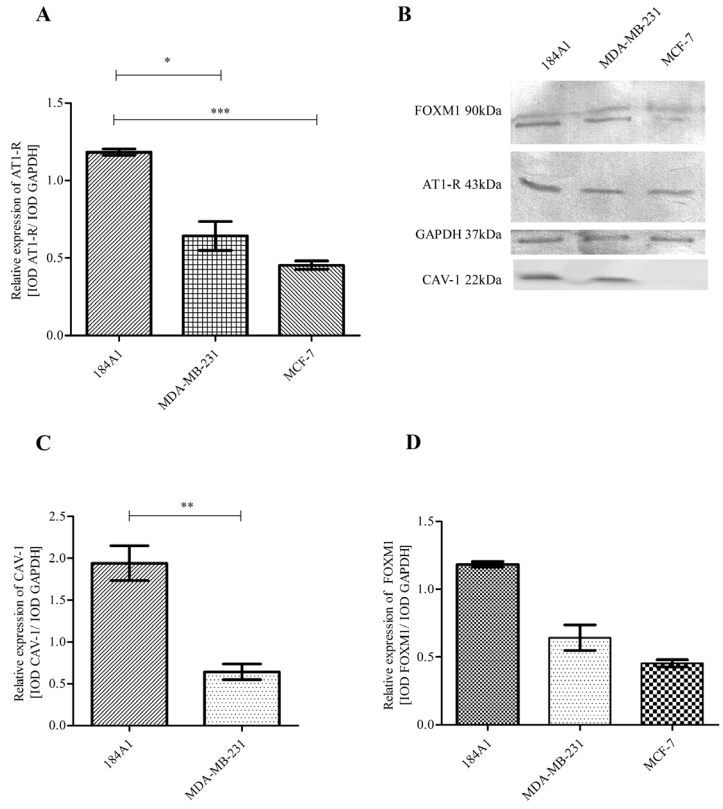

Figure 2. Expression of AT1-R, CAV-1 and FOXM1 in normal breast and breast cancer cell lines, as obtained by western blotting. (**B**) Representative images of western blot analysis. The relative expression of (**A**) AT1-R, (**C**) CAV-1 and (**D**) FOXM1 was determined by comparing their protein expression levels to those of GAPDH. **P*<0.05; ***P*<0.01; ****P*<0.001. The experiment was conducted in triplicate. AT1-R, angiotensin II type 1 receptor; CAV-1, caveolin-1; FOXM1, forkhead box M1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

between the mRNA and protein expression patterns may be associated with post-transcriptional modifications in gene expression profiles. The pattern of CAV-1 protein expression was similar to its mRNA expression pattern, with no detectable expression in MCF-7 cells (Fig. 2C). The difference in CAV-1 expression between 184A1 and MDA-MB-231 cells was significant ($P<0.01$). The highest protein expression levels of FOXM1 were detected in the normal breast cell line 184A1, compared with decreased expression levels observed in MDA-MB-231 and the lowest level in the MCF-7 cells (Fig. 2D).

DISCUSSION

Since its identification, the function of CAV-1 in tumorigenesis has been disputable. It is well-documented that CAV-1 has a dual role in regulating cancerogenesis (Grande-Garcia & Del Pozo, 2008; Senetta *et al.*, 2013). It may act as a tumour suppressor in breast cancer, where it downregulates numerous oncogenes such as Src or Ras (Senetta *et al.*, 2013). In contrast, tumour promoter properties of CAV-1 have been associated with the regulation of cyclin D1 expression and cell cycle progression via interaction with Stat3 oncoprotein, EGF signalling or as a transducer of cell signalling by Rho-guanosine triphosphatases (Grande-Garcia & Del Pozo, 2008). Furthermore, as a part of caveolae, CAV-1 also participates in endocytosis and signal transduction in cells (Senetta *et al.*, 2013). Interactions between CAV-1, MMP2 and MMP9 are key factors in cell motility and dynamics, which, simultaneously with VEGF-induced angiogenesis, may lead to tumour metastasis (Goetz *et al.*, 2008; Han & Zhu, 2010). The antimetastatic function of CAV-1 has been also reported for brain tumours, where CAV-1 suppressed the process of metastasis via inhibition of the Stat3 oncoprotein (Chiu *et al.*, 2011). Thus, CAV-1 is considered to act as a tumour suppressor in the early stages of tumour development, whereas in more advanced stages it promotes tumorigenesis (Senetta *et al.*, 2013). In prostate cancer, CAV-1 has been reported to be a potential high-risk marker (Gumulec *et al.*, 2012). In the study presented here, an increase in the CAV-1 expression at the protein and mRNA levels was observed to be associated with the metastatic potential of the prostate cell lines. The DU145 cell line, which is derived from a metastatic central nervous system lesion, and is known to possess a higher metastatic potential than LNCaP or PC3 cells (Dominska *et al.*, 2012), exhibited the highest expression levels of CAV-1 in this study. This observation may indicate that high CAV-1 expression may be one of the factors contributing to the development of increased cell invasiveness. However, CAV-1 expression in the PC3 and LNCaP cancer cells was significantly lower than in normal PNT1A cells. This fact may indicate that CAV-1 is required for the correct functioning of normal prostate cells, but the oncogenic transformation of these cells may result in the enhancement of the tumour promoter properties of CAV-1. Contrary to the findings in the aforementioned prostate cell line, a negative correlation between CAV-1 expression and metastatic potential was observed for breast cell lines, with the exception of 184A1, the normal breast cell line tested in the present study, which exhibited the highest expression levels of CAV-1, both at the mRNA and protein levels, supporting the hypothesis that CAV-1 is required for the correct functioning of normal cells.

In breast cancer, CAV-1 was reported to potentially increase angiogenesis and metastasis by activation of the protein kinase C, MAPKs and phosphoinositide 3-kinase/Akt (Uemura *et al*., 2008b). Contrary to this assumption, western blotting and RT-qPCR results of the study presented here have demonstrated relatively high expression levels of CAV-1 in normal breast cells and the less aggressive MDA-MB-231 cancer cell line, which were higher than in the MCF-7 cancer cells, indicating that CAV-1 does not exert a tumorigenic role in breast cancer. These observations are consistent with the fact that CAV-1 has been reported to act as a tumour suppressor in breast cancer (Chiu *et al*., 2011).

In the study presented here, the expression of other genes assumed to co-participate in the development of the invasiveness potential of cancer cells and possibly participating in CAV-1 signalling, was also investigated. One of these candidate genes is Ang II, which has been suggested to participate in cancer metastasis and invasiveness (Dominska *et al*., 2012). Ang II increases the proliferation of breast cells and influences angiogenesis *via* AT1-R (Jethon *et al*., 2012). Ang II was also reported to modulate the migration of LNCaP cells (Dominska *et al*., 2012). Furthermore, AT1-R is known to modulate the viability of prostate cancer cells (Dominska *et al*., 2009), and its blockers may cause a beneficial effect on tumour progression and metastasis (Uemura *et al*., 2008b). Our study revealed here a positive correlation between CAV-1 and AT1-R expression, and observed a similar expression profile for CAV-1 and AT1-R in the two types of tumour cell lines evaluated. The highest expression levels of AT1-R were observed in the androgen-positive PC3 and DU145 cell lines, while the lowest expression levels of AT1-R were observed in androgen-negative LNCaP cells (Cariaga-Martinez *et al*., 2013). In the normal cell line PNT1A, the expression of AT1-R was ~3-fold lower than in the highly metastatic cell lines PC3 and DU145. The protein expression levels of AT1-R were the highest in the LNCaP cells when compared with PC3, PNT1A and DU145 cells, which displayed lower expression levels of AT1-R. The relatively high expression levels of AT1-R in the prostate cancer cell line LNCaP may not be associated with proliferation and migration. Kosaka and coworkers (2007) *et al*. observed that blockers of AT1-R did not alter the proliferation of LNCaP cells, indicating that AT1-R in this prostate cancer cell line is not associated with proliferation. In breast cancer, the expression pattern of AT1-R was similar to that of CAV-1, indicating that the expression of AT1-R decreases with increasing metastatic potential, although it should be considered whether the expression levels of AT1-R correlate with the response to Ang II in these cells.

The FOXM1 transcription factor appears to be associated with the AT1-R and CAV-1 genes, according to the results presented here. In the two types of cell lines investigated, a positive and significant correlation between FOXM1 and CAV-1 expression, and a strong coexpression tendency with AT1-R, was noticed. The expression pattern of FOXM1 according to metastatic potential is consistent with that observed for AT1-R and CAV-1, with a significant increase in expression in prostate cancer cell lines in relation to metastasis, and a decrease in expression in breast cancer cell lines in relation to metastasis, while normal breast and prostate cell lines exhibited relatively high FOXM1 expression. FOXM1 was demonstrated to be involved in the regulation of cellular processes characteristic of cancer (Halasi and Gartel, 2013). In addition, numerous correlations between FOXM1 and cancer-associated genes have been identified (Xu *et al*., 2015), including direct coregulation between FOXM1 and CAV-1 (Huang *et al*., 2012). Huang *et al* (Huang *et al*., 2012) reported that the expression levels of CAV-1 directly correlate with those of FOXM1 in pancreatic cancer cell lines and tumour samples. Notably, this correlation is direct and positive, indicating that overexpression of FOXM1 leads to upregulation of CAV-1, while knockdown of FOXM1 produces the opposite effect (Huang *et al*., 2012). The results of the study presented here confirm the existence of this FOXM1-CAV-1 signalling pathway in the two types of cell lines investigated, in spite of the correlation with metastatic properties being adverse. This discrepancy may be due to the dual functional properties of CAV-1, but the present results confirm the close functional connection that exists between the FOXM1 and CAV-1 genes. Furthermore, the strong tendency in association of FOXM1 and AT1-R expression, shown here for the first time, suggests that AT1-R may be another target gene for FOXM1, and may possibly be a third player in the FOXM1-CAV-1 axis, thus influencing the functioning of these genes. However, this assumption must be further investigated to determine if the correlation is direct or indirect.

The study presented here also revealed that all the investigated genes (CAV-1, FOXM1 and AT1-R) exhibited comparable high expression levels in normal prostate and breast cell lines. This may indicate that all of these genes are required for proper cell functioning, and during the initiation of cancerogenesis they are timely inactivated, thus enabling the cell to be redirected to the cancer progression pathway. However, these observations may be also due to cell line specificity, as a result of the immortalization method employed to establish the different cell lines.

In conclusion, the results presented here indicate that CAV-1, FOXM1 and AT1-R are potential markers of tumorigenesis, depending on the type of cancer and its characteristics *in vitro*. The relative expression pattern of these genes appears to influence the metastatic potential of the cells. However, the complexity of their molecular functions appears to influence these features. CAV-1, FOXM1 and AT1-R exhibit similar, although contradictory expression pattern in breast and prostate cancer cell lines, and AT1-R seems to be part of the CAV-1 and FOXM1 signalling pathway, although the association between FOXM1 and AT1-R must be further investigated.

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